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The Role of Quantitative CT Image Analysis in Understanding COPD

by

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Abstract

COPD is a heterogeneous disease with multiple clinical phenotypes and biological endotypes and the association between these are poorly understood. Novel quantitative analysis techniques permit objective measurements of pulmonary and extra-pulmonary manifestations of the disease. This thesis examines the role of quantitative CT analysis in exploring COPD, with the aim of showing that these techniques can potentially give important insights into the disease and explore the heterogeneity and underlying biology of the condition.

CT image analysis was performed on two different cohorts of COPD subjects, which included quantification of emphysema, small airways disease and bronchial wall dimensions. Furthermore, novel analysis was also performed to quantify emphysema sub-types and body composition parameters. One of the cohorts was a small cross-sectional study of mild-moderate COPD subjects involving CT imaging and bronchoscopic sampling of the airways. BAL samples were analysed for inflammatory cytokines, white cell differential, matrix-metalloproteinases (MMPs) and microbiology. The other cohort was a larger 2 year longitudinal study of moderate-very severe COPD subjects who had CT imaging at enrolment and at 2 years. They also had careful characterisation with pulmonary function testing, exercise testing, sputum and blood analysis and clinical follow-up.

The findings confirmed that CT image analysis could successfully measure emphysema and small airways disease with both having significant relationships with pulmonary function. Using these measures, I demonstrated a novel relationship between multiple MMPs and CT measured small airways disease, suggesting these proteases play an important role in the small airway remodelling that occurs in COPD. There were also associations between emphysema and MMPs, providing further evidence for their role in parenchymal destruction. In the larger cohort emphysema was independently associated with desaturation on exertion, but neither emphysema nor small airways disease had significant associations with blood or sputum inflammatory markers, sputum bacterial detection or exacerbation rate. On longitudinal analysis, emphysema progressed over a two period, although the causes for this were not apparent in my study. Quantification of airway dimensions were variable and unreliable and did not provide significant information about disease in either of my two cohorts.

The findings from this study suggest that CT image analysis can be used to explore the disease and provides further rationale for future investigation into the use of image analysis in assessing different features of COPD.

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DECLARATION OF AUTHORSHIP

I, Kristoffer Ostridge declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The Role of Quantitative CT Image Analysis in Understanding Disease Mechanisms of COPD

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as.

Signed: .....

Date: .....

Authorship

My thesis consists of work from two different studies. For the bronchoscopy study (MICA study) I was involved in the conception and design of the study, data acquisition, analysis and interpretation. Specifically, I screened and enrolled subjects and completed multiple study visits including clinical and physiological assessments. I also performed all CT analysis and bronchoscopies and analyzed bronchoalveolar lavage samples by quantitating proteinases and inflammatory cytokines. The second study was a large collaboration between GSK, the university of Southampton and university hospital Southampton. I was involved in data capture, by running study visits, capturing exacerbation data and performing clinical and physiological assessments on study subjects. I was responsible for the CT image analysis developed and performed throughout the study and the automated CT analysis was performed either by myself or the VIDA imaging laboratory. For both studies I was integrally involved in the data analysis and the analysis presented in this thesis is all my own work.

To develop my skills in CT image analysis I was awarded a Scadding-Morriston Davies joint fellowship in respiratory medicine in order to spend a 5-week period at the Harvard chest imaging laboratory led by Dr George Washko. This led to the novel CT analysis presented in this thesis on emphysema sub-types and body composition in COPD.

Acknowledgements

I wish to thank my supervisor Professor Tom Wilkinson for the invaluable support, supervision and guidance provided to me throughout my PhD. I am also very grateful to Dr Karl Staples who also provided much-needed guidance and helped with sample collection and processing in the bronchoscopy study.

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samples for microbiological assessment. I am also grateful for the statistical support provided to me by Dr Ngaire Coombs.

Funding for this work was provided by GSK and I am therefore extremely grateful for this important collaboration. They also provided resources and expertise as well as analysing sputum samples for PCR detection of bacteria and blood samples for novel biomarkers. I would like to thank VIDA for the collaboration and use of their image analysis software, on which the whole of this project was based. I would also like to thank the Harvard chest imaging laboratory run by Dr George Washko for welcoming me to Boston and their collaboration on CT measured emphysema sub-types and body composition.

I am also thankful for the generous participation of all the research volunteers involved in my studies and hope we strive to improve their lives with our discoveries and research. Finally to my wife, Jen for all the love and support that she has offered without which none of this would have been achieved.

Definitions and Abbreviations

$\%LAA_{<-950}$	low attenuation area under -950 Hounsfield units as a measure of emphysema
$\%LAA_{<-910}$	low attenuation area under -910 Hounsfield units as a measure of emphysema
$\%LAA_{<-856}$	low attenuation area under -910 Hounsfield units as a measure of emphysema
$\%LAA_{EXP<-856}$	low attenuation area under -856 Hounsfield units on an expiratory CT as a measure of gas trapping
$\%LAA_{EXP<-856-950}$	low attenuation area between -856 and -950 Hounsfield units on an expiratory CT as a measure of gas trapping
A1AT	alpha-1-antitrypsin
BAL	bronchoalveolar lavage
CLE	centrilobular emphysema
COPD	Chronic Obstructive Pulmonary Disease
CT	computed tomography
CXCL1	chemokine ligand-1
ECM	extracellular matrix
E/I MLD	mean lung attenuation in expiration/mean lung attenuation in inspiration on CT as a measure of gas trapping
FEV1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GOLD	global initiative for obstructive lung disease
IL	interleukin
LAA	low attenuation area
LTB4	leukotriene B4
LVRS	lung volume reduction surgery

MCAT	Moraxella catarrhalis
MCP-1	monocyte chemoattractant protein 1
MMP	matrix metalloproteinase
NE	neutrophil elastase
NTHI	non-typeable Haemophilus influenza
PA	Pseudomonas aeruginosa
PCR	polymerase chain reaction
$PERC_{15}$	the 15^{th} percentile in the CT attenuation histogram
Pi10	A standardised measure of airway wall dimensions
PLE	panlobular emphysema
PLF	preserved lung function
PPM	potentially pathogenic microorganisms
PSE	paraseptal emphysema
ROS	reactive oxygen species
RV	residual volume
RVC	residual volume change, a CT measure of gas trapping
SA	Staphylococcus auras
SP	Streptococcus pneumoniae
TGF- β	Transforming growth factor-β
TIMP	Tissue inhibitor of MMPs
TLC	total lung capacity
TLCO	Carbon monoxide gas transfer
TNF-α	tumour necrosis factor-α

Chapter 1: Review of the Literature

1.1 Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is characterised by persistent airflow obstruction that is usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung to noxious particles or gases[1]. It is now the third most common cause of death worldwide[2] and is associated with significant morbidity and economic burden. Worldwide prevalence is estimated to be around 10%[3].

Exposure to cigarette smoke is the principle environmental risk factor and cause for COPD[4] and is estimated to account for up to 80 – 90% of cases within the developed world. There are also a number of other risk factors including the burning of biofuels, occupational exposure and pollution[5,6]. Despite being the main risk factor only 25% of smokers develop COPD[7], leading to the idea that the disease results from a number of complex host-environment interactions.

1.2 Disease Phenotypes

Although COPD is defined by airflow obstruction it is a heterogeneous disease with multiple pulmonary and extra pulmonary manifestations. The clinical features and natural history of COPD vary considerably and even within individuals there is marked variation in disease distribution throughout the lungs, with preserved lung juxtaposed to regions of severe damage. Conventional methods of measuring airflow obstruction do not reflect the complexity of the condition.

The concept that there are various forms of COPD is not a new one as chronic bronchitis and emphysema were described long before the term COPD was used[8]. More recently there has been the growing recognition of the need to subtype COPD into clinically meaningful phenotypes. It has been suggested that a phenotype in COPD should be a single or combination of disease attributes that describe differences between individuals with COPD as they relate to clinically meaningful outcomes[9]. A variety of methods have been used to explore the different phenotypes with a number of large epidemiological studies being performed[10–12]. Many different phenotypes have been suggested in COPD, although in reality there has only been a limited amount of success at identifying clinically meaningful ones. Two examples of clinical phenotypes are hypoxic patients

who have a survival benefit with long-term oxygen therapy[13] and patients with upper lobe emphysema and poor exercise tolerance who benefit from lung-volume reduction surgery (LVRS)[14].

The limited amount of success in defining clinically important phenotypes is down to a number of different issues. Firstly there is not a consensus within the COPD community about what constitutes a phenotype and phenotypic trait. Some of the characteristics used to define phenotypes can be a symptom, sign or outcome marker of disease. There can also be considerable overlap between phenotypes with patients belonging to more than one group. One of the biggest limitations is that we do not really understand the underlying pathobiological mechanisms of the disease and certainly not how these contribute and lead to the different phenotypic traits. For example, we describe the group with severe upper lobe emphysema but have little understanding of the underlying mechanisms of emphysema or why some patients develop this heterogeneous distribution. The term endotypes has been coined for these different mechanisms and it is the understanding of how different endotypes cause the varying phenotypic traits that are key for understanding the heterogeneity of COPD. It would be hoped that phenotypes have similar pathobiologic mechanisms with which to target treatment.

Currently, treatment options for COPD have encompassed using inhaled bronchodilators and corticosteroids relatively indiscriminately. Treatment has not been targeted at specific phenotypes or endotypes and this is mainly because of our poor understanding of the underlying causes of the disease and the associated phenotypic traits. The heterogeneity of disease has also led to challenges in designing clinical studies, which has impaired drug discovery. Despite this, novel drugs that are more specific to particular disease endotypes are being developed and it is therefore vital that we are able to define the population that will benefit from these medications and bring COPD into the era of personalised medicine.

Imaging techniques such as computed tomography (CT) along with automated quantitative analysis techniques have the potential to provide significant information about COPD phenotypes. By combining this with investigation of underlying disease mechanisms and outcome markers we may further our understanding of the association between endotypes and phenotypic traits.

1.3 Natural History of COPD

The characteristic clinical features of COPD consists of chronic progressive dyspnoea, cough and sputum production[1]. The natural history is of progression of symptoms and deterioration in lung

function and functional ability[15]. COPD is also a systemic condition with extra-pulmonary manifestations leading to multiple comorbidities, coronary artery disease and malnutrition.

The chronic symptoms of COPD are punctuated by acute deteriorations termed exacerbations, which are responsible for significant morbidity and mortality. There are a number of different causes for exacerbations, although a significant proportion are attributed to viral or bacterial infection[16,17]. Exacerbations are associated with increased airways inflammation[17,18] and recurrent exacerbations are associated with increased airway inflammation and physiological deterioration[18].

As already stated COPD is heterogeneous disease and therefore the clinical features vary considerably. The prognosis and disease trajectories can also be very different with some subjects remaining stable and others having rapid deterioration. It is obviously very important that the reasons for this are understood more fully than at present.

1.4 Pathogenesis of COPD

There are a number of key underlying mechanisms leading to the characteristic changes seen within COPD. These include inflammation, proteinases, chronic infection and oxidative stress, which will be briefly discussed in this section.

1.4.1 Inflammation

COPD is characterised by an abnormal inflammatory response in the lung to noxious particles or gases, resulting in remodelling of the airways and tissue destruction. Inhalation of Cigarette smoke is associated with inflammation and results in the influx of a number of different inflammatory cells within the lungs[19–22]. In susceptible smokers this is up regulated or enhanced leading to the chronic inflammatory changes seen in COPD subjects. Infiltrating inflammatory cells in COPD include neutrophils, macrophages and CD8+ T lymphocytes and are likely to drive a number of pathways responsible for lung damage including the release of pro-inflammatory cytokines, proteinases and mediators of oxidative stress. It has been demonstrated that in COPD subjects this chronic inflammation persists despite smoking cessation[23].

Neutrophils have been implicated as the dominant infiltrating inflammatory cell in COPD as they are found in the airways in increased numbers and are related to disease severity[19,20,24] and smoking history. A number of mediators are involved in recruiting neutrophils including leukotriene B4 (LTB4), interleukin-8 (IL-8) and chemokine ligand-1 (CXCL1). Although neutrophils have an

important function for fighting infection they are also thought to contribute to the typical lung damage seen in COPD by releasing inflammatory cytokines and proteinases. Alveolar macrophages have also been implicated in the tissue damage that occurs in COPD. They are found in increased concentrations in the airways and lung parenchyma in COPD[25] and their numbers correlate with COPD severity[24]. It is thought that they are recruited from circulating monocytes and can release inflammatory mediators including tumour necrosis factor-α (TNF-α), IL-8, CXC-chemokines, monocyte chemoattractant protein 1 (MCP-1) and LTB4 and reactive oxygen species. They also release a number of elastolytic enzymes including matrix metalloproteinases (MMPs) and cathepsins[25]. Subjects with COPD have also been shown to have increased T cells which are mainly of the CD8+ cells[21,22]. These activated T cells release perforins and granzymes which cause cell death and can activate apoptotic pathways[22] and also release chemotactic factors to further amplify the inflammatory response.

1.4.2 Proteinases

The lungs are given their structure by the pulmonary extracellular matrix (ECM), which is composed of collagen, elastin, proteoglycans, fibronectin and laminin. These molecules form a mesh, providing cell support along with multiple other functions and are found throughout the lungs and bronchial tree. Proteinases have the potential to cause significant lung damage as they are able to degrade key components of the pulmonary ECM and so a proteinase/antiproteinase imbalance has been postulated as a key contributor to emphysema, with a number of candidate mechanisms being proposed[26]. The classic example is alpha-1-antitrypsin (A1AT) where unopposed neutrophil elastase (NE) activity causes tissue destruction. A number of different proteinases can be found within the human lungs, including NE, cathepsin, proteinase-3 (PR-3) and MMPs. All of these can be produced by inflammatory cells such as macrophages and neutrophils and also bronchial epithelial cells in COPD.

NE is able to degrade all components of the pulmonary ECM and given its role in A1AT it has been proposed as key contributor of lung damage seen in COPD. However the evidence for this is not overwhelming with only a small number of studies showing increased NE concentration or activity in COPD[27,28]. One of the complexities about measuring NE in vivo is that it is rapidly inhibited by A1AT and so Carter et al measured a specific breakdown product from NE and found this was related to radiological and physiological markers of disease[29]. Neutrophil elastase inhibitors have not been particularly successful at preventing disease progression or lung damage[30], supporting the idea that other proteinases may be more important in COPD. PR-3, another serine protease has been found in significant quantities in sputum from COPD subjects and was found to have more activity than NE[31]. Cathepsins are another group of proteases that have also been implicate in the

tissue destruction seen in COPD as they have been shown to be increased in COPD[32] and inversely associated with airflow obstruction[33].

There has been particular recent interest in the role of MMPs in COPD. These are a large family of zinc-dependent proteolytic enzymes, which have the ability to degrade the pulmonary ECM and have therefore been implicated in COPD[26]. These enzymes are broadly grouped depending on their substrate specificity, including collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11) and elastases (MMP-7 and -12)[34], although there is considerable overlap. MMPs are not normally expressed in healthy tissue but in disease can be produced by alveolar macrophages, neutrophils and bronchial epithelial cells[34].

In animal models, transgenic mice over expressing MMP-1 develop emphysema at an accelerated rate[35]. Selective inhibition of MMP-9 and -12 in guinea pigs reduced the extent of emphysema when these animals were exposed to smoke[36]. Human studies have demonstrated increased expression of MMP-1[37,38], -8[38,39], -9[37–42] and -12[37,43,44] in the sputum or bronchoalveolar lavage (BAL) of COPD subjects. MMP-9 and -12 have been shown to have significant correlations with quantitative measures of emphysematous change on CT[40,43].

MMP biology is however complex with multi-step activation and numerous molecular interactions[45], and the most important MMPs have yet to be determined in COPD. In addition, MMPs have multiple other functions that are independent of their ability to degrade the ECM, including facilitating cell migration and activating growth factors and cytokines[34]. Most studies investigating MMPs in COPD have only investigated a subset of MMPs. Given their complexity, novel multiplex-based arrays provide the opportunity to profile a broad spectrum of MMPs in a carefully characterised clinical cohort. The key sites of MMP activity in the lung are unknown and it is likely that matrix turnover and remodelling differs between regions. Detailed analysis of the MMP profile has not been integrated with systematic characterisation of lung pathology by HRCT and lung function

A further complicating factor in understanding the role of MMPs in emphysema is the role of proteinase inhibitors. MMPs are tightly regulated by endogenous inhibitors, the four Tissue inhibitors of MMPs (TIMPs)[34]. TIMP-3 null mice develop emphysema[46] while human studies show TIMP-1 and -2 are raised in the airways of COPD subjects[37,38,42,43] and TIMP-2 polymorphisms are associated with CLB emphsyema[47]. Sputum MMP-9/TIMP-1 ratio has been found to be significantly raised in COPD[42], although other studies do not show increased airway ratios of MMPs/TIMPs[37,43,48]. There is however, a school of thought which states that MMPs act in the immediate pericellular environment and are therefore not inhibited by TIMPs[49].

1.4.3 Pulmonary Infection

Chronic pulmonary infections are an important feature of COPD as the innate defences of the lungs which protect against invading organism are disrupted by exposure to cigarette smoke and other environmental irritants. In the stable state 29-70% of sputum samples from COPD patients culture potentially pathogenic microorganism/bacteria (PPM)[16,50–55]. Originally this was felt to represent chronic colonisation however this term implies no lung damage or ill effect with the organism being just an innocent by-stander. Chronic bacterial growth in the airways of COPD subjects is associated with higher airway inflammation[16,53–58], more exacerbations[16,54] and rapid decline in lung function[53,59]. The term chronic infection therefore seems more appropriate. The most commonly cultured PPMs are Streptococcus pneumoniae (SP)[16,51,54,60–62]., Moraxella catarrhalis (MCAT)[16,51,60,61] and non-typeable Haemophilus influenzae (NTHI)[16,51,54,59–62]. Of these the most interest has revolved around NTHI, as it has been shown to persistently infect the airways and is linked to more intense airway inflammation, decline in lung function and exacerbations[53,55].

A significant number of exacerbations are linked to bacterial infection with PPMs being cultured in the airways of patients in over 50% of exacerbations[51,63,64]. Given the high rates of bacteria in the chronic state it has been difficult to prove a definitive link between bacteria and exacerbations. However, exacerbations associated with bacterial infection are associated with a greater inflammatory response[64,65]. More advanced microbiology techniques, such as polymerase chain reaction (PCR) and molecular techniques have also been utilised and have found evidence for increased bacterial load at exacerbation as well as strain changes[65,66]. Viruses are also a significant causative organism of COPD exacerbations, with respiratory viruses detected in 20-50% of acute exacerbations of COPD[64,67,68] .

1.4.4 Oxidative Stress

Oxidative stress is felt to be a key feature of COPD as the epithelial surface of the lung is particularly vulnerable to oxidant damage and cigarette smoke contains free radicals and other oxidants. Chronic smoking exposes the lungs to these reactive oxygen species (ROS), leading to oxidative stress and injury[69]. This in turn triggers production of other ROS, which act as direct messengers to propagate the inflammatory response in COPD[69]. ROS are capable of activating the nuclear transcription factors NF-κB and other signal transduction pathways which can activate pro-inflammatory cytokines[70].

1.5 Lung Compartments

COPD is characterised by destruction of the lung parenchyma, small airways disease and chronic bronchitis.

1.5.1 Small Airways Disease

The human airway consists of approximately 23 generations of branching airways from the trachea to the alveoli (Figure 1)[71]. The first 15 generations are conducting airways and are not involved in gaseous exchange and beyond this are the respiratory bronchioles or acinar airways. The small airways are defined as airways smaller than 2mm in diameter and are generally located after the 8th generation. They include a portion of the conducting airways as well as the acinar airways.

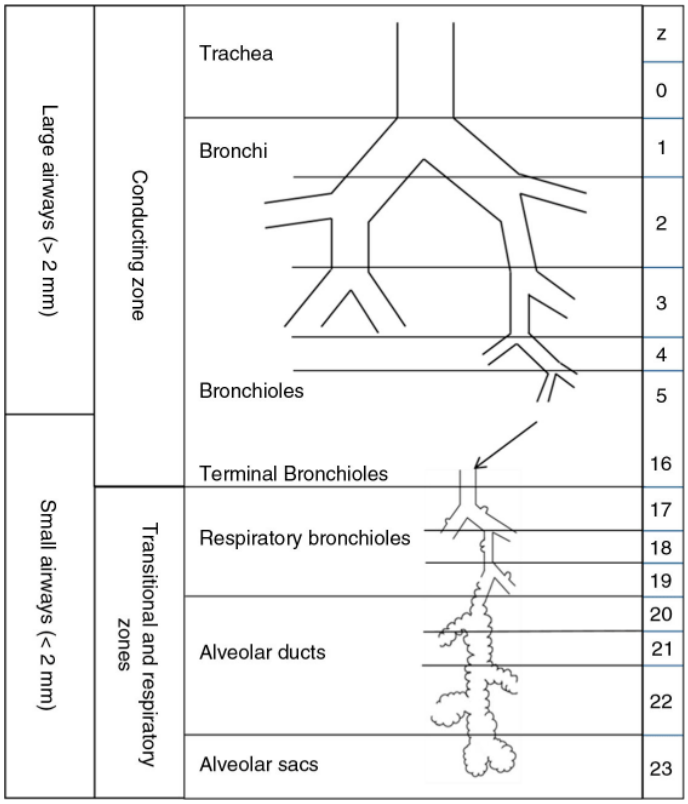


Figure 1. Diagrammatic representation of the airways (adapted from reference 71)

Unlike the larger airways the small airways lack cartilaginous support and therefore depend on the outward tensile strength provided by surrounding lung parenchyma to maintain patency. They also lack mucous glands and are lined by surfactant, which reduces surface tension, preventing the lungs from closing on expiration. There is an exponential increase in airway numbers with each successive generation leading to a massive increase in cross-sectional area and therefore in health there is

little resistance to flow from the small airways. In disease however there can be significant resistant to flow and the small airways are thought to be the major sight of airflow obstruction in COPD[72].

In COPD the abnormalities in the small airways are caused by squamous metaplasia, goblet cell hyperplasia and peribronchial fibrosis, resulting in airway wall thickening and luminal obstruction by mucoinflammatory infiltrates[73]. Hogg demonstrated that the small airways in COPD were thickened and this occurred throughout the epithelium, lamina propria and adventitia[73]. He also demonstrated a mild increase in smooth muscle in the small airway walls. Small airway wall thickening was associated with worsening airflow obstruction. The number of muco-inflammatory cells were also increased causing luminal occlusion which were associated with disease progression. Abnormalities of the ECM within small airway walls are not fully understood, however decreased elastin has been described[74,75], although another study found no difference with controls[76]. Peribronchial fibrosis is another key component of small airways disease in COPD[77] and increased collagen has been shown in the small airway walls[74]. The mechanisms of this are still unclear, however it is thought that cigarette smoke drives this small airways remodelling by causing the up-regulation and release of pro-fibrotic growth factors such as TGF-B[21,78].

1.5.2 Emphysema

Emphysema is traditionally a histological diagnosis, characterised by permanent enlargement of the air spaces distal to the terminal bronchiole accompanied by the destruction of their walls without obvious fibrosis. Emphysema causes airflow obstruction due to the loss of the alveolar supporting structure leads to loss of elastic recoil. The parenchymal destruction also leads to decline in alveolar surface area for gaseous exchange leading to ventilation/perfusion mismatches.

Emphysema is caused by the processes and mechanisms discussed in the previous section with the combination of oxidative stress, inflammation, proteases and chronic infection likely to be particularly important. These cause destruction of the pulmonary architecture and especially the pulmonary ECM. In the distal parenchyma and alveolar regions, studies have shown decreased elastin fibres in COPD subjects[74–76,79], although one other study showed the opposite in emphsyema[80]. Another study also showed increased alveolar gene expression of elastin in COPD and that the elastin fibres that were present were abnormal and less densely packed than in controls[81]. There is convincing evidence that collagen is increased in the lung parenchyma around emphysematous lesions[74,80,82,83], but this is not universal as another study showed no difference between COPD and controls[76]. The general consensus of these results is that emphysema is caused by destruction of the pulmonary ECM with aberrant remodelling, which may help explain these inconsistent results described above.

Three distinct pathological sub-types of emphysema are classified according to the distribution around the secondary pulmonary lobule and are termed centrilobular (CLE), panlobular (PLE), and paraseptal (PSE) emphysema[84]. All sub-types are found in COPD patients[85,86] although how they are distributed in individuals and populations and hence contribute to the disease is uncertain. CLE is the commonest form[86,87] and is associated with older age[87], smoking history[87,88] and lower FEV1[86,88]. It results from dilatation of or destruction of the respiratory bronchiole with the surrounding lung parenchyma often being normal[8]. PLE is common in younger age[87] and is associated with lower BMI[86,88] and more severe GOLD stage[86]. It is commonly associated with alpha-1-antitrypsin deficiency, but also occurs in typical COPD and it results in more even dilatation and destruction of the entire acinus[89]. PSE is the least common form and involves the distal airway structures and tends to be localised to the septae of the lungs or pleura. It is associated with male sex[86], older age, worse respiratory symptoms and interstitial abnormalities[90]. Although these are district emphysema sub-types, there is considerable overlap between these and it is common for individual patients to have multiple forms. Limited work has assessed the relationship between emphysema sub-types and underlying mechanisms of disease and consequently our understanding of the aetiology of these sub-types is poor. PLE in the context of alpha-1-antitrypsin deficiency (A1ATD), is perhaps the most well understood as it is the predominant sub-type in this condition where it is encoded by the Serpina 1 gene, resulting in unopposed neutrophil elastase activity[91]. In subjects without A1ATD, PLE has been linked with polymorphisms of the Serpina 2 gene[92], whilst CLE has been associated with MMP-9 and Transforming growth factor-β (TGF- β) polymorphisms[47] and PSE with TIMP-2 and TNF polymorphisms[47].

1.5.3 Chronic Bronchitis

Chronic bronchitis is a clinical definition of sputum production on a daily basis for at least three consecutive months over two consecutive years[1]. This is characterised pathologically by inflammation and mucus hypersecretion leading to airway wall remodelling and thickening in the central airways. The sub-epithelial layer is particularly affected with deposition of increased collagen and other ECM proteins[93,94]. This airway thickening is associated with worsening airflow obstruction[95]. Some studies have also reported that airway smooth muscle is thickened in the large airways in COPD[93]. The large airways are also the dominant site of mucus hypersecretion. This is due to chronic smoke exposure and inflammation causing squamous metaplasia, increased number of goblet cells and increased size of bronchial sub-mucosal glands[96]. Inflammatory cells also release proteinases that are potent secretagogues for mucus and oxidants.

1.6 Systemic Manifestations of Disease

COPD is a systemic disease with many extra-pulmonary manifestations that include among others skeletal muscle wasting and cachexia, cardiovascular disease and osteoporosis. These manifestations can result in impaired functional ability, morbidity and mortality. The cause for them is uncertain as many of these abnormalities share common risk factors with COPD. However it does seem that they are associated with disease severity independent of these risk factors[97–100], although this is not a universal finding[101,102]. One hypothesis is that many of these systemic manifestations are as a result of systemic inflammation and studies have shown higher levels of systemic inflammatory cytokines and inflammatory markers[103,104].

Cachexia and skeletal muscle wasting are a significant problem in COPD and low body mass index (BMI) is associated with airflow obstruction, disease severity and mortality[105,106]. However there is growing recognition that other markers of body composition, such as fat-free mass index (FFMI) may be more important as they are a better marker of skeletal muscle. FFMI has been shown to be a predictor of mortality independently of fat mass or BMI[107,108]. Osteoporosis is also very common in COPD[102] although it can be quite difficult to determine the cause as COPD shares a number of risk factors with osteoporosis in the form of smoking, low BMI, and use of corticosteroids. However, bone mineral density has been identified as being independently associated with airflow obstruction[98,99]. The precise mechanisms for both skeletal muscle wasting and osteoporosis are currently poorly understood.

There is also significant cardiovascular disease within COPD. In the lung health study of mild-moderate COPD patients, 25% of the mortality was due to cardiovascular disease and airflow obstruction was shown to be an independent predictor of deaths due to cardiovascular disease[97]. The cause for this is unknown however, it is likely to be a combination of systemic inflammation, oxidative stress, chronic hypoxia and shared risk factors. Pulmonary vascular disease and pulmonary hypertension are also well described in COPD[109–111]. It is often only mild or moderate but there does seem to be a sub-group who develop more severe pulmonary vascular disease[111] and pulmonary artery hypertension is associated with mortality[112].

1.7 Investigations for COPD

The following investigations are common measurements performed in patients with COPD.

1.7.1 Spirometry

Post-bronchodilator spirometry is the current test used to diagnose COPD by demonstrating airflow obstruction[1]. It is a physiological test that measures the volume of air an individual can expel from his lungs after a maximum inspiration. The standard spirometry manoeuvre is maximal exhalation after a maximum deep inspiration. A number of indices can be derived from this:

- *Forced vital capacity (FVC)*
  The volume delivered during an expiration made as forcefully and completely as possible, starting from full inspiration.
- *Forced Expiratory Volume in one Second (FEV1)*
  Maximum volume of air exhaled in the first second of a forced expiration.
- *FEV1/FVC ratio*
  Spirometric criteria for airflow obstruction is a FEV1/FVC ratio of under 0.7.
- *FEF25%–75%*
  The mean forced expiratory flow rate between 25% and 75% of the FVC.

Predicted values for spirometry have been calculated from large sample groups and vary with age, height, sex and race. A diagnosis of airflow obstruction in COPD is made when the ratio of FEV1/FVC is less than 0.7 on post-bronchodilator spirometry. However, in certain populations this absolute cut-off may be unreliable as it can over diagnose in the elderly and under diagnose in the young. There are therefore advocates of using the Lower limit of normal value for these populations, which involves taking the bottom 5% as abnormal.

The FEV1 can also be used to severity grade COPD.

- Mild COPD — FEV1/FVC ratio <0.7; FEV1 ≥ 80% predicted
- Moderate COPD — FEV1/FVC ratio <0.7; 50% ≤ FEV1 < 80%
- Severe COPD — FEV1/FVC ratio <0.7; 30% ≤ FEV1 < 50%
- Very severe COPD — FEV1/FVC ratio <0.7; FEV1 < 30%

Spirometry is cheap, easy to perform and highly reproducible in most populations and has therefore been utilised as the investigation of choice for diagnosing and severity grading COPD. It is for this

reason that it is so widely used in COPD. Reduced FEV1 has been associated with higher mortality[113,114]. However in the large NETT trial FEV1 was not associated with mortality on multivariate analysis[115] and other studies have also not found this link with mortality[116]. There are also only weak correlations between FEV1 and change of quality of life and health status[117]. There are also concerns that spirometry is an effort dependent test and can be insensitive to early disease. As previously discussed it cannot explain the significant heterogeneity that exists within the disease and within an individual's lungs. FEV1 provides little information about the distal airways and so FEF25%–75% is often cited as a measure of small airways pathology. However it is not very sensitive and has poor reproducibility[118].

1.7.2 Static lung volumes

Although spirometry is the most commonly used test to measure lung function, it cannot provide information about total lung capacity (TLC) and residual volume (RV). The most commonly used methods for measuring static lung volumes are plethysmography (Figure 2)[119] and nitrogen/helium dilution. These procedures have an added benefit over spirometry in that they are not forced manoeuvres.

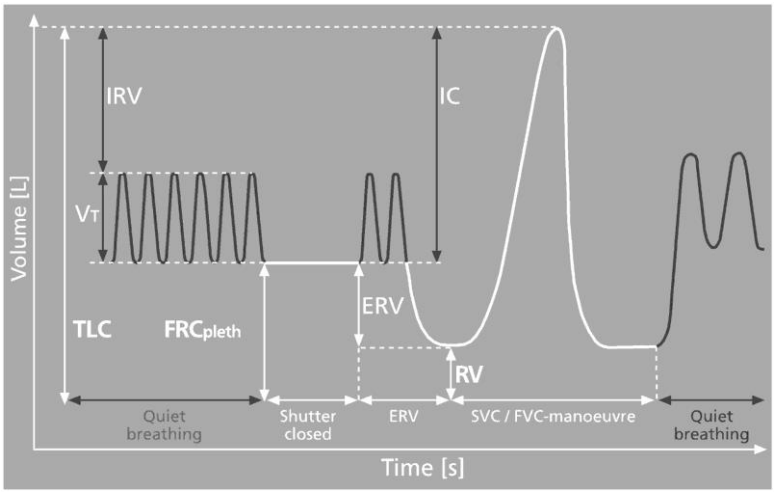


Figure 2. Volume-time display of a plethysmography sequence. Expiratory Reserve volume (ERV), functional residual capacity (FRC), inspiratory capacity (IRC), inspiratory reserve volume (IRV), residual volume (RV), total lung capacity (TLC).

In COPD airflow obstruction and narrowing, especially of the small airways results in prolonged expiratory phase and airways may close early resulting in hyperinflation and gas trapping, causing an increase in TLC and RV. It is therefore felt that these measures can act as an indirect marker of small airways disease. Hyperinflation measured by increased residual volume and TLC are

associated with increased mortality in COPD[115], although this is not a consistent finding amongst all studies[116]. These measures and techniques are undoubtedly useful in COPD but they are not specific markers of hyperinflation or small airways disease.

1.7.3 Gas transfer

The diffusing capacity of the lung for carbon monoxide is a measure of carbon monoxide transfer from the airspace to pulmonary capillary blood. It can reflect lung damage in the alveolar region such as what occurs in emphysema and represents a good marker of emphysema. TLCO has been shown to be associated with mortality in COPD[116]. Once again it is not a specific marker of emphysema as other disorders can affect the alveolar-capillary membrane.

1.7.4 The Six Minute Walk Test

The six-minute walk test (6MWT) is used to assess the functional status of patients with COPD by measuring the distance walked in 6 minutes (6MWD). It is a potentially useful marker of disease severity and measures both lung function and extra pulmonary manifestations of disease including muscle weakness and pulmonary vascular disease. It is an important predictor of survival in observational studies[120–125] and exacerbation-related hospitailasation[121]. Studies have suggested various distances from 340 to 395 metres as a prognostic markers for increased mortality in COPD[120,123–126]. There is however only a modest correlation between 6MWT and FEV1[120] and in longitudinal studies the change in 6MWT correlated poorly with change in FEV1[122]. Importantly, change in 6MWD independent is an independent predictor of mortality[122].

1.7.5 Pulse Oximetry

The finger-tip pulse oximeter is a probe that attaches to a patients finger and utilises the differential absorption of infrared light by oxygenated and deoxygenated haemoglobin in capillaries. It is therefore able to give a measure of the oxygenation of an individuals blood. Subjects with COPD who desaturate on exertion have been shown to have increased mortality[125–127]. A number of different cut-offs, between 3-5% have been used to define significant desaturation during the 6MWT[125–127].

1.8 CT Imaging in COPD

Medical CT imaging utilises x-rays to produce tomographic images of the body. The basic components of a standard diagnostic CT scanner include an x-ray source rotating around a patient, producing x-rays that are recorded by a detector at the opposite end of the patient (Figure 3). A cross-sectional image consisting of multiple pixels is created by mathematical reconstruction of the measured x-ray intensities received by the detector at different positions. Different tissues have different x-ray attenuation coefficient and so the digital image will reflect this with a grey scale representation for each pixel. The attenuation coefficient can be normalised with respect to the coefficient for water and is expressed in terms of Hounsfield units (Figure 4). Modern multi-detector CT technology have multiple rows of detectors meaning it is possible to scan the entire lungs in one breath hold, producing a contiguous volumetric image.

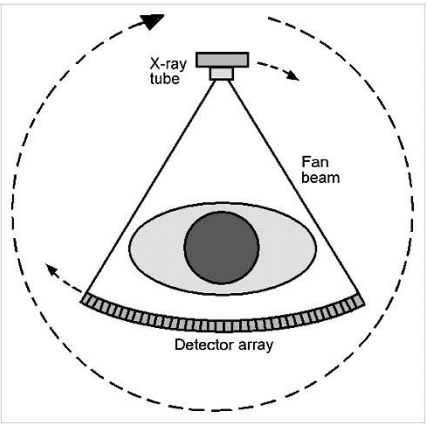


Figure 3. Diagramatic representation of the basic components of a CT scanner.

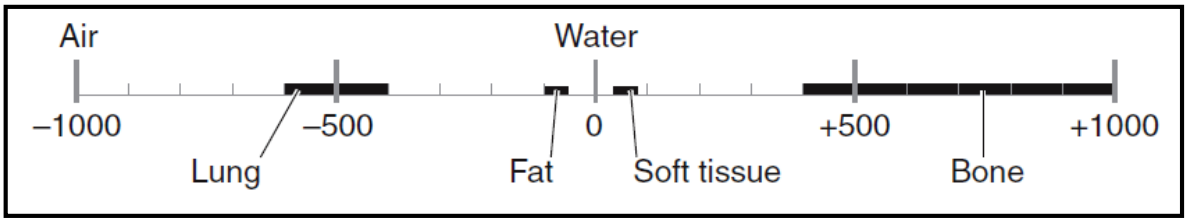


Figure 4. Scale of CT attenuation values and typical values for body tissues.

A number of factors affect the images a CT scanner produces. The electrical power supplied to the X-ray tube can be altered which affects the number of x-rays produced and radiation dose. The current can be varied to control the radiation dose and modern scanners now use dose modulation to detect the appropriate current for each individual. The speed of the scanner rotation and the speed with which the subject moves through the scanner can also be varied and these parameters are labelled as the pitch. Pitch is defined as being the speed of table movement in mm per complete 360° rotation divided by the product of the number of detector rows and the slice thickness.

The slice thickness of the scan can also be varied and the advantage of thin slices is that it can produce images with more detail. If the slices are reduced to approximately the size of a pixel (usually 0.6mm) then the voxels of the image (3D pixels) are isotropic (have each side the same length) and enable accurate three-dimensional images to be produced. Once the image data is acquired the CT scanner software reconstructs the data image using pre-set algorithms often referred to as kernels. Different kernels can be used to reconstruct each scan each with different spatial and contrast resolution. These reconstruction kernels can affect the quantitative analysis results.

1.8.1 CT Imaging in Current Clinical Practise

Prior to the 1970s histological and post mortem studies were required to study structural changes of the lung. The introduction of CT made it possible to visualise the thorax and assess lung structure non-invasively. In COPD CT can identify key morphological features, including emphysema, bronchial wall thickening and gas trapping. These pathologies contribute directly to airflow obstruction and therefore CT has the potential to provide vital insights into the underlying pathophysiological changes of COPD. Despite this, routine CT imaging has not necessarily been widely adopted in clinical practise and its place in the management and investigation of COPD has not been firmly established. The GOLD guidelines do not routinely recommend CT scanning in COPD and only advise that it may be helpful in differential diagnosis or when surgical options are being considered[128]. This general ambivalence is highlighted by a study of attitudes in respiratory physicians and surgeons in the UK where only 32% thought it necessary for patients with severe COPD to have a CT scan[129].

There are a number of reasons why the use of CT has not become routine in COPD. One of the most important is the perceived notion that using CT does not alter management, although this is not strictly true. The National Emphysema Treatment Trial (NETT) demonstrated that patients with advanced upper lobe emphysema and limited exercise ability improved after lung volume reduction surgery[14]. There has also been considerable progress and experience in using endobronchial treatments for severe emphysema including coils, valves, sealant and airway bypass. In the correct patients with severe heterogeneous emphysema these treatments can lead to significant improvement in pulmonary physiology and functional capacity[130,131]. CT imaging can also identify bullous disease, which may be amenable to surgery and bullectomy can lead to improved health status[132]. It therefore seems clear that patients with severe COPD require CT imaging to determine which patients are appropriate for these techniques.

CT imaging can also detect concomitant pulmonary pathology in COPD. Bronchiectasis is particularly prevalent, with studies demonstrating 50% of COPD patients having CT evidence of the disease[133–135]. When present bronchiectasis is associated with worse airflow obstruction[135], increased exacerbations[133,135], higher airways and systemic inflammation[133–135] and higher mortality[133]. COPD is also an independent risk factor for lung cancer[136] and higher cancer rates have been associated with worsening airflow obstruction[137,138] and emphysema[139,140]. Fibrotic change has also been shown to be common in COPD and seems to confer a poor prognosis[141]. In addition it can be difficult to diagnose due to the fact that pulmonary physiology may be normalised by the opposing effects of hyperinflation and fibrosis. As these concomitant pathologies are common and often associated with poorer outcomes and would require a change management it is vital that CT imaging is used to detect them.

A major limitation to the routine use of CT in COPD has been the subjective reporting, making it difficult to interpret the results and guide management plans or give insights into the disease. Objective methods have been targeted to provide numerical data on key disease facets. Initially, simple visual scoring systems were developed, which showed early promise at quantifying the disease. These techniques were unable to detect subtle disease and were liable to intra and inter observer errors as well as being insensitive to longitudinal change. To address these issues automated quantitative analysis techniques have been developed, which are able to segment the lung parenchyma and airways from the chest wall and surrounding structures. Early software analysed 2-dimensional axial slices but coupled with advances in CT technology a number of different analysis software exist that allows volumetric reconstructions (Figure 5). This raises the prospect that quantitative CT analysis can be used to understand the disease and phenotypes in more detail.

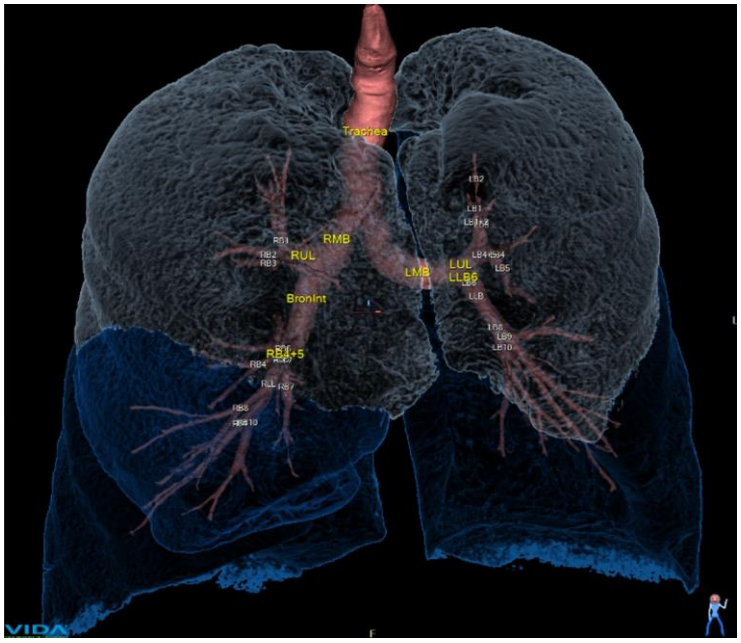


Figure 5. Volumetric computed tomography reconstruction of bronchial tree and lung parenchyma using Apollo software (VIDA Diagnostics Inc., Coralville, IA, USA)

1.8.2 Emphysema

As previously discussed emphysema is traditionally a histological diagnosis, although emphysematous changes can be identified on CT. In 1978 Rosenblum[142] described areas of lower lung attenuation and mean lung density in patients with a clinical diagnosis of COPD. To provide numerical data on emphysema, Goddard developed a visual scoring system for emphysema by estimating the amount on axial CT slices[143], which showed a strong association with airflow obstruction and with histological specimens[144–146]. Basic quantitative techniques were developed[147] and then automated techniques were developed with which to segment the lung parenchyma and quantitate emphysema. The two main techniques both use the principle that emphysematous regions are represented by low attenuation areas on CT. The first, termed CT densitometry involves applying a density mask to the lung parenchyma and setting a threshold below which all voxels are assumed to be emphysema, giving a low attenuation percentage (%LAA) (Figure 6). Initially a threshold of -910 Hounsfield Units (HU) was used which showed a strong correlation with pathological measures of emphysema[148]. Subsequently, LAA under -950HU ($\%LAA_{<-950}$) was shown to have the strongest associations with both microscopic and macroscopic emphysema[149–152], leading to the common adoption of this threshold. The second method termed percentile densitometry involves choosing a threshold percentile in the attenuation distribution curve, which provides the density value in HU under which n% of the voxels are distributed. A number of different thresholds between 1 and 18% have been used and correlate strongly with microscopic emphysema

on histological specimens[152]. The most commonly used threshold is 15% based on a number of studies in A1ATD[153–155]. There is still no definite consensus about which of these methods is best and even now different studies use different thresholds. Given the fact that emphysema is an all or nothing phenomenon it seems more intuitive to use CT densitometry, which uses an absolute cut-off to quantify it.

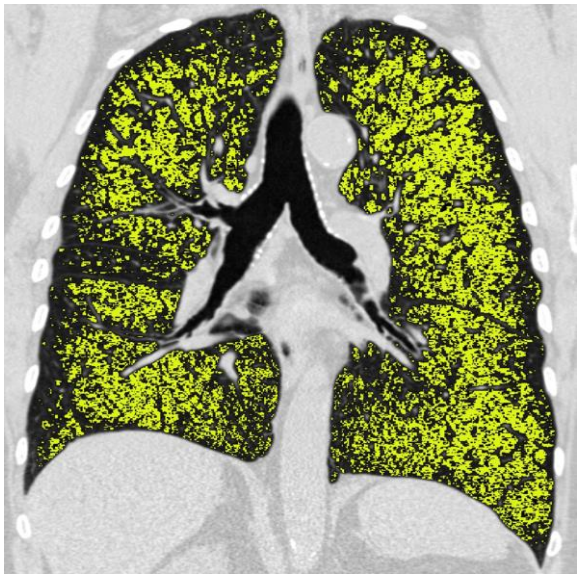


Figure 6. Coronal reconstruction of chest CT showing low attenuation areas below -950HU.

Studies have confirmed that CT densitometry demonstrates higher levels of emphysema in individuals with COPD as opposed to healthy smokers[156–159] There is also convincing evidence that CT densitometry has strong associations with airflow obstruction[134,157–166] and analysis of over 4000 CT scans from the COPDgene study showed a strong negative correlation between $\%LAA_{<-950}$ and forced expiratory volume in 1 second (FEV1) and demonstrated increased emphysema with worsening GOLD severity[158]. CT densitometry also has strong associations with gas transfer (TLCO)[134,160–165], six minute walk distance (6MWD)[157,161,165,167], BODE score[161,164,168–170] and body composition[162,164,171,172].

The above densitometry techniques do not take into account the distribution or different sub-types of the disease, which may have important implications. Emphysema does not affect the lung in a uniform way and it is unclear how this affects the clinical presentation of the disease. As previously discussed, it has been shown to be clinically important in determining which patients benefit from lung volume reduction surgery[14]. A small study found that LAA% was higher in central and upper regions within the lung[173]. Interestingly, airflow limitation correlated best with LAA% in the central and lower regions and also found that more homogenous emphysema was associated with worse airflow obstruction. Other studies have shown accelerated decline in lung function in those with

homogenous emphysema[174] and those with more upper zone emphysema[175]. With advances in quantitative CT analysis, lobar variation of disease can be assessed and significant differences in emphysema quantity between the lobes has been found[176,177].

Three distinct patterns of emphysema have been described in histological studies, based on the distribution within the secondary pulmonary lobule; centrilobular, panlobular and paraseptal emphysema. CT imaging can visualise these different sub-types. One study using a visual scoring system found that centrilobular emphysema was the most common sub-type but over half of patients with emphysema had multiple sub-types[86]. An automated method, called local histogram-based emphysema patterns involves dividing the lung into small cubes and analysing the sub-type of emphysema present in each cube (Figure 7). This was found to be more predictive of lung function than $\%LAA_{<-950}$[85].

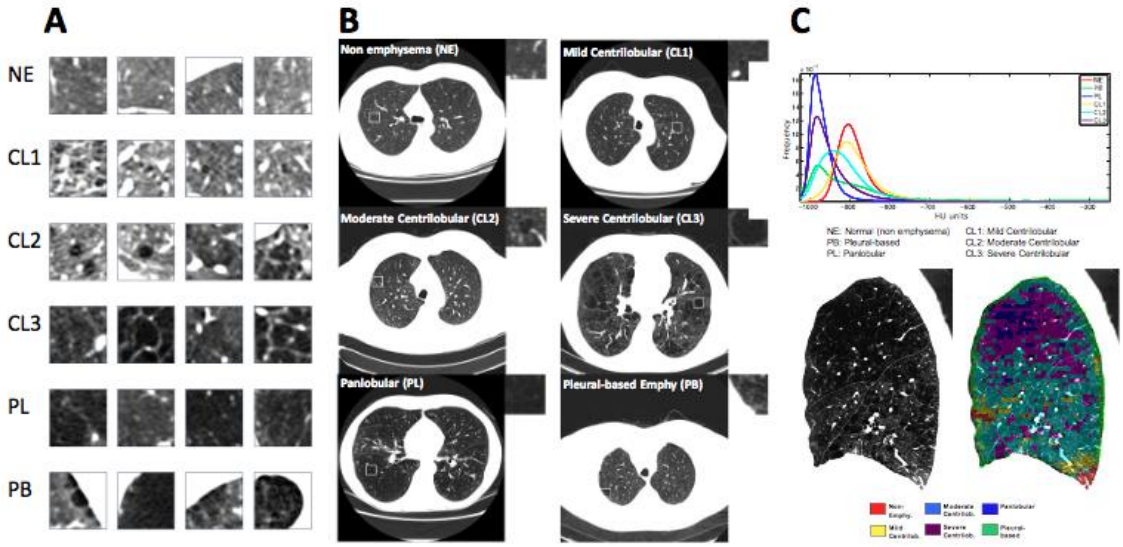


Figure 7. Prototypic lung CT patches for each local histogram emphysema pattern as regions of interest (ROI) (A) and in context (B). Representative ROI local histograms (C, top) and local histogram emphysema classification results for a sagittal slice of a COPD subject (C, bottom). CL1 mild centrilobular; CL2 moderate centrilobular; CL3 severe centrilobular; NE nonemphysematous; PB pleural-based; PL panlobular. Adapted with permission from reference 85.

The above data points to CT densitometry and other methods being able to accurately quantify emphysema and its association with physiological markers of disease. To validate CT imaging as a biomarker of disease it is necessary to compare it to underlying disease mechanisms, outcome measures and track longitudinal change. CT densitometry has been found to correlate with mortality in patients with COPD[178] and analysis from the GENKOLs trial[179] found that LAA% had a better predictive value for respiratory and cardiac mortality than GOLD staging. Emphysematous

change on CT has also been associated with higher exacerbation rate[180–182] and increased mortality from exacerbations[182].

A potentially important application of CT imaging is to track the changes of emphysema over time and see how it contributes to functional decline. Until recently the majority of these studies were conducted in patients with A1ATD and have shown that CT densitometry can accurately detect progression of emphysema[183–186] and correlates with decline in lung function[154,183]. Furthermore, CT lung density parameters are more sensitive in detecting progression of disease than traditional lung function[183] and have therefore been accepted as the primary end-point in prospective studies on treatment effects for A1ATD. In COPD, it has been demonstrated that patients with higher quantities of emphysema on CT have an increased rate of decline of FEV1[159,175]. CT emphysema progression is also associated with frequent exacerbations[187] and current smoking status[188]. The largest longitudinal study to date assessed CT scans from the ECLIPSE cohort[189] where they found lung density and low attenuation areas progressed on an annual basis. There were however significant variation between subjects and this did not convincingly correlate with lung function decline.

When comparing CT measures of emphysema with underlying markers of disease it has to be considered that our knowledge of these mechanisms is imprecise. Emphysematous change has been associated with various MMPs[37,40,43,156] as well as raised sputum neutrophils[190] and eosinophils[164,191]. A number of airway inflammatory cytokines such as IL-8, LTB4 and myeloperoxidase (MPO) have been associated with emphysema progression[23,52,164] and emphysema has also been associated with raised serum fibrinogen[192]. Some work has been performed at associating novel biomarkers with emphysema and in the ECLIPSE cohort Surfactant protein D and sRAGE were both associated with decline in lung densitometry[189]. Little is known about bacterial colonisation and emphysema however Bafadhel[193] did not find any difference in bacteria culture between patients who had emphysema on CT and those who did not.

In summary emphysema quantitation is relatively simple and can be performed with minimal user-intervention. CT densitometry is associated with a number of physiological and clinical parameters. However, further work is required to evaluate emphysema distribution and sub-types and also to assess the longitudinal changes of emphysema and the association with outcome markers of disease and underlying disease mechanisms.

1.8.3 Bronchial Wall Measurements

Airway wall remodelling is an important feature in COPD and histological specimens confirm airway wall thickening throughout the bronchial tree[73,194]. The limited resolution of CT means only large

and intermediate sized airways can be visualised directly. Early measurements of the airway relied on manual tracing[195], however a number of automated methods have been developed. One of these involves using the "full-width-at-half-maximum" principle (Figure 8), which uses CT attenuation values to identify the inner and outer airway wall by projecting rays from the centre of the airway. These and other algorithms have been refined and using region-growing approaches it is now possible to generate three-dimensional reconstructions of the bronchial tree down to the fifth or sixth generation airway. Multiple airway dimensions can be measured from these reconstructions including bronchial wall thickness (T), wall area (Aaw), lumen area (Ai), total airway area (Ao) wall area percentage (WA%) and internal perimeter (Pi) (Figure 9). A standardised parameter called Pi10 has been developed which predicts the square root of the wall area for a hypothetical airway with an internal perimeter of 10mm.

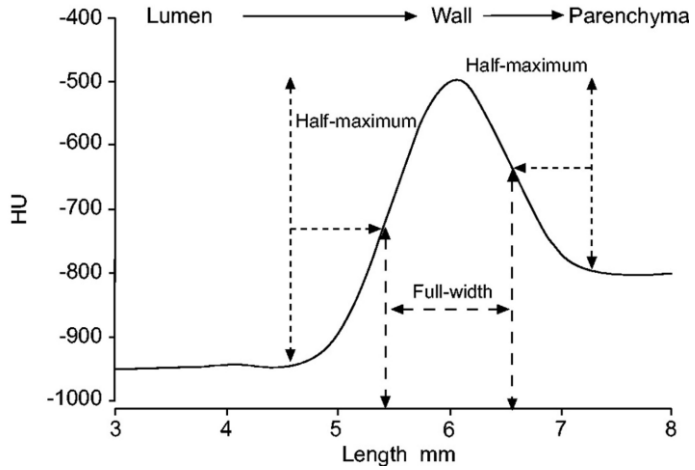
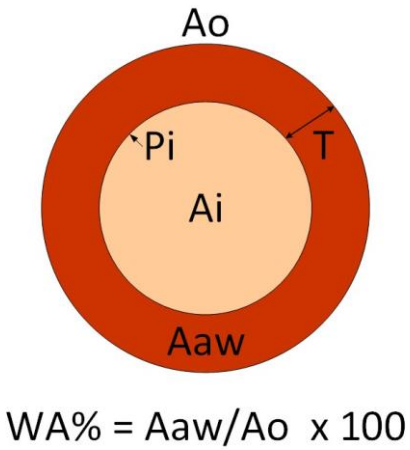


Figure 8. Full-width-at-half-maximum method for determine airway wall dimensions



$$WA\% = Aaw/Ao \times 100$$

Figure 9. Airway wall dimensions. Airway wall area (Aaw), wall thickness (T), total airway area (Ao), Inner area (Ai), internal perimeter (Pi) and wall area % (WA%)

Early studies found that markers of bronchial wall thickening on CT were increased in COPD[171,196–198]. However, more recent studies have shown that both airway wall and lumen size are reduced in COPD, although proportionally the lumen more so, resulting in a larger WA%[158,196,199]. The reasons for the discrepancies are unclear although the later studies tended to make more complete measurements of the bronchial tree and spatially matched the airways. By not doing this there is a chance of introducing bias into results by inadvertently selecting more proximal airways in COPD, which have thicker walls. This highlights the current difficulties in measuring the airways, with the sheer number of variables presenting significant challenges and it is clear that further research is needed.

Taking the above limitations into account, measures of increased bronchial wall thickness are associated with FEV1[166,198,200,201] and this is stronger with more distal airways[166]. Bronchial wall markers also correlate with functional markers in the form of BODE index[169,170], exercise capacity[157,161] and body composition[171]. Although statistically significant many of these associations tended to be quite weak. In the GENKOLS study there was generally no increase in mortality with increased Pi10 but in a sub-group with severe emphysema, Pi10 was associated with mortality[179]. Another study demonstrated that increased bronchial wall dimensions were associated with mortality from exacerbations[182]. It has been hypothesised that increased bronchial wall thickness is a marker of airway inflammation and therefore would be associated with symptoms of chronic bronchitis or frequent exacerbations. Han[180] confirmed this by showing that markers of airway wall thickness were increased in frequent exacerbators, although another study failed to find this association[181]. These measures also show associations with symptoms of chronic bronchitis[201,202]. Few studies have investigated the link between CT markers of bronchial wall thickening and sputum inflammatory markers or bacterial culture, although Bafadhel[193] did not find any association.

Simply measuring markers of bronchial wall thickness may be insufficient to describe the airway remodelling that occurs in COPD. Other methods have therefore been developed and one of these uses peak wall attenuation, which is a marker likely to reflect thickening and density within the airway wall. This has been shown to have a negative correlation with FEV1[166,203].

Minimal work has assessed the change of airway dimensions over time. A small study looked at a lower lobe segmental airway changes between scans a year apart and did not find a significant difference, although did find a correlation between annual change in WA% and FEV1 decline[204]. Regional variability in bronchial wall thickness has not been systematically analysed. Ohara did not

find any difference between the apical upper lobe segmental bronchus and lower lobe segmental bronchus[205].

In theory CT should be a useful tool at assessing the morphology of the large and intermediate sized airways. However there are still many uncertainties regarding this technology and measurements and no definite evidence has shown that this is a particularly useful tool in COPD.

1.8.4 Small Airways Disease

The small airways are the main contributor to the airflow obstruction seen in COPD[73] and histological studies confirm epithelial metaplasia, goblet cell hyperplasia and peri-bronchial fibrosis. There is currently no gold standard for measuring this small airways disease and so better methods are required and considerable research is on-going in this area. CT cannot image the small airways directly as they are beyond the maximum resolution. The indirect sign of gas trapping can be used as a surrogate marker, which is defined as lack of volume reduction after expiration and is seen as low attenuation areas on expiratory CT.

A number of quantification methods have been developed to measure gas trapping and the simplest of these involve calculating the number of voxels below -856 HU or the mean lung density (MLD) on expiratory CT. These methods do not compensate for the amount of emphysema and so in effect will be a combined score for gas trapping and emphysema. To correct for this, techniques using paired inspiratory and expiratory scans have been developed which include using the ratio of MLD in expiration to inspiration. Matsuoka[206], developed a more complex technique using the relative volume change between -856HU and -950HU between inspiratory and expiratory scans. This was validated in a study of 36 patients and found strong associations with pulmonary function test markers of gas trapping and was superior to the other methods described above. When comparing these techniques to single breath nitrogen washout, expiratory to inspiratory MLD ratio exhibited the best performance[207]. A large trial investigating gas trapping in COPDgene subjects found that paired (inspiratory and expiratory) samples were more predictive of pulmonary function in those with severe emphysema than just using the expiratory scan alone[208]. It therefore seems logical that any CT marker investigating gas trapping excludes emphysema by using paired inspiratory/expiratory scans.

Most studies investigating gas trapping have simply associated it to physiological parameters of disease where it has been found to correlate with FEV1 and other lung function parameters[156–158,161,163,209–211] and also with worsening GOLD status[157,209]. Few studies have investigated the link between gas trapping on CT and underlying mechanisms of disease. The longitudinal changes and the distribution of gas trapping throughout the lungs are unknown. More recently novel CT

methods of investigating small airways disease have been developed termed parametric response mapping (PRM), which involves co-registering inspiratory and expiratory scans so that voxel by voxel comparison can be performed (Figure 10)[212]. This allows determination of emphysema and functional small airways disease and has been shown to subgroup patients effectively which correlated with pulmonary function testing. Functional small airways disease measured in the COPDgene cohort was independently associated with decline in FEV1 and this was especially the case with milder patients.[213] This and other similar techniques offer the opportunity to characterise disease phenotypes and has the potential to monitor disease status and so considerable research is ongoing.

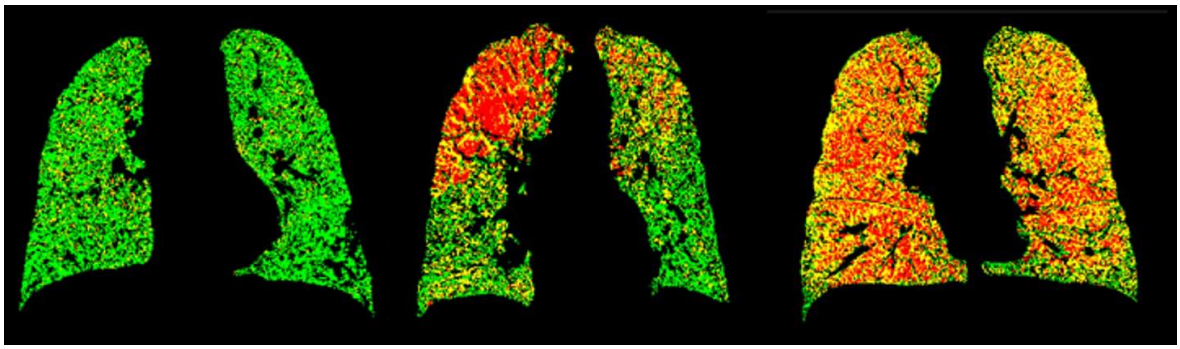


Figure 10. Parametric Response Mapping from CT scans in three individuals. Normal lung tissue is denoted green, functional small airways disease denoted yellow and emphysema denoted red. Adapted with permission from reference 210.

In summary, using paired inspiratory and expiratory scans CT imaging can effectively measure gas trapping, although the optimum method has yet to be defined. Valid questions exist about whether gas trapping is truly a surrogate marker of small airways disease, although it does show strong associations with pulmonary function measures of small airways disease. Further work is needed to see how underlying mechanisms of disease associate with gas trapping and whether this parameter reflects changes in lung function over time.

1.8.5 Other Pulmonary Morphological Features

There have been efforts to develop quantitative analysis for pulmonary features other than the ones discussed above. An automated method for bronchiectasis has been developed where the ratio of airway inner diameter to adjacent vessel diameter can be quantified[214]. This has shown the burden of bronchiectasis is higher with increasing airflow obstruction and is associated with increased exacerbations. Further work is required to refine and automate this technique.

There has also been interest in using CT measured lung density as a marker of pulmonary inflammation. Increased lung density has been found in current smokers as opposed to never/ex-smokers[215–217] and has been associated with greater smoking history[216,217] and inflammatory cells in BAL[215]. Interestingly, in a longitudinal study, lung density decreased after smoking cessation[217], suggesting smoking causes increased lung density possibly through pulmonary inflammation. This may prove to be a potential tool in evaluating early lung disease prior to structural changes occurring in COPD.

Pulmonary vascular imaging is an active area of research as pulmonary vascular disease is well described in COPD and is a significant predictor of mortality[109–111]. The pulmonary artery diameter and ratio of pulmonary artery diameter to aorta diameter is associated with direct measures of pulmonary artery pressures[218] and higher exacerbation rates[219]. Cross sectional area of small pulmonary vessels on CT is correlated strongly with invasive measures[220]. A technique to automatically segment and create a three-dimensional model of the pulmonary vasculature, allowing blood volume to be calculated has been developed[221] and subjects with more emphysema had less blood volume. Further work is required to demonstrate the clinical utility of this.

1.8.6 Extra-Pulmonary Manifestations

COPD has multiple systemic manifestations and CT chest is able to capture some of this information. Cachexia and skeletal muscle wasting are a significant problem in COPD and BMI is associated with airflow obstruction, disease severity and mortality[105,106]. There is growing recognition that other markers of body composition, such as FFMI may be more important as they are a better marker of skeletal muscle dysfunction. FFMI has been shown to be a predictor of mortality independently of fat mass or BMI[107,108]. A technique to assess pectoralis muscle area on CT showed significantly reduced muscle area in COPD versus healthy controls and was associated with GOLD staging[222]. This was also associated with functional markers of disease including BODE index, MRC dyspnoea score and 6MWD[222]. Various fat compartments can also be assessed including anterior chest wall subcutaneous fat and intra-abdominal fat, which have shown some clinical correlations. These methods are all relatively crude and still require significant user input.

Osteoporosis is also common in COPD[102] and bone mineral density has been identified as being independently associated with airflow obstruction[98,99]. Vertebral body attenuation values can be measured by CT and is associated with bone mineral density on DEXA[223] and also show associations with airflow obstruction[224] and exacerbation rate[225].

1.9 Other Imaging Modalities

1.9.1 PET-CT and SPECT

Although CT is the imaging modality of choice for providing structural images of the lung one of the major drawbacks is its inability to provide functional imaging. CT can however be combined with other imaging modalities to provide functional data in COPD. Positron emission tomography-CT (PET-CT) and single-photon computed tomography (SPECT) are nuclear medicine scans which combine radioisotopes with CT imaging, allowing both functional and molecular imaging studies with anatomical co-registration. Small molecules, peptides or proteins are labelled with radioactive atoms that emit high-energy photons which can be detected by PET or SPECT. For PET-CT, 18F-FDG is the most commonly used radioisotope and is taken up by metabolically active tissues and can be used as a marker of pulmonary neutrophilic inflammation. Uptake is significantly increased in COPD[226] and is associated with FEV1 and emphysema severity[226,227]. With SPECT, perfusion and ventilation imaging can be performed with either a vascular or inhaled tracer.

1.9.2 MRI

Magnetic resonance imaging (MRI) is another modality that can provide functional images of the lungs in COPD[228,229]. There are a number of challenges in using MR to image the lungs including the low concentrations of protons, multiple air tissue interfaces and relatively poor spatial resolution. Until recently the main pulmonary application was in evaluating the pulmonary vasculature using contrast enhanced imaging. Advances in technology, especially using oxygen-enhanced or hyperpolarized noble gases to increase the signal allows functional imaging of ventilation and have shown ventilation defects in COPD[230]. Some studies have combined functional MR imaging with the structural images seen on CT. One such study assessed pulmonary microvascular blood flow and found it was reduced in COPD and was associated with degree of emphysematous change[231]. Another study compared ventilation and perfusion defects measured on MRI with PRM measurements of emphysema and small airways disease on CT. Ventilation defects were associated with emphysema and small airways disease and diffusion measurements were significantly raised in areas of gas trapping[232]. MR technology is still in early development but does provide an advantage over CT by allowing functional imaging and importantly being radiation free. Despite this MR is limited by availability, cost, the complexity of image analysis required and relatively poor spatial resolution.

1.10 Technical limitations and variability of CT analysis

There are a number of limitations that need to be recognised when using CT image analysis to investigate COPD. A number of different factors have the potential to affect the Quantitative analysis. Radiation dose can significantly affect the images produced and therefore the quantitative analysis. Decreasing the radiation dose means the signal to noise ratio increases, leading to increases in emphysema detection[233]. There is very little evidence stating how lower scan dosing effects airway wall measurements and that off small airways disease. Slice thickness and distance between the scans have an influence on the images produced. The ideal scenario is for the slice thickness to be the same size as the in-slice pixel dimension, which is 0.6mm. This allows voxels to be isometric and three-dimension reconstructions can be accurately produced. Once CT images are acquired the CT scanner software reconstructs the images using pre-set algorithms. These CT reconstruction algorithms can affect quantitative assessments of emphysema, gas trapping and bronchial wall dimensions[234,235]. Different CT scanners also have minor variations built into them and this can affect quantitative CT results in COPD[233].

Another significant issue when performing quantitative analysis of the airways is the limited spatial resolution of current CT imaging. Voxels have dimensions of approximately 6mm, meaning it is not possible to image the small airways directly. In addition, partial volume artefact can also cause significant inaccuracies. This occurs when adjacent tissues with different absorption are encompassed within the same voxel producing an attenuation in that voxel which is proportional to the average values of these tissues.

Lung attenuation changes during the respiratory cycle and the volume at which the scan is taken can have a significant effect on quantitative analysis parameters[233,236]. Other conditions such as fibrotic lung disease and active inflammation within the lung can also have an effect on densitometry measurements and in addition to this smoking can also cause increased lung attenuation, potentially confounding results[215]. CT measure of emphysema and gas trapping are derived and are not direct measurements of these structural lung abnormalities. $\%LAA_{<-950}$ has been shown to have strong correlations with histological measures of emphysema but these are far from a perfect associations[150]. In non-smoking individuals $\%LAA_{<-950}$ has been shown to be as high as 8%, suggesting this measure over-diagnoses emphysema[177,237]. There is no gold-standard investigation to measure the small airways and so it is unknown whether CT gas trapping measures accurately reflect small airways disease.

1.11 Unknowns of CT Analysis in COPD

Further work is required to confirm quantitative CT analysis as an imaging biomarker of disease that can be used practically to manage patients with COPD and as a useful research tool to give insights into the disease. From a technical point of view CT protocols need standardising as radiation dose, slice thickness and reconstruction algorithms can all effect analysis. Methods for quantifying the various morphological features need optimising and this is especially the case for airway measurements and gas trapping. More sophisticated tools may be needed that do not just simply quantify results but also take into account other features, including emphysema sub-types and airway wall attenuation. Measures at detecting extra-pulmonary manifestations are also in early development and further research is required to optimise and validate these.

CT analysis needs to be assessed against outcome measures and markers of disease activity. To date there have been relatively few longitudinal studies and these have shown variable results. It is vital studies are performed to track CT changes over time to get an accurate picture of the progression of the disease and how effective CT imaging is at capturing this. There have also been relatively few studies assessing quantifiable CT markers of disease and markers of underlying biological disease activity, which includes inflammatory markers, microbiology, proteases and other biomarkers. Studies need to be conducted in order to further our understanding of the active disease processes and endotypes occurring in different compartments and regions of the lung, hopefully allowing the development of novel targeted therapies.

Enhancing CT technology will also increase its application in COPD. A major limiting factor is the radiation dose and associated cancer risks. Dose reduction strategies are vital if longitudinal scans are to be considered. Dual-energy CT technology is being developed which allows functional imaging of the pulmonary vasculature and will have further applications. CT imaging could also be augmented with other imaging tools to provide more information about disease activity. PET-CT/SPECT are current examples, but these could be enhanced with development of novel molecular probes targeting key features of COPD. CT could also be co-registered with MR imaging to combine structural and functional imaging.

One of the most important questions is how to utilise CT analysis to define clinically important phenotypes. It is unlikely that simply subgrouping patients into emphysema and airway predominant disease will be sufficiently helpful to guide treatment options. Cluster analysis and other statistical modelling techniques may provide interesting results but are more likely to be research tools with few practical clinical applications. Emphysema and bronchial wall thickening on CT have already been associated with increased mortality[178,179,182] and refining this information and

combining it with clinical parameters may help enhance multi-dimensional prognostic tools such as the BODE index.

This technology may have a significant impact in clinical trial and drug discovery work in COPD. Quantitative CT has already been used as an outcome marker for emphysema progression in A1ATD and this could be extended for drugs targeting airway inflammation and small airways disease. The heterogeneity of the disease causes significant challenges in clinical trial design. Most trials include unselected populations of patients and therefore have to enrol large numbers and last considerable time to identify sufficient responders. Improving knowledge of patient heterogeneity is vital for optimising drug trials and quantitative CT may play an important role. Potentially, CT could be used to stratify study subjects into phenotypic groups that would be most likely to respond to the investigational drug. For example, roflumilast targets airway inflammation but study results have been underwhelming. CT could be used to define which patients have evidence of airway thickening/inflammation and this subject group could be used in any interventional study. Improving stratification of study subjects may increase treatment effects and reduce variability in response, thereby increasing the chances of novel drugs being developed. This will in turn lead to further clinical applications for CT imaging in determining which patients may benefit from novel pharmaceutical agents.

Chapter 2: Hypothesis and Aims

COPD is a complex, heterogeneous condition with varying clinical features, consisting of a number of different phenotypes. The origins of clinical phenotypes, and the associations of disease processes with endotypes along with the drivers to heterogeneity between individuals have not been fully explored and are likely to have important implications for understanding disease progression and the future management of this condition. Reliance on using spirometric markers to diagnose and characterise the disease has limited both our understanding of COPD and the individualised clinical management of the condition. Other tools are required in addition to spirometry to help explain the heterogeneity and provide further insights into approaches to study and treat COPD. CT can image the pathological changes that directly contribute to the airflow limitation that defines the condition and the development of novel analysis techniques has the potential to provide further details about the pulmonary and extra-pulmonary manifestations of COPD. However, the application of this has not been adequately demonstrated with further work required to assess longitudinal changes of the disease on CT and the relationship with disease activity and underlying biology.

The primary goal of my study is to further understand the role of CT imaging in COPD by investigating its relationship with disease activity and to assess longitudinal changes in COPD. By systematically investigating the use of quantitative CT imaging in COPD I hope to further understand the association between underlying endotypes and phenotypic traits.

My hypothesis is that quantitative CT image analysis can accurately measure the key features seen in COPD and these have associations with physiological and functional markers of disease. Furthermore I propose that CT image analysis can be used to explore the association between emphysema, gas trapping and bronchial wall thickening and airway inflammation, proteases and bacterial colonisation of the airways. Finally I hypothesise that CT image analysis will show significant anatomical variation throughout the lungs and this will correspond with regional markers of disease activity.

In order to test my hypothesis my specific aims are;

1. To characterise the systemic and airway inflammatory profile, airway bacteria presence and airway proteases in a cohort of COPD subjects undergoing bronchoscopy.
2. To demonstrate that quantitative CT analysis can be used to adequately measure the main features of COPD and determine the optimum methods for measuring these.

3. To show that quantitative CT imaging can be used to further our understanding of disease by comparing the structural abnormalities of COPD with airway and inflammatory status, airway bacterial presence, airway proteases and longitudinal change of clinical and physiological measures of disease.
4. To explore the anatomical regional variation of disease, both in terms of disease mechanisms and imaging abnormalities and understand how these correspond to disease severity and clinical features.
5. Use CT imaging to investigate how structural features of COPD change over time by analysing CT scans taken two years apart in a COPD cohort and identifying other disease components which can predict progressions of these.

Chapter 3: Methods

3.1 Ethics

All subjects gave written informed consent and the two studies (ClinicalTrials.gov:NCT01701869 and ClinicalTrials.gov:NCT01360398) were approved by the South Central - Southampton B NRES Committee (12/SC/0304).

3.2 Study Design

My PhD thesis comprised two separate studies;

1. *Microbiology and Immunology of the Chronically-inflamed Airway (MICA) study.* A cross-sectional study in which mild-moderate COPD and ex/current smokers with preserved lung function underwent CT imaging and bronchoscopy.
2. *Acute exacerbations and Respiratory Infections in COPD (AERIS) study*[238]*.* An observational two year study where subjects with moderate to very severe COPD underwent CT imaging at enrolment and year two as well as in-depth phenotyping, using clinical, physiological and laboratory investigations.

3.2.1 Microbiology and Immunology of the Chronically-inflamed Airway (MICA)

This is a bronchoscopy study investigating microbiology, inflammation and immunology within the airways of COPD patients. All subjects underwent CT imaging to characterise their structural and morphological features of COPD. CT was also used to assess the regional variability of disease in COPD and guided bronchoscopic sampling from diseased and less-diseased lobes. At bronchoscopy BAL was performed in these two separate lobes and the inflammatory profile, bacterial colonisation and MMP concentration were analysed.

Previous studies have not taken into account the regional variability of the disease within the lungs and have relied on composite measures in the form of lung function, sputum inflammatory markers or 'random' bronchial sampling from poorly characterised regions of the lung. The consequence is a loss of discriminatory power to determine key disease mechanisms and a characterisation of disease based upon physiological severity rather than disease activity. Combining CT analysis with multiplex profiling of targeted lavage fluid has potential to define the disease process much more effectively.

Volunteers each completed four visits.

1. *Screening.* Subjects underwent informed consent, Medical History and Examination and Pre and post bronchodilator spirometry.
2. *Enrolment and Sample collection.* Volunteers underwent sputum induction with hypertonic saline and bloodletting.
3. *CT.* All subjects underwent CT imagining.
4. *Bronchoscopy.* Bronchoscopy was undertaken to collect brushings and BALF, in two regions of the lung identified by subjective reporting of the CT scan as being “most diseased” and “least diseased”.

3.2.2 Acute exacerbations and Respiratory Infections in COPD (AERIS)

AERIS was funded and sponsored by GlaxoSmithKlines Biologicals SA. It was an observational two-year study to explore the determinants of COPD and acute exacerbations of COPD in a moderate to very severe cohort of patients. Subjects had detailed clinical, radiological, functional and laboratory analysis performed over the two year period and it therefore represents a unique resource with which to phenotype COPD. CT imaging was performed at enrolment and at conclusion of the study and quantitative image analysis was applied and then correlated with the various data collected throughout the study to explore disease phenotypes. One of the main focuses of this study was the longitudinal CT data and how they related to other outcome measures of disease.

Subjects were seen at enrolment and monthly for two years and within 72 hours of exacerbation. Exacerbations were defined as a worsening of at least two major symptoms (dyspnoea, sputum volume and sputum purulence) or worsening of at least one major and one minor symptom (wheeze, sore throat, cold, cough and fever). They were identified by means of electronic diary card that were monitored by the study team. The samples, investigations and data collected over the study period are shown in Table 1.

Table 1. Study protocol for visits and sampling over the two-year period.

Physical examination, medical history, medication review and smoking status	Monthly and at exacerbation
Spirometry	Monthly and at exacerbation
Plethysmography	Enrolment and final visit
Transfer factor (TLCO)	Every 6 months and at exacerbation
6 minute walk Test	Every 6 months
Chest CT scan	Enrolment and final visit
Blood for Biochemistry	Study entry
Blood for cell-medicated immunity	Quarterly and at exacerbation
Blood for biomarkers, blood counts and haematology	Quarterly and at exacerbation
Blood for vitamins, antioxidants and nutrients	Every 6 months and at exacerbation
Sputum Sampling	Monthly and at exacerbation
mMRC	Every 6 months
CAT, NEADL and CNAQ questionnaires	Quarterly and at exacerbation
Healthcare use questionnaire	Monthly and at exacerbation
EXACT-PRO and symptom diary	Daily via electronic diary

As this study was a large collaboration involving a pharmaceutical company, I had little influence over analysis timelines and as such data analysis is not yet complete. I have performed analysis on CT scans at enrolment and at month 24 but unfortunately for the rest of the study variables only year one data was made available to me for this thesis. Therefore the longitudinal work is incomplete as I was only able compare longitudinal CT changes with enrolment parameters.

3.3 Study Populations

3.3.1 The MICA study

Subjects with stable mild and moderate COPD as defined by GOLD guidelines[1] were recruited into the study. Post-bronchodilator spirometry was used to assess airflow obstruction with a FEV1/FVC ratio of <0.7 and an FEV1 of ≥50% predicted value required for enrolment. A group of eight current or ex-smokers, with at least a 10 pack year history but preserved lung function (PLF) were also recruited into the study. Detailed inclusion and exclusion criteria are shown in Table 2.

Table 2. Inclusion and exclusion criteria for the MICA study

Inclusion Criteria	Exclusion Criteria
Written informed consent obtained from the participant	A confirmed diagnosis of asthma, cystic fibrosis, pneumonia risk factors or other respiratory disorders (eg, tuberculosis, lung cancer, etc)
History of ≥10 pack-years of cigarette	History of lung surgery α-1 antitrypsin deficiency as underlying cause of COPD
Male or female aged 40–85 years	α-1 antitrypsin deficiency as underlying cause of COPD
COPD subjects must have a confirmed diagnosis of mild/moderate COPD based on post bronchodilator spirometry with FEV1 >50% of predicted normal and FEV1/FVC <0.7 [239]	Moderate or severe COPD exacerbation not resolved at least 1 month prior to enrolment and less than 30 days following the last dose of oral corticosteroids
Control subjects must have an FEV1/FVC >0.7.	Long-term corticosteroid or antibiotic therapy Use of any antibacterial, antiviral or respiratory investigational drug or vaccine within 30 days of the enrolment visit
Subjects must be fit to undergo bronchoscopy	Presence of other conditions that the principal investigator judges may interfere with the study findings
	Evidence of alcohol or drug abuse

3.3.2 The AERIS Study

Subjects with stable moderate to very severe COPD as defined by GOLD guidelines[1] were recruited into the study. Post-bronchodilator spirometry was used to assess airflow obstruction with a FEV1/FVC ratio of <0.7 and an FEV1 of <80% predicted value required for enrolment. Detailed inclusion and exclusion criteria are shown in Table 3.

Table 3. Inclusion and exclusion criteria for the AERIS study

Inclusion Criteria	Exclusion Criteria
Participants who the investigator believes can and will comply with the requirements of the protocol	A confirmed diagnosis of asthma, cystic fibrosis, pneumonia risk factors or other respiratory disorders (eg, tuberculosis, lung cancer, etc)
Written informed consent obtained from the participant	History of lung surgery α-1 antitrypsin deficiency as underlying cause of COPD
History of ≥10 pack-years of cigarette	α-1 antitrypsin deficiency as underlying cause of COPD
Male or female aged 40–85 years	Evidence of alcohol or drug abuse
Confirmed diagnosis of COPD based on post bronchodilator spirometry with FEV1 ≤80% of predicted normal and FEV1/FVC <0.7 [239]	Moderate or severe COPD exacerbation not resolved at least 1 month prior to enrolment and less than 30 days following the last dose of oral corticosteroids
Moderate, severe or very severe COPD, according to GOLD staging[1]	Long-term corticosteroid or antibiotic therapy Use of any antibacterial, antiviral or respiratory investigational drug or vaccine within 30 days of the enrolment visit
Documented history of ≥1 exacerbation requiring antibiotics and/or oral corticosteroids or hospitalisation in las 12 months	Presence of other conditions that the principal investigator judges may interfere with the study findings
	Risk of non-compliance or inability to comply with the study procedures

3.4 Protocols

3.4.1 Spirometry

Spirometry was performed with a Micro-Lab Carefusion spirometer in accordance with ATS guidelines by a trained individual following the SOP. Briefly, forced spirometry was selected on the spirometer and the flow volume graph screen appeared. The subject was seated and a nose clip was applied during the procedure. The patient was instructed to take a deep breath in filling their lungs to total lung capacity, making a good seal around the mouthpiece and blowing out as hard and as fast as possible, and for as long as possible. This continued until their lungs were completely empty and they were coaxed through the process. The blows were repeated, or rejected as necessary and up to 8 blows were allowed to ensure repeatability or 3 blows with the best blow. Data was only used if it was deemed to be off sufficient quality as defined by the ATS guidelines. Spirometry results recorded were FEV1, FEV1% predicted, FVC, FVC% predicted, FEV1/FVC ratio, FEF25%–75% and FEF25%–75% % predicted.

3.4.2 Transfer Factor

Gas transfer was only performed in the AERIS study, using a Collins CPL machine or HDpft4000 by a trained member of staff following the SOP. Briefly, tidal breathing was traced to ensure there are no irregularities. The patient was then instructed to exhale slowly to residual volume and tap their leg when they had blown out all their air. At this point the space bar on the computer software was pressed and the patient instructed to immediately take a deep and full breath into TLC. When TLC was reached the V key was pressed which shut off the valve thus preventing air going back into the bell spirometer. The patient was then asked to hold their breath for 10 seconds until the red sample line reaches the vertical lines at which point they were asked to breathe out with medium force until RV (the red sample line touches/plateaus with the RV dotted blue line). The space bar was pressed at RV. 4 minutes were given between tests, allowing adequate washout of the gases from the lungs. Tests were acceptable if SOT (complete inspiratory breath) were within 2 seconds, breath hold were between 8 and 12 seconds and complete exhalation was within four seconds. A maximum of 5 tests were performed and two DLCO results needed to be within 10% of each other.

3.4.3 Plethysmography

This was performed in the AERIS study using a Body Plethysmograph HDpft 4000 (inspire Healthcare Ltd.) Briefly, a sterile mouthpiece was placed on the on the end of the disposable viral filter and it was adjusted so that it was the correct height for the patient. The plethysmograph door was closed and the patient was instructed to place the mouthpiece in their mouth (ensuring a tight seal and no leaks) and attach their nose clip and breathe normally. After 5-6 breaths a vertical line appeared on the computer software, after which on exhalation the space bar was pressed, causing the shutter to close on the subject's next inspiration. The subject was coached to pant against the shutter aiming for a frequency of 30-60 breaths/minute (0.5-1Hz) for 3-4 breaths. Following the panting manoeuver the subject was asked to perform an SVC manoeuver by breathing into TLC and then slowly exhaling to RV and holding for 2-3 seconds before returning to tidal breathing. The space bar was pressed to end the test. The VTG graph and alveolar pressure versus plethysmograph pressure loops were viewed and assessed for technical acceptability. If there was more than one loop then the VTG values of those loops needed to be within 5% of each other. A minimum of three acceptable measurements of FRC_{pleth} were obtained and two measurements of FRC_{pleth} must have been within 5% (Highest-lowest/mean) of each other and the mean reported. The two best IC measurements must have been within 150mls or 5% of each other. The two best VC measurements must have been within 150mls or 5% of each other.

3.4.4 6 minute walk test

The 6MWT was performed by a suitably qualified member of staff, on a 30 metre pre-prepared course. A finger-tip pulse oximeter was attached to the patient's finger and the oximeter checked to make sure that it is gave a good signal. The oximeter readings were observed for at least five minutes to ensure stability and to ensure the patient was rested. Baseline measurements at rest were recorded (heart rate, oxygen saturation). The Patient was instructed to walk round the pre-prepared course at their own pace for 6 minutes and were informed they could stop to rest if necessary. The number of laps walked in 6 minutes, end $Sp0_2$, lowest saturations and end heart rate were noted. Measurements of SpO2 and heart Rate were recorded at 1 and 2 minutes post exercise.

3.4.5 Body composition measurements

Body composition measurements were performed in the AERIS study. Height and weight were documented and the BMI was calculated using the equation height/weight^2. Triceps skinfold thickness was measured using skinfold callipers. The measurement was performed at the half-way point between the acromion (shoulder) and olecranon (elbow) on the posterior aspect of the arm.

FFMI was calculated from body impedance, measured by using a bodystat quadscan 4000. Different components of the body have varying levels of resistance in response to different frequencies of electrical signals. The *Bodystat QuadScan 4000*, provide actual resistance/impedance values from measurements taken at frequencies of 5, 50, 100 and 200 kHz and additionally calculate several *estimates* of body composition. It was performed by a trained member of the study team. Briefly, the patient was placed in the supine position and two electrodes were placed on the right foot and two on the right hand. They were connected to alligator clips from wires attached to the bodystat machine. The machine was switched on and the measurements were calculated and FFMI was recorded.

3.4.6 Sputum Induction

Sputum induction was performed using an Ultrasonic Nebulizer (Devilbiss Ultraneb U3000). Prior to the procedure subjects were given 200mcg of inhaled Salbutamol and then underwent spirometry to determine the post-bronchodilator FEV1. Based on FEV1 (L) subjects were divided into two groups; those with FEV1 ≥1L and those with FEV1<1L. Subjects with FEV1≥1L were administered 3 cycles of nebulized 3% Sodium Chloride solution at a rate of 2-4ml/min each lasting 7 minutes. After each 7-minutes cycle subjects attempted to expectorate sputum. All subjected were instructed to perform an oral cleansing technique prior to the official cough attempts. If no adequate sample was obtained, two further cycles could be attempted. Repeat spirometry was

performed between cycles to ensure no more than a 20% FEV1 drop from the baseline. A modified protocol was applied for the "at risk" group with FEV1 <1L and 0.9% Sodium Chloride was administered at a low output nebulisation (≤1ml/min). Unlike a group with FEV1>1L a cut off of 10% FEV1 drop was applied at post-inhalation spirometry checks. For both protocols sputum induction was terminated if subjects developed troublesome symptoms, FEV1 dropped beyond the safety cut-off or when a sufficient sputum sample was obtained.

3.4.7 Sputum Processing

Sputum was collected in a petri dish and processed within 2 hours from expectoration. Sputum plugs were separated from saliva using tweezers and sputum samples were weighed in polypropylene tubes. Based on the weight the sputum samples were divided for biomarkers and microbiology according to the study protocol. In cases when a very small sample was obtained (less than 0.1g) the sample for microbiology was prioritised. Sputum was filtered through 100µm filters and centrifuged at 400g for 10 minutes at 4^{o}C. The supernatant was aliquoted and stored at -80^{o}C for analysis, while the resulting cell pellet was resuspended in PBS at a concentration of 5x10^{5} cells/ml. Cells were loaded onto poly-L-lysine coated glass slides using a cytocentrifuge as previously described[240].

3.4.7.1 Sputum cell counts

Cells on poly-L-lysine coated glass slides were stained with Rapid Romanowsky stain (Raymond Lamb Ltd, Eastbourne, UK). Differential cell counts were obtained by counting 400 cells using light microscopy. For quality control purposes, only sputum samples with fewer than 30% squamous cells were included in the analysis.

3.4.7.2 Sputum Culture for PPMs

Sputum was cultured to identify the main PPMs in COPD; NTHI, MCAT, SP, SA and PA. A biomedical scientist in the Public Health England Laboratory processed microbiology sputum samples within 6 hours from expectoration. Samples were diluted with DTT in 1:1 ratio and these were then inoculated to a selection of agar plates and incubated in 5% CO2 @37.0 for 24-48hrs. The plate selections included; Blood agar (horse blood) - a general plate that will grow all bacteria except NTHI, Chocolate agar (lysed horse blood) - nutritionally rich and grows all bacteria, Bac – a chocolate agar plate containing Bacitracin, which is an antibacterial agent that eliminates all gram positive bacteria leaving only gram negative (NTHI and MCAT); CAN – a blood agar plate containing antibiotics against gram negative bacteria, leaving only gram positive organisms (SP and SA); CFC – a plate containing various antibacterial agents and where PA is the only organism that grows on it.

Suspect colonies were then selected off the plate and subjected to series of tests to confirm the identity of isolates. NTHI isolates were confirmed by applying the factor XV assessment as NTHI requires X+V factors to grow after 24 hours of incubation. MCAT isolates were confirmed via gram film (negative cocci); oxidase test (chemical colour change), Tributyrin test (colour change) and DNase (zone of clearing around colony). SP isolates were confirmed by a positive optochin test (zone of growth inhibition of more than 14mm). PA isolates were confirmed by growth on CFC plate (bright green colonies and gram negative rods). SA isolates are confirmed by Gram positive cocci and morphologically distinct white colonies and coagulase/Staphylococcus latex test was performed to confirm the isolate.

3.4.7.3 Sputum for PCR

This work was performed by GSK. Briefly, nucleic acids were extracted using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche Diagnostics), as per the manufacturer's instructions. A triplex real-time quantitative PCR assay was used for the detection and quantification of the lipo-oligosaccharide glycosyltransferase encoding gene (*lgtC*) of NTHi, the CopB outer membrane protein encoding gene (*copB*) of MCAT, and the autolysin encoding gene (*lytA*) of SP. The presence of SA and PA was determined using a triplex real-time PCR assay targeting conserved regions of the CDS23 gene, the clumping factor A encoding gene (*clfA*), and the GDP mannose dehydrogenase encoding gene (*algD*), respectively. The concentration of bacterial DNA in each sample, expressed in copy/mL, was inferred from the calibration curve (made of serial dilutions of a plasmid containing the sequences targeted by the PCR assays) present in each PCR plate and corrected against the dilution factors at each step of the process (DNA extraction and PCR reaction).

3.4.8 Phlebotomy

Blood samples were analysed by the University of Southampton pathology laboratory. They were analysed for inflammatory markers (CRP, fibrinogen, pro-calcitonin) and full blood count. For the AERIS study five novel serum biomarkers were analysed by GSK using ELISA assays; high sensitivity troponin (hs-trop), IL-6, IP-10, n-terminal pro b-type natriuretic peptide (NT-proBNP) and surfactant protein D (Sp-D).

3.4.9 Bronchoscopy

Bronchoscopy was performed in the MICA study. Prior to bronchoscopy the subjects CT scans was reviewed by a thoracic radiologist and a "diseased lobe" and "preserved lobe" were chosen according the estimated quantity of emphysema. Both lobes were targeted at bronchoscopy, which was performed on an outpatient basis. Subjects were asked not to eat and drink four hours prior to

the procedure. All subjects were given 2.5mg of nebulised salbutamol and underwent spirometry. A combination of intravenous alfentynyl (100-500mcg) and midazolam (1-10mg) were given as sedation. Lignocaine spray and gel were used as local anaesthetic to the nose and pharynx. 10mls 1% lignocaine was used as local anaesthetic for the larynx and bronchial tree and were given via the bronchoscope channel. The bronchoscope was passed through the nose in all subjects. The two lobes were then sampled.

Microbiology samples were taken using a protected brush technique. This is a brush that has a wax bung at the tip, preventing contamination from the upper airways. When the brush was in the lobe that was to be sampled, the wax tip was expelled and the brush advanced. The brush was then gently rubbed against the mucosal surface of the airway and then withdrawn. Four protected brushes were taken from each lobe. BAL was performed by advancing and wedging the bronchoscope into a segmental bronchus. 100mls of 0.9% saline in 20ml aliquots were introduced into each lobe and recovered by gentle aspiration.

3.4.10 Microbiological analysis of protected brushes

The brushes were collected in PBS and then centrifuged and the resulting pellet resuspended in 250µl of PBS. For culture, this liquid was used for sweeps on plates as described above. For PCR, 1ml of STGG was added and the samples frozen at -80oc until required. RNA extraction and PCR for the relevant bacteria was performed as already described.

3.4.11 BAL analysis

BAL fluid was poured through 100µm filters and cells removed by 400g centrifugation for 10 minutes at 4^{o}C. The supernatant was aliquoted and stored at -80^{o}C prior to analysis, while the resulting cell pellet was resuspended in PBS and cytocentrifuge slides were prepared. Differential cell counts were performed as previously described for sputum samples.

3.4.11.1 MMP Analysis

MMP concentrations in BAL were quantitated using a microparticle based multiplex immunoassay (R&D systems, Abingdon, UK) developed by Luminex corp. MMP beads were used to analyse MMP-1, -2, -3, -7, -8, -9, -10, -12 -13, extracellular matrix metalloproteinase inducer (EMMPRIN), an adhesion molecule that induces MMPs.

The assays were prepared using an adapted protocol from the manufacturers guidelines. BAL supernatant was diluted 1 in 5 in calibrator diluent. Standards were made up according to

manufacturer instructions. Microparticles were spun at 1000g for 30 seconds and then gently vortexed. 17µl of each microparticle was mixed with 5.2ml of microparticle diluent. Each well of the 96-well plate was pre-wet plate with 100µl wash buffer and vacuumed off. The microparticle solution was vortexed and 50µl was then added to each well. 50µl standard or sample were added to each well. The standard curve was loaded vertically down the plate. The plate was covered with a foil plate sealer and shaken at full speed for 30 seconds and then at 500rpm for 2 hours. The biotinylated secondary antibody was then made up by spinning at 1000g for 30 seconds and then gently vortexed. 17µl of each antibody was into the biotin antibody diluent bottle. After the two hours the fluid was vacuumed off the plate and washed with 100µl of wash buffer three times. 50µl of diluted biotinylated antibody was added to each well and the plate was covered and incubate on a rocker at full speed for 30 seconds and then for 1 hour at 500rpm. In the meantime the diluted streptavadin-PE was made up by spinning the vial at 1000g for 30 seconds and gently vortexed. 55µl was added to 5.5ml of wash buffer in a brown mixing bottle. The plate was once again washed with 100µl of wash buffer three times. 50µl of streptavadin-PE was added to each well and the plate was covered and incubated on a rocker for 30 minutes. The plate was then washed with 100µl of wash buffer for three final times. The contents of each well were suspended in 80µl of wash buffer and shaken on a plate shaker for two minutes at full speed. The plate was then analysed on the Luminex 200 platform (Biorad Bioplex 200, Hemel Hempstead, UK), as per manufacturer's instructions.

3.4.11.2 TIMP analysis

TIMP 1-4 concentrations in BAL were also quantitated using a microparticle based multiplex immunoassay (R&D systems, Abingdon, UK). The method was exactly the same as for the MMP assay apart from the TIMP pack were used and the beads were already pre-mixed.

3.4.11.3 Cytokine Analysis

Cytokine analysis was performed for the following cytokine; IL-1β, IL-2, IL-6, IL-8, IL-10, GM-CSF, IFNγ and TNFα. Once again a microparticle based multiplex immunoassay (R&D systems, Abingdon, UK) was used and the protocol was the same apart from using the pre-mixed cytokine beads.

3.4.12 CT Protocol

CT scanning for the two studies was undertaken on a Siemens Sensation 64 CT scanner (Siemens Medical Solutions, Erlangen Germany). The MICA and AERIS enrolment scans comprised of a helical scan in inspiration and one in held expiration. Due to wanting to limit cumulative radiation exposure the AERIS follow-up scan at month 24 compromised a full inspiratory scan and only high-resolution slices in expiration. During the scan each subject was given strict instructions about position, which

consisted of having the arms raised above their heads. The imaging protocol consisted of; slice thickness 0.75mm, slice separation 0.5mm, tube voltage 120KV, effective mAs 90mAs (using dose modulation), collimation 0.6mm and a pitch of 1. A number of different reconstruction algorithms were stored and for the purposes of Apollo analysis the CT images were reconstructed to a slice thickness of 0.75mm and a reconstruction increment of 0.5mm on a sharp kernel reconstruction algorithm (B35f).

The scan data was anonymised to a study number on the CT scanner. This data was transferred directly from the scanner to a DVD burning robot in the UHS PACS office, which automatically burnt the data onto DVD. The images were then backed up onto the hard drive of the image processing workstation and the original DVDs were then stored in a secure location distant to the workstation. All CT images were reviewed by a thoracic radiologist to determine if there were any clinically important abnormalities, which were then managed as per local clinical practise.

3.4.13 Image Analysis

CT scans were analysed using a number of different methods in this study.

3.4.13.1 Visual Scoring

For emphysema, gas trapping and bronchiectasis a visual scoring system adapted from previous studies was used (Table 4). A thoracic radiologist, who was blinded to patient details, performed this scoring. For each patient three slices were taken and a total score given.

Table 4. Visual score for emphysema and gas trapping.

Estimated emphysema	Estimated gas trapping	Score
<5%	<5%	0
5-25%	5-25%	1
26-50%	26-50%	2
51-75%	51-75%	3
>75%	>75%	4

Score estimated for each slice. 3 slices used (caudal, middle and cranial) to give a cumulative score out of 12.

3.4.13.2 Apollo Software Analysis

Quantitative analysis of the CT scans was undertaken with Apollo software by VIDA® Diagnostics, Inc., Iowa, USA. This is a commercially available three-dimensional analysis software tool for pulmonary CT scans. It automatically segments the bronchial tree, lungs and pulmonary vasculature and from this data the dimensions and geometry of the bronchial tree can be ascertained and analysis can be performed on the lung parenchyma and airway tree.

For the MICA study CT analysis was performed by myself using the Apollo software. For the AERIS study the image analysis was performed by the VIDA image-processing lab. The protocol for CT analysis was developed by direct tuition from VIDA and by consulting the Apollo user Manual. The B35f reconstruction was used for image analysis by the software and both inspiratory and expiratory scans were analysed using the same method. The CT scan was imported into the VIDA Processing Server Case Library Scheduler (VPS/CLS) and the CT files with the B35f reconstruction algorithm were selected for analysis. Automated analysis was performed and the segmentation of the airways and lungs were inspected and checked. Each pulmonary segment branch was visually checked to ensure that it was correctly labelled and that no significant airway branches have been missed in the segmentation process. Missed branches and missing/incorrect labels were manually added. Once this was complete and everything correct the analysis was updated. The rendered overlay of the lobes were then visualised and checked and if necessary manual corrections were made. On some occasions the anatomy of the airway tree or lung did not conform to the standard upon which the accepted nomenclature is based and therefore investigator judgement was used. When the segmentation of the airway and lobes were correct the final update was performed and the data reports were produced. The data from the scan was extracted as two CSV files; airways and histogram.

3.4.13.2.1 Emphysema measurements

Three different methods were used to quantitate emphysema using Apollo software and all of these used the inspiratory CT scan. Two of these techniques applied CT densitometry to the inspiratory scan and assessed different thresholds; -950HU, and -910HU. The other technique assessed the attenuation value of the inspiratory image below which lie 15% of the voxels ($PERC_{15}$). Emphysema was quantitated in the entire lungs and for each of the five lobes.

3.4.13.3 Dimensions of large and intermediate Airways

The Apollo software uses a region growing approach to segment the bronchial tree and for my study this was done for all airways down to the segmental level. More distally than this only airways in five paths were segmented; RB1, RB4, RB10, LB1 and LB10. Airways under 2mm in diameter were

excluded from the analysis as these are too small to be accurately measured by CT imaging. The analysis software measures a number of dimensions for each airway it visualises and I used inner and outer perimeter, wall thickness, lumen area, outer wall area and total airway area. From this the wall area % could be derived by calculating the ratio of the outer wall area against the area of the entire airway. These dimensions were analysed using four different methods as the bronchial tree is a complex structure with multiple ways of labelling it. The first method consisted of averaging these dimensions for the whole airway. The second method measured these dimensions in each airway generation. The third method measured these dimensions in each segmental generation airways. This method involves labelling the segmental airways as the segmental generation 3 and the next airway distal to these are segment al generation 4 and so on. Finally, the last method involved measuring these dimensions in each of the five airway paths in each segmental generation.

I also analysed another measure calculated by the Apollo software termed Pi10. This represents the square root of the wall area for a hypothetical airway with a perimeter of 10mm. The software only uses airways with a perimeter of less than 20mm to derive this value.

3.4.13.4 Gas Trapping

Gas trapping is thought to represent an indirect measure of small airways disease in COPD. A number of different techniques can be used to calculate gas trapping either directly or indirectly from the Apollo software. I assessed four of these parameters in this study. EXP_{-856} is the simplest measurement and represents the % of voxels on the expiratory scan below -856HU. $EXP_{856-950}$ represents the percentage of voxels between -856HU and -950HU on the expiratory scan. E/I MLD represents the ratio of the mean lung density on expiratory and inspiratory scan. $RVC_{856-950}$ is a more complex measure and is calculated using the technique described previously[209].

3.4.13.5 Local histogram-based Emphysema analysis

Emphysema sub-types were measured using custom software designed by the chest-imaging laboratory at Brigham and Women's hospital and Harvard medical school. Characterisation of the emphysema pattern was achieved using local histogram information. Briefly, the algorithm was trained using 267 CT scans from the COPD subjects. Each scan was processed into ROIs of size 24.18 3 24.18 mm2 and each of these were labelled by a physician with expertise in chest radiology. These labels were then used to teach a classifier that would classify new ROIs into one of six categories; NE, mild CLE, moderate CLE, severe CLE, PLE and PSE. Areas of lung with normal-appearing architecture were labelled NE. CLE labels were applied to regions with emphysematous destruction within the secondary pulmonary lobule with overall preservation of architecture (mild), more

confluent emphysema but preservation of the bronchovascular bundle (moderate), or confluent emphysema with obliteration of the bronchovascular bundle but preservation of septa (severe). Regions with complete destruction of the parenchyma including the septa and bronchovascular bundles were labelled as PLE. Regions of emphysema abutting a pleural surface were labelled as PSE. The local histogram for each ROI was computed using a kernel density estimator and each emphysema pattern was matched to a ROI local histogram. The computer algorithm was therefore able to determine which emphysema pattern was located in each region of interest on a chest CT and when a whole CT was analysed could produce six continuous measures representing the lung volume percentage that was classified into each of the six patterns.

In this study, images reconstructed with the B30 kernel were used for image analysis. These were uploaded into the computer analysis software by an analyst with no manual intervention required.

3.4.13.6 Body Composition CT analysis

Body composition on CT was measured using custom software designed by the chest-imaging laboratory at Brigham and Women's hospital and Harvard medical school. Pectoralis muscle area (PMA) and anterior chest fat (ACF) were measured on a single-axial slice of the chest CT (B30f reconstruction). The superior aspect of the aortic arch was identified on each scan and the image was scrolled toward the apex of the lungs to identify the first axial image above the arch, which was used for analysis. The left and right pectoralis major and minor muscles were then identified on the anterior chest, and their edges manually segmented using a predefined attenuation range of between -50 and 90 HU (Figure 11). ACF area was measured in the region between the pectoralis major muscles and skin surface on the same axial slices and, their edges were manually determined using a range between -200 and 0 HU (Figure 11). Both PMA and ACF area were measured in square millimetres and the right and left pectoralis major and minor and the right and left fat areas were combine to give one result for each.

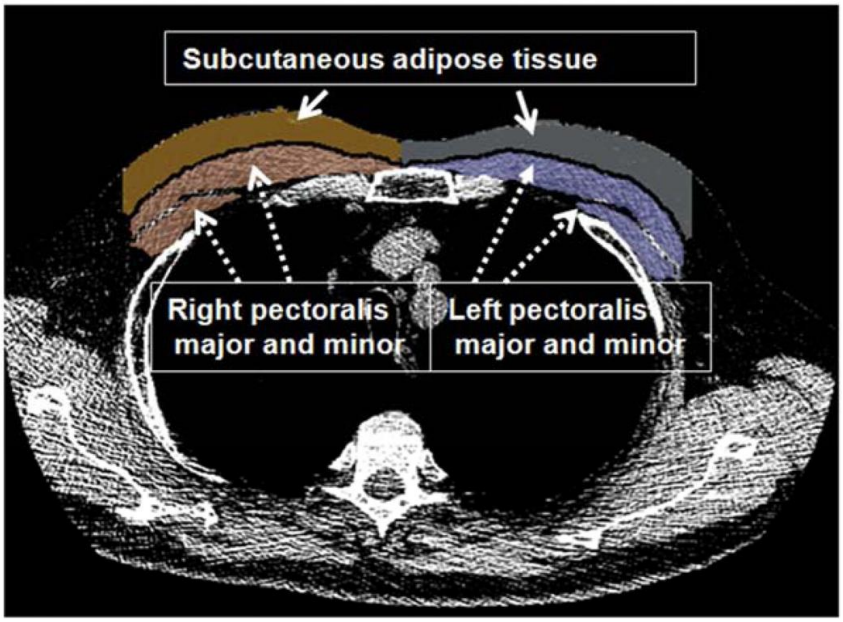


Figure 11. Axial CT slice at aortic arch demonstrating muscles and fat segmentation. Reproduced with permission by Elsevier Publishing.

3.4.13.7 Optimisation of Image Analysis

As described in the introduction section there are a number of possible technical and methodological limitations in CT image analysis. For these studies I attempted to limit these as much as possible. All of the scans were performed on the same CT scanner using the same reconstruction algorithms. This CT scanner was calibrated using the same technique on a daily basis, thereby reducing variability caused by CT scanner factors. The scans were performed to an optimum protocol by having thin slices and inter-slice distance, meaning accurate three-dimension reconstructions could be produced. Dose modulation was also used, which means that the scanner delivers a uniform radiation dose to the lungs depending on patient size and shape. During the scan each subject was given strict positional and breathing instructions in an effort to perform the scans at the correct moment in the breathing cycle and thereby reduce variability.

3.5 Statistical Analysis

All statistical analyses in this study were performed using SPSS version 21. All data are presented as medians and inter-quartile range unless stated otherwise.

3.5.1 The MICA study

This was a proof of concept study and so was pragmatically powered. As for the most-part data was not normally distributed, parametrical statistical analyses were performed. Mann-Whitney U test and Fisher's exact tests compared data between COPD and control groups. The Wilcoxon signed-rank test and related samples McNemar test were used to compare related data between two lobes. Friedman's two-way analysis of variance was used to test CT data between all five lobes. Associations were tested using spearman's correlation with rho and p values presented. Partial Spearman's correlation was used to conduct multivariate analysis. For the purpose of statistical analysis, values that were below the lower limit of detection were given the value of half the concentration of detection. A p-value of <0.05 was considered statistically significant.

3.5.2 The AERIS study

The AERIS study was powered in order to assess the primary end-point, which was the aetiology of exacerbations in COPD. As with the MICA study, data was for the most-part not normally distributed and therefore parametrical statistical tests were performed. Associations were tested using Spearman's correlation. Mann Whitney U test compared data between two groups. The independent samples Kruskal-Wallis test was used to test differences between multiple groups. Friedman's two-way analysis of variance was used to test CT data between all five lobes. Multivariate analysis was performed using multiple linear regression, logistic regression and partial correlation. For linear regression, if residuals were not normally distributed then logarithmic transformation was performed in order to achieve this.

For the longitudinal part of the study, the Wilcoxon signed-rank test was used to assess differences in CT data between enrolment and month 24. The independent samples Kruskal-Wallis test was used to test differences between multiple groups and the Mann Whitney U-test was used to compare data between two groups. Spearman's correlation was used to test associations and linear regression was used for multivariate.

3.5.3 Multiple associations

Due to the large number of CT, clinical, physiological and biological variables measured in this study there were a substantial number of comparisons made throughout the analysis. I chose not to use a post hoc multiple comparison test such as the Bonferroni correction as the cut-point for statistical significance would be prohibitively, and unrealistically low. Although this increases the chance of

false positive results, the number and strength of associations as well as the direction of associations were assessed and described within the discussion section.

Chapter 4: Inflammatory markers, MMPs and microbiology measured by targeted BAL in COPD

It has been hypothesised that inflammation, proteinases and chronic bacterial infection are key mechanisms in the lung damage that occurs in COPD, ultimately leading to clinic features of the disease. However, these mechanisms have not been fully defined, thanks in part due to the heterogeneity of the disease as well as the significant variability that exists within an individuals lungs. Previous studies have not taken into account the regional variability of the disease within the lungs and have relied on sputum sampling or 'random' bronchial sampling from poorly characterised regions of the lung.

This study aims to investigate these mechanisms by performing targeted bronchoscopic sampling on a cohort of COPD and PLF subjects. The method I have employed is novel as it utilises CT imaging to guide sampling from two different lobes; one with the most evidence of disease and another with the least evidence of disease. This ensured that results were not affected by random sampling as they were averaged between the two lobes. Furthermore, it allowed me investigate the variability of these mechanisms within an individuals lungs.

This chapter describes my findings in this cross-sectional study and specifically discusses the concentrations of inflammatory markers, MMPs and bacterial colonisation within the airway of COPD subjects and their relationships with spirometric marker of disease.

4.1 Subject Characteristics

24 COPD subjects with stable disease and 8 ex/current smokers with preserved lung function (PLF) were successfully recruited into the study. They were well matched for age, sex, current smoking status, pack years and BMI (Table 5). There were however more males in both groups. As expected FEV1% and FEF75-25% were significantly reduced in the COPD group. In the COPD cohort the median number of exacerbations in the 12 months prior to enrolment was 1.5.

Table 5. Characteristics of participants included in the study

	COPD (n=24)	PLF (n=8)	P value
Age	66.0 (12.0)	56.0 (18.0)	0.064
Male	16	6	>0.999
Current smoker	11	5	0.685
Pack Years	39.0 (39.8)	32.3 (23.8)	0.268
FEV1%	69.0 (21.0)	108.0 (20.3)	**<0.001***
FVC%	98.0 (22.8)	119.0 (26.3)	0.147
FEV1/FVC	54.5 (10.8)	78.0 (9.5)	**<0.001***
FEF75-25%	21.5 (16.0)	77.5 (45.3)	**<0.001***
BMI	28.3 (4.1)	26.1 (5.6)	0.252
Exacerbations in last year	1.5 (2.1)	-	

Values are given as medians (IQR). Male and current smokers given as number of subjects. *p<0.05. Fishers Exact test for male and current smoker. Mann-Whitney U-test for all other variables. PLF – preserved lung function.

4.2 Blood Results

To characterise the systemic inflammatory status of the cohort, blood analysis was performed. White cell counts were successfully measured in 30 of the subjects (22 COPD and 8 PLF) and for fibrinogen 31 of the subjects (23 COPD and 8 PLF). There was no significant difference between total leukocyte count, white cell differential or fibrinogen concentrations between subjects with COPD and those with PLF (Figure 12).

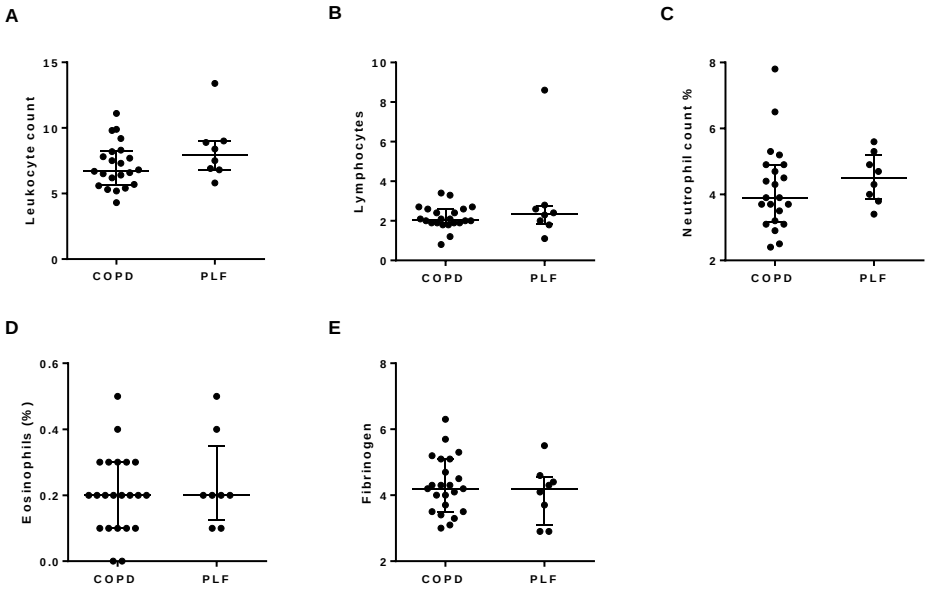


Figure 12. Serum concentrations of (A) Leukocyte (B) Lymphocytes (C) Neutrophils (D) eosinophils (E) fibrinogen. Data represents median with IQ range. Each dot represents serum concentration of individual value in a specific patient. For white cell counts n=22 for COPD and 8 PLF. For fibrinogen n=23 for COPD and 8 for preserved lung function (PLF).

When assessing the association of these blood markers with spirometric markers of disease the only significant association was a negative one between FEV1% and fibrinogen (Table 6). When only including COPD subjects, the negative association between fibrinogen and FEV1% remained and there was also a negative association between fibrinogen and FEF75-25% and also between lymphocytes and FEF25–75%.

Table 6. Correlation between blood White cell counts, fibrinogen and spirometric markers of disease.

	FEV1%	FVC%	FEV1/FVC	FEF25%–75%
Whole Cohort				
Leukocytes	0.07	0.12	0.07	-0.03
Lymphocytes	-0.14	0.01	-0.07	-0.19
Neutrophils	0.09	0.17	0.02	-0.12
Eosinophils	-0.05	-0.16	0.05	0.07
Fibrinogen	**-0.49****	-0.31	-0.28	-0.32
COPD subjects				
Leukocytes	-0.08	0.16	-0.14	-0.34
Lymphocytes	-0.30	0.03	-0.23	**-0.44***
Neutrophils	-0.06	0.12	-0.19	-0.24
Eosinophils	-0.11	-0.14	-0.02	0.05
Fibrinogen	**-0.59****	-0.23	-0.38	**-0.46***

Spearman's correlation coefficient. For white cell counts n = 30 (n=22 for COPD) and for fibrinogen n = 31 (n=23 for COPD). *p<0.05, **p<0.001.

4.3 BAL inflammatory Profile and MMP and TIMP concentrations

It is unlikely that blood inflammatory markers reflect the localised conditions within the lung compartment, especially in such a mild cohort of subjects. Bronchoscopy and BAL were therefore used to study localised inflammation and proteases within the lungs of subjects with COPD and PLF. This was successfully performed in all 32 subjects. As previously described each subject had two lobes sampled and for initial analysis the mean concentrations between the two lobes were used.

4.3.1 BAL Cell Differential

BAL was prioritised for other assessment and so adequate quantities for cell differentials were only achieved in 26 subjects (19 COPD and 7 PLF). As opposed to the blood results there were significantly increased neutrophils in the BAL of COPD subjects compared to subjects with PLF (Figure 13). Conversely there were significantly increased BAL macrophages in PLF subjects as opposed to those with COPD. Relative numbers of eosinophils and lymphocytes in BAL were low and there were no significant differences between groups.

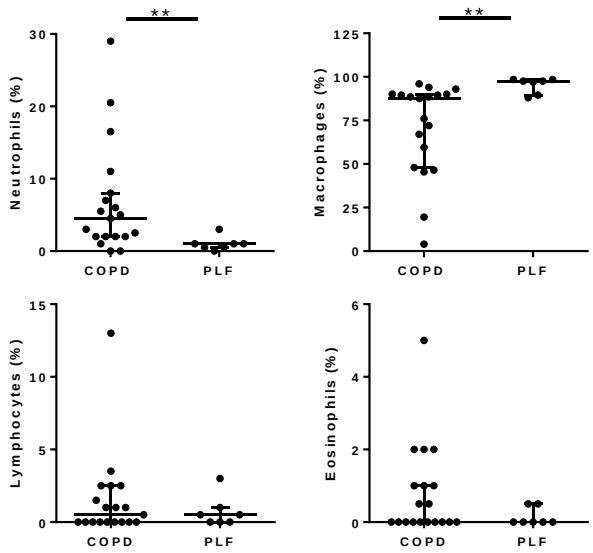


Figure 13. BAL expression of white blood cells in subjects with COPD and preserved lung function (PLF). (A) neutrophils (B) Macrophages (C) lymphocytes (D) eosinophils. Data represents median with IQ range. Each dot represents BAL concentration of individual value in a specific patient, n=19 for COPD and 7 PLF. **p<0.01 using Mann-Whitney U test.

Next I assessed the relationship between BAL white cell differential and spirometric markers of disease (Table 7). In the whole cohort there were significant associations between neutrophil counts and FEV1%, FEV1/FVC ratio and FEF75%-25%. Conversely there was a positive association between macrophages and these spirometric parameters. However, when analysing only subjects with COPD the significance of these associations disappeared. There were no associations between eosinophils or lymphocytes and spirometry results.

Table 7. Correlation between BAL white cell differential and spirometry

	FEV1%	FVC%	FEV1/FVC	FEF75-25
Whole Cohort				
Neutrophils	**-0.59****	-0.30	**-0.57****	**-0.51****
Macrophages	**0.46***	0.13	**0.62****	**0.56****
Lymphocytes	0.19	0.03	0.12	-0.01
Eosinophils	-0.07	-0.01	-0.03	-0.27
COPD subjects				
Neutrophils	-0.40	-0.19	-0.34	-0.18
Macrophages	0.15	0.00	0.32	0.22
Lymphocytes	0.31	-0.03	0.28	0.04
Eosinophils	0.11	0.07	0.27	-0.13

Spearman's correlation coefficient. N = 26 (N= 19 for COPD) *p<0.05, **p<0.001.

4.3.2 Cytokine Analysis

To further characterise airway inflammation, BAL cytokine concentrations were successfully measured in all 32 subjects. BAL IL-6 and IL-8 concentrations were significantly higher in COPD subjects than subjects with PLF (Figure 14). There were no significant differences in IL-1β and GM-CSF between groups. IL-2, IL-10, IFN and TNF-α were only detected at very low concentration in all samples and so further analysis was not performed.

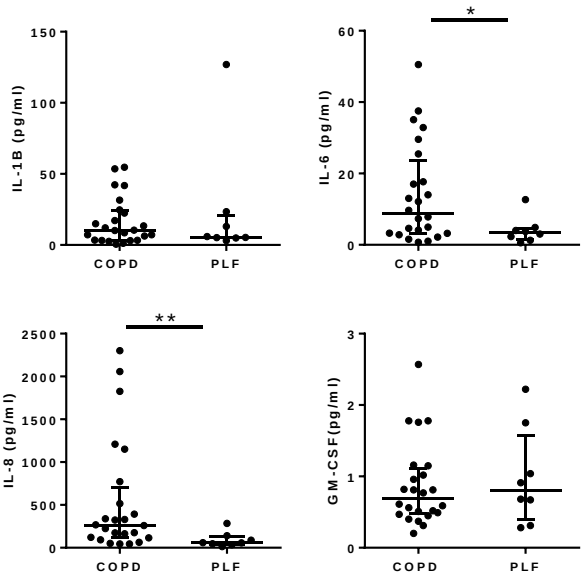


Figure 14. BAL expression of cytokines in subjects with COPD and preserved lung function (PLF). (A) IL-1B (B) IL-6 (C) IL-8 (D) GM-CSF. Data represents median with IQ range. Each dot represents BAL concentration of individual value in a specific patient, n=24 for COPD and 8 PLF. * $p<0.05$ **$p<0.01$ using Mann-Whitney U test

Concentrations of BAL cytokines were compared to spirometric markers of disease, where strong negative correlations were found between IL-6 and IL-8 and FEV1%, FEV1/FVC and FEF75%-25% in the whole cohort (Table 8). Within COPD subjects only there were significant associations between IL-1B and FEV1/FVC and FEF75%-25%. IL-8 also had negative associations with FEV1%, FEV1/FVC and FEF75%-25% while IL-6 only had associations with FEV1/FVC.

Table 8. Correlations between spirometric markers and inflammatory cytokines in whole cohort and COPD subjects alone.

	FEV1%	FVC%	FEV1/FVC	FEF75-25
Whole Cohort				
IL-1B	-0.22	0.21	-0.35	-0.35
IL-6	**-0.51****	-0.16	**-0.56****	**-0.51****
IL-8	**-0.58*****	-0.08	**-0.75*****	**-0.68*****
GM-CSF	-0.00	0.09	-0.15	-0.10
COPD subjects				
IL-1B	-0.16	0.37	**-0.47***	**-0.44***
IL-6	-0.39	-0.09	**-0.50***	-0.37
IL-8	**-0.41***	0.10	**-0.74*****	**-0.57****
GM-CSF	-0.05	0.03	-0.31	-0.18

Spearman's rho values given. N=32 (N=24 for COPD). * p<0.05 **p<0.01 ***p<0.001

4.3.3 MMP Analysis

MMPs have complex mechanisms of actions with multistep activation and so initially the association between the individual MMPs were investigated. There were multiple strong associations between the MMPs measured and this was particularly strong between MMP-8 and MMP-9 (Table 9).

Table 9. Spearman's correlation analysis between MMPs measured in whole cohort.

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12	MMP-13
MMP-1	-								
MMP-2	0.27	-							
MMP-3	0.53**	0.36*	-						
MMP-7	0.26	0.15	0.33	-					
MMP-8	0.52**	0.12	0.41*	0.59***	-				
MMP-9	0.43*	-0.03	0.30	0.57**	0.93***	-			
MMP-10	0.35	0.49**	0.57**	0.56**	0.56**	0.56**	-		
MMP-12	0.63***	-0.01	0.57**	0.39*	0.54**	0.47**	0.22	-	
MMP-13	0.03	0.44*	-0.08	0.15	0.03	-0.07	0.14	-0.21	-
EMMPRIM	0.27	0.10	0.10	0.34	0.28	0.34	0.43*	0.30	-0.29

Spearman's correlation coefficient. N=32. * p<0.05 **p<0.01 ***p<0.001

Median MMP-1, -2, -3, -8, -9 and -10 were significantly higher in the BAL of COPD subjects compared to those with PLF (Figure 15). There was no significant difference in MMP-7, -12, -13 and EMMPRIN between the groups.

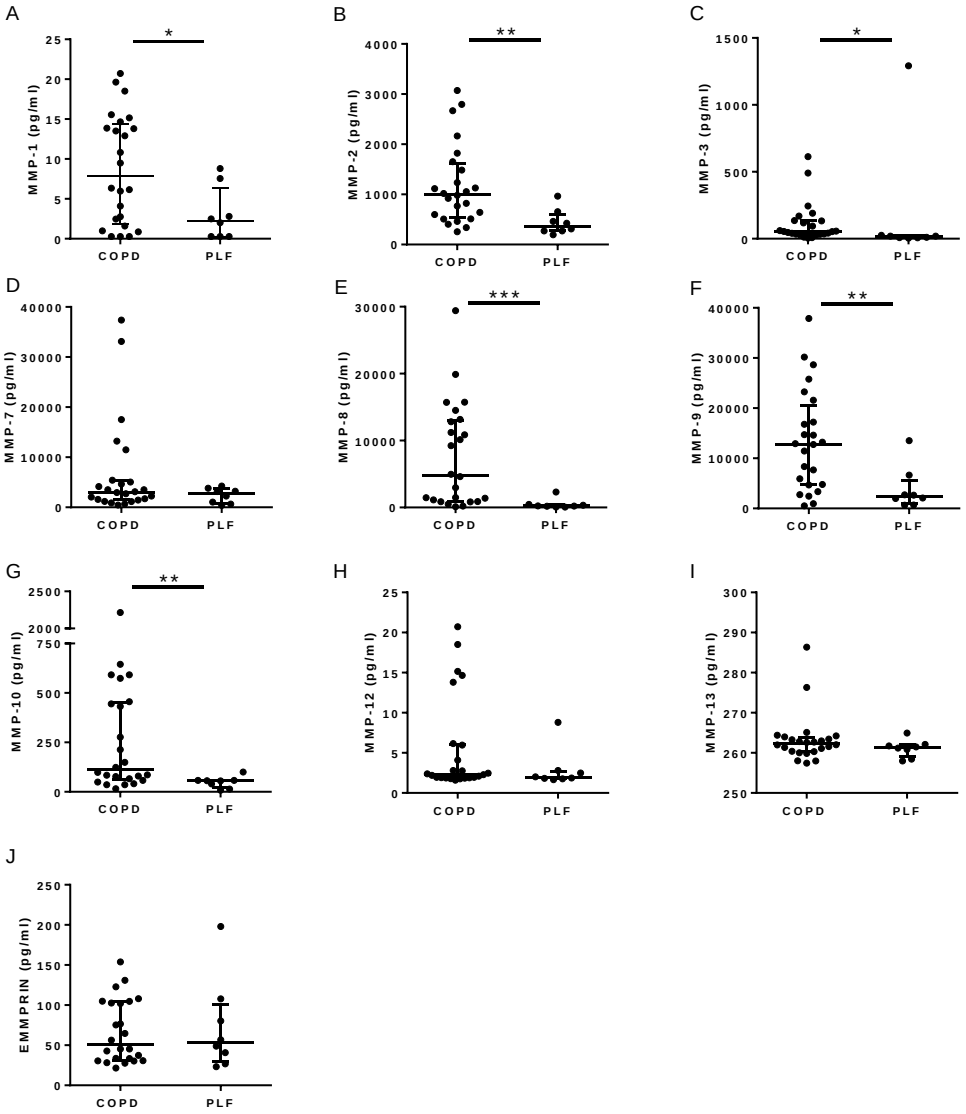


Figure 15. BAL expression of MMPs in COPD and preserved lung function (PLF) subjects (HC). (A) MMP-1 (B) MMP-2 (C) MMP-3 (D) MMP-7 (E) MMP-8 (F) MMP-9 (G) MMP-10 (H) MMP-12 (I) MMP-13 (J) EMMPRIN. Data represents median with IQ range. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for PLF. * $p<0.05$ ** $p<0.01$ ***$p<0.001$ using Mann-Whitney U test.

MMP concentrations were compared with lung function markers of disease severity using Spearman's correlation (Table 10). In the whole cohort there were significant associations between airflow obstruction (FEV1%) and MMP-1, -3, -7, -8, -9 -10 and -12. There were also significant associations between FEV1/FVC and MMP-1, -2, -3 -7, -8, -9, -10 and -12 and between FEF75%-25% and MMP-1, -3, -8, -9, -10 and -12. There were no associations between MMPs and FVC. When analysing COPD subjects only there were fewer associations between MMPS and spirometric markers of disease, although MMP-8, -9 and -12 remained strongly associated with these.

Table 10. Spearman's correlation analysis between MMPs and spirometry

	FEV1%	FVC%	FEV1/FVC	FEF75-25
Whole Cohort				
MMP-1	**-0.50****	-0.07	**-0.56****	**-0.55****
MMP-2	-0.18	0.09	**-0.37***	-0.20
MMP-3	**-0.45****	-0.25	**-0.44***	**-0.35***
MMP-7	**-0.40***	-0.07	**-0.52****	-0.30
MMP-8	**-0.74*****	-0.31	**-0.79*****	**-0.75*****
MMP-9	**-0.65*****	-0.31	**-0.65*****	**-0.65*****
MMP-10	**-0.47****	-0.11	**-0.53****	**-0.43***
MMP-12	**-0.48****	-0.18	**-0.48****	**-0.44***
MMP-13	-0.10	0.05	-0.18	-0.03
EMMPRIM	-0.19	-0.01	-0.24	-0.14
COPD subjects				
MMP-1	-0.35	0.04	**-0.50***	**-0.47***
MMP-2	0.28	0.27	0.05	0.39
MMP-3	-0.34	-0.30	-0.21	-0.03
MMP-7	-0.35	0.00	**-0.57****	-0.18
MMP-8	**-0.60****	-0.15	**-0.69*****	**-0.61****
MMP-9	**-0.59****	-0.19	**-0.56****	**-0.58****
MMP-10	-0.19	0.08	-0.31	-0.11
MMP-12	**-0.51***	-0.21	**-0.52****	**-0.44***
MMP-13	0.02	0.04	-0.06	0.22
EMMPRIM	-0.20	0.11	**-0.44***	-0.22

Spearman's correlation coefficient. N=32 (N=24 for COPD). * $p<0.05$ **$p<0.01$ ***$p<0.001$

4.3.4 TIMP Analysis

To understand the role of MMPs in COPD it is also important to understand the role of their inhibitors the TIMPs. TIMPs were measured successfully in the BAL of all 32 subjects. TIMP-1 and -2 were found in abundant concentrations within BAL (Figure 16), while TIMP-4 was found in much lower concentrations. TIMP-2 and -4 were significantly increased in COPD subjects while there was no significant difference between groups for TIMP-1 and TIMP-3.

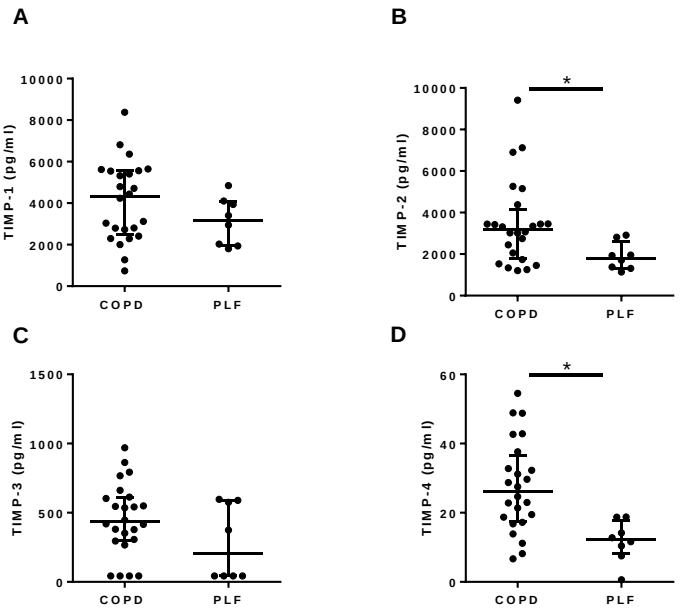


Figure 16. BAL expression of TIMPs in subjects with COPD and preserved lung function (PLF). (A) TIMP-1 (B) TIMP-2 (C) TIMP-3 (D) TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function. * p<0.05 using Mann-Whitney U test.

When analysing the association between TIMPs and spirometry in the whole cohort, TIMP-2, -3 and -4 had significant associations with FEV1%, FEV1/FVC ratio and FEF75%-25% (Table 11). However these associations did not reach significance when investigating COPD subjects alone.

Table 11. Spearman's correlation analysis between TIMPs and spirometry

	FEV1%	FVC%	FEV1/FVC	FEF75-25
Whole Cohort				
TIMP-1	-0.07	0.11	-0.19	-0.09
TIMP-2	**-0.38***	0.03	**-0.51****	**-0.40***
TIMP-3	**-0.38***	-0.15	**-0.42***	**-0.36***
TIMP-4	**-0.56****	-0.18	**-0.62*****	**-0.50****
COPD subjects				
TIMP-1	0.25	0.25	0.03	0.22
TIMP-2	-0.11	0.18	-0.34	-0.11
TIMP-3	-0.26	-0.07	-0.40	-0.26
TIMP-4	-0.26	-0.02	-0.39	-0.13

Spearman's correlation coefficient. N=32 (N=24 for COPD). * p<0.05 **p<0.01 ***p<0.001

4.3.5 MMP/TIMP Ratio Analysis

As it has been hypothesised that a proteinase/antiproteinase is implicated in the development of COPD and emphysema the ratio of MMP/TIMPs in BAL were calculated. 36 different ratios were calculated for each subject (figures 17-25). Ratios were significantly increased in COPD subjects for MMP-8/TIMP-1, -2, -3, -4, MMP-9/TIMP-1 and MMP-10/TIMP-1 and -2.

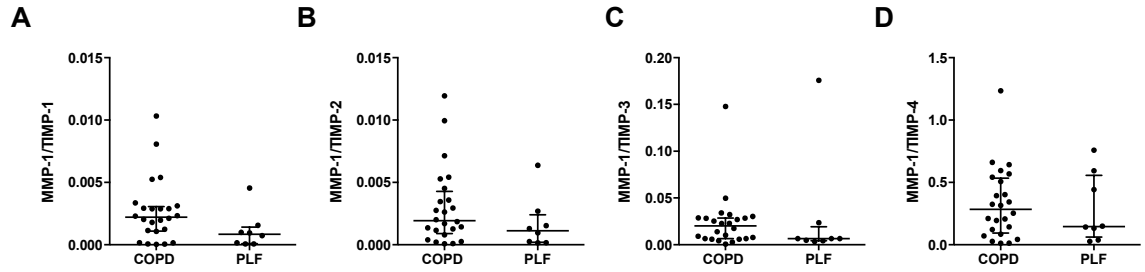


Figure 17. BAL expression of MMP-1/TIMPs in subjects with COPD and preserved lung function. (A) MMP-1/TIMP-1 (B) MMP-1/TIMP-2 (C) MMP-1/TIMP-3 (D) MMP-1/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function.

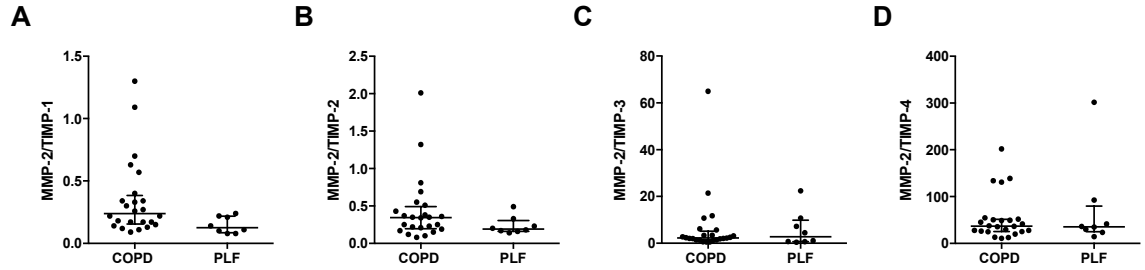


Figure 18. BAL expression of MMP-2/TIMPs in subjects with COPD and preserved lung function. (A) MMP-2/TIMP-1 (B) MMP-2/TIMP-2 (C) MMP-2/TIMP-3 (D) MMP-2/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function.

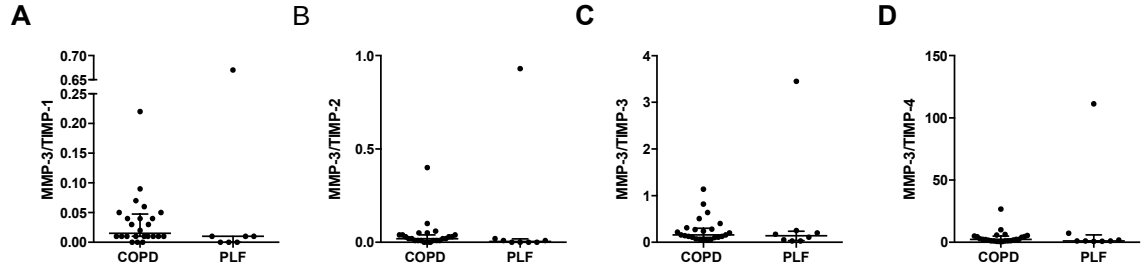


Figure 19. BAL expression of MMP-3/TIMPs in subjects with COPD and preserved lung function. (A) MMP-3/TIMP-1 (B) MMP-3/TIMP-2 (C) MMP-3/TIMP-3 (D) MMP-3/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function.

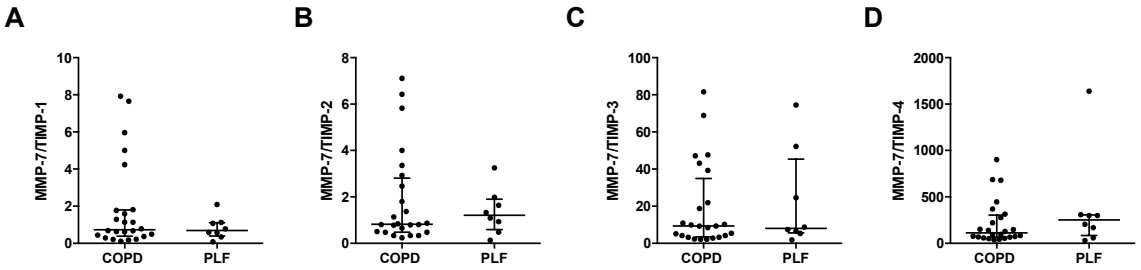


Figure 20. BAL expression of MMP-7/TIMPs in subjects with COPD and preserved lung function. (A) MMP-7/TIMP-1 (B) MMP-7/TIMP-2 (C) MMP-7/TIMP-3 (D) MMP-7/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function.

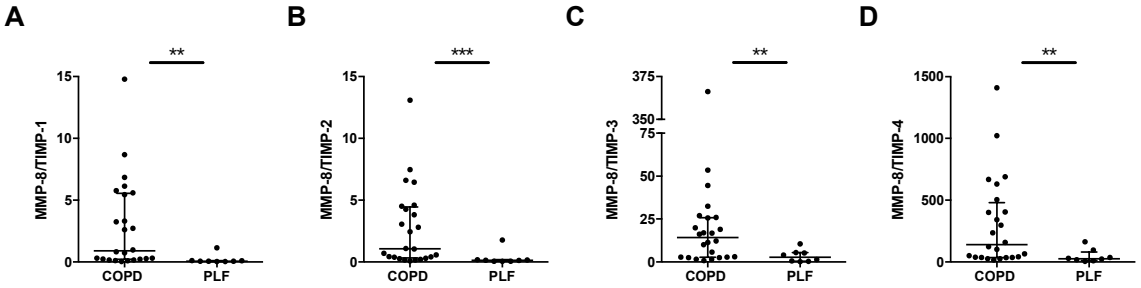


Figure 21. BAL expression of MMP-8/TIMPs in subjects with COPD and preserved lung function. (A) MMP-8/TIMP-1 (B) MMP-8/TIMP-2 (C) MMP-8/TIMP-3 (D) MMP-8/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function. ** p<0.01, *** p<0.001 using Mann-Whitney U test.

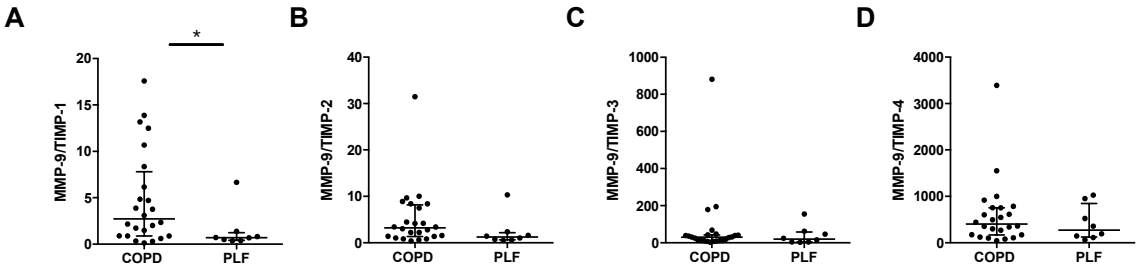


Figure 22. BAL expression of MMP-9/TIMPs in subjects with COPD and preserved lung function. (A) MMP-9/TIMP-1 (B) MMP-9/TIMP-2 (C) MMP-9/TIMP-3 (D) MMP-9/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function. * p<0.05 using Mann-Whitney U test.

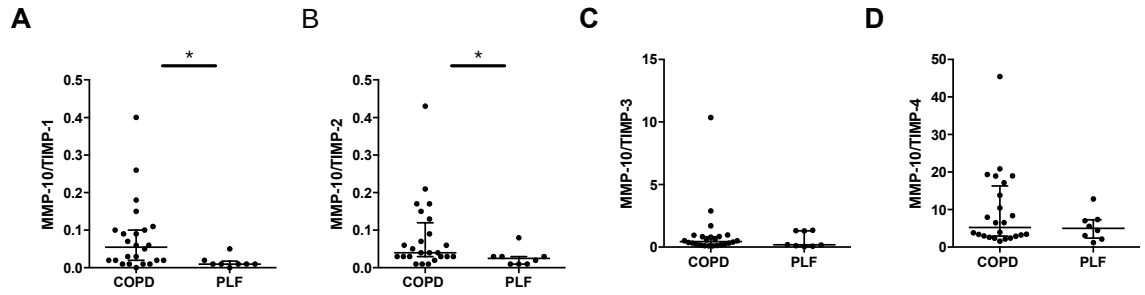


Figure 23. BAL expression of MMP-10/TIMPs in subjects with COPD and preserved lung function. (A) MMP-10/TIMP-1 (B) MMP-10/TIMP-2 (C) MMP-10/TIMP-3 (D) MMP-10/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function. * $p<0.05$ using Mann-Whitney U test.

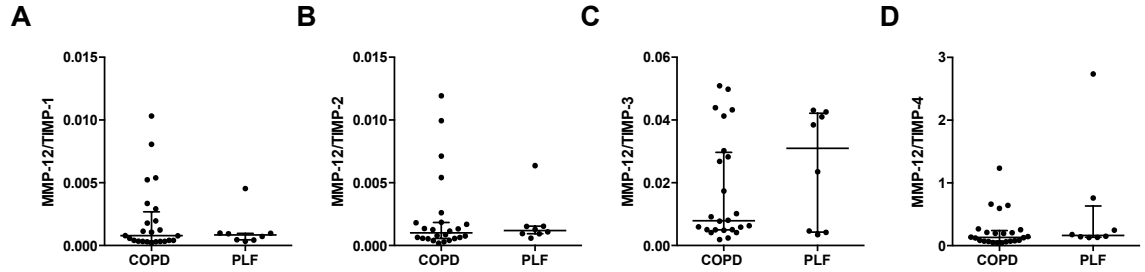


Figure 24. BAL expression of MMP-12/TIMPs in subjects with COPD and preserved lung function. (A) MMP-12/TIMP-1 (B) MMP-12/TIMP-2 (C) MMP-12/TIMP-3 (D) MMP-12/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function.

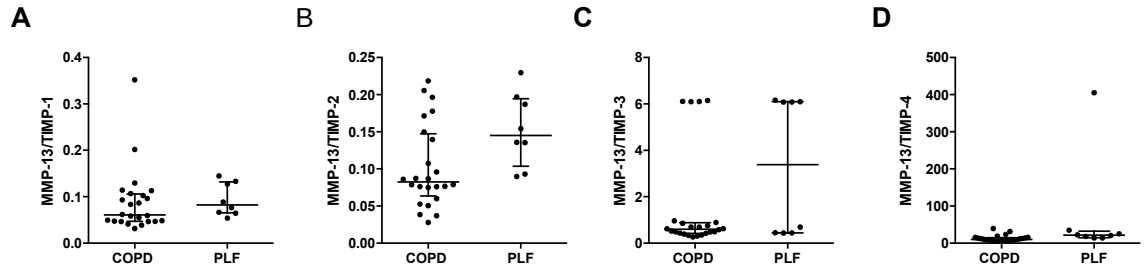


Figure 25. BAL expression of MMP-13/TIMPs in subjects with COPD and preserved lung function. (A) MMP-13/TIMP-1 (B) MMP-13/TIMP-2 (C) MMP-13/TIMP-3 (D) MMP-13/TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for preserved lung function.

4.4 Airway Bacteria

Bacteria presence in the airways was detected using traditional culture techniques and quantitative PCR in sputum and bronchial brushing samples. In this study the three main pathogens known to cause infection in COPD were analysed.

4.4.1 Sputum

Results for sputum culture are shown in Table 12. In 50% of sputum samples, one of the three main bacteria (PPM) was cultured, irrespective of whether airflow obstruction was present or not. There was no significant difference in culture positive samples for NTHI or MCAT between subjects with COPD and PLF. SP was cultured from 1/3 of subjects with COPD, whereas no subjects with PLF cultured this and this trended towards significance.

Next I analysed the results of the sputum quantitative PCR to assess if there were any differences using this method. This resulted in a reduction in the number of sputum positive samples. Both MCAT and SP were found less frequently in sputum using PCR than by traditional culture. The reverse was true for NTHI where more samples were positive using PCR. There was still no significant difference between bacterial detection using PCR results in COPD subjects and those with PLF. Due to the low numbers of samples with bacterial DNA detected, quantitative results (ie number of copies) were not analysed.

Table 12. Number of subjects where bacteria was detected in sputum using traditional culture and quantitative PCR.

	COPD	PLF	P value
Traditional Culture			
PPM	12	4	>0.999
SP	8	0	0.081
MCAT	6	3	0.654
NTHI	4	1	>0.999
Quantitative PCR			
PPM	8	2	>0.999
SP	5	1	>0.999
MCAT	2	0	>0.999
NTHI	6	2	>0.999

Values given as number of subjects. N=24 with COPD and 8 with preserved lung function. Fishers exact test used for p value. PPM - Potentially pathogenic microorganisms, SP - Streptococcus pneumoniae, MCAT – Moraxella catarrhalis, NTHI – Non-typeable Haemophilus influenza.

4.4.2 Bronchial Brushings

Bacterial presence was also assessed using protected brush sampling from the lobes targeted at bronchoscopy, with both traditional culture and quantitative PCR techniques used. For the initial analysis the results from the two lobes were combined, meaning if either lobe was positive the subject was treated as positive. When using traditional culture, results for the protected brushes were similar to those for sputum culture (Table 13). There remained no significant difference in any of the bacteria cultured between COPD subjects and those with PLF. When using quantitative PCR, positive results for bacteria were found much less frequently (Table 13). Only four COPD subjects and no PLF subjects were found to have bacterial DNA present. As the detection rates for bacterial DNA was so low, quantitative results were not analysed.

Table 13. Number of subjects where bacteria was detected in bronchial brushings using traditional culture and quantitative PCR.

	COPD	PLF	P value
Traditional Culture			
PPM	12	3	0.691
SP	7	2	>0.999
MCAT	6	1	0.646
NTHI	5	1	>0.999
Quantitative PCR			
PPM	4	0	0.550
SP	0	0	-
MCAT	1	0	0.750
NTHI	3	0	0.555

Values given as number of subjects. PPM - Potentially pathogenic microorganisms, SP - Streptococcus pneumoniae, MCAT – Moraxella catarrhalis, NTHI – Non-typeable Haemophilus influenzae. N=24 with COPD and 8 with PLF. Fishers exact test used.

4.5 Regional/lobar results

One of the major aims of this particular study was to assess the anatomical regional variation of disease. To achieve this, the study was designed so that CT imaging guided sampling of a “diseased” and “preserved/spared” lobe, as previously described. This next section describes the lobar results for white cell differential, inflammatory cytokines, MMPs, TIMPs and bacterial detection. For obvious reasons when performing the analysis on anatomical variation only the COPD subjects were included in the analysis.

4.5.1 Lobar Values of cytokines and neutrophils

There was no significant difference between BAL cytokine concentrations and neutrophils in diseased and preserved lobes (Figure 26).

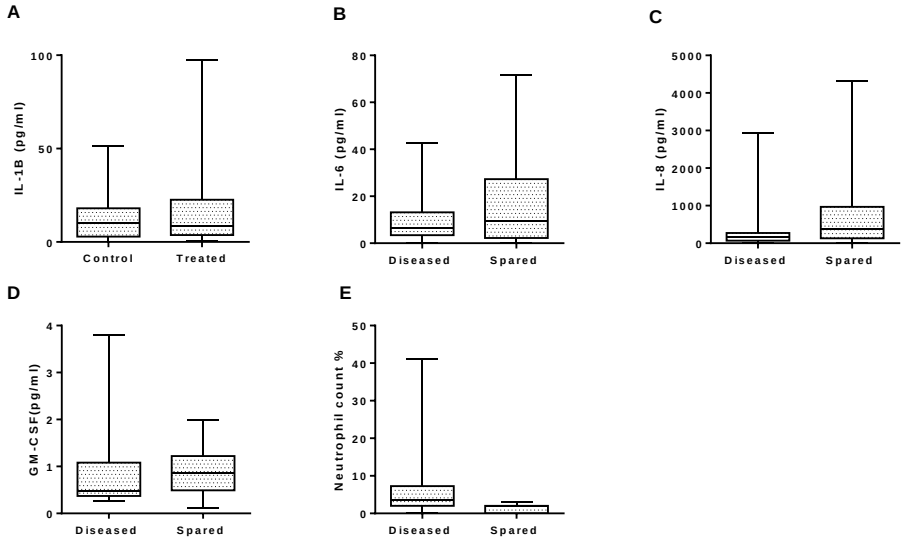


Figure 26. BAL expression of cytokines and neutrophils in diseased and preserved lobes. (A) IL-1B (B) Il-6 (C) IL-8 (D) GM-CSF (E) neutrophil count. Data represents median upper and lower quartiles. N=24 for cytokines. N = 19 for neutrophils. Significance calculated using related samples McNemar test.

4.5.2 Lobar Values of MMPs and TIMPs

Next I assessed the expression of MMPs in the diseased and healthy lobes (Figure 27). MMP-7 and -8 was significantly higher in the spared lobes compared to the diseased lobes. For the other MMPs there was no significant difference between lobes. There was no difference in expression of TIMPs between diseased and spared lobes (Figure 28).

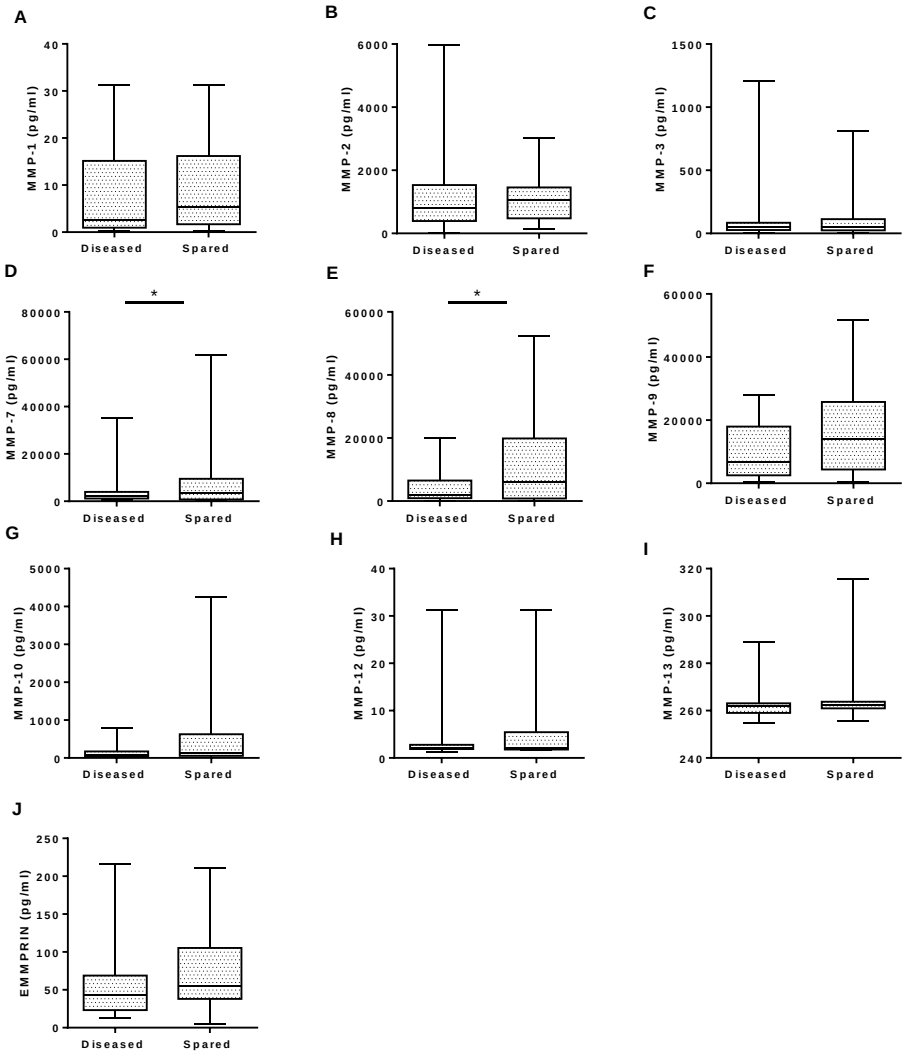


Figure 27. BAL expression of MMPs in diseased and preserved lobes. (A) MMP-1 (B) MMP-2 (C) MMP-3 (D) MMP-7 (E) MMP-8 (F) MMP-9 (G) MMP-10 (H) MMP-12 (I) MMP-13 (J) EMMPIRIN. Data represents median upper and lower quartiles. N=24 for COPD. * $p<0.05$ using related samples McNemar test.

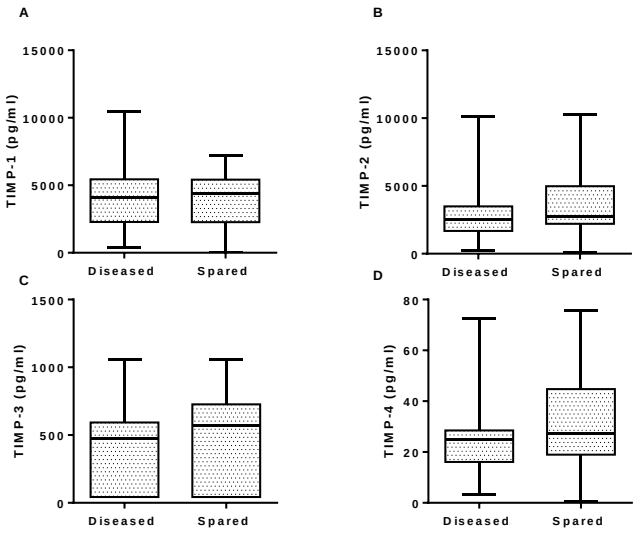


Figure 28. BAL expression of TIMPs in diseased and preserved lobes. (A) TIMP-1 (B) TIMP-2 (C) TIMP-3 (D) TIMP-4. Data represents median, upper and lower quartiles. N=24. Significance calculated using related samples McNemar test.

4.5.3 Lobar differences of bacterial detection

There was no significant difference in bacterial detection between diseased and preserved lobes using either traditional culture techniques or PCR (Table 14). Detection rates were remarkably consistent in both lobes.

Table 14. Number of subjects where bacteria was detected in bronchial brushings from disease and spared lobes using traditional culture and quantitative PCR.

	Diseased Lobe	Spared Lobe	P value
Traditional Culture			
PPM	10	8	>0.999
SP	5	5	>0.999
MCAT	5	2	>0.999
NTHI	4	4	>0.999
Quantitative PCR			
PPM	3	4	>0.999
SP	0	0	>0.999
MCAT	1	1	>0.999
NTHI	2	3	>0.999

Values given as number of subjects. PPM - Potentially pathogenic microorganisms, SP - Streptococcus pneumoniae, MCAT – Moraxella catarrhalis, NTHI – Non-typeable Haemophilus influenzae. N = 24. P value determined using Related samples McNemar Test.

4.6 Discussion

In this chapter I have carefully characterised the inflammatory profile, protease concentrations and bacterial presence in the airways of a cohort of COPD and ex/current smokers with PLF using a targeted bronchoscopic approach. The most significant and novel finding were demonstrating multiple raised MMPs and TIMPs in COPD subjects, as well as finding further evidence for neutrophilic airway inflammation.

4.6.1 Blood Inflammatory Markers

COPD has long been associated with systemic inflammation with a number of studies demonstrating raised neutrophils[241–243], fibrinogen[244–248], CRP[247–252] and inflammatory cytokines[247–250,252]. In this study blood white cell differential and fibrinogen concentrations were measured and no significant difference between subjects with COPD and those with PLF were found. There was also was no association between blood neutrophils and airflow obstruction which is also inconsistent with previous work[241]. The reason for these discrepancies is unknown, however previous studies have generally included a much more severe cohort of patients than my study. It may be suggested that there is less likely to be as much systemic inflammation in milder cohorts and therefore the difference with control subjects will be less pronounced. The small sample size of my study also meant that potential confounders could not be corrected for and this is especially important for smoking status, which can potentially increase systemic inflammatory markers[253–255]. This study did show some evidence of higher systemic inflammation in COPD by demonstrating a significant inverse relationship between fibrinogen and FEV1, which is in keeping with a number of other studies[256–258]. Fibrinogen is a glycoprotein that is marker of blood clotting and also involved in the acute phase response, where it has a number of pro-inflammatory effects[259]. This association therefore suggests that those with more severe COPD have higher circulating systemic inflammation.

The underlying mechanisms of systemic inflammation in COPD are poorly understood. A number of different theories have been suggested including over-spill from the lungs, smoking-induced inflammation, extra-pulmonary sites of inflammation and effects of ageing. However, there is no definite evidence to suggest which of these is the most important, although in likelihood all are involved. Unfortunately my study was not conceived to answer this question. Whatever the aetiology of systemic inflammation in COPD, it is important as it is associated with increased exacerbations[248,251], mortality[248] and it has been hypothesised that many of the extra-pulmonary manifestations of the condition are mediated by systemic inflammation[103,250,260,261].

4.6.2 Airway Inflammatory Indices

There was much stronger evidence for increased airway inflammation in COPD in my study. I found significantly raised BAL neutrophils in COPD subjects as opposed to subject with PLF. There was also an inverse association between airflow obstruction and neutrophil count, although this only reached significance in the whole cohort and not when only assessing COPD subjects. Multiple studies have demonstrated raised neutrophils in the airways of COPD patients[20,56,243,262–266] and have also shown a similar negative association between airway neutrophils and FEV1%[20,58,243,264]. I also found increased concentration of IL-8 in COPD subjects compared to those without airflow obstruction and there was also an inverse relationship between IL-8 and airflow obstruction. This is consistent with multiple studies demonstrating raised IL-8 in the airways of COPD subjects[20,56,241,263,266–270] and have also shown a similar relationship with airflow obstruction [58,269,270]. This all points to significant neutrophilic airway inflammation in COPD. Neutrophils are phagocytic cells and are involved in ingestion and killing and digestion of microorganisms, especially bacteria. Neutrophils are rapidly recruited to sites of tissue injury recruited by various chemotactic factors, such as IL-8, LTB4 and TNF-α. In COPD it is thought that exposure to cigarette smoke, repeat infections and hypoxia contribute to recruitment of neutrophils from the systemic circulation to the lungs. Although neutrophils have important antibacterial function they can also induce tissue damage by the release of proteases and reactive oxygen species. The fact that I found both raised neutrophils and the chemokine IL-8 in COPD subjects and found they both correlated with airflow obstruction supports previous studies, suggesting neutrophillic airway inflammation plays a key role in the pathogenesis of COPD.

I also found increased BAL concentrations of IL-6 in COPD and this had an inverse association with FEV1. Evidence for the role of IL-6 in COPD is weaker than for IL-8, although previous studies have shown increased IL-6 in the airways of COPD[267] and that it is inversely associated with FEV1[58]. IL-6 is produced from inflammatory cells and bronchial epithelial cells as a result of cell stress and damage. It has pro-inflammatory effects and is thought to contribute to the inflammatory cascade in the lungs of patients with COPD.

There was no significant difference in IL-1β and GM-CSF concentrations in BAL between subjects with COPD and PLF. There was however a moderate inverse relationship between airflow obstruction and IL-1β in COPD subjects. Both of these cytokines are pro-inflammatory however few studies have assessed their role within the airways of COPD. One such study that did, found no difference in IL-1β concentration in induced sputum between COPD and controls[271]. In contrast to my results sputum concentrations of GM-CSF were raised in COPD compared to controls[272]. Sputum cells, produced higher concentrations of GM-CSF in COPD subjects but interestingly this cytokine

could not be detected from bronchial epithelial cells harvested at bronchoscopy[273]. This could explain the discrepancy with my results as these were from bronchoscopy samples.

What is clear is that the inflammatory process in COPD is complex with a number of different inflammatory mediators present. My study has added to the evidence that neutrophilic airway inflammation and other pro-inflammatory cytokines are involved in the disease process.

4.6.3 MMPs and TIMPs

MMPs are structurally similar proteolytic enzymes that have been implicated in the tissue remodelling, matrix destruction and inflammation seen in COPD[26]. They are broadly grouped depending on their substrate specificity, including collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11) and elastases (MMP-7 and -12)[34], although there is considerable overlap. In disease they are produced by inflammatory cells and bronchial epithelial cells. MMP-1, -2, -3, -8, -9 and -10 were significantly elevated in the airways of COPD subjects compared to subjects with PLF. This is in keeping with previous studies which have demonstrated that MMP-1[37,38], -8[38,39] and -9[37–42] are increased in COPD subjects. Few previous studies have investigated the role of MMP-2, -3 and -10. A histological study of lung resection subjects also found that MMP-2 was up-regulated in the lung periphery in COPD[274]. However, Culpitt et al found no difference between MMP-2 and -3 in sputum between COPD and controls[38]. My results may diverge from these due to the difference in sampling locations between sputum and BAL. Previous studies have also found that MMP-12 is increased in COPD[37,43,44], although I did not replicate this observation in my cohort. I also found that MMP-1, -3, -7, -8, -9, -10 and -12 were significantly associated with airflow obstruction in the whole cohort. When analysing the COPD subjects this was only significant for MMP-8, -9 and -12. This is consistent with and adds to previous studies showing associations between MMP-1, -8, -9 and -12 and airflow obstruction[38,44,48,275] and therefore adds to the evidence that MMPs may be implicated in COPD.

MMPs are complex, with multiple mechanisms of actions and multiple interactions with one another. My results show that MMPs have significant strong associations with one another, making it difficult to elicit which of these is the most important in COPD. This demonstrates the importance of measuring a large array of MMPs when investigating their function and role in lung diseases, something which many previous studies have failed to do. The MMPs that were raised in COPD subjects and also had significant associations with airflow obstruction were MMP-1, -3, -8, -9 and -10. Interestingly none of these have significant elastolytic properties and instead these MMPs have the ability to degrade collagens and proteoglycans and therefore may be responsible for destruction of the ECM and the resultant abnormal tissue remodelling that occurs in COPD.

MMPs are inhibited by four endogenous inhibitors, the TIMPs which bind with MMPs in a 1:1 manner[276]. This potentially means that in order to understand the mechanisms of action of MMPs it is important to measure TIMPs. Previous studies are more limited than for MMPs, although they have shown elevated concentrations of TIMP-1 and -2 are in the airways of COPD subjects[37,38,42,43]. I found significantly increased TIMP-2 and TIMP-4 in COPD subjects and also found that TIMP-2, -3 and -4 had significant inverse associations with airflow obstruction. To understand this further I also investigated the MMP/TIMP ratio, which may be more important than absolute concentrations of individual MMPs and TIMPs. Previous studies have not demonstrated increased airway MMP/TIMP in COPD[43,48]. In my study a number of MMP/TIMP ratios were increased in COPD and this adds to the evidence that an imbalance between MMPs and their inhibitors is an important feature of COPD. However the MMP/TIMP ratios did not seem to provide further information than measuring MMPs alone. The activity and specificity of TIMPs are poorly understood and there is even a suggestion that MMPs act in the immediate pericellular environment and are therefore not inhibited by TIMPs[49].

4.6.4 Airway Microbiology

In COPD patients, studies have demonstrated that 29-70% of sputum samples in the stable state[16,50–55] and 25-43% of bronchoscopy specimens[56,58,60–62,277] are culture positive for PPMs. This has led to the belief that chronic bacterial infection is an important feature of COPD and this is further supported by the fact that it has been associated with higher airway inflammation[16,53–58], more exacerbations[16,54] and decline in lung function[53,59]. My results confirm this by finding that 50% of sputum samples with COPD cultured one of the three main organisms known to cause disease in COPD. The three most common bacteria found in the airways of COPD subjects both in the stable state and at exacerbation are SP, MCAT and NTHI. In this present study SP was cultured in 33.3% sputum samples, NTHI 16.7% and 25% had MCAT. SP is a common respiratory pathogen and has been detected in the airways of COPD subjects 7.5-33% of the times[16,51,54,60–62]. MCAT is another respiratory pathogen that has been found in the airways of COPD subjects 3-14% of times[16,51,60,61]. NTHI is perhaps the organism in which most importance and interest has been attached in COPD. It has been cultured in 9-53% of occasions[16,51,54,59–62].

Perhaps most interesting was the finding that 50% of sputum samples from subjects with preserved lung function also cultured one of the three main bacteria known to cause disease in COPD. Previous studies have shown much lower levels of detection of bacteria in sputum of healthy controls and even smokers[56,61,62]. This indicates that the mechanisms leading to bacterial colonisation in COPD subjects are also occurring in ex/current smokers with PLF. It may be that in this supposedly healthy groups substantial damage to the airways has already occurred,

predisposing to bacterial infection. Another interpretation may be that the acute effects of smoking predispose subjects to infection or colonisation with bacteria.

Methods of sampling the airways for bacterial presence may affect results and therefore I used both sputum and protected endobronchial brushings to determine whether bacteria was present. There has been some suggestion that sputum samples may be contaminated by the upper airways and results may reflect bacterial presence in the upper airway. My results do not show this as culture results for sputum and endobronchial brushes were remarkably consistent. It has also been suggested that traditional culture techniques are not optimal for analysing bacteria within the airways as specificity is limited. Therefore quantitative PCR was also used in this study and interestingly reduced the frequency of detection of the three main organisms in both sputum and endobronchial brushes. This is in contrast to another study which showed significantly increased detection of PPMs with PCR compared to standard culture[55]. However this was in a much more severe cohort. One explanation for this is that the quoted lower limit of detection (provided by GSK) was too high and therefore there were a number of false negatives.

4.6.5 Regional Difference

It has long been recognised that COPD does not affect the lung uniformly. The most obvious example of this is with emphysema where significant heterogeneity has been described and even more important can effect management and outcome in lung volume reduction surgery[14]. However the underlying mechanisms for this heterogeneity is poorly understood and it is for this reason that I chose to sample two separate lobes with differing degrees of disease in each individual. There were no significant differences between inflammatory cytokines, white cell differential and bacterial detection between the two lobes. When assessing MMPs there were significantly raised concentrations of MMP-7 and -8 in the preserved lobes compared to the diseased lobes. This is a rather surprise finding which is difficult to explain. There was no other significant differences between the remaining MMPs and TIMPs between the lobes.

Although there have been a number of studies describing the heterogeneity of imaging abnormalities there have been few describing the intra-patient heterogeneity of mechanisms of disease. Most bronchoscopy studies only sample one region of the lung and so cannot describe these variations. One study assessed the differences in regional microbiome using BAL techniques in different lung regions and found there were significant differences between regions[278]. Another study looked at MMP-9 production from macrophages in cores of lungs removed from COPD subjects prior to transplant and this showed significant variability[279]. One of the main limitations in trying to understand the regional variation in my study was the fact that a subjective opinion was

used to decide which represented the diseased and preserved lobes. As this was a mild cohort evidence of disease was subtle and therefore it was difficult to discriminate between the different lobes. This may well have affected the results when looking at regional differences and so techniques which can quantitative the morphological features of disease may be helpful.

4.6.6 Strengths and Limitations of the study

One of the main strengths of this study was the sampling techniques I employed. By utilising CT imaging to guide sampling from two different lobes I was able to ensure that results were not affected by random sampling. This technique also allowed me to investigate the variability of these mechanisms within an individuals lungs, although unfortunately this did not show any significant results. Unlike most previous studies I measured a broad array of MMPs which is important as they are complex and activate one another, meaning measuring them in isolation is not appropriate.

One of the main limitations of this study was the small sample size and associated limited statistical power, meaning I was unable to correct for certain aetiological factors such as smoking. Despite this I found significant differences in airway inflammation and proteases between subjects with COPD and preserved lung function and found strong evidence for associations between airway inflammation and MMPs and spirometry. Another limitation was the multiple comparisons made in this study. However, I found many more significant associations than would have been expected to be significant just by chance at the 5% level, suggesting the presence of genuine associations. I also found a number of correlations that were significant in the whole cohort but were not significant in the COPD cohort. This is likely to represent inter-group differences rather than truly significant associations and the validity of these associations have to be treated with caution. Due to the need to perform bronchoscopy, this study consisted of mild and moderate COPD patients and it is unknown whether these results would be the same in more severe patients. In this study I measured concentrations of MMPs and inflammatory cytokines rather than activity and therefore cannot be sure that elevated levels of these biological markers reflect activity and therefore further mechanistic work is required to understand them in more detail.

4.6.7 Summary

In summary, I have added to the evidence for a number of key underlying features of COPD, including suggesting a role for MMPs. I also demonstrated evidence for neutrophilic airway

inflammation and confirmed high rates of bacterial presence in the airways of COPD subjects. So far I have only compared these with spirometric markers of disease, which does not provide any information on the morphological features contributing to airflow obstruction. There is also significant variability and heterogeneity with FEV1 and so it can be difficult to interpret results and provide a clear insight into the underlying pathology of disease. Another potential problem when only using FEV1 to characterise and define the condition is that there is likely to be early lung damage occurring prior to airflow obstruction. Therefore simply comparing COPD subjects to ex/current smokers without airflow obstruction may not give accurate results. Further investigations, such as quantitative analysis of CT imaging are required which can provide information about the structural and morphological features of disease. In the next chapter I plan to use novel CT analysis to explore the key morphological features of COPD and assess their associations with the disease activity and mechanisms. This CT analysis will also allow me to explore the anatomical variability of disease in an objective manor.

Chapter 5: Quantitative CT imaging in COPD and the relationship with inflammatory markers, proteases and microbiology

The previous chapter highlighted some of the problems of characterising and assessing COPD by using spirometry alone. Novel CT analysis has the potential to quantitate emphysema, gas trapping and large/intermediate airway dimensions, which will allow further understanding of the underlying structural changes that contribute to airflow obstruction. Furthermore, exploring the relationship between CT parameters and biological samples will increase our understanding of endotypes of disease and provide further insights into the pathology of the condition.

In this chapter I have applied quantitative analysis to the CT scans that were performed as part of the MICA study, to describe emphysema, small airways disease and large airway morphology in mild/moderate COPD subjects and those with PLF. The relationship between these morphological features and inflammatory status, protease concentrations and microbiology biological samples will be investigated to understand the underlying disease process more effectively. Finally the anatomical regional variability of disease was assessed using CT analysis.

5.1 Emphysema

Segmentation and quantitative analysis was successfully achieved in 31 subjects. One of the control CT scans could not be analysed for technical reasons.

5.1.1 Assessing the Different Methods

Emphysematous regions can be identified on CT as low attenuation areas and this can be used to provide quantitative estimates of the amount of emphysema present. There is currently no definite consensus as to which is the best method for determining emphysema on CT and so initially, I chose to investigate three automated methods and a visual score. Two of these methods used different densitometry thresholds ($\%LAA_{<-950}$ and $\%LAA_{<-910}$), while the other automated method represented the 15^{th} percentile in the CT attenuation histogram ($PERC_{15}$). Figure 29 shows a typical coronal reconstruction of a subject with COPD using the two different LAA thresholds. All of the quantitative analysis methods showed very strong associations with one another (Table 15). The visual emphysema score did not have any significant associations with the quantitative methods. When assessing the differences in these measures of emphysema between COPD subjects and those with PLF, only $\%LAA_{<-950}$ and the visual emphysema score showed significant differences (Figure 30).

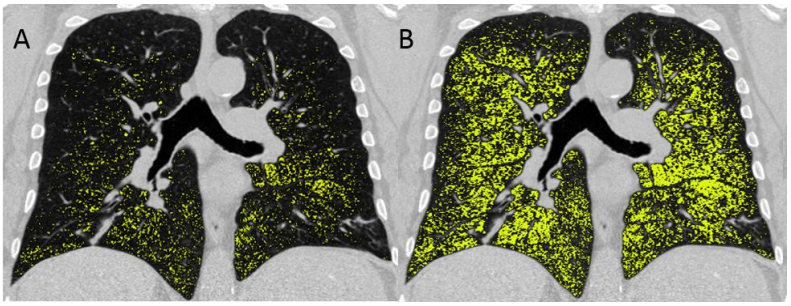


Figure 29. Coronal reconstruction of a CT from a subject with COPD showing low attenuation areas below (A) -950HU (B) -910HU.

Table 15. Association between different methods of estimating emphysema on CT.

	%LAA$_{<-950}$	**%LAA$_{<-910}$**	**PERC$_{15}$**
%LAA$_{<-910}$	**0.94*** **	**-**	
PERC$_{15}$	**-0.98*** **	**-0.97*** **	
Emphysema Score	0.24	0.08	-0.16

Spearman's correlation. N=31. *** p <0.001.

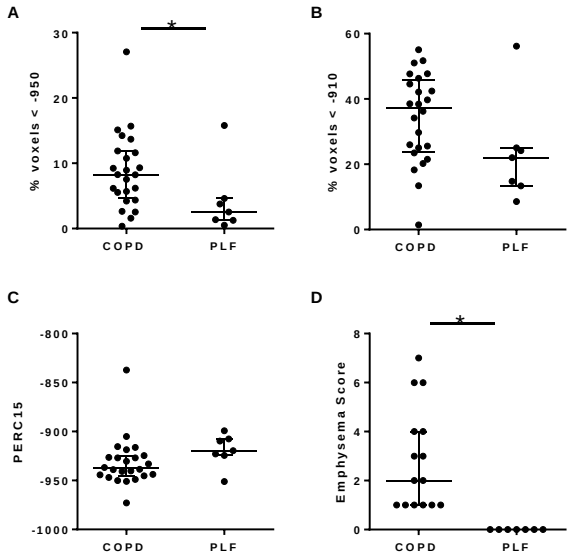


Figure 30 FigureEmphysema quantitation in COPD and preserved lung function (PLF) using four different methods (A) % voxels below -950HU (B) % voxels below -910HU (C) CT attenuation value below which lie 15% of the voxels (D) visual emphysema score. Data represents median with IQ range. Each dot represents individual CT measure in a specific patient, n=24 for COPD and 7 for controls. * p<0.05 using Mann-Whitney U test.

Next the associations between these emphysema estimates and spirometric markers of disease were analysed in the whole cohort and COPD subjects. For the quantitative markers there were no associations with FEV1%, FVC% and FEF75-25% (Table 16). There was only one relatively weak

significant association between FEV1/FVC ratio and $\%LAA_{<-950}$ for the whole cohort, which lost significance when assessing COPD subjects only. The visual emphysema score had and inverse relationships with FEV1, FEV1/FVC ratio and FEF75-25, although the relationship with FEV1 lost significance when only analysing COPD subjects.

Table 16. Association between emphysema and spirometric markers of disease.

	FEV1%	FVC%	FEV1/FVC	FEF75-25%
Whole Cohort				
$\%LAA_{<-950}$	-0.27	0.08	**-0.39***	-0.33
$\%LAA_{<-910}$	-0.22	0.05	-0.27	-0.25
PERC15	0.19	-0.10	0.31	0.26
Emphysema Score	**-0.39***	0.09	**-0.67*****	**-0.61*****
COPD subjects				
$\%LAA_{<-950}$	-0.10	0.16	-0.19	-0.14
$\%LAA_{<-910}$	-0.09	0.09	-0.06	-0.08
PERC15	0.02	-0.19	0.10	0.05
Emphysema Score	-0.14	0.25	**-0.54****	**-0.44***

Spearman's correlation coefficient. N=31 for automated analysis and n=32 for visual score. N=24 for COPD subjects. * p<0.05 **p<0.01 ***p<0.001

All of the automated measures had strong associations with one another and they also exhibited similar associations with spirometric markers of disease. This suggests that all of these measures provide similar estimates of emphysema. Of the automated measures $\%LAA_{<-950}$ was the only one which showed a significant difference between subjects with COPD and PLF. I therefore elected to use this measure throughout the rest of the study when analysing quantitative measures of emphysema.

5.1.2 Emphysema and blood results

Next I investigated the association between the emphysema measurements and blood inflammatory markers in the form of white cell differential and fibrinogen (Table 17). There were no significant associations between any of these and measures of emphysema, although $\%LAA_{<-950}$ trended towards a significant association with fibrinogen (p value 0.06).

Table 17. Correlation between emphysema and blood inflammatory markers.

	WCC	Neutrophils	Lymphocytes	Eosinophils	Fibrinogen
Whole Cohort					
$\%LAA_{<-950}$	-0.26	-0.28	-0.10	-0.01	-0.35
COPD subjects					

%LAA$_{<-950}$	-0.17	-0.28	-0.07	0.12	-0.24

Spearman's correlation coefficient. For automated analysis N=29 for white cells and N= 30 for fibrinogen. For visual score N=30 for white cells and 31 for fibrinogen. For COPD subjects N=22 for white cells and N=23 for fibrinogen.

5.1.3 Emphysema and BAL cell differential

There were significant associations between BAL neutrophils and %LAA$_{<-950}$, although this association lost significance when analysed in the COPD group only (Table 18). Conversely there was an inverse association between BAL macrophages and %LAA$_{<-950}$. Within the COPD subjects there was also an inverse association between BAL eosinophils and BAL lymphocytes and %LAA$_{<-950}$.

Table 18. Correlation between emphysema and BAL white cell differentials.

	Neutrophils	**Macrophages**	**Eosinophils**	**Lymphocytes**
Whole Cohort				
%LAA$_{<-950}$	**0.44***	**-0.67*****	-0.36	-0.24
COPD subjects				
%LAA$_{<-950}$	0.23	**-0.71****	**-0.51***	**-0.50***

Spearman's correlation coefficient. N=26 for automated analysis and n=27 for visual score. N=19 for COPD subjects. * p<0.05 **p<0.01 ***p<0.001

5.1.4 Emphysema and BAL cytokines

When assessing the association between the emphysema estimations and BAL cytokines there were no significant associations between emphysema measures and BAL inflammatory cytokines (Table 19). The association between %LAA$_{<-950}$ and Il-1B trended towards significance (p value 0.06).

Table 19. Correlation between emphysema and BAL cytokines.

	IL-1B	**IL-6**	**IL-8**	**GM-CSF**
Whole Cohort				
%LAA$_{<-950}$	-0.34	0.24	0.24	-0.15
COPD subjects				
%LAA$_{<-950}$	-0.30	0.28	0.22	0.36

Spearman's correlation coefficient. N=31 for whole cohort and N=24 for COPD subjects.

5.1.5 Emphysema and MMPs

There has been particular interest regarding the role of MMPs in emphysema development and so next I investigated the association between emphysema measurements and MMPs. %LAA$_{<-950}$ had significant associations with MMP-3, -7, -8 and -10 in the whole cohort, although the association with MMP-8 lost significance when analysed only in the COPD cohort (Table 20).

Table 20. Correlation between emphysema and BAL MMPs.

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12	MMP-13
Whole cohort									
LAA%	0.05	0.15	**0.50****	**0.42***	**0.38***	0.33	**0.50****	0.10	-0.03
COPD cohort									
LAA%	-0.03	-0.05	**0.49***	**0.52****	0.35	0.33	**0.52***	0.09	-0.24

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD. * p<0.05 **p<0.01 ***p<0.001

5.1.6 Emphysema and TIMPs

When assessing the associations between emphysema and TIMPs, $\%LAA_{<-950}$ had significant associations with TIMP-2 and -4, although this lost significance in the COPD cohort (Table 21).

Table 21. Correlation between emphysema and BAL TIMPs.

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Whole Cohort				
$\%LAA_{<-950}$	0.03	**0.36***	0.19	**0.37***
COPD subjects				
$\%LAA_{<-950}$	-0.11	0.35	0.34	0.13

Spearman's correlation coefficient. N=31 for the whole cohort and n=24 for COPD. * p<0.05

5.1.7 Emphysema and Bacterial Colonisation

There was no significant difference in the amount of emphysema between subjects who cultured bacteria in their sputum and those that did not (Figure 31). This remained the case when dividing the analysis into the individual bacteria cultured. When using PCR results there were significantly greater quantities of emphysema in those where SP DNA was not detected in sputum than those where it was (Figure 32). There were no other significant differences for PCR results and emphysema estimation.

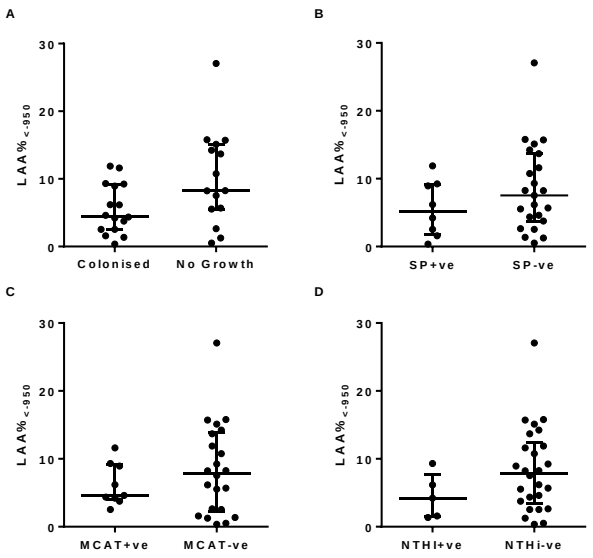


Figure 31. Emphysema quantities in Sputum culture positive and negative subjects (A) PPM - Potentially pathogenic microorganism (B) SP - streptococcus Pneumoniae (C) MCAT- Moraxella catarrhalis (D) NTHI - non-typeable Haemophilus influenzae. Data represents median with IQ range. Each dot represents individual CT measure in a specific patient, n=31.

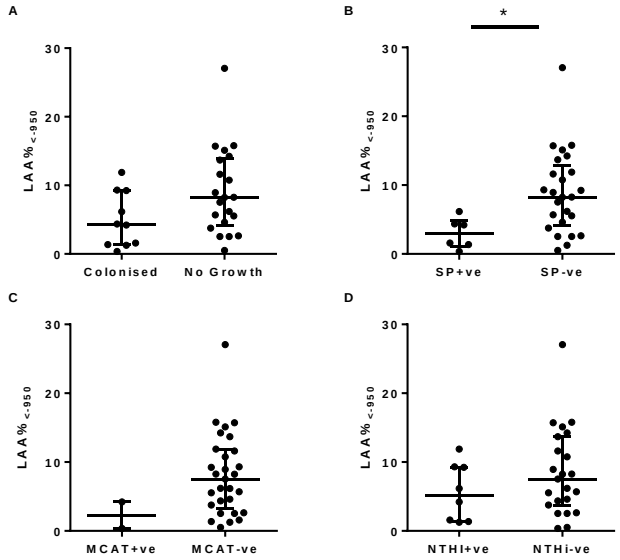


Figure 32. Emphysema quantities in subjects with bacteria detected in sputum using PCR (A) PPM - Potentially pathogenic microorganism (B) SP - streptococcus Pneumoniae (C) MCAT - Moraxella catarrhalis (D) NTHI - non-typeable Haemophilus influenzae. Data represents median with IQ range. Each dot represents individual CT measure in a specific patient, n=31. * $p<0.05$ using Mann Whitney U test.

5.1.8 Emphysema Sub-types

The above methods for quantifying emphysema do not take into account the different emphysema sub-types. These different sub-types can be identified on CT imaging, although very few attempts have been made to quantitate them. In this next section I present results for a novel automated analysis that calculates local histogram data in regions of interest within the lungs to quantify the proportion of emphysema sub-types within an individuals lung.

CT analysis defining specific emphysema sub-types was successfully achieved in 31 subjects. Figure 33 shows a typical LHE reconstruction of a subject with COPD and PLF. One scan from a subject with PLF could not be analysed for technical reasons. All emphysema sub-types apart from mild CLE had very strong associations with one another (Table 22).

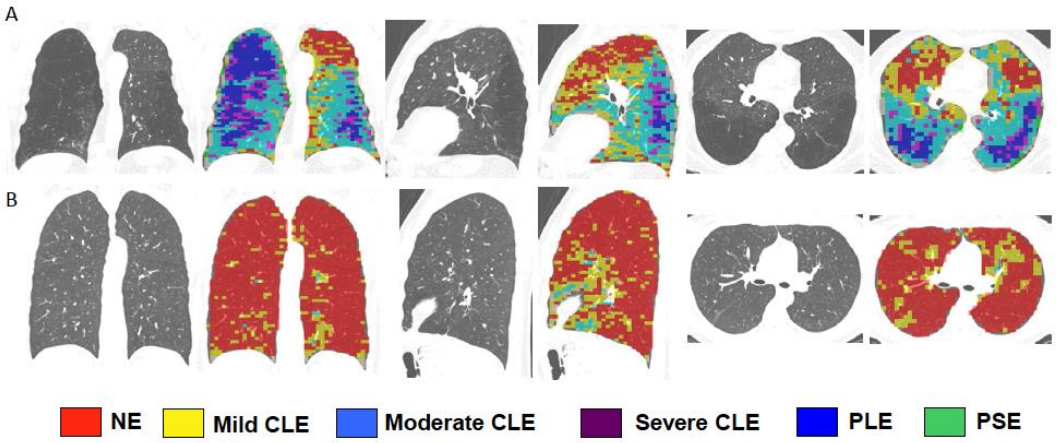


Figure 33. Local histogram emphysema (LHE) classification results for a coronal, sagittal and axial slice corresponding to (A) COPD subject and (B) subject with preserved lung function. NE – non-emphysematous tissue, CLE – centrilobular emphysema, PLE- panlobular emphysema, PSE – paraseptal emphysema.

Table 22. Correlation between different tissue sub-types.

	Mild CLE	Moderate CLE	Severe CLE	PLE	PSE
Moderate CLE	0.04				
Severe CLE	-0.12	**0.89*** **			
PLE	-0.17	**0.81*** **	**0.89*** **		
PSE	0.04	**0.91*** **	**0.91*** **	**0.85*** **	
NE	-0.16	**-096*** **	**-0.87*** **	**-0.79*** **	**-0.93*** **

Spearman's correlation coefficient. N = 31. ***p<0.001.

All subjects (COPD and PLF) had non-emphysematous tissue, mild CLE, moderate CLE and PSE. Only eight subjects had >1% of PLE. 10 subjects had >1% of severe CLE. NE tissue was the most common in 15 subjects. Mild CLE was the most common in 12 subjects and moderate CLE the most common in 4 subjects.

The median percentage of each LHE pattern is shown in Figure 34. Moderate CLE, severe CLE, PLE and PSE were all significantly raised in COPD subjects compared to those with PLF. Non-emphysematous tissue was significantly higher in those with preserved lung function. Median percentage for mild CLB was over 30% for subjects with and without airflow obstruction with no significant difference between the two.

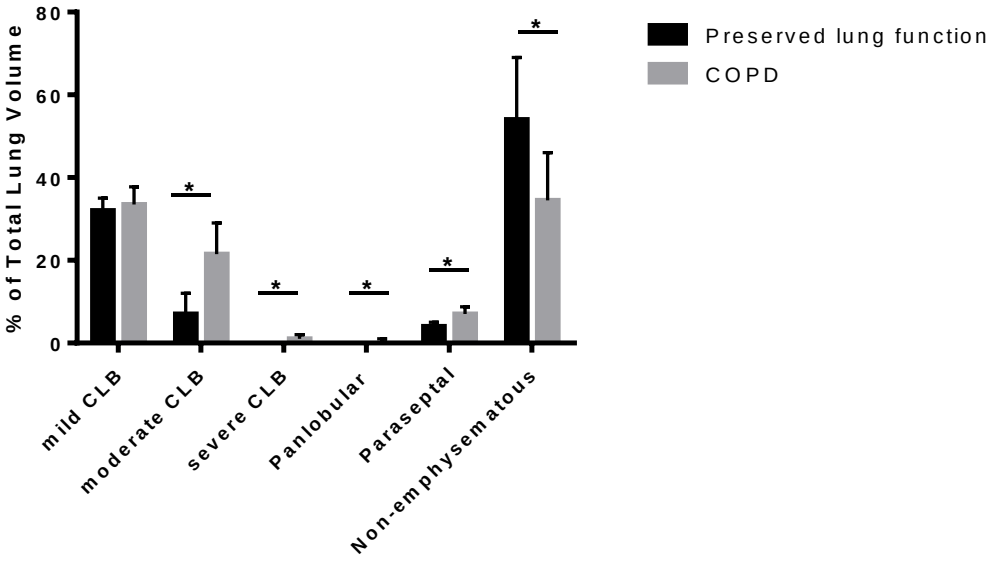


Figure 34. Emphysema sub-types and non-emphysematous tissue in COPD subjects and subjects with preserved lung function as a percentage of lung volume. Data represents median with upper quartile. N = 31. * $p<0.05$ using Mann-Whitney U test.

5.1.8.1 Association between Emphysema sub-types and spirometry

When analysing the association between emphysema sub-types and spirometry, there were significant associations between FEV1% and FEF75-25% and moderate CLE and severe CLE (Table 23). FEV1/FVC ratio had significant associations with moderate and severe CLE as well as PSE and NE. All of these significances disappeared when performing the analysis in COPD subjects only. There was no association between mild CLE and any spirometric marker of disease.

Table 23. Correlation between emphysema sub-types and spirometry.

	Mild CLE	Moderate CLE	Severe CLE	PLE	PSE	NE
Whole Cohort						
FEV1%	0.07	**-0.39***	**-0.37***	-0.30	-0.31	0.32
FVC%	-0.17	0.08	0.04	0.04	-0.01	-0.06
FEV1/FVC	0.09	**-0.54****	**-0.46***	-0.35	**-0.40***	**0.44***
FEF75-25	0.04	**-0.48****	**-0.44***	-0.34	**-0.34**	**0.42***
COPD Subjects						
FEV1%	0.16	-0.29	-0.06	-0.03	-0.06	0.09
FVC%	0.27	-0.33	0.14	-0.06	-0.13	0.16
FEV1/FVC						
FEF75-25	0.25	-0.30	-0.16	-0.07	-0.07	0.15

Spearman's correlation coefficient. N=31 for whole cohort and N=24 for COPD. * p<0.05 **p<0.01.

5.1.8.2 Association between emphysema sub-types and BAL Cytokines

Next, I analysed the association between emphysema sub-types and BAL cytokines. There was a negative association between Mild CLE and IL-1B and IL-6 and a positive association between moderate CLE and IL-8 (Table 24). When performing the analysis in only COPD subjects there were negative associations between mild CLE and IL-6 and IL-8 as well as an association between PLE and IL-1B.

Table 24. Correlation between emphysema sub-types and BAL cytokines.

	Mild CLE	Moderate CLE	Severe CLE	PLE	PSE	NE
Whole Cohort						
IL-1B	**-0.36***	-0.17	-0.20	-0.35	-0.27	0.22
IL-6	**-0.54****	0.31	0.34	0.30	0.33	-0.21
IL-8	-0.34	**0.38***	0.29	0.20	0.33	-0.29
GM-CSF	-0.14	-0.12	-0.25	-0.14	-0.10	0.10
COPD Subjects						
IL-1B	-0.25	-0.07	-0.14	**-0.41***	-0.21	0.13
IL-6	**-0.63****	0.32	0.33	0.26	0.37	-0.17
IL-8	**-0.42***	0.37	0.22	0.09	0.29	-0.25
GM-CSF	-0.02	-0.12	-0.31	-0.19	-0.09	0.09

Spearman's correlation coefficient. N=31 for whole cohort and N=24 for COPD. * p<0.05 **p<0.01 ***p<0.001

5.1.8.3 Association between emphysema sub-types and MMPs and TIMPs

I performed a systematic analysis of associations between emphysema sub-types and MMPs and TIMPs in the whole cohort. MMP-3 and -10 had significant associations with all emphysema sub-types apart from mild CLE (Table 25). MMP -7 and -8 had significant associations with all emphysema sub-types apart from mild CLE and PLE. MMP-9 had significant associations with

moderate CLE and PSE. MMP-1 and emmprim had a negative association with mile CLE. MMP-2, -12 and -13 did not exhibit any significant associations with emphysema sub-types. There were fewer associations when performing the analysis in COPD subjects. MMP-3 continued to have significant associations with moderate CLE, PLE and PSE. MMP-7 had association with moderate CLE, severe CLE and PSE, while MMP-8 had significant associations with moderate CLE. MMP-1 had a negative association with mild CLE and MMP-13 had a negative association with PLE.

The only association between TIMPs and emphysema sub-types were those between TIMP-4 and moderate and severe CLE and PSE (Table 26).

Table 25. Correlation between emphysema sub-types and BAL MMPs.

	Mild CLB	**Moderate CLB**	**Severe CLB**	**PLB**	**PSE**	**NE**
Whole Cohort						
MMP-1	**-0.42***	0.18	0.18	0.12	0.09	-0.05
MMP-2	0.04	0.13	0.18	0.14	0.10	-0.11
MMP-3	-0.09	**0.45***	**0.52****	**0.56****	**0.50****	**-0.45***
MMP-7	-0.11	**0.43***	**0.39***	0.34	**0.49****	**-0.39***
MMP-8	-0.10	**0.49****	**0.40***	0.33	**0.46****	**-0.41***
MMP-9	-0.08	**0.42***	0.33	0.29	**0.42***	**-0.36***
MMP-10	0.05	**0.41***	**0.38***	**0.43***	**0.44***	**-0.45***
MMP-12	-0.30	0.15	0.11	0.22	0.12	-0.04
MMP-13	0.27	0.00	-0.05	-0.24	-0.03	0.03
Emmprim	**-0.37***	-0.19	-0.18	-0.09	-0.19	0.19
COPD Subjects						
MMP-1	**-0.52****	0.09	0.06	-0.02	-0.00	0.09
MMP-2	-0.05	-0.15	-0.06	-0.02	-0.17	0.17
MMP-3	-0.24	0.35	**0.42***	**0.54****	**0.44***	-0.31
MMP-7	-0.22	**0.51***	**0.44***	0.40	**0.60****	**-0.42***
MMP-8	-0.32	**0.44***	0.26	0.23	0.40	-0.32
MMP-9	-0.22	0.39	0.21	0.22	0.39	-0.31
MMP-10	-0.07	0.33	0.24	0.39	0.35	-0.37
MMP-12	-0.39	0.10	0.03	0.19	0.09	0.04
MMP-13	0.29	-0.19	-0.24	**-0.42***	-0.22	0.23
Emmprim	-0.35	0.02	-0.02	0.08	0.06	-0.00

Spearman's correlation coefficient. N=31 for whole cohort and N=24 for COPD. * p<0.05 **p<0.01 ***p<0.001

Table 26. Correlation between emphysema and BAL TIMPs.

	Mild CLB	Moderate CLB	Severe CLB	PLB	PSE	NE
Whole Cohort						
TIMP-1	-0.04	0.03	0.05	-0.15	-0.01	-0.07
TIMP-2	-0.21	0.31	0.29	0.22	0.31	-0.31
TIMP-3	-0.23	0.20	0.18	0.21	0.24	-0.19
TIMP-4	-0.19	**0.37***	**0.36***	0.28	**0.37***	-0.36
COPD Subjects						
TIMP-1	0.05	-0.09	-0.11	-0.31	-0.15	0.04
TIMP-2	-0.24	0.25	0.15	0.10	0.22	-0.23
TIMP-3	-0.32	0.30	0.20	0.24	0.37	-0.23
TIMP-4	-0.27	0.34	0.25	0.18	0.34	-0.31

Spearman's correlation coefficient. N=31 for whole cohort and N=24 for COPD. * p<0.05 **p<0.01 ***p<0.001

5.1.9 Regional Variation in Emphysema

To investigate the distribution of emphysema throughout the five different lobes, $\%LAA_{<-950}$ was measured in each individual lobe. Out of the 31 subjects, the RUL was most severely affected in 3 cases (9.7%), the RML in 18 cases (58.1%), the RLL in 3 cases (9.7%), the LUL in 4 cases (12.9%) and the LLL in 3 cases (9.7%). When repeating this analysis in only COPD subjects the RUL was most severely affected 3 cases (12.5%), the RML in 13 cases (54.2%), the RLL in 2 cases (8.3%), the LUL in 4 cases (16.7%) and the LLL in 2 cases (8.3%). In both the whole cohort and COPD sub-cohort the distribution of emphysema was relatively even throughout the lobes apart from between the RUL and RML which showed significant differences in $\%LAA_{<-950}$ (Figure 35). There were no other significant differences in the quantities of emphysema between the other lobes.

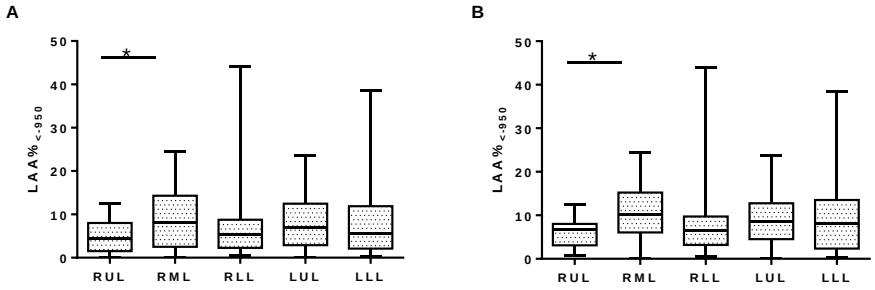


Figure 35. $\%LAA_{<-950}$ in each of the five lobes (A) whole cohort (N=31) (B) COPD only (N=24). *p<0.05 Friedman's Two-way analysis of variance by ranks.

There were few associations between $\%LAA_{<-950}$ in the individual lobes and spirometric markers of disease (Table 27). $\%LAA_{<-950}$ in the RUL had significant associations with FEV1/FVC and FEF75-25% while $\%LAA_{<-950}$ had significant associations with FEF75-25%. These associations were lost significance in when only assessing COPD subjects.

Table 27. Correlation between %LAA$_{<-950}$ in individual lobes and spirometry.

	FEV1%	FVC%	FEV1/FVC	FEF75-25%
Whole Cohort				
RUL	-0.31	0.06	**-0.38***	**-0.39***
RML	-0.14	-0.01	-0.17	-0.15
RLL	-0.234	0.07	-0.32	-0.26
LUL	-0.24	0.07	**-0.37***	-0.34
LLL	-0.19	0.05	-0.28	-0.23
COPD Subjects				
RUL	-0.06	0.18	-0.04	-0.10
RML	-0.06	-0.03	0.015	-0.02
RLL	-0.16	0.09	-0.20	-0.14
LUL	0.00	0.18	-0.12	-0.11
LLL	-0.12	0.02	-0.15	-0.12

Spearman's correlation coefficient. N=31 for whole cohort and N=24 for COPD. * p<0.05

To further evaluate the regional variation of disease I analysed the association between lobar values of emphysema and the lobar concentrations of cytokines, neutrophils, MMPs and TIMPs (two lobes were sampled for each patient). In the previous chapter I used the subjective opinion of a radiologist to define the diseased and preserved lobe. In this section I reclassified these lobes with the disease lobe having the highest %LAA$_{<-950}$ and the preserved lobe having the lowest value.

5.1.9.1 Lobar Emphysema and BAL Neutrophils and cytokines

There was a significant negative association between lobar %LAA$_{<-950}$ and lobar IL-1B (Table 28). There were no other associations between lobar concentrations of neutrophils and other cytokines and lobar %LAA$_{<-950}$. There was no significant difference in BAL neutrophils or cytokines between lobes with the most emphysema and lobes with the least (Figure 36).

Table 28. Associations between Lobar %LAA$_{<-950}$and lobar cytokines and neutrophils

	Lobar %LAA$_{<-950}$
Neutrophils	0.22
IL-1B	**-0.43*** **
IL-6	0.04
IL-8	0.08
GM-CSF	-0.23

Spearman's correlation coefficient. 31 subjects, but 64 associations. *** p<0.005

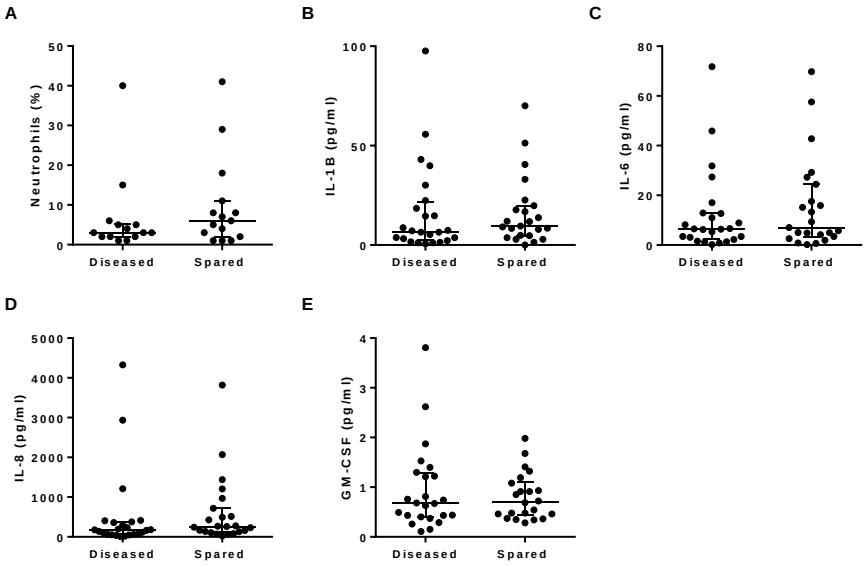


Figure 36. BAL expression of cytokines and neutrophils in diseased and preserved lobes as defined by $\%LAA_{<-950}$. (A) IL-1B (B) Il-6 (C) IL-8 (D) GM-CSF (E) neutrophil count. Data represents median upper and lower quartiles. N=24 for cytokines (x2). N = 19 for neutrophils (x2).

5.1.9.2 Lobar Emphysema association with MMPs

There were significant positive associations between lobar $\%LAA_{<-950}$ and lobar concentrations of MMP-3 and -10 (Table 29). There was also a significant negative association between $\%LAA_{<-950}$ and Emmprim. There were no other associations between lobar emphysema and MMPs. When reclassifying the diseased and spared lobes according to emphysema severity there were significantly higher concentrations of BAL MMP-2, -10 and -13 in the spared lobes compared to diseased lobes (Figure 37).

Table 29. Associations between Lobar $\%LAA_{<-950}$ and lobar MMPs.

	Lobar $\%LAA_{<-950}$
MMP-1	-0.05
MMP-2	0.05
MMP-3	**0.35****
MMP-7	0.16
MMP-8	0.23
MMP-9	0.17
MMP-10	**0.25***
MMP-12	0.02
MMP-13	0.06
Emmprim	**-0.28***

Spearman's correlation coefficient. N=31 subjects, 62 samples. *p<0.05, ** p<0.01.

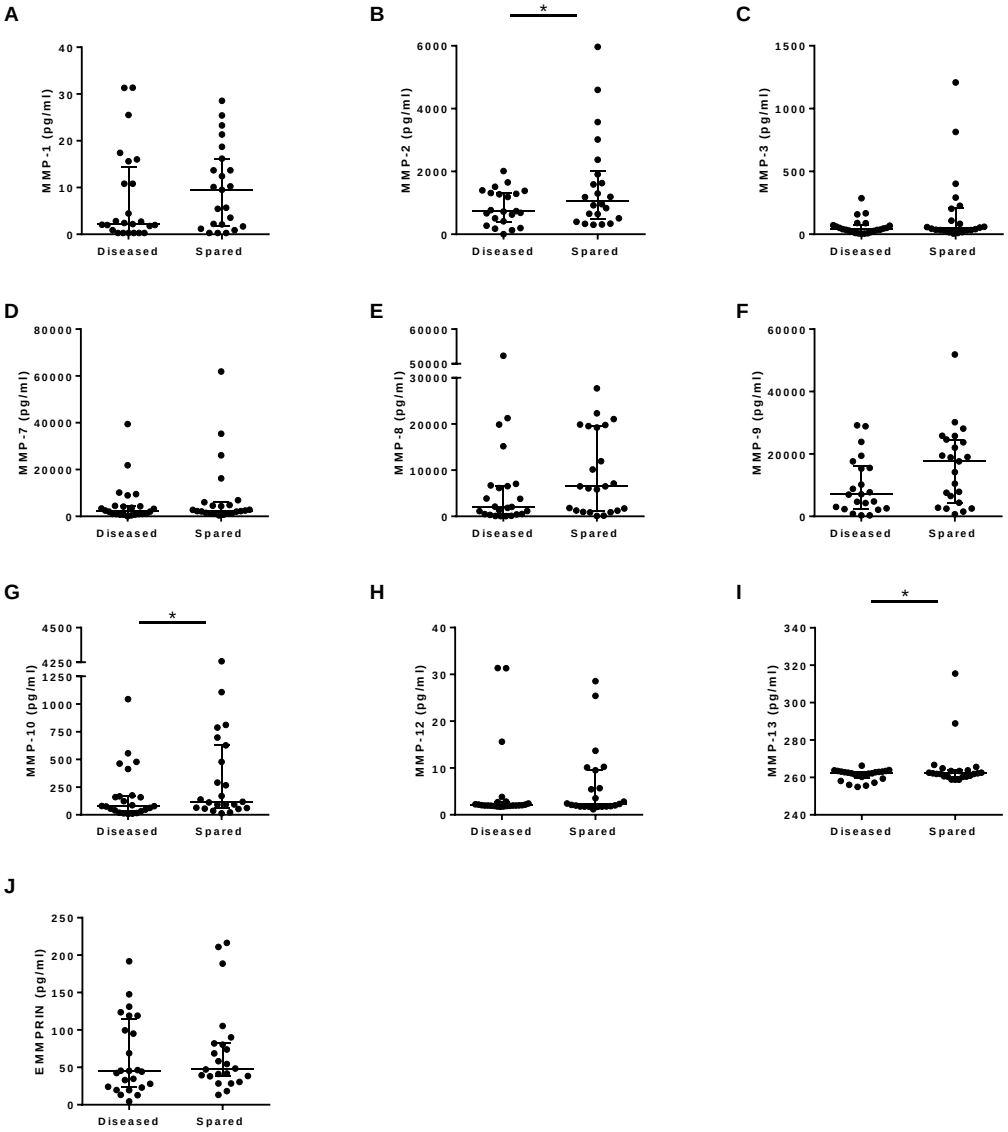


Figure 37. BAL expression of MMPs in diseased and preserved lobes as defined by $\%LAA_{<-950}$. (A) MMP-1 (B) MMP-2 (C) MMP-3 (D) MMP-7 (E) MMP-8 (F) MMP-9 (G) MMP-10 (H) MMP-12 (I) MMP-13 (J) EMMPIRIN. Data represents median upper and lower quartiles. N=24 (x2). * $p<0.05$ using Wilcoxon signed-rank test.

5.1.9.3 Lobar Emphysema and TIMPs

There were no associations between lobar $\%LAA_{<-950}$ and lobar TIMP concentrations (Table 30). There was also no significant difference in TIMP concentrations between the lobes most and least affected by emphysema (Figure 38).

Table 30. Associations between Lobar %LAA$_{<-950}$ and lobar TIMPs.

	Lobar %LAA$_{<-950}$
TIMP-1	-0.00
TIMP-2	0.01
TIMP-3	0.02
TIMP-4	0.21

Spearman's correlation coefficient. N=31, 62 samples.

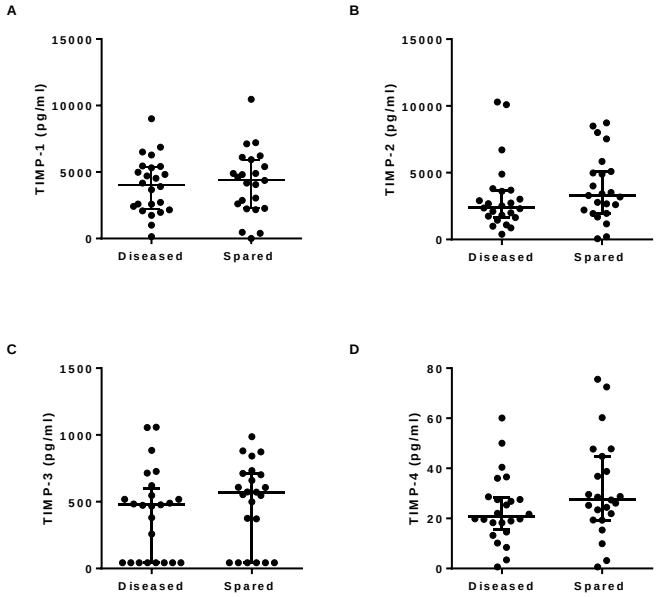


Figure 38. BAL expression of TIMPs in diseased and preserved lobes as defined by %LAA$_{<-950}$. (A) TIMP-1 (B) TIMP-2 (C) TIMP-3 (D) TIMP-4. Data represents median, upper and lower quartiles. N=24 (x2).

5.2 Gas Trapping/small airways disease

Gas trapping is thought to be an indirect measure of small airways disease in COPD and is therefore an important variable to measure. This analysis was successfully performed in a sub-cohort of 22 subjects (16 with COPD and 6 with PLF) who had both inspiratory and expiratory CT scans.

5.2.1 Assessing the best method

As with emphysema there are a number of different methods to measure gas trapping and the best one has yet to be determined. I assessed four different automated measures and a visual score. Figure 39 shows a typical coronal reconstruction of a subject with COPD demonstrating the four

different automated methods for calculating gas trapping/small airways disease. There were strong associations between all four automated measures of gas trapping (Table 31). There was however, no association between the automated measures of gas trapping and the visual score. All four automated measures showed significantly more gas trapping in COPD subjects than those with PLF (Figure 40). Interestingly, visual gas trapping score was raised in subjects with PLF compared to those with COPD, although this did not reach statistical significance.

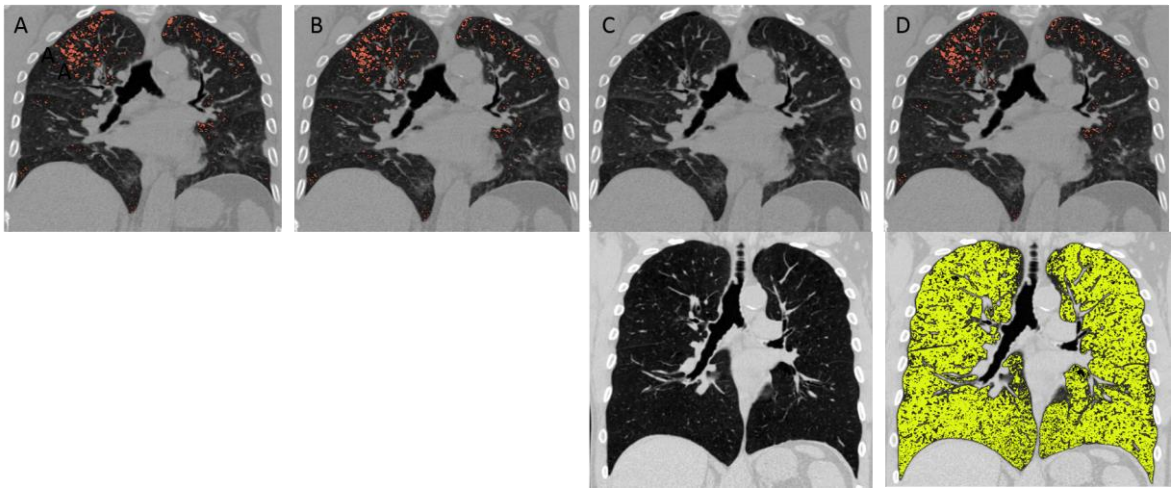


Figure 39. Coronal reconstruction of a CT from a subject with COPD showing different methods for calculating small airways disease/gas trapping (A) red shading is low attenuation area on expiratory scan below -856HU (B) red shading is attenuation area on expiratory scan between -950HU and -856HU (C) upper image is expiratory image and lower image is inspiratory image and higher attenuation can be seen on expiratory scan. E/I MLD calculated by dividing mean lung density on expiratory scans by MLD on inspiratory scan (D) upper image is expiratory scan and red shading represents attenuation area on between -950HU and -856HU. Bottom image is inspiratory scan and yellow shadowing represents attenuation between -950HU and -856HU. RVC is calculated as the difference between these shaded volumes.

Table 31. Correlation between gas trapping markers.

	$\%LAA_{EXP<-856}$	**$\%LAA_{EXP<-856-950}$**	**RVC**	**E/I MLD**
$\%LAA_{EXP<-856-950}$	**0.99*** **	-		
RVC	**0.79*** **	**0.75*** **	-	
E/I MLD	**0.85*** **	**0.84*** **	**0.78*** **	
Gas trapping score	-0.10	-0.08	0.00	0.04

Spearman's correlation coefficient. N=22. *** p<0.005

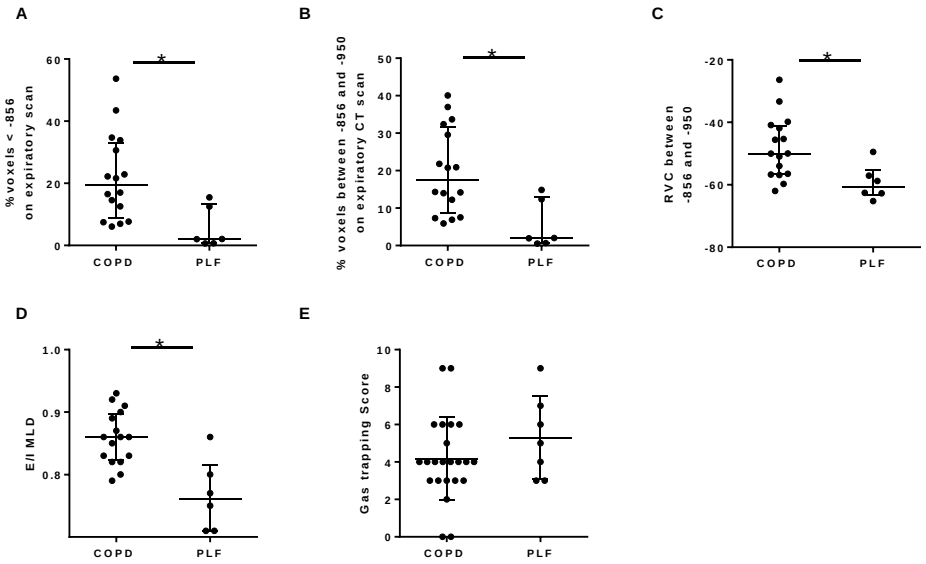


Figure 40. Gas trapping/small airway quantitation in COPD and preserved lung function (PLF) using five different methods (A) % voxels below -856HU on expiratory CT (B) % voxels between -856HU and -950HU on expiratory CT (C) Relative volume change between -856HU and -950HU between inspiratory and expiratory CT (D) Ratio of expiratory to inspiratory mean lung density on CT (E) Gas trapping score. Data represents median with IQ range. Each dot represents individual CT measure in a specific patient. For automated scores n=16 for COPD and 6 for PLF. For visual score n=24 for COPD and 8 for PLF. * p<0.05 using Mann-Whitney U test.

5.2.2 Association between gas trapping and spirometry

There were a number of strong significant associations between markers of gas trapping and spirometric markers. In the whole cohort all automated measures of gas trapping had significant associations with FEV1%, FEV1/FVC and FEF75-25% (Table 32). In COPD subjects the associations with FEF75-25% lost significance, apart from E/I MLD. The gas trapping visual emphysema score did not have any associations with spirometric markers of disease, although in COPD subjects it did show an association with FEF75-25%.

Table 32. Correlation between gas trapping and spirometry.

	FEV1%	FVC%	FEV1/FVC	FEF75-25%
Whole Cohort				
%LAA$_{EXP<-856}$	**-0.63****	0.07	**-0.79*****	**-0.66****
%LAA$_{EXP<-856-950}$	**-0.60****	0.07	**-0.73*****	**-0.63****
RVC	**-0.66****	-0.23	**-0.68*****	**-0.53***
E/I MLD	**-0.77*****	-0.12	**-0.81*****	**-0.73*****
Gas trapping Score	-0.20	-0.23	0.04	0.08
COPD Subjects				
%LAA$_{EXP<-856}$	**-0.55***	0.10	**-0.68****	-0.43
%LAA$_{EXP<-856-950}$	**-0.56***	0.08	**-0.62***	-0.41
RVC	**-0.50***	-0.06	**-0.55***	-0.21
E/I MLD	**-0.72****	-0.05	**-0.73****	**-0.54***
Gas trapping Score	**-0.50***	-0.26	-0.36	**-0.44***

Spearman's correlation coefficient. N=22 for all subjects and N=16 for COPD. *** p<0.005

As E/I MLD had the strongest association with FEV1% and FEF75-25% I decided to use this as the measure for gas trapping throughout the rest of this study.

5.2.3 Gas trapping and blood results

There were no significant associations between blood white cell differentials and fibrinogen and markers of gas trapping (Table 33).

Table 33. Correlation between gas trapping and blood inflammatory markers.

	WCC	Neutrophils	Lymphocytes	Eosinophils	Fibrinogen
Whole Cohort					
E/I MLD	0.07	-0.19	0.44	-0.11	-0.10
COPD subjects					
E/I MLD	0.10	-0.10	0.44	-0.13	0.03

Spearman's correlation coefficient. N=20 for WCC (N=14 for COPD) and N=21 for fibrinogen (N=15 for COPD).

5.2.4 Gas Trapping and cell differential

Next I assessed the relationships between sputum cell differentials and gas trapping. There was a significant positive association between E/I MLD and neutrophils (Table 34). There were consequently negative associations between macrophages and E/I MLD. In the COPD sub-cohort there were also negative associations between BAL lymphocytes and E/I MLD.

Table 34. Correlation between emphysema and BAL white cell differentials.

	Neutrophils	Macrophages	Eosinophils	Lymphocytes
Whole Cohort				
E/I MLD	**0.55***	**-0.49***	0.05	-0.29
COPD subjects				
E/I MLD	0.43	-0.20	-0.37	**-0.67***

Spearman's correlation coefficient. N=17 for all subjects and N=12 for COPD. * $p<0.05$.

5.2.5 Gas trapping and cytokines

IL-8 had significant associations with E/I MLD in both the entire cohort and COPD subjects aloneTable 35). Gas trapping visual score also had significant associations with IL-6 in the whole cohort and with IL-6 and IL-8 in the COPD subjects only.

Table 35. Correlation between gas trapping and cytokines.

	IL-1B	IL-6	IL-8	GM-CSF
Whole Cohort				
E/I MLD	0.23	0.38	**0.53***	-0.22
COPD subjects				
E/I MLD	0.12	0.29	**0.53***	-0.19

Spearman's correlation coefficient. N=22 for all subjects and N=16 for COPD. * p<0.05.

5.2.6 Gas trapping and MMPs and TIMPs

Next I assessed the association between gas trapping and MMPs and found multiple associations. E/I MLD had significant associations with MMP-3, -7, -8, -9, -10 and -12 in the whole cohort and COPD subjects (Table 36 and Figure 41). E/I MLD also had significant associations with TIMP-2 and -4 (Table 37), however this lost significance when the analysis was repeated in the COPD subjects only.

Table 36. Correlation between gas trapping and BAL MMPs.

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12	MMP-13
Whole Cohort									
E/I MLD	0.39	0.28	**0.70*****	**0.53***	**0.76*****	**0.67****	**0.72*****	**0.58****	0.03
COPD subjects									
E/I MLD	0.16	-0.14	**0.52***	**0.52***	**0.60***	**0.56***	**0.55***	**0.50***	-0.03

Spearman's correlation coefficient. N=22 or all subjects and N=16 for COPD. * p<0.05, ** p<0.01, ***p<0.001.

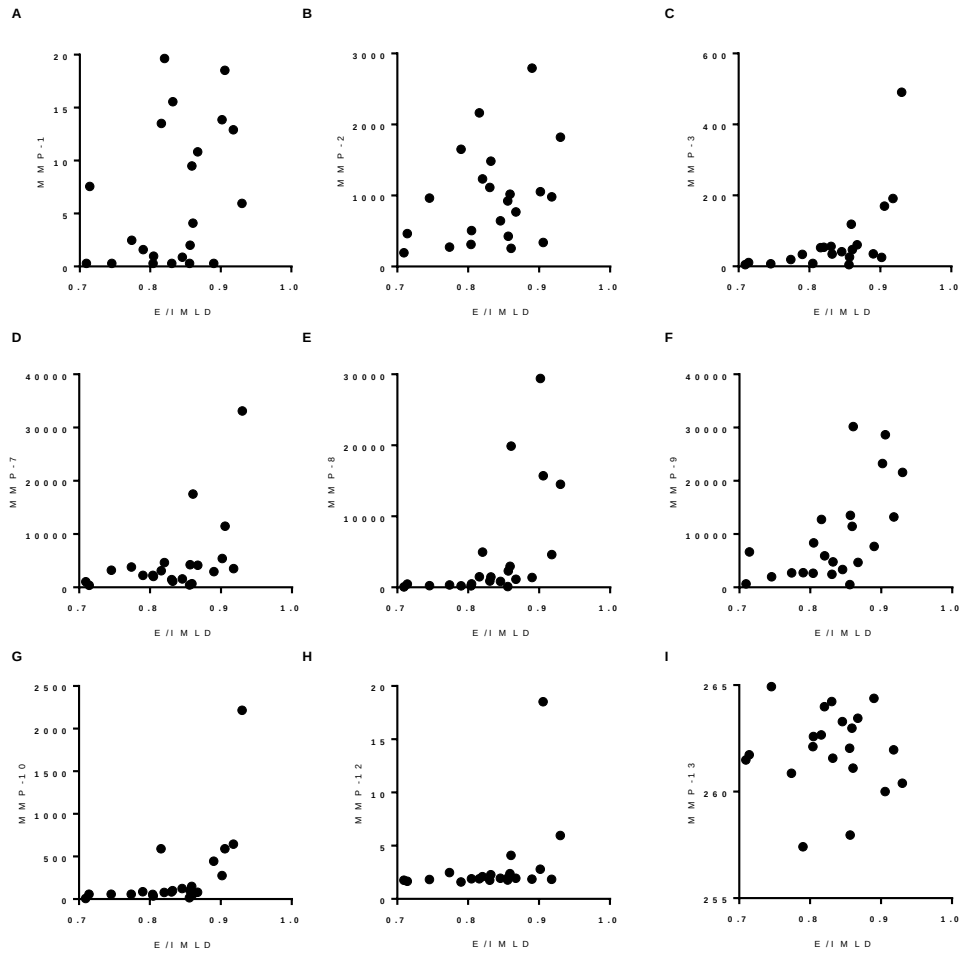


Figure 41. Scatterplots for E/I MLD against BAL expression of MMPs. (A) MMP-1 (B) MMP-2 (C) MMP-3 (D) MMP-7 (E) MMP-8 (F) MMP-9 (G) MMP-10 (H) MMP-12 (I) MMP-13

Table 37. Correlation between gas trapping and BAL TIMPs.

	E/I MLD
Whole cohort	
TIMP-1	0.14
TIMP-2	**0.55****
TIMP-3	0.41
TIMP-4	**0.60****
COPD subjects	
TIMP-1	-0.23
TIMP-2	0.31
TIMP-3	0.20
TIMP-4	0.32

Spearman's correlation coefficient. N=22 or all subjects and N=16 for COPD. ** $p<0.01$.

5.2.7 Gas trapping and microbiology

There was no significant difference in E/I MLD between those whose sputum cultured bacteria and those whose did not (Figure 42). This remained the case when assessing the individual bacteria. There were not enough PCR positive samples in order to perform any meaningful analysis with gas trapping.

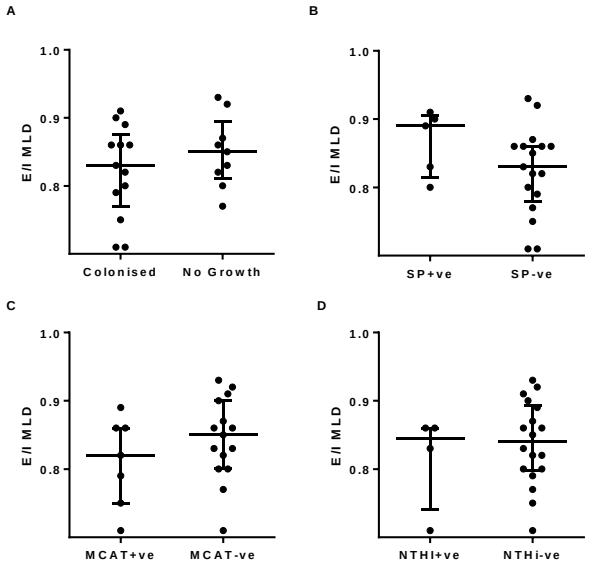


Figure 42. E/I MLD quantities in Sputum culture positive and negative subjects (A) PPM - Potentially pathogenic microorganism (B) SP -streptococcus Pneumoniae (C) MCAT- Moraxella catarrhalis (D) NTHI- non-typeable Haemophilus influenzae. Data represents median with IQ range. Each dot represents individual CT measure in a specific patient, n=22.

5.2.8 Lobar Gas trapping

Next I analysed the distribution of gas trapping in each of the five lobes. There was a significant difference in E/I MLD between the five lobes (Figure 43). The middle and upper lobes were most severely affected and there were significant differences between these and the lower lobes. There were no significant difference in the amount of gas trapping between both upper lobes and the middle lobe. E/I MLD in all lobes had significant associations with FEV1%, FEV1/FVC and FEF75-25% (Table 38). The associations with FEF75-25% lost significance when only analysing the COPD cohort.

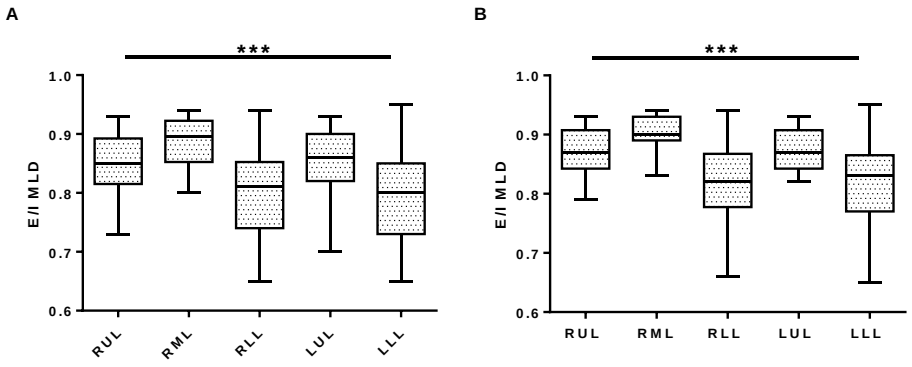


Figure 43. E/I MLD in each of the five lobes (A) whole cohort, N=22 (B) COPD only, N=16. Data represents medians, IQR and range. *** p<0.001 Friedman's Two-way analysis of variance by ranks.

Table 38. Correlation between E/I MLD in individual lobes and spirometry.

	FEV1%	FVC%	FEV1/FVC	FEF75-25%
Whole Cohort				
RUL	**-0.73*** **	-0.19	**-0.69** **	**-0.66** **
RML	**-0.49***	0.083	**-0.58** **	**-0.47***
RLL	**-0.76*** **	-0.16	**-0.75*** **	**-0.70*** **
LUL	**-0.64** **	0.044	**-0.75*** **	**-0.66** **
LLL	**-0.68** **	-0.11	**-0.73*** **	**-0.66** **
COPD subjects				
RUL	**-0.69** **	-0.14	**-0.53***	-0.44
RML	**-0.45**	0.14	**-0.45**	-0.26
RLL	**-0.68** **	-0.15	**-0.63** **	-0.49
LUL	**-0.54***	0.26	**-0.69** **	-0.50
LLL	-0.62*	-0.05	-0.64**	-0.46

Spearman's correlation coefficient. N=22 or all subjects and N=16 for COPD. *p<0.05, **p<0.01, ** p<0.01.

To further evaluate the regional variation of disease I analysed the association between lobar values of gas trapping and different disease mechanisms. I then reclassified the diseased and preserved lobes for the quantitative markers of gas trapping.

5.2.8.1 Lobar gas trapping and BAL neutrophils and cytokines

There were significant positive associations between the lobar value of E/I MLD and IL-6 and IL-8 (Table 39). There was also a significant negative association between lobar E/I MLD and GM-CSF. When reclassifying the diseased and healthy lobes according to severity of gas trapping, there were significantly raised concentrations of IL-1B, IL-8 and GM-CSF in the preserved lobes (Figure 44).

Table 39. Associations between Lobar %$LAA_{<-950}$and lobar neutrophils and cytokines.

	Lobar E/I MLD
Neutrophils	0.25
IL-1B	0.11
IL-6	**0.36***
IL-8	**0.35***
GM-CSF	**-0.33***

Spearman's correlation coefficient. N=17 for neutrophils and N=22 for cytokines. *p<0.05.

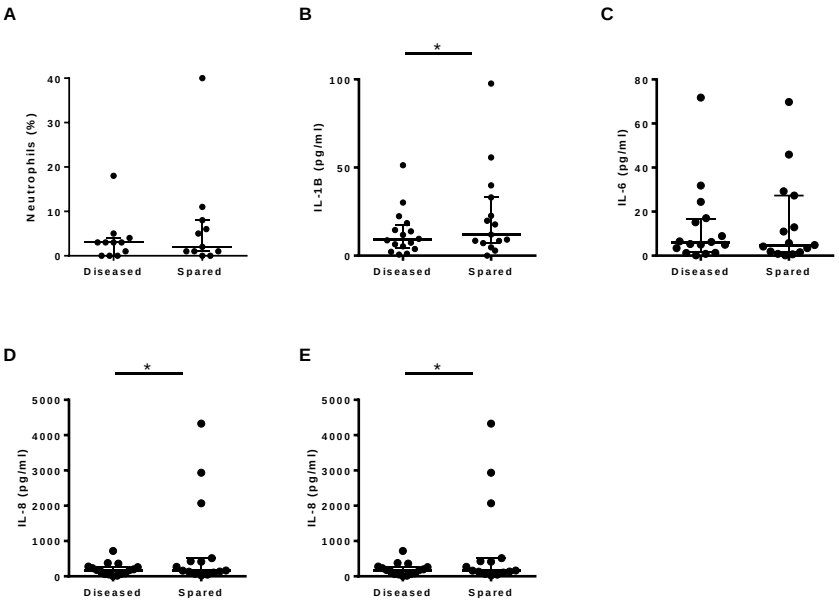


Figure 44. BAL expression of cytokines and neutrophils in diseased and preserved lobes as defined by E/I MLD. (A) IL-1B (B) Il-6 (C) IL-8 (D) GM-CSF (E) neutrophil count. Data represents median upper and lower quartiles. N=22 for cytokines and N=17 for neutrophils. *p<0.05 using related samples McNemar test.

5.2.8.2 Lobar gas trapping and MMPs.

There were significant associations between lobar E/I MLD and MMP-3, -7, -8, -9, -10 and -12 (Table 40). When reclassifying the diseased and healthy lobes according to severity of gas trapping there were no significant difference in any of the MMPs between lobes (Figure 45).

Table 40. Associations between Lobar %$LAA_{<-950}$and lobar MMPs.

	Lobar E/I MLD
MMP-1	0.34
MMP-2	0.31
MMP-3	**0.53*** **
MMP-7	**0.37***
MMP-8	**0.62*** **
MMP-9	**0.51****
MMP-10	**0.52****
MMP-12	**0.46****
MMP-13	-0.01

Spearman's correlation coefficient. N=22. *p<0.05, **p<0.01, ***p<0.001.

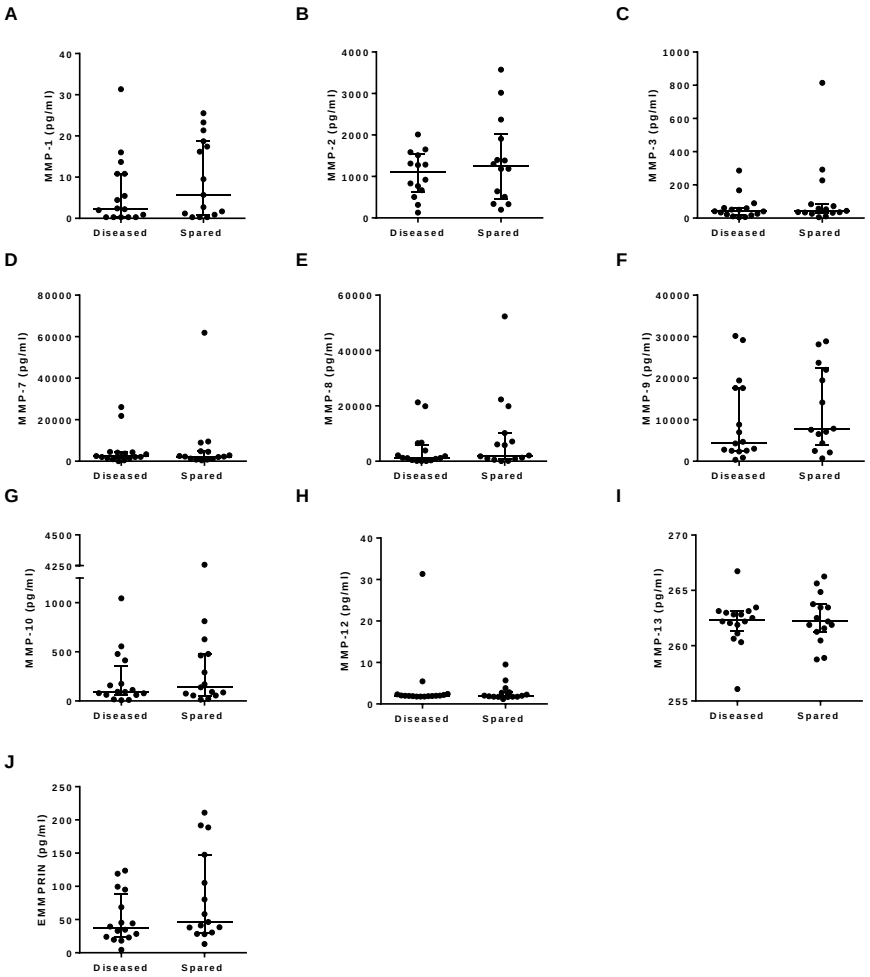


Figure 45. BAL expression of MMPs in diseased and preserved lobes as defined by E/I MLD. (A) MMP-1 (B) MMP-2 (C) MMP-3 (D) MMP-7 (E) MMP-8 (F) MMP-9 (G) MMP-10 (H) MMP-12 (I) MMP-13 (J) EMMPIRIN. Data represents median upper and lower quartiles. N=16.

5.2.8.3 Lobar Gas Trapping and TIMPs

There were significant associations between lobar E/I MLD and TIMP-3 and -4 (Table 41). When reclassifying the diseased and healthy lobes according to severity of gas trapping there were no significant difference in any of the TIMPs between lobes (Figure 46).

Table 41. Associations between Lobar %$LAA_{<-950}$and lobar MMPs.

	Lobar E/I MLD
TIMP-1	0.08
TIMP-2	0.23
TIMP-3	**0.33***
TIMP-4	**0.42****

Spearman's correlation coefficient. N=22. *p<0.05, **p<0.01.

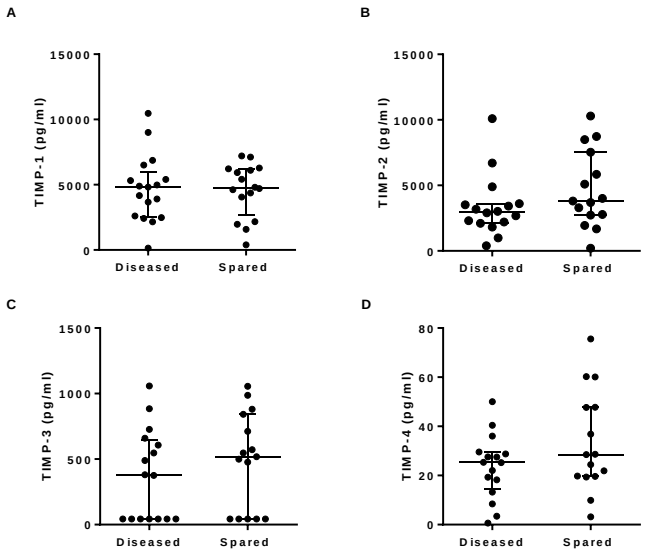


Figure 46. BAL expression of TIMPs in diseased and preserved lobes as defined by E/I MLD. (A) TIMP-1 (B) TIMP-2 (C) TIMP-3 (D) TIMP-4. Data represents median, upper and lower quartiles. N=16.

5.3 Bronchial Wall Morphology

The bronchial tree is a complex structure, with multiple branches and generations. Each airway is essentially a hollow cylinder and therefore there are a number of different measurements that can be made. Using Apollo software I measured the following airway dimensions; internal and external perimeter, wall thickness, lumen area, wall area, total airway area and wall area% (WA%). To add

to the complexity there are also a number of different ways to label the airway tree and all of these features make it difficult to accurately reflect bronchial wall morphology quantitatively.

5.3.1 Global measures

Initially, I measured the median for all of the measurement parameters for all airways combined, giving a global measurements for airway morphology. When doing this significantly more airways were measured in subjects with PLF than COPD (Table 42). There were significantly increased internal and external perimeters, wall thickness, lumen area, wall area, and total airway area in subjects with COPD compared to those with PLF (Table 42). There was no significant difference in WA% between subjects with and without airflow obstruction.

In this initial analysis I also used another global measures of airway wall morphology, termed Pi10. Pi10 represents the square root of the wall area of a hypothetical airway with a perimeter of 10mm. It only includes airways with an internal perimeter of less than 20mm and has the benefit of standardising the results for airway size and for giving one figure for airway wall size for each scan. There was no significant difference in Pi10 between COPD subjects and those with preserved lung function (Table 42).

Table 42. Median airway dimensions for the whole airway tree.

	COPD	PLF	P value
Number of airway	85.3 (26.0)	124.0 (53.0)	**0.003****
Internal Perimeter	12.7 (2.5)	11.7 (1.7)	**0.042***
External Perimeter	21.0 (3.9)	19.6 (2.3)	**0.023***
Wall Thickness	1.35 (0.18)	1.18 (0.13)	**0.002****
Lumen area	11.5 (5.6)	8.5 (1.8)	**0.006****
Wall Area	21.8 (8.1)	17.5 (2.8)	**0.008****
Total Airway area	33.3 (12.5)	25.9 (5.0)	**0.008****
WA%	65.6 (2.0)	65.2 (2.0)	0.962
Pi10	3.74 (0.15)	3.74 (0.04)	0.502

Data represents medians and IQ range. N=24 for COPD and N=7 for PLF. *p<0.05, **p<0.01 using Mann Whitney U test.

5.3.1.1 Association between Global airway Dimensions and Spirometry

Next I analysed the association between these global measures of airway morphology and spirometric markers of disease (Table 43). There were significant positive associations between the number of airways measured and FEV1%, FEV1/FVC ratio and FEF75-25%, although for COPD subjects the association with FEF75-25% lost significance. There were also negative associations between internal and external perimeters, wall thickness, lumen area, wall area, total airway wall

area and the spirometric markers FEV1%, FVC% and FEV1/FVC. The same airway parameters apart from internal perimeter also had significant associations with FEF75-25%. Some of these associations lost significance when performing the analysis in the COPD group only, especially with FEV1/FVC and FEF75-25%. There were no association between WA% and spirometry, while the only significant association with Pi10 was in the COPD cohort and a negative association with FEV1%.

In an attempt to correct the analysis for the number of airways measured, partial correlation was performed and when doing this there were no associations between global CT measures of airway dimensions and FEV1% (Table 44).

Table 43. Associations between airway dimensions and spirometry.

	FEV1%	FVC%	FEV1/FVC	FEF75-25%
Whole Cohort				
Number of airway	**0.73*** **	0.30	**0.74*** **	**0.62*** **
Internal Perimeter	**-0.46** **	**-0.53** **	**-0.40***	-0.32
External Perimeter	**-0.51** **	**-0.52** **	**-0.46** **	**-0.36***
Wall Thickness	**-0.61*** **	**-0.40***	**-0.60*** **	**-0.45***
Lumen area	**-0.59** **	**-0.55** **	**-0.50** **	**-0.43***
Wall Area	**-0.59** **	**-0.50** **	**-0.53** **	**-0.42***
Total Airway area	**-0.61** **	**-0.52** **	**-0.54** **	**-0.44***
WA%	0.03	0.22	-0.02	-0.00
Pi10	-0.31	0.05	-0.30	-0.11
COPD subjects				
Number of airway	**0.53** **	0.15	**0.58** **	0.34
Internal Perimeter	-0.38	**-0.57** **	-0.28	-0.10
External Perimeter	**-0.43***	**-0.56** **	-0.34	-0.15
Wall Thickness	**-0.43***	**-0.41***	-0.36	-0.07
Lumen area	**-0.47***	**-0.60** **	-0.29	-0.15
Wall Area	**-0.46***	**-0.52** **	-0.35	-0.13
Total Airway area	**-0.51***	**-0.54** **	-0.36	-0.18
WA%	0.09	0.30	-0.03	0.02
Pi10	**-0.41***	0.10	-0.35	-0.22

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

Table 44. Partial correlation between airway dimensions and FEV1% correcting for number of airways.

	FEV1%
Whole Cohort	
Internal Perimeter	-0.13
External Perimeter	-0.12
Wall Thickness	-0.21
Lumen area	-0.26
Wall Area	-0.16
Total Airway area	-0.22
WA%	0.11
COPD Subjects	
Internal Perimeter	-0.20
External Perimeter	-0.21
Wall Thickness	-0.22
Lumen area	-0.30
Wall Area	-0.22
Total Airway area	-0.30
WA%	0.19

Spearman's partial correlation, correcting for number of airways. N=31 for whole cohort and n=24 for COPD.

5.3.2 Analysis by Airway Generation

Although global measures of airway wall morphology offer a rather neat solution to analysing airway morphology it is unlikely to be sufficient to reflect the complexity of the bronchial tree. Therefore I elected to perform a more systematic analysis into the different measurements in each airway generation. In this analysis the airways are labelled from generation 0 (the trachea) and increase by a generation at every branch point.

Table 45 shows the median number of airways measured for each airway generation in COPD subjects and those with PLF and the number of subjects these measurements came from. In generation 0-6 all subjects contributed to the airway measurements. In more distal generations fewer subjects contributed to airway measurements and this was especially the case for COPD subjects in generation 9 and above. There was no significant difference between the median number of airways measured in COPD and PLF subjects in generation 0, 1, 2, 3, 4, 5, 9, 10, 11 and 12. There were significantly fewer airways analysed in COPD subjects in the 6^{th}, 7^{th} and 8^{th} generation. There were significant associations between the number of airways measured in generation 5, 6, 7, 8 and 9 (as well as 10, in the COPD cohort only) and airflow obstruction (Table 46).

Table 45. Number of airways measured in each generation

	COPD		PLF		
Generation	No	Airways	No	Airways	P Value
0	24	1.0 (0.0)	7	1.0 (0.0)	>0.999
1	24	2.0 (0.0)	7	2.0 (0.0)	>0.999
2	24	4.0 (0.0)	7	4.0 (0.0)	>0.999
3	24	13.0 (1.0)	7	12.0 (3.0)	0.400
4	24	14.0 (0.0)	7	14.0 (1.0)	0.108
5	24	14.0 (2.0)	7	14.0 (1.0)	0.703
6	24	13.0 (11.0)	7	20 (2.0)	**0.015***
7	23	8.0 (11.0)	7	28.0 (14.0)	**0.002****
8	21	4.0 (7.0)	7	16.0 (11.0)	**0.003****
9	11	4.0 (7.0)	7	8.0 (9.0)	0.294
10	6	5.5 (12.0)	4	7.0 (9.0)	0.667
11	4	1.5 (3.0)	3	4.0 (0)	0.354
12	1	2.0 (0)	1	2.0 (0)	>0.999
13	0	0	1	1.0 (0)	-

No. represents number of subjects contributing to airway measurements. Number of airways represents medians and IQR. *p<0.05, **p<0.01 using Mann Whitney U Test.

Table 46. Association between FEV1% and number of airways measured in each generation.

	Whole Cohort FEV1	COPD FEV1
3	-0.10	0.13
4	0.29	0.22
5	**0.37***	**0.45***
6	**0.57****	**0.44***
7	**0.70*****	**0.48***
8	**0.70*****	**0.47***
9	**0.73****	**0.79****
10	0.52	**0.91***
11	0.57	0.78

Spearman's correlation coefficient. For number of subjects see previous table.*p<0.05, **p<0.01, ***p<0.001.

I only included airway generation 0-6 in my main analysis as all subjects CT scans contributed to the airway data. When comparing the measurements between subjects with COPD and PLF there were few significant differences (Table 47). In generation 4 and 6 airways, WA% was significantly higher in COPD than in PLF. In generation 5 airways lumen area was significantly smaller in COPD compared to those with PLF.

Table 47. Airway wall Dimensions in different generations

	COPD	PLF	P value
Generation 0			
Internal Perimeter	62.3 (15.7)	59.2 (13.5)	0.479
External Perimeter	80.6 (17.6)	77.4 (13.4)	0.479
Wall Thickness	2.76 (0.32)	2.67 (0.20)	0.962
Lumen area	285.7 (144.2)	268.8 (120.2)	0.603
Wall Area	208.4 (63.1)	192.0 (38.4)	0.450
Total Airway area	483.8 (210.7)	463.7 (158.6)	0.603
WA%	40.9 (6.0)	41.4 (4.0)	0.741
Generation 1			
Internal Perimeter	45.2 (11.4)	43.4 (14.3)	0.671
External Perimeter	57.8 (13.6)	56.2 (18.7)	0.571
Wall Thickness	2.15 (0.51)	2.15 (0.59)	0.321
Lumen area	149.4 (66.8)	141.9 (104.0)	0.395
Wall Area	106.9 (45.6)	101.9 (70.4)	0.369
Total Airway area	256.0 (119.6)	243.8 (172.9)	0.369
WA%	42.3 (3.0)	42.8 (2.0)	0.156
Generation 2			
Internal Perimeter	33.8 (7.2)	35.8 (11.1)	0.571
External Perimeter	45.3 (8.9)	47.4 (11.9)	0.508
Wall Thickness	1.86 (0.30)	1.92 (0.19)	0.345
Lumen area	78.9 (36.3)	94.5 (59.2)	0.539
Wall Area	71.5 (24.6)	75.6 (22.2)	0.345
Total Airway area	151.4 (66.8)	170.1 (81.9)	0.422
WA%	47.5 (4.0)	45.8 (5.0)	0.422
Generation 3			
Internal Perimeter	21.0 (4.5)	20.6 (5.9)	0.741
External Perimeter	32.2 (5.5)	31.5 (6.9)	0.539
Wall Thickness	1.75 (0.13)	1.74 (0.21)	0.741
Lumen area	32.4 (12.9)	32.3 (18.6)	0.508
Wall Area	47.5 (14.2)	46.2 (14.8)	0.395
Total Airway area	78.7 (26.1)	76.8 (33.3)	0.422
WA%	58.4 (5.0)	58.8 (6.0)	0.508
Generation 4			
Internal Perimeter	14.9 (4.0)	16.0 (3.6)	0.089
External Perimeter	24.8 (5.5)	26.7 (4.4)	0.119
Wall Thickness	1.49 (0.25)	1.51 (0.22)	0.450
Lumen area	15.8 (8.8)	18.7 (7.7)	0.059
Wall Area	30.3 (11.4)	35.0 (13.8)	0.089
Total Airway area	45.5 (21.5)	53.4 (18.3)	0.073
WA%	63.7 (5.0)	62.3 (3.0)	**0.042***
Generation 5			
Internal Perimeter	11.6 (2.6)	12.8 (2.2)	0.065
External Perimeter	20.1 (4.0)	21.0 (3.5)	0.219
Wall Thickness	1.21 (0.22)	1.25 (0.12)	0.479
Lumen area	9.4 (4.7)	12.1 (4.6)	**0.047***
Wall Area	19.8 (7.9)	20.7 (5.8)	0.321
Total Airway area	30.4 (12.7)	32.5 (10.3)	0.131
WA%	65.7 (3.0)	65.3 (3.0)	0.143
Generation 6			
Internal Perimeter	9.7 (2.0)	10.3 (2.8)	0.257
External Perimeter	16.9 (2.9)	17.7 (2.9)	0.345
Wall Thickness	1.09 (0.13)	1.05 (0.15)	0.422
Lumen area	6.0 (2.9)	7.1 (3.4)	0.369
Wall Area	14.1 (3.3)	13.6 (5.2)	0.741
Total Airway area	20.3 (6.5)	19.6 (8.6)	0.777
WA%	68.4 (3.0)	66.0 (3.0)	**0.038***

Data represents medians and IQR. N=31. *p<0.05 using Mann Whitney U Test. PLF – preserved lung function.

5.3.2.1 Association between airway generation morphology and spirometry

Next I assessed the association between airway morphology at each generation and FEV1% (Table 48). There were no associations for generation 0-3 airways. In generation 4 there were significant positive associations between FEV1% and internal and external perimeters, lumen area and total airway area. In generation 5 airways lumen area had a significant positive association with FEV1%. All of these associations lost significance when performing the analysis in COPD subjects only. WA% had negative association with FEV% in generations 4, 5 and 6, although this only remained significant in generation 4 for COPD subjects only.

As it appears that the number of airways measured influences the results I used partial correlation to correct for this (Table 49). When doing so WA% had a significant negative association with FEV1% association in airway generation 4 and 5. There also continued to be associations between FEV1% and internal perimeter and lumen area in generation 4. When repeating the analysis in the COPD subjects only WA% had a significant inverse relationship with FEV1% in generation 4.

Table 48. Association between airway dimensions in different generations and FEV1%

Generation	0	1	2	3	4	5	6
Whole Cohort							
Internal Perimeter	-0.02	0.04	0.17	0.26	**0.46****	0.35	0.26
External Perimeter	-0.04	0.13	0.24	0.27	**0.39***	0.31	0.20
Wall Thickness	0.12	0.29	0.28	0.11	0.11	0.15	-0.09
Lumen area	0.01	0.12	0.20	0.31	**0.50****	**0.37***	0.19
Wall area	-0.04	0.23	0.29	0.30	0.34	0.27	0.03
Total wall Area	-0.01	0.17	0.26	0.29	**0.39***	0.34	0.12
Wall area %	0.02	0.32	-0.10	-0.35	**-0.60*****	**-0.45***	**-0.47****
COPD subjects							
Internal Perimeter	0.06	-0.10	0.18	0.23	0.31	0.13	0.08
External Perimeter	0.05	0.05	0.24	0.20	0.23	0.17	0.07
Wall Thickness	0.19	0.19	0.20	0.13	0.01	0.04	-0.10
Lumen area	0.09	-0.02	0.20	0.25	0.34	0.14	0.02
Wall area	0.02	0.15	0.25	0.27	0.15	0.17	-0.01
Total wall Area	0.07	0.07	0.25	0.21	0.19	0.18	0.01
Wall area %	0.02	0.14	-0.11	-0.32	**-0.46***	-0.35	-0.29

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

Table 49. Partial correlation of airway dimensions with FEV1%, correcting for airway numbers.

	Gen.3	Gen.4	Gen.5	Gen. 6
Whole Cohort				
Internal Perimeter	0.25	**0.40***	0.29	0.08
External Perimeter	0.25	0.32	0.23	0.11
Wall Thickness	0.09	0.05	0.09	-0.10
Lumen area	0.30	**0.44***	0.31	0.04
Wall area	0.29	0.28	0.20	-0.04
Total wall Area	0.28	0.33	0.28	0.03
Wall area %	-0.35	**-0.55****	**-0.38***	-0.31
COPD subjects				
Internal Perimeter	0.28	0.25	0.05	-0.05
External Perimeter	0.27	0.17	0.07	0.01
Wall Thickness	0.18	-0.03	-0.05	-0.12
Lumen area	0.30	0.28	0.04	-0.07
Wall area	0.35	0.10	0.07	-0.05
Total wall Area	0.27	0.13	0.09	-0.02
Wall area %	-0.35	**-0.41***	-0.25	-0.15

Spearman's partial correlation. N=31 for whole cohort and 24 for COPD. *p<0.05, **p<0.01.

5.3.3 Analysis by segmental generation

As previously mentioned there are a number of methods to label the airways and one potential problem with labelling the airways by generation number is that they do not compare structurally similar airways. For example RLL7 and RB1 are both third generation airway. One way to combat this is to use a different method which consists of labelling segmental generation airways as segmental generation 3 and then each subsequent generation is labelled as one higher.

Table 50 shows the median number of airways measured for each segmental airway generation in COPD subjects and those with PLF and the number of subjects these measurements came from. In generation 3-5 all subjects contributed to the airway measurements. More distally, fewer subjects contributed to airway measurements. There was no significant difference between the median number of airways measured in COPD and PLF subjects in segmental generation 3, 4, 8, 9 and 10. There were significantly fewer airways analysed in COPD subjects in the 5^{th}, 6^{th} and 7^{th} segmental generation. There were significant associations between the numbers of airways measured in segmental generation 3, 5, 6, and 7 and airflow obstruction. In COPD subjects there was an association between the number of airways measured and FEV1% in segmental generation 5, 7, 8 and 10 (Table 51).

I only included segmental airway generations 3-5 in this analysis as these were the only ones where all subjects CT scans contributed to the airway data. The only parameter that was significantly different between COPD and PLF subjects was WA% in segmental generation 5. There was a trend

towards a smaller lumen area in in COPD in segmental generation 3 and 5 and for larger WA% in COPD in segmental generation 3 and 4 (Table 52).

Table 50. Number of airways measured in each segmental generation

	COPD		PLF		
Generation	No	Airways	No	Airways	P Value
3	24	19.0 (0.0)	7	19.0 (0.0)	0.197
4	24	10.0 (0.0)	7	10.0 (0.0)	0.796
5	24	16.0 (6.0)	7	19.0 (2.0)	**0.012***
6	22	11.5 (15.0)	7	26.0 (15.0)	**0.003****
7	20	4.0 (9.0)	7	18.0 (8.0)	**0.005****
8	8	6.5 (8.0)	7	10.0 (10.)	0.292
9	4	7.0 (10.0)	4	5.5 (14.0)	>0.999
10	2	2.5 (0.0)	3	3.0 (0.0)	>0.999
11	0		1	2.0 (0.0)	-
12	0		1	2.0 (0.0)	-

No. represents number of subjects contributing to airway measurements. Number of airways represents medians and IQR. *p<0.05, **p<0.01 using Mann Whitney U Test.

Table 51. Association between FEV1% and number of airways measured in each generation.

	Whole Cohort FEV1	COPD FEV1
3	**0.43***	0.39
4	0.15	0.17
5	**0.58****	**0.47***
6	**0.63*****	0.34
7	**0.78*****	**0.58****
8	0.62*	**0.90****
9	0.33	0.78
10	0.00	**1.00*****

Spearman's correlation coefficient. For number of subjects see previous table. *p<0.05, **p<0.01, ***p<0.001.

Table 52. Airway wall Dimensions in different generations

	COPD	PLF	P value
Segmental Generation 3			
Internal Perimeter	16.0 (4.3)	16.8 (4.7)	0.156
External Perimeter	25.6 (4.8)	26.9 (5.9)	0.156
Wall Thickness	1.57 (0.23)	1.56 (0.18)	0.777
Lumen area	18.7 (8.7)	21.7 (8.7)	0.089
Wall Area	33.9 (11.2)	34.7 (11.8)	0.257
Total Airway area	50.2 (18.8)	55.9 (25.7)	0.131
WA%	63.7 (5.0)	61.4 (5.0)	0.080
Segmental Generation 4			
Internal Perimeter	11.5 (3.3)	12.0 (2.3)	0.202
External Perimeter	20.0 (4.3)	20.2 (3.0)	0.299
Wall Thickness	1.23 (0.19)	1.25 (0.12)	0.143
Lumen area	9.4 (5.9)	10.7 (4.4)	0.156
Wall Area	20.0 (7.9)	19.9 (5.61)	0.299
Total Airway area	28.9 (13.6)	30.6 (9.9)	0.219
WA%	66.7 (4.0)	65.3 (5.0)	0.080
Segmental Generation 5			
Internal Perimeter	9.2 (2.5)	10.6 (2.5)	0.059
External Perimeter	16.6 (3.2)	18.7 (2.6)	0.080
Wall Thickness	1.07 (0.23)	1.12 (0.17)	0.422
Lumen area	5.6 (3.6)	7.4 (3.9)	0.080
Wall Area	12.9 (5.9)	16.1 (5.6)	0.219
Total Airway area	18.5 (9.3)	23.8 (8.2)	0.131
WA%	68.1 (4.0)	65.6 (4.0)	**0.023***
Segmental Generation 6			
Internal Perimeter	9.0 (1.7)	10.0 (3.0)	0.169
External Perimeter	16.0 (2.3)	17.5 (4.3)	0.221
Wall Thickness	1.01 (0.07)	1.02 (0.21)	0.838
Lumen area	5.4 (1.9)	6.4 (2.4)	0.221
Wall Area	12.3 (2.5)	13.1 (4.2)	0.386
Total Airway area	17.9 (4.5)	19.8 (8.6)	0.359
WA%	69.0 (3.0)	67.3 (1.0)	0.114

Data represents medians and IQR. N=31. *p<0.05 using Mann Whitney U Test. PLF – preserved lung function.

5.3.3.1 Association between segmental airway generation Dimensions and Airflow Obstruction

There were significant associations between FEV1% and WA% in all segmental generations (Table 53). This only remained significant for segmental generation 3 and 6 in the COPD subjects only. There were also positive associations between internal perimeter, external perimeter, lumen area, wall, total airway area and FEV1% in segmental generation 3. There were also positive associations between internal perimeter and lumen area and FEV1% in segmental generation 6. The only one of these that remained significant when the analysis was repeated in COPD subjects was between FEV1% and lumen area in segmental generation 3.

When correcting for the number of airways measured using partial correlation only WA% in segmental generation remained significantly associated with FEV1% (Table 54).

Table 53. Association between airway dimensions in different segmental generations and FEV1%.

	Gen.3	Gen.4	Gen.5	Gen. 6
Whole cohort				
Internal Perimeter	**0.48****	0.30	0.33	**0.45***
External Perimeter	**0.43***	0.24	0.31	0.36
Wall Thickness	0.23	0.17	0.07	0.21
Lumen area	**0.53****	0.31	0.32	**0.37***
Wall area	**0.40***	0.20	0.22	0.30
Total Airway Area	**0.56****	0.27	0.29	0.31
Wall area %	**-0.59*****	**-0.45***	**-0.45***	**-0.50****
COPD Subjects				
Internal Perimeter	0.39	0.21	0.08	0.36
External Perimeter	0.32	0.14	0.05	0.20
Wall Thickness	0.20	-0.02	-0.14	0.14
Lumen area	**0.43***	0.19	0.08	0.24
Wall area	0.34	0.07	-0.04	0.18
Total Airway Area	0.34	0.13	0.06	0.17
Wall area %	**-0.51***	-0.33	-0.22	**-0.43***

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

Table 54. Partial correlation of airway segmental dimensions with FEV1%, correcting for airway numbers.

	Gen.5	Gen. 6
Whole Cohort		
Internal Perimeter	0.20	0.36
External Perimeter	0.25	0.29
Wall Thickness	0.11	0.22
Lumen area	0.18	0.32
Wall area	0.19	0.27
Total Airway Area	0.22	0.30
Wall area %	-0.30	**-0.41***
COPD Subjects		
Internal Perimeter	0.02	0.31
External Perimeter	0.05	0.16
Wall Thickness	-0.05	0.14
Lumen area	0.01	0.22
Wall area	-0.01	0.19
Total Airway Area	0.06	0.17
Wall area %	-0.10	-0.38

Spearman's partial correlation correcting for number of airways. N=31 for whole cohort and n=24 for COPD. *p<0.05.

5.3.4 Association between Airway wall morphology and Biological Markers

Next I assessed the various biological samples measured with morphological features of COPD. In an attempt to limit the number of comparisons I decided to only include a couple of different methods for measuring airway morphology. For this I chose Pi10 and dimensions for segmental generation 3 and 4.

5.3.4.1 Airway wall morphology and BAL neutrophils and cytokines

There was no association between Pi10 and BAL white cell differential or BAL cytokines (Table 55). There was also no significant association between BAL neutrophils or cytokines and airway dimensions in segmental generation 3 (Table 56). In segmental generation 4 there was an association between BAL neutrophils and wa%, although this lost significance when only analysing COPD subjects (Table 57).

Table 55. Correlation between Pi10 and BAL neutrophils and cytokines.

	BAL Neutrophils	IL-1B	IL-6	IL-8	GM-CSF
Whole Cohort					
Pi10	0.13	0.02	-0.05	0.09	-0.01
COPD subjects					
Pi10	0.10	0.05	-0.10	0.10	0.09

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD.

Table 56. Correlation between airway dimensions in segmental generation 3 and neutrophils and cytokines in BAL.

	Neutrophil	IL-1B	IL-6	IL-8	GM-CSF
Whole Cohort					
Internal Perimeter	-0.18	-0.19	0.15	-0.09	-0.02
External Perimeter	-0.15	-0.16	0.17	-0.08	0.05
Wall Thickness	0.10	-0.17	0.16	-0.01	0.21
Lumen area	-0.24	-0.14	0.11	-0.14	-0.02
Wall area	-0.11	-0.22	0.12	-0.14	0.03
Total Airway Area	-0.17	-0.18	0.14	-0.12	0.03
Wall area %	0.33	0.16	0.02	0.20	0.15
COPD Subjects					
Internal Perimeter	0.04	-0.18	0.32	0.01	0.10
External Perimeter	0.04	-0.13	0.36	0.03	0.15
Wall Thickness	0.16	-0.09	0.36	0.08	0.31
Lumen area	-0.01	-0.12	0.29	-0.04	0.08
Wall area	0.02	-0.16	0.30	-0.04	0.15
Total Airway Area	0.04	-0.15	0.32	-0.02	0.13
Wall area %	0.11	0.15	-0.08	0.13	0.10

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD.

Table 57. Correlation between airway dimensions in segmental generation 4 and neutrophils and cytokines in BAL.

	Neutrophil	IL-1B	IL-6	IL-8	GM-CSF
Whole Cohort					
Internal Perimeter	-0.28	-0.30	0.01	-0.16	-0.09
External Perimeter	-0.25	-0.33	0.02	-0.15	-0.09
Wall Thickness	-0.27	-0.26	0.00	-0.19	-0.05
Lumen area	-0.29	-0.29	0.02	-0.16	-0.09
Wall area	-0.24	-0.33	0.02	-0.15	-0.08
Total Airway Area	-0.29	-0.31	0.01	-0.16	-0.09
Wall area %	**0.40***	0.20	-0.05	0.15	0.16
COPD Subjects					
Internal Perimeter	-0.14	-0.33	0.11	-0.14	-0.07
External Perimeter	-0.16	-0.35	0.13	-0.12	-0.06
Wall Thickness	-0.19	-0.33	0.08	-0.17	-0.07
Lumen area	-0.13	-0.33	0.14	-0.12	-0.06
Wall area	-0.21	-0.33	0.12	-0.13	-0.06
Total Airway Area	-0.21	-0.34	0.11	-0.15	-0.05
Wall area %	0.18	0.22	-0.20	0.04	0.11

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD. *p<0.05.

5.3.4.2 Airway Wall Morphology and MMPs

There were no associations between Pi10 and any MMPs in either the whole cohort or the COPD cohort (Table 58). There were few associations between airway wall dimensions in segmental generation three and four and MMPS (Table 59 and Table 60)

Table 58. Correlation between Pi10 and MMPs

	Pi10	
	Whole Cohort	COPD Subjects
MMP-1	0.15	0.12
MMP-2	0.05	0.08
MMP-3	0.14	0.04
MMP-7	0.08	0.11
MMP-8	0.23	0.23
MMP-9	0.21	0.21
MMP-10	0.15	0.13
MMP-12	0.17	0.17
MMP-13	0.10	0.20
EMMPRIN	0.23	0.15

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD

Table 59. Correlation between airway dimensions in segmental generation 3 and MMPs.

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12	MMP-13
Internal Perimeter	-0.23	-0.30	-0.12	-0.12	-0.18	-0.12	-0.15	-0.26	-0.27
External Perimeter	-0.24	-0.27	-0.12	-0.06	-0.16	-0.12	-0.10	-0.23	-0.24
Wall Thickness	-0.32	-0.14	-0.01	0.04	-0.05	-0.09	-0.03	-0.08	-0.08
Lumen area	-0.27	-0.29	-0.19	-0.15	-0.26	-0.19	-0.18	-0.32	-0.25
Wall area	-0.34	-0.28	-0.13	-0.08	-0.17	-0.14	-0.13	-0.27	-0.22
Total Airway Area	-0.28	-0.25	-0.13	-0.08	-0.21	-0.17	-0.11	-0.30	-0.20
Wall area %	0.22	0.30	0.27	0.32	0.34	0.24	0.30	**0.38***	0.31
COPD									
Internal Perimeter	-0.14	-0.15	0.09	0.09	0.02	-0.01	0.01	-0.10	-0.31
External Perimeter	-0.13	-0.10	0.11	0.16	0.07	0.04	0.09	-0.04	-0.29
Wall Thickness	-0.23	-0.04	0.12	0.23	0.15	0.07	0.11	0.07	-0.17
Lumen area	-0.17	-0.10	0.04	0.07	-0.06	-0.08	0.01	-0.18	-0.29
Wall area	-0.26	-0.12	0.04	0.10	0.02	-0.03	0.01	-0.14	-0.26
Total Airway Area	-0.18	-0.07	0.10	0.15	0.00	-0.04	0.08	-0.13	-0.22
Wall area %	0.13	0.19	0.07	0.18	0.18	0.16	0.17	0.23	0.37

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD

Table 60. Correlation between airway dimensions in segmental generation 4 and MMPs.

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12	MMP-13
Internal Perimeter	-0.16	-0.34	-0.09	-0.07	-0.16	-0.08	-0.017	-0.15	-0.06
External Perimeter	-0.16	-0.30	-0.04	-0.05	-0.11	-0.04	-0.13	-0.10	-0.03
Wall Thickness	-0.15	**-0.40***	-0.07	-0.04	-0.16	-0.11	-0.19	-0.04	-0.02
Lumen area	-0.17	**-0.36***	-0.10	-0.07	-0.17	-0.10	-0.21	-0.14	-0.07
Wall area	-0.14	-0.32	-0.03	-0.05	-0.10	-0.03	-0.12	-0.08	-0.01
Total Airway Area	-0.14	-0.30	-0.07	-0.08	-0.15	-0.08	-0.16	-0.11	-0.02
Wall area %	0.20	**0.38***	0.20	0.18	0.19	0.07	0.18	0.28	0.29
COPD									
Internal Perimeter	-0.11	-0.21	0.14	0.09	-0.03	0.02	-0.05	0.02	-0.09
External Perimeter	-0.08	-0.15	0.17	0.11	0.02	0.07	-0.01	0.09	-0.05
Wall Thickness	-0.01	-0.25	0.20	0.06	-0.01	0.05	-0.06	0.14	-0.07
Lumen area	-0.09	-0.21	0.16	0.10	-0.01	0.03	-0.06	0.05	-0.10
Wall area	-0.03	-0.18	0.17	0.08	0.03	0.08	-0.02	0.11	-0.01
Total Airway Area	-0.04	-0.14	0.15	0.07	-0.01	0.04	-0.04	0.09	-0.04
Wall area %	0.13	0.23	-0.09	-0.03	-0.02	-0.08	-0.05	0.10	0.28

Spearman's correlation coefficient. N=31 for whole cohort and n=24 for COPD

5.3.4.3 Airway Wall morphology and Bacterial detection in Sputum

There was no significant difference in Pi10 between those with PPM cultured in their sputum and those that did not. This remained the case when the individual bacteria were analysed (Figure 46).

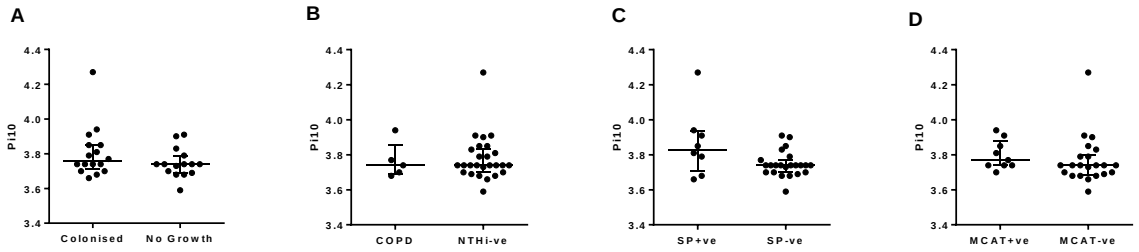


Figure 47. Pi10 in subjects with bacteria detected in sputum or not (A) Any PPM - Potentially pathogenic microorganism (B) NTHI - non-typeable Haemophilus influenzae. (C) SP - streptococcus Pneumoniae (D) mcatt - Moraxella catarrhalis.

5.3.5 By specific airway paths

The above analysis does not take into account the possible anatomical variation between the different lobes and so in this next section I analysed dimensions in five different airway paths (RB1, RB4, RB10, LB1 and LB10). For each of these airway paths I measured dimensions in segmental generations 3-6. In the RB1, RB4 and RB10 path at each segmental generation there were no significant difference in airway dimensions between subjects with COPD and those with PLF. In the LB1 pathway WA% in segmental generation 6 was significantly higher in the COPD subjects than those with PLF (Table 64), although this was the only difference between the two groups in this pathway. The LB10 pathway exhibited the most significant differences in airway wall dimensions between subjects with and without airflow obstruction (Table 65). In LB10 itself, internal and external perimeter, lumen area, wall area and total airway wall area were all significantly higher in subjects without airflow obstruction than those with COPD. In addition in segmental generation 6 of the LB10 pathway WA% was significantly higher in COPD subjects than those with PLF.

There were significant differences between the different airway paths in segmental generation 3 for internal and external perimeter, wall thickness, inner area, wall area, outer area and WA% ($p<0.001$). In generation 4 there were significant differences between airway paths for inner and outer perimeter, wall thickness, lumen area, wall area and total airway area ($p<0.001$) and WA% (p 0.017). For generation 5 there were also significant differences in the airway paths for all of the parameters ($P<0.001$, apart from WA% $p=0.039$ and total airway area $p=0.001$). For generation 6 there were also significant differences between airway paths for IP ($p=0.004$), EP ($p=0.013$), lumen

area (p=0.005), wall area (p=0.007), total airway area (p=0.0010 and WA% (p=0.039). There was no significant difference between wall thicknesses in the different airway paths for segmental generation 6 airways.

Table 61. Airway wall Dimensions in different segmental generations in the RB1 Path.

	COPD	PLF	P value
RB1	N=24	N=7	
No. Airways	1.0 (0.0)	1.0 (0.0)	-
Internal Perimeter	16.9 (6.0)	17.4 (4.7)	0.705
External Perimeter	27.3 (7.0)	27.8 (4.8)	0.741
Wall Thickness	1.67 (0.21)	1.64 (0.17)	0.813
Lumen area	21.5 (15.3)	23.2 (13.6)	0.777
Wall Area	36.5 (16.4)	37.9 (6.5)	0.479
Total Airway area	56.9 (29.5)	60.1 (21.4)	0.637
WA%	63.8 (9.0)	61.4 (8.0)	0.813
Generation 4	(n=24)	(N=7)	
No. Airways	2.0 (0.0)	2.0 (0.0)	-
Internal Perimeter	12.5 (4.9)	14.0 (2.8)	0.317
External Perimeter	21.2 (5.6)	22.9 (4.0)	0.502
Wall Thickness	1.30 (0.24)	1.32 (0.21)	0.695
Lumen area	11.2 (9.6)	14.7 (5.6)	0.274
Wall Area	22.0 (8.6)	25.1 (9.1)	0.473
Total Airway area	32.9 (17.2)	39.8 (14.4)	0.444
WA%	65.8 (5.0)	64.5 (3.0)	0.253
Generation 5	(n=22)	(n=7)	
No. Airways	4.0 (0.0)	4.0 (0.0)	-
Internal Perimeter	9.1 (2.5)	10.7 (2.0)	0.098
External Perimeter	15.5 (4.4)	18.4 (3.1)	0.110
Wall Thickness	0.98 (0.32)	1.14 (0.15)	0.110
Lumen area	5.8 (3.8)	8.0 (3.3)	0.098
Wall Area	11.9 (8.5)	17.5 (5.6)	0.098
Total Airway area	17.1 (11.6)	25.3 (9.4)	0.110
WA%	67.5 (5.0)	67.1 (2.0)	0.500
Generation 6	(N=18)	N=7	
No. Airways	4.0 (6.0)	8.0 (2.0)	0.064
Internal Perimeter	9.1 (1.7)	8.8 (0.92)	0.790
External Perimeter	15.9 (2.2)	15.7 (2.0)	0.615
Wall Thickness	1.01 (0.18)	1.00 (0.18)	0.357
Lumen area	5.6 (2.0)	5.3 (0.6)	0.745
Wall Area	12.1 (2.6)	11.6 (2.9)	0.326
Total Airway area	18.1 (5.4)	16.9 (2.9)	0.495
WA%	69.9 (6.0)	68.9 (3.0)	0.534

Data represents medians and IQR. N=31. *p<0.05 using Mann Whitney U Test. PLF – preserved lung function.

Table 62. Airway wall Dimensions in different segmental generations in the RB4 Path.

	COPD	PLF	P value
RB4	N=24	N=7	
No. Airways	1.0 (0.0)	1.0 (0.0)	>0.999
Internal Perimeter	14.1 (4.0)	15.3 (3.2)	0.257
External Perimeter	23.0 (5.2)	25.2 (5.9)	0.238
Wall Thickness	1.37 (0.28)	1.40 (0.17)	0.479
Lumen area	14.7 (7.6)	18.0 (7.3)	0.131
Wall Area	24.7 (9.3)	29.0 (11.3)	0.131
Total Airway area	39.5 (16.9)	48.5 (22.7)	0.131
WA%	63.4 (4.0)	62.9 (6.0)	0.479
Generation 4	N=24	N=7	
No. Airways	2.0 (0.0)	2.0 (0.0)	>0.999
Internal Perimeter	9.5 (2.3)	10.7 (2.8)	0.115
External Perimeter	16.8 (4.0)	17.9 (3.6)	0.104
Wall Thickness	1.00 (0.22)	1.10 (0.18)	0.167
Lumen area	6.5 (4.0)	8.5 (4.9)	0.104
Wall Area	14.2 (6.1)	16.2 (5.7)	0.094
Total Airway area	20.6 (10.3)	24.7 (10.6)	0.104
WA%	66.4 (4.0)	65.6 (5.0)	0.365
Generation 5	N=19	N=7	
No. Airways	3.0 (2.0)	4.0 (0.0)	0.035*
Internal Perimeter	9.1 (2.1)	10.3 (2.6)	0.063
External Perimeter	16.4 (3.0)	17.2 (3.5)	0.107
Wall Thickness	1.02 (0.29)	0.94 (0.20)	>0.999
Lumen area	5.9 (2.8)	7.4 (4.4)	0.120
Wall Area	13.2 (4.7)	13.8 (5.1)	0.364
Total Airway area	18.9 (6.5)	21.5 (9.7)	0.188
WA%	67.4 (5.0)	65.3 (5.0)	0.209
Generation 6	N=13	N=7	
No. Airways	2.0 (4.0)	5.0 (3.0)	0.395
Internal Perimeter	9.6 (2.6)	10.7 (2.9)	0.799
External Perimeter	16.6 (4.8)	17.8 (3.5)	0.879
Wall Thickness	0.94 (0.28)	1.03 (0.16)	0.935
Lumen area	5.4 (2.4)	6.1 (3.2)	0.335
Wall Area	11.7 (4.8)	13.1 (3.0)	0.457
Total Airway area	17.1 (7.4)	20.4 (4.3)	0.380
WA%	67.5 (2.0)	67.7 (3.0)	0.278

Data represents medians and IQR. N=31. *p<0.05 using Mann Whitney U Test. PLF – preserved lung function.

Table 63. Airway wall Dimensions in different segmental generations in the RB10 Path.

	COPD	PLF	P value
RB10	N=23	N=7	
No. Airways	1.0 (0.0)	1.0 (0.0)	
Internal Perimeter	15.8 (4.5)	16.5 (6.0)	0.292
External Perimeter	25.0 (6.2)	26.3 (5.9)	0.292
Wall Thickness	1.54 (0.19	1.53 (0.10)	0.713
Lumen area	16.7 (11.2)	20.6 (18.2)	0.315
Wall Area	31.5 (13.4)	33.6 (10.4)	0.364
Total Airway area	48.6 (25.8)	52.6 (27.9)	0.315
WA%	62.9 (7.0)	60.8 (8.0)	0.292
Generation 4	N=22	N=7	
No. Airways	2.0 (0.0)	2.0 (0.0)	0.862
Internal Perimeter	12.5 (4.7)	12.4 (6.2)	0.636
External Perimeter	20.7 (5.4)	20.9 (8.1)	0.784
Wall Thickness	1.25 (0.18)	1.32 (0.27)	0.469
Lumen area	11.7 (8.8)	11.6 (13.3)	0.636
Wall Area	22.2 (10.0)	22.3 (12.6)	0.600
Total Airway area	33.7 (18.4)	33.9 (28.0)	0.280
WA%	65.3 (6.0)	64.2 (5.0)	0.862
Generation 5	N=22	N=7	
No. Airways	3.0 (2.0)	4.0 (2.0)	0.328
Internal Perimeter	10.1 (2.6)	11.1 (3.8)	0.135
External Perimeter	17.7 (3.9)	19.0 (4.9)	0.122
Wall Thickness	1.12 (0.22)	1.16 (0.25)	0.237
Lumen area	6.8 (4.8)	8.6 (6.7)	0.110
Wall Area	15.4 (7.8)	16.6 (8.6)	0.110
Total Airway area	22.5 (13.2)	25.2 (15.2)	0.122
WA%	67.8 (6.0)	67.4 (5.0)	0.600
Generation 6	N=17	N=7	
No. Airways	2.0 (3.0)	7.0 (6.0)	0.099
Internal Perimeter	9.7 (1.5)	10.0 (4.8)	0.418
External Perimeter	16.5 (2.5)	17.5 (6.1)	0.455
Wall Thickness	1.07 (0.30)	1.12 (0.33)	0.951
Lumen area	6.1 (2.6)	6.5 (7.8)	0.804
Wall Area	12.7 (4.3)	14.7 (8.6)	0.418
Total Airway area	19.5 (5.9)	21.1 (16.4)	0.534
WA%	68.1 (3.0)	69.0 (8.0)	>0.999

Data represents medians and IQR. N=31. PLF – preserved lung function.

Table 64. Airway wall Dimensions in different segmental generations in the LB1 Path.

	COPD	PLF	P value
LB1	N=23	N=7	
No. Airways			
Internal Perimeter	12.9 (3.4)	16.0 (5.6)	0.162
External Perimeter	22.4 (5.1)	26.7 (8.4)	0.148
Wall Thickness	1.36 (0.26)	1.57 (0.43)	0.135
Lumen area	12.1 (7.7)	18.7 (13.0)	0.135
Wall Area	24.9 (11.3)	34.7 (19.5)	0.162
Total Airway area	37.0 (19.0)	53. 4 (31.3)	0.122
WA%	64.7 (5.0)	63.5 (2.0)	0.364
Generation 4	N=23	N=7	
No. Airways	2.0 (0.0)	2.0 (0.0)	>0.999
Internal Perimeter	10.1 (3.0)	11.8 (5.6)	0.086
External Perimeter	17.6 (4.2)	19.4 (7.5)	0.086
Wall Thickness	1.12 (0.27)	1.18 (0.29)	0.131
Lumen area	7.3 (4.9)	10.8 (10.8)	0.077
Wall Area	15.8 (8.2)	19.6 (13.6)	0.077
Total Airway area	22.8 (12.0)	30.5 (25.5)	0.086
WA%	66.9 (3.0)	64.7 (6.0)	0.207
Generation 5	N=21	N=7	
No. Airways	3.0 (2.0)	4.0 (1.0)	0.272
Internal Perimeter	8.7 (2.6)	11.4 (4.5)	0.126
External Perimeter	16.1 (3.7)	18.5 (6.0)	0.189
Wall Thickness	1.00 (0.19)	1.11 (0.31)	0.348
Lumen area	5.0 (3.1)	9.3 (7.4)	0.113
Wall Area	12.3 (5.1)	16.8 (10.3)	0.296
Total Airway area	18.0 (8.2)	25.9 (16.1)	0.155
WA%	69.3 (3.0)	66.7 (6.0)	0.055
Generation 6	N=13	N=6	
No. Airways	4.0 (4.0)	5.0 (3.0)	0.152
Internal Perimeter	8.7 (2.3)	10.4 (1.9)	0.087
External Perimeter	16.7 (2.6)	17.5 (2.7)	0.179
Wall Thickness	0.96 (0.12)	0.97 (0.12)	0.521
Lumen area	4.9 (2.2)	6.7 (3.1)	0.087
Wall Area	12.1 (3.3)	13.5 (4.3)	0.323
Total Airway area	17.7 (4.4)	20.4 (8.0)	0.244
WA%	69.4 (3.0)	66.5 (3.0)	**0.046***

Data represents medians and IQR. N=31. *p<0.05 using Mann Whitney U Test. PLF – preserved lung function.

Table 65. Airway wall Dimensions in different segmental generations in the LB10 Path.

	COPD	PLF	P value
LB10	N=24	N=7	
No. Airways	1.0 (0.0)	1.0 (0.0)	
Internal Perimeter	16.8 (5.2)	20.2 (7.5)	**0.047***
External Perimeter	26.9 (5.4)	31.0 (5.9)	**0.038***
Wall Thickness	1.58 (0.18)	1.69 (0.12)	0.098
Lumen area	21.0 (13.5)	30.2 (22.0)	**0.047***
Wall Area	34.7 (10.9)	43.1 (9.0)	**0.026***
Total Airway area	54.9 (23.1)	73.3 (29.4)	**0.030***
WA%	61.6 (6.0)	58.8 (8.0)	0.156
Generation 4	N=24	N=7	
No. Airways	2.0 (0.0)	2.0 (0.0)	0.872
Internal Perimeter	12.1 (4.5)	14.1 (5.9)	0.182
External Perimeter	20.7 (5.9)	23.5 (6.9)	0.094
Wall Thickness	1.24 (0.28)	1.35 (0.18)	0.127
Lumen area	11.4 (9.1)	14.8 (13.7)	0.167
Wall Area	22.0 (8.5)	27.6 (11.3)	0.167
Total Airway area	33.6 (15.7)	42.4 (27.1)	0.104
WA%	65.7 (7.0)	64.5 (7.0)	0.216
Generation 5	N=23	N=7	
No. Airways	3.0 (2.0)	4.0 (0.0)	0.131
Internal Perimeter	10.9 (2.3)	11.1 (4.4)	0.962
External Perimeter	18.8 (3.7)	19.1 (6.8)	0.774
Wall Thickness	1.19 (0.17)	1.22 (0.42)	0.666
Lumen area	8.2 (3.6)	9.0 (6.3)	0.924
Wall Area	16.7 (6.6)	18.7 (10.0)	0.666
Total Airway area	24.7 (10.4)	27.7 (15.7)	0.631
WA%	67.8 (5.0)	65.7 (4.0)	0.107
Generation 6	N=20	N=7	
No. Airways	4.0 (3.0)	8.0 (4.0)	**0.006****
Internal Perimeter	9.5 (2.1)	10.2 (3.3)	0.162
External Perimeter	16.5 (2.8)	18.3 (5.1)	0.288
Wall Thickness	1.06 (0.27)	1.03 (0.31)	0.570
Lumen area	5.8 (2.3)	6.7 (2.9)	0.464
Wall Area	13.2 (4.5)	14.2 (5.5)	0.850
Total Airway area	19.4 (6.4)	20.7 (8.7)	0.725
WA%	69.9 (3.0)	67.6 (2.0)	**0.041***

Data represents medians and IQR. N=31. *p<0.05, **p<0.001 using Mann Whitney U Test. PLF – preserved lung function.

Next I assessed the association between these airway dimensions in the different airway paths and segmental generations and FEV1%. For segmental generation 3 airways there were significant associations between FEV1% and internal perimeter, external perimeter, lumen area, wall area, total airway area and WA% in RB10 and LB10 (Table 66). When repeating the analysis in the COPD cohort wall thickness and wall area in RB10 and external perimeter and total airway wall area in RB4 had positive associations with FEV1%. In segmental generation 4 airways FEV1% had significant associations with internal perimeter and lumen area in RB4, RB10 and LB10 (Table 67). There were also associations with internal perimeter and total airway wall area in RB4 and LB10. FEV1% also had significant associations with WA% in RB10, LB1 and LB10. When repeating the analysis in COPD

subjects, FEV1% only had significant associations with airway dimensions in RB10. In generation 5 airways FVE1% had significant associations between IP, EP, lumen, wall and total airway area in RB4 (Table 68). There was also an association with WA% in LB1. In COPD subjects all of the associations lost significance. In segmental generation 6 airways there were significant associations between FEV1% and IP and EP in LB1 and LB10 as well as lumen area in LB1 (Table 69). These once again all lost significance in the COPD cohort.

Table 66. Association between airway dimensions in different airway paths in segmental generation 3 and FEV1%.

	RB1	RB4	RB10	LB1	LB10
Whole cohort					
Internal Perimeter	0.01	0.22	**0.43***	0.23	**0.38***
External Perimeter	0.03	0.23	**0.47****	0.19	**0.41***
Wall Thickness	0.02	0.14	0.33	0.08	0.15
Lumen area	0.02	0.30	**0.44***	0.24	**0.38***
Wall area	0.09	0.33	**0.46***	0.15	**0.37***
Total Airway Area	0.05	0.32	**0.47****	0.22	**0.40***
Wall area %	0.06	-0.30	**-0.37***	-0.22	**-0.41***
COPD subjects					
Internal Perimeter	-0.11	0.38	0.03	-0.06	0.20
External Perimeter	-0.07	**0.42***	0.06	-0.13	0.25
Wall Thickness	0.03	0.03	**0.46***	-0.26	0.00
Lumen area	-0.07	0.07	0.39	-0.07	0.20
Wall area	0.03	0.12	**0.43***	-0.19	0.21
Total Airway Area	-0.06	**0.42***	0.10	-0.13	0.26
Wall area %	0.17	-0.26	-0.32	-0.04	-0.27

Spearman's correlation. N=31 for whole cohort and N=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

Table 67. Association between airway dimensions in different airway paths in segmental generation 4 and FEV1%.

	RB1	RB4	RB10	LB1	LB10
Whole cohort					
Internal Perimeter	0.10	**0.37***	**0.38***	0.26	**0.40***
External Perimeter	0.10	**0.36***	0.33	0.21	**0.40***
Wall Thickness	0.02	0.23	0.14	0.11	0.29
Lumen area	0.13	**0.37***	**0.40***	0.27	**0.41***
Wall area	0.10	0.33	0.33	0.21	0.35
Total Airway Area	0.09	**0.36***	0.36	0.22	**0.41***
Wall area %	-0.18	-0.25	**-0.54****	**-0.38***	**-0.45***
COPD Subjects					
Internal Perimeter	-0.08	0.22	**0.51***	-0.05	0.31
External Perimeter	-0.02	0.14	**0.46***	-0.12	0.29
Wall Thickness	-0.04	-0.04	0.29	-0.19	0.13
Lumen area	-0.03	0.19	**0.52***	-0.05	0.30
Wall area	-0.00	0.10	**0.46***	-0.16	0.25
Total Airway Area	-0.03	0.17	**0.51***	-0.14	0.31
Wall area %	-0.01	-0.29	**-0.57****	-0.30	-0.37

Spearman's correlation. N=31 for whole cohort and N=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

Table 68. Association between airway dimensions in different airway paths in segmental generation 5 and FEV1%.

	RB1	RB4	RB10	LB1	LB10
Whole cohort					
Internal Perimeter	0.23	**0.40***	0.17	0.34	0.08
External Perimeter	0.25	**0.42***	0.14	0.24	0.11
Wall Thickness	0.26	0.28	0.06	0.11	0.04
Lumen area	0.23	**0.42***	0.19	0.35	0.10
Wall area	0.25	**0.40***	0.13	0.14	0.10
Total Airway Area	0.24	**0.44***	0.16	0.22	0.07
Wall area %	-0.06	-0.15	-0.37	**-0.50****	-0.21
COPD Subjects					
Internal Perimeter	-0.08	0.21	-0.22	0.01	0.02
External Perimeter	-0.02	0.30	-0.25	-0.11	0.14
Wall Thickness	0.02	0.39	-0.31	-0.27	0.03
Lumen area	-0.08	0.25	-0.16	0.03	0.02
Wall area	-0.05	0.37	-0.26	-0.23	0.15
Total Airway Area	-0.03	0.32	-0.16	-0.20	0.12
Wall area %	0.01	0.17	-0.37	-0.32	0.00

Spearman's correlation. N=31 for whole cohort and N=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

Table 69. Association between airway dimensions in different airway paths in segmental generation 6 and FEV1%.

	RB1	RB4	RB10	LB1	LB10
Whole cohort					
Internal Perimeter	0.20	0.03	0.32	**0.60****	**0.48***
External Perimeter	0.15	0.08	0.34	**0.56***	**0.42***
Wall Thickness	-0.20	0.20	0.11	0.37	0.04
Lumen area	0.13	0.04	0.18	**0.53***	0.37
Wall area	-0.03	0.12	-0.25	0.41	0.26
Total Airway Area	0.07	0.03	0.23	0.43	0.28
Wall area %	-0.20	0.25	-0.25	-0.46	-0.37
COPD Subjects					
Internal Perimeter	0.23	-0.25	0.17	0.55	0.47
External Perimeter	0.24	-0.13	0.29	0.54	0.43
Wall Thickness	-0.26	-0.08	0.07	0.25	0.10
Lumen area	0.16	-0.24	0.09	0.38	0.43
Wall area	-0.01	-0.13	0.21	0.17	0.36
Total Airway Area	0.13	-0.12	0.05	0.26	0.35
Wall area %	-0.18	0.29	-0.45	-0.15	-0.26

Spearman's correlation. N=31 for whole cohort and N=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

5.3.5.1 Lobar values and association with biological markers

Next I investigated the association between the airway dimensions in the different segmental generations and cytokines and MMPs in the specific location where the BAL was taken (Table 70). For cytokines there were significant negative associations between all the dimensions and IL-1B in generation 6. There was also negative associations between lumen area in segmental generation 6 and IL-6 and IL-8 concentrations. In segmental generation 3 there was a weak association between WA% and IL-1B. There were no other significant associations.

For MMPs there were significant associations between MMP-2 and all of the airway dimensions in segmental generation 3 (Table 71). In segmental generation 4 and 5 all apart from WA% had associations with MMP2 while in segmental generation 6 only lumen area and WA% had significant associations with this MMP. MMP-1 had significant association with wall thickness and wall area in segmental generation 5 and lumen area, wall area and total airway area in segmental generation 6. MMP-3 had negative associations with wall thickens, wall area and total airway area in segmental generation 5 only. MMP-12 had significant association with lumen area and total airway area in segmental generation 6 while MMP-13 had significant association with WA% in segmental generation 3.

Table 70. Association between lobar concentrations of cytokines and airway dimensions in the corresponding lobe in different segmental generation paths.

	Wall thickness	Lumen area	Wall area	Total airway area	WA%
Segmental Gen 3					
IL-1B	0.03	-0.24	-0.16	-0.20	**0.26***
IL-6	-0.06	-0.14	-0.15	-0.16	0.03
IL-8	-0.00	-0.05	-0.07	-0.06	0.02
GM-CSF	0.13	-0.09	0.02	-0.05	0.34
Segmental Gen 4					
IL-1B	-0.06	-0.15	-0.10	-0.13	0.14
IL-6	-0.01	-0.05	-0.02	-0.04	0.00
IL-8	-0.06	-0.08	-0.06	-0.07	0.01
GM-CSF	0.04	-0.02	0.02	0.00	0.05
Segmental Gen 5					
IL-1B	-0.17	-0.16	-0.17	-0.17	-0.11
IL-6	-0.13	0.02	-0.03	-0.02	-0.04
IL-8	-0.13	-0.07	-0.11	-0.11	-0.01
GM-CSF	0.15	0.19	0.17	0.17	-0.10
Segmental Gen 6					
IL-1B	**-0.47****	**-0.55*****	**-0.50*****	**-0.50*****	**-0.52*****
IL-6	-0.17	**-0.30***	-0.23	-0.27	0.13
IL-8	-0.23	**-0.30***	-0.22	-0.26	0.17
GM-CSF	-0.10	-0.23	-0.13	-0.19	0.20

Spearman's correlation. N=31 for whole cohort and N=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

Table 71. Association between lobar concentrations of MMPs and airway dimensions in the corresponding lobe in different segmental generation paths.

	Wall thickness	Lumen area	Wall area	Total wall area	WA%
Segmental Gen 3					
MMP-1	-0.09	-0.20	-0.20	-0.21	0.12
MMP-2	**-0.26***	**-0.38****	**-0.35****	**-0.37****	**0.37****
MMP-3	0.01	-0.07	-0.06	-0.08	0.05
MMP-7	-0.02	-0.14	-0.06	-0.11	0.17
MMP-8	0.00	-0.03	-0.03	-0.03	0.01
MMP-9	-0.02	0.07	0.00	0.04	-0.11
MMP-10	-0.06	-0.13	-0.10	-0.12	0.16
MMP-12	0.13	-0.09	-0.02	-0.06	0.12
MMP-13	-0.07	-0.21	-0.16	-0.18	**0.33****
Segmental Gen 4					
MMP-1	-0.09	-0.15	-0.12	-0.14	0.15
MMP-2	**-0.44****	**-0.37****	**-0.39****	**-0.38****	0.22
MMP-3	-0.06	-0.10	-0.08	-0.09	0.09
MMP-7	-0.08	-0.16	-0.09	-0.13	0.19
MMP-8	-0.05	-0.09	-0.07	-0.08	0.07
MMP-9	-0.08	-0.07	-0.06	-0.07	0.00
MMP-10	-0.20	-0.18	-0.18	-0.19	0.10
MMP-12	0.08	-0.02	0.03	0.01	0.06
MMP-13	**-0.29***	-0.24	-0.23	-0.22	0.18
Segmental Gen 5					
MMP-1	**-0.33***	-0.21	**-0.28***	-0.26	0.14
MMP-2	**-0.36****	**-0.42****	**-0.42****	**-0.44****	0.17
MMP-3	**-0.27***	-0.23	**-0.27***	**-0.27***	0.23
MMP-7	-0.15	-0.05	-0.06	-0.06	-0.03
MMP-8	-0.19	-0.12	-0.17	-0.17	0.04
MMP-9	-0.21	-0.10	-0.17	-0.15	-0.12
MMP-10	-0.21	-0.25	-0.24	-0.25	0.15
MMP-12	-0.21	-0.09	-0.17	-0.15	0.01
MMP-13	0.07	-0.16	-0.06	-0.10	0.22
Segmental Gen 6					
MMP-1	-0.21	**-0.37***	**-0.33***	**-0.35***	0.21
MMP-2	0.03	**-0.30***	-0.13	-0.21	**0.33***
MMP-3	0.08	-0.21	-0.06	-0.12	0.22
MMP-7	-0.06	-0.27	-0.17	-0.20	0.17
MMP-8	-0.05	-0.11	-0.02	-0.06	0.10
MMP-9	-0.17	-0.09	-0.07	-0.08	-0.02
MMP-10	0.07	-0.13	0.08	-0.00	0.24
MMP-12	-0.14	**-0.34***	-0.27	**-0.31***	0.15
MMP-13	0.10	-0.00	0.04	0.01	0.20

Spearman's correlation. N=31 for whole cohort and N=24 for COPD. *p<0.05, **p<0.01, ***p<0.001.

5.4 Discussion

In this chapter I have successfully used quantitative CT analysis to explore the key morphological features of COPD. Many studies rely on spirometry to characterise the condition, but this represents a global measure of disease and does not necessarily tell us much about the underlying pathological features. This causes obvious errors and leads to lack of sensitivity in determining important underlying mechanisms of disease. I have demonstrated that quantitative CT analysis can objectively measure emphysema and small airways disease and these can be used to investigate and provide further information about key mechanisms of disease. The most important findings were that I demonstrated novel associations between MMPs and emphysema and gas trapping/small airways disease. I also performed novel analysis in determining emphysema sub-types.

5.4.1 Emphysema

Up until relatively recently emphysema was viewed as a histological diagnosis and as such required histological specimens in order to carry out any meaningful research into the condition. The advent of CT imaging has changed this and has enabled direct visualisation of the low attenuation lesions that represent emphysema. Subjective visual reporting has not particularly helped inform our knowledge of the disease and so more objective ways of quantifying the condition has been sought. Emphysema is the simplest of the morphological features to quantify and measure using CT as in its simplest form involves simple thresh-holding methodology. The optimum methods and thresholds have not been determined and in this study I investigated three of these, along with a visual score of emphysema. Of the automated methods two of these measures ($\%LAA_{<-950}$, and $\%LAA_{<-910}$) involved different CT densitometry thresholds below which all voxels are assumed to be emphysema, giving a low attenuation percentage. The other method uses percentile densitometry ($PERC_{15}$), which involved measuring the density value in HU under which 15% of the voxels are distributed. I found that all of the automated methods had strong association with one another, which is unsurprising given they are all ultimately based on thresh-holding techniques. Importantly, the automated techniques did not have any associations with visual scoring. This is inconsistent with previous studies, which have usually shown good associations between quantitative analysis and visual scoring[148,151,160,176]. The reason for these inconsistencies is uncertain, however many of these previous studies used multiple readers to perform the scoring whereas I only used a single radiologist, which may have introduced errors. Also my study was in a milder cohort than what most of the other studies have used and visual scoring may be less discriminatory for milder disease.

Of the automated emphysema scores, only $\%LAA_{<-950}$ was significantly higher in COPD than in subjects with PLF. This is in agreement with multiple previous studies which have also shown significant differences in $\%LAA_{<-950}$ between subjects with COPD and without[157–159]. Schroeder et al investigated all three techniques finding significant differences between subjects with COPD and controls for all three measurements and furthermore found that $\%LAA_{<-950}$ showed the most consistent progressive change across GOLD groups[158]. Unlike my data another study demonstrated a significant differences between COPD and controls for PEC_{15}[159]. I did not find any association between FEV1% and any of the automated markers of emphysema which is also inconsistent with previous studies which have provided significant evidence that $\%LAA_{<-950}$ has an inverse correlation with airflow obstruction and other spirometric markers of disease[158–161,163,164,167]. $PERC_{15}$ has also been shown to have significant associations with airflow obstruction[158,159]. Once again the reason for this apparent inconsistency may be the milder cohort used in my study, meaning emphysema quantities were relatively low.

Visual score of emphysema was significantly raised in subjects with COPD compared to subjects with PLF. However all subjects with PLF had an emphysema score of 0, suggesting they had no emphysema whatsoever. The automated scores suggest otherwise and it therefore seems likely that visual scores do not have the sensitivity to detect subtle disease. Visual score did however, have a significant association with FEV1%, although this disappeared when analysed in the COPD sub-cohort, which suggests this represents inter-group differences.

Like previous studies my work still does not provide definite evidence, as too which of the methods are best at measuring emphysema. Previous pathological studies have shown strong associations between $\%LAA_{<-910}$[148–150,152], $\%LAA_{<-950}$[149–152] and $PERC_{15}$[152] and histological measurements of emphysema, although Gevenois showed that $\%LAA_{<-950}$ had the strongest association with microscopic and macroscopic emphysema measured on lung tissue[149,150]. Visual scoring has also been shown to have good associations with macroscopic emphysema, although it overestimates emphysema and has inter-observer errors[151]. My results showed that of the automated measures only $\%LAA_{<-950}$ showed a significant difference between those with COPD and those with PLF and I therefore elected to use this as the marker of emphysema for my study. $\%LAA_{<-950}$ also has the benefit of being a direct measure of emphysema, whereas $\%LAA_{<-910}$ gave much higher rates of emphysema that were unlikely to reflect true amounts. For example, $\%LAA_{<-910}$ had median values of over 20% in both COPD and PLF subjects. However, some groups advocate that $\%LAA_{<-910}$ should be the preferred measurement in cohorts with mild emphysema. $PERC_{15}$ did not show significant differences between COPD and PLF subjects and one of the reasons may be that this measure can be affected by areas of higher attenuation. Evidence suggest that inflammation can cause higher attenuation values in smokers which could confound the results[215]. On the face of it visual

emphysema estimation seems to be a good marker of emphysema as it showed both a significant difference between subjects with and without airflow obstruction and had significant associations with spirometry. However multiple subjects had the same emphysema score and a 12 point scale is likely to be less sensitive to subtle differences between patients. Also all of the subjects with PLF scored 0, whereas the automated methods showed a range of emphysema, which is more likely to reflect what is actually occurring within the lung.

In order to further understand possible causative mechanisms of emphysema I investigated the relationship between $\%LAA_{<-950}$ and a number of different biological markers. There were no associations between emphysema and blood inflammatory cells or fibrinogen. Very few studies have analysed systemic inflammatory markers in emphysema and there have often been equivocal results. A previous study showed increased fibrinogen in emphysematous subjects but no difference in serum CRP, IL-6 or TNF-α[192]. Another study showed that in ex or current smokers without airflow obstruction there was significantly lower concentrations of IL-1, IL-8 and IL-15 and no difference in TNF-α[280]. The only study investigating quantitative estimates of emphysema and cytokines showed a positive association between $\%LAA_{<-950}$ and IL-6 and a negative association with TNF-α, and no association with many other inflammatory cytokines[281]. My evidence along with these other studies does not really provide any significant evidence for an association between emphysema and systemic inflammation.

The evidence for an association between emphysema and airway inflammation was equally unconvincing, with none of the inflammatory cytokines showing associations with $\%LAA_{<-950}$. Relatively few studies have investigated the association of emphysematous change and the expression of inflammatory cytokines within the airways in COPD. Two previous studies have shown an association between sputum IL-8 and automated emphysema and this was associated with progression of emphysema[23,52]. However one of these studies was very small with only 10 study subjects. They also both used sputum sampling rather than BAL and both had sub-optimal CT analysis compared to my study. In exhaled breath condensate, TNF-α levels were found to be no different between those with and without emphysema[192]. Animal models have suggested that inflammatory cytokines may be involved in emphysema as airway IL-6 in mice were associated with increase air spaces consistent with emphysema change[282]. BAL neutrophils had significant associations with $\%LAA_{<-950}$, suggesting subjects with higher quantities of emphysema had higher concentrations of neutrophils in their airways. However this results has to be treated with caution as the significance disappeared when only subjects with COPD were analysed. Previous studies have given inconsistent results when investigating the link between emphysema and airway neutrophils. One study has demonstrated an association between emphysema and airway neutrophil[190] although multiple other have not found this association[163,164,191,193,283]. It may be that it is the cell

population within the alveolar tissue and lung parenchyma that is important for emphysema development and BAL and sputum samples may not reflect this. However even histological specimens give conflicting results, with one study showing reduced neutrophils in emphysematous tissue[284] and another showing neutrophils in biopsy specimens were associated with emphysema[285]. Interpreting these results along with my own study does not provide any definite evidence of an association between emphysema and airway inflammation in COPD and therefore the role of inflammation in the development of emphysema remains unknown.

There is considerable interest in the role of MMPs in emphysema due to their ability to degrade the pulmonary ECM and a significant amount of work has been performed to assess this. In animal studies, transgenic mice overexpressing MMP-1 develop emphysema at an accelerated rate[35] while MMP-12 knockout mice are protected from cigarette induced lung damage[286]. In the SMAD3 mouse model of emphysema, genetic blockade of MMP-9 reduced the amount of emphysema[287]. Selective inhibition of MMP-9 and -12 in guinea pigs reduced the extent of emphysema when exposed to smoke[36]. In human genetic studies, polymorphisms of MMP-9 genes have been linked to emphysematous change[47,288], while polymorphisms of MMP-12 have been linked to airflow obstruction[275]. Previous work using lavage fluid found that MMP-1, -9 and -12 were significantly raised in emphysematous patients when compared to healthy non-smokers but not current smokers[37]. In two prior studies using quantitative CT analysis, $\%LAA_{<-950}$ correlated with sputum MMP-9 and -12[40,43]. Unlike these two studies, I did not find any associations between CT-measured emphysema and MMP -9 or -12, which may be due to the differences between sampling sputum or BAL. However, I found significant associations between $\%LAA_{<-950}$ and MMP-3, -7, -8 and -10, which have not been previously investigated. When only analysing COPD subjects there were significant association for MMP-3, -7 and -10. MMP-10 had the strongest association with emphysema and interestingly this and the other significantly associated MMPs have limited elastin degradation properties and mainly degrade collagens and proteoglycans. Studies show there is increased collagen around emphysematous lesions[82] and therefore a key mechanism may be MMP-induced collagen breakdown and subsequent abnormal remodelling and aberrant deposition of collagen. Further mechanistic work is required to investigate this link. TIMPs are inhibitors of MMPs and are therefore a complicating factor when investigating the role played by MMPs in emphysema and need to be considered. Previous studies have suggested they may have an important role to play in emphysema as TIMP-3 null mice develop emphysema[46] while TIMP-2 polymorphisms are associated with CLE emphsyema[47]. I found that TIMP-2 and -4 were significantly associated with $\%LAA_{<-950}$, although this did not remain significant when assessing only COPD subjects and so it is difficult to define their precise role in emphysema.

When assessing the effect that emphysema had on microbiology within the lungs, no difference in $\%LAA_{<-950}$ was found between subjects who cultured bacteria in their sputum and those who did not. The PCR results showed significantly lower $\%LAA_{<-950}$ in those that had evidence of SP DNA in their sputum than those who did not. It is perhaps, unsurprising that there were no substantial differences in emphysema quantities between those who had bacteria in their sputum and those that did not as bacterial colonisation or invasion is likely to be due to abnormalities in the epithelial mucosal surface in the airways rather than parenchymal abnormalities. My findings are also supported by a study by Bafadhel[193], who also found no significant difference in sputum bacterial culture between patients who had emphysema on CT and those who did not.

One of the benefits of using quantitative analysis techniques is that it allows the degree of emphysema to be calculated on a lobar basis. The distribution of emphysema has already been shown to be clinically important in determining which patients benefit from lung volume reduction surgery[14]. I found that that the degree of emphysematous change was relatively consistent throughout the lobes. $\%LAA_{<-950}$ was on average greatest in the RML although this only was only significantly different from the RUL. There were no other significant differences between lobes. This is inconsistent with other work which have shown significant differences in emphysema quantity between the lobes[176,177]. However, one of these studies was in a much more severe cohort of patients who had been referred for a LVRS, while the other study also showed that emphysematous change was greatest in the RML[177]. I found no significant associations between lobar $\%LAA_{<-950}$ and airway obstruction, which is not surprising given there was no association with overall $\%LAA_{<-950}$. Although insignificant the strength of the associations with FEV1% were similar across the different lobes.

When comparing lobar values of emphysema to lobar values of inflammatory cytokines there was only a negative association between IL-1B, but no association with IL-6, IL-8 or neutrophil count. There was also no difference in these inflammatory cytokines in lobes sampled with the most emphysema compared to lobes sampled with the least emphysema. This provides further evidence that there is no significant association between airway inflammation and emphysema. Lobar $\%LAA_{<-950}$ had significant associations with lobar BAL concentrations of MMP-3 and MMP-10. However, rather confusingly MMP-2, -10 and -13 were significantly higher in lobes sampled with the least emphysema compared to lobes sampled with the most emphysema. This is difficult to explain but may be due to the fact that this was a very mild cohort and often there were minimal differences between lobar values of emphysema and defining one lobe as emphysematous and the other as preserved does not reflect this. Another explanation may also be that I did not use a protected catheter when sampling the individual lobes meaning there could have been contamination from other lobes.

So far I have discussed results using $\%LAA_{<-950}$ as a measure of emphysema, which gives an overall estimate of emphysema but does not give any information about specific emphysema sub-types. I used LHE analysis to automatically determine the distribution of emphysema sub-types on CT imaging in my cohort. In my mild-moderate COPD subjects, the predominant emphysema sub-types were mild and moderate CLE, with only small quantities of severe CLE present, which is in keeping with previous work[85,86]. CLE emphysema is defined as an abnormal enlargement of air-spaces centred on the respiratory bronchiole and is the classical form associated with smoking[86,88]. Only small amounts of PLE and PSE were present in COPD subjects which is also consistent with other studies[85,86,90]. Panlobular emphysema is an abnormal enlargement of airspaces distributed throughout the pulmonary lobule and has been associated with A1ATD, lower BMI[86,88], lower FEV1[88] but not with smoking history[86]. As PLE is associated with more severe disease it is unsurprising it was found in such low quantities in our cohort. Paraseptal emphysema refers to emphysematous change adjacent to a pleural surface and is the least well understood form of emphysema and shows no relationship with COPD symptoms[86] or smoking history[87].

The underlying mechanisms driving the evolution of emphysema sub-types are poorly understood. I have previously reported that overall emphysema had associations with MMP-3, -7 and -10 and MMP-9 polymorphisms have been associated with CLB[47], but this is the first study evaluating the contribution of MMPs to different emphysema sub-types in detail. MMP-3 and -10 had significant associations with all forms of emphysema (apart from mild CLB) while MMP-7, -8 and -9 had associations with multiple sub-types. I also compared TIMP concentrations to emphysema sub-types and found TIMP-4 had positive associations with several emphysema sub-types. A previous study showed TIMP-2 polymorphisms are associated with CLB emphsyema[47], although their activity and specificity are poorly understood.

Perhaps the most interesting results from this part of my study were those relating to mild CLE. All subjects, irrespective of whether they had airflow obstruction, had evidence of mild CLE with median values above 30%. This is consistent with other studies which have demonstrated emphysematous change in smokers without airflow obstruction[86,87]. Using LHE CT analysis, Castaldi also described high rates of mild CLE in healthy smokers, linking it with degree of airflow obstruction and functional capacity[85]. This suggests that mild CLE is a genuine tissue abnormality and occurs prior to the development of airflow obstruction and a key question is whether this progresses into more significant disease. Unlike more severe forms of emphysema I found no associations between MMPs and mild CLE (apart from a negative on with MMP-1). When correcting for the other tissue sub-types, using partial correlation no associations between mild CLE and MMPs were found, suggesting mild CLE genuinely does not have any associations with MMPs. I propose a possible explanation for this may be that mild emphysema is ubiquitous in smokers and ex-smokers and

occurs via a non-MMP derived mechanism, such as oxidative stress secondary to cigarette smoke exposure. This however is speculative and further work is required to study this in detail. It may be that only subjects with significant MMP activity develop more advanced emphysema and my results suggest MMP-3 and -10 are the most important in this process. These MMPs are stromelysins, mainly degrading collagen and proteoglycans, important constituents of the pulmonary ECM[34]. In addition MMP-7, -8 and -9 had associations with moderate CLE and PSE and MMP-7 and -8 also had associations with severe CLE. MMP-7 is an elastase, MMP-8 a collagenase and MMP-9 a gelatinase and together can degrade all components of the pulmonary ECM[34]. Given that individual MMPs have different substrate specificity, it may be proposed that particular MMPs are responsible for each sub-type of emphysema. However, my results do not support this as the profile of MMP expression is broadly similar across all emphysema sub-types, apart from mild CLE. These significant associations do not prove causation and further mechanistic work is required to understand this is in more detail and longitudinal studies are needed to understand how mild CLE and the other emphysema sub-types progress and whether this can predicted by MMP concentrations.

5.4.2 Gas Trapping

Small airways disease is felt to be one of the key features of COPD and has been shown to be an important site of airflow limitation. However, there is no gold standard for measuring the small airways, with all methods having significant limitations. It is therefore vital that other techniques are found to investigate the small airways. CT cannot visualise the small airways directly due to limited resolution but gas trapping can be used as an indirect marker. In this study, using CT I have assessed four different automated measures of gas trapping and a visual scoring system. $\%LAA_{EXP<-856}$ is the simplest of these automated measures and represent the number of voxels below -856HU on the expiratory CT scan. There is concern that this measure may include emphysematous voxels and so I also assessed the measure $\%LAA_{EXP-856-950}$, which is the percentage of voxels between -856 and -950 HU on the expiratory scan. The benefit of this technique is that it excludes voxels under -950HU which are likely to represent emphysema. However it is argued that to get an even more accurate estimate of gas trapping paired inspiratory/expiratory scans need to be performed. E/I MLD is the ratio of the mean lung density on the expiratory scan to the inspiratory scan, with results closer to one indicating more gas trapping. RVC is the most complex of these techniques and represents the relative volume change between -856HU and -950HU between inspiratory and expiratory scans.

All four of the automated techniques had strong associations with one another, although RVC seemed to have slightly weaker associations. Hersh et al demonstrated that three of these automated methods (not $\%LAA_{EXP-856-950}$) had associations with one another and also observed the association was slightly weaker for RVC[208]. The four automated measures also showed significant differences between subjects with COPD and those with PLF. This is consistent with a number of studies which have demonstrated raised $\%LAA_{EXP<-856}$[157,158,210], RVC[210] and E/I MLD[210] in COPD compared to controls. There were strong negative associations between these CT measures of gas trapping and FEV%, FEV1/FVC and FEF75-25%. These remained significant when the analysis was repeated in only the COPD cohort apart for FEF75-25%, which only had a significant association with E/I MLD. The strongest associations with spirometry were with the marker E/I MLD. A number of previous studies have also demonstrated that $\%LAA_{EXP<-856}$[206], $\%LAA_{EXP-856-950}$[206], RVC[206] and E/I MLD all have associations with spirometric markers of disease[161].

I found no association between any of the automated CT measures of gas trapping and visual scoring of gas trapping. Unlike automated methods, there was no significant difference in visual gas trapping scores between subjects with COPD and PLF and if anything demonstrated raised scores in PLF. There was also no association between spirometry and visual gas trapping, although when the analysis was performed in the COPD subjects only there were significant negative associations with FEV1% and FEF75-25%. This suggests that the gas trapping visual score was more accurate in the COPD cohort and on reviewing the data it seemed that rather high scores were given for some of the subjects with PLF. It therefore seems that visual scoring of gas trapping is not particularly accurate in mild subjects and those without airflow obstruction.

Given that this was a small cohort of relatively mild patients it is not possible to ascertain whether one method for measuring gas trapping with CT is superior to another. This is especially the case, as complex pulmonary function tests were not performed in this study, which are better markers of the small airways than simple spirometry. Neither FEV1 nor FEF75-25% are specific for small airways dysfunction, although FEF75-25% represents an adequate estimate with which to compare my CT analysis. Although all the automated measures had associations with spirometric measures of disease, E/I MLD had the strongest association. Unlike the other measures, the associations between FEVF75-25% and E/I MLD remained significant when analysing the COPD cohort. A number of studies have investigated which of the automated methods is the best at quantifying gas trapping in COPD. One study demonstrated that a modified version of RVC was a better predictor for FEF75-25% than techniques using a single expiratory scan or not excluding emphsyema[209]. Matsuoko found that $\%LAA_{EXP<-856}$, $\%LAA_{EXP-856-950}$ and RVC had strong associations with mild emphysematous change but when severe emphysematous change was present, RVC performed better, being the only marker exhibiting significant associations with RV/TLC[206]. Mets also investigated $\%LAA_{EXP<-856}$,

RVC and E/I MLD and found that E/I MLD predicted raised RV/TLC most accurately in COPD subjects[211]. A subsequent study by Mets, demonstrated that all four automated techniques had strong associations with RV/TLC, however $\%LAA_{EXP<-856}$ and E/I MLD had the strongest associations. In the COPDgene cohort, $\%LAA_{EXP<-856}$, RVC and E/I MLD all had equally strong associations with RV/TLC and FEF75-25[208]. In another study, Bommart showed that E/I MLD had a strong association with single breath nitrogen washout, one of the more accurate pulmonary function test markers of small airways disease[207]. Along with my results this all suggests that all of the automated measures are good at measuring gas trapping on CT imaging in COPD. However it seems reasonable to conclude that techniques utilising paired inspiratory and expiratory scan are the most accurate and theoretically this seems logical as doing this will account for emphysematous change and intra-patient variability. In my study E/I MLD exhibited stronger associations with spirometric markers of disease than RVC and E/I MLD is also a much more simple technique. I therefore chose to use E/I MLD for the rest of the study.

The role of inflammation in the distal airways is poorly understood and using CT parameters of gas trapping can help provide information on this. There were significant associations between E/I MLD and BAL neutrophils and conversely a negative association with macrophages. This is consistent with a previous study demonstrating a significant association between E/I MLD and sputum neutrophils[163]. Up until recently the only way to investigate the role of inflammation in the peripheral airways would have been to use biopsy specimens from lung resections. A number of studies have demonstrated increased neutrophils in the walls of the peripheral airways in COPD as opposed to controls[73,289]. However, in contrast to this another did not demonstrate any difference in the number of neutrophils in the airway wall in COPD and control subjects[290]. These inconsistencies may be explained by the rapid migration of neutrophils through the airway walls and so BAL may therefore be the best method with which to measure them. The only inflammatory cytokine to have significant associations with E/I MLD was IL-8. To the best of my knowledge this is the first study to analyse the role of inflammatory cytokines in small airways disease in COPD. These results in combination suggest an important role for neutrophilic inflammation the distal airway abnormalities and remodelling that occur in COPD.

Few studies have investigated the role of MMPs in small airways disease. In guinea pigs, inhibition of MMP-9 and -12 protected the animals from small airways fibrosis in response to cigarette smoke exposure[36]. A study of symptomatic smokers with normal lung function found that MMP-8, -9 and -12 in induced sputum correlated with FEF75-25[48]. My results found that MMP-1, -3 -8, -9, -10 and -12 had associations with FEF75-25%. However this is an insensitive and non-specific marker of small airways disease. When using the CT marker of E/I MLD I found strong associations with the concentrations of MMP-3, -7, -8, -9, -10 and -12. These also remained significant when performing

the analysis in COPD subjects only. MMP-8 had the strongest association with small airways disease, which remained significant when adjusting for the other MMPS in turn using partial correlation. These associations tended to be stronger than those seen with emphysema, suggesting that MMPs may play a significant role in the development of small airway remodelling and the associated airflow obstruction. MMP-8 and these other associated MMPs are mainly produced by neutrophils and macrophages and between them are able to degrade all components of the ECM. In the small airways it has been demonstrated that elastin and collagen are reduced in COPD patients[76] and this degradation may be driven by MMPs leading to deranged remodelling and fibrosis. Further mechanistic work is required to investigate this is in more detail. TIMP-2 and -4 had significant associations with E/I MLD. All of these however lost significance when applying them to the COPD cohort. No previous studies have assessed the role of TIMPs in small airways disease in COPD and my study does not really provide any mechanistic explanations for their actions.

There was no significant difference in the amount of gas trapping in subjects who had bacteria detected in their sputum and those that did not. Studies have not specifically assessed the role of chronic bacterial infection in COPD. My study would suggest that subjects with significant small airways disease are not any more likely to have bacteria in their airways.

There is limited previous work on the regional variability of CT measured gas trapping in COPD and so I measured E/I MLD in each of the five lobes. When doing so I found significant differences between the lobes, with increased gas trapping in the upper and middle lobes compared to the lower lobes. The lobe most severely affected was the right middle lobe. This is consistent with work from Bhatt et al, who used the CT marker $\%LAA_{EXP<-856}$ and showed that upper and middle lobes were most affected by gas trapping in COPD. Another study assessed E/I MLD at four different slices throughout the lung and found significant differences[207]. I found all of the different lobar values of E/I MLD exhibited significant associations with FEV1%, FEV1/FVC ratio and FEF75-25%. The associations were all relatively similar apart from for the RML, suggesting that small airways disease in each lobe contributes equally to spirometric abnormalities. The association between E/I MLD in the RML and spirometry was weaker suggesting small airways disease in this lobe contributes less to airflow obstruction than the other lobes. This is consistent with a previous study[177] and it may be due to the relatively smaller size of the RML.

There were associations between lobar values of E/I MLD and IL-6 and IL-8, providing further evidence of a link between small airways disease and inflammation. There were also associations between lobar E/I MLD and lobar values of MMP-3, -7, -8, -9, -10 and -12, once again demonstrating the importance of these proteases in the small airways disease seen in COPD. However, there were no significant differences in MMP concentrations in lobes classified as most severely affected by

gas trapping compared to those classified as preserved and there was significantly raised IL-1B and IL-8 in preserved lobes. In the previous section I have already discussed the possible limitations of this approach.

5.4.3 Dimensions of Large and intermediate Airways

Bronchial wall measures of the large and intermediate airways are perhaps the most technically demanding and most difficult to analyse due to the sheer number of measurements, airways and generations to measure. In this study I elected to assess a number of different methods to measure airway dimensions. Firstly I analysed the medians for the airway measurements of all the airways in each subject's lungs. This showed significantly larger internal and external perimeter, wall thickness, lumen area, wall area and total airway area in COPD subjects as opposed to subjects with PLF. These parameters also showed significant inverse associations with airflow obstruction suggesting larger airway dimensions in COPD subjects. However, these results were influenced by the number of airways measured in each individual as there were significantly more airways measured in subjects with PLF than those with COPD. There was also a strong positive association between the number of airways measured in each subject and airflow obstruction, suggesting that fewer airways can be measured in those with worse airflow obstruction. Lung resection specimens using micro-CT demonstrate fewer terminal bronchioles in COPD compared to controls[291]. There is also evidence to suggest that it is not only the terminal bronchioles that are reduced in COPD as Diaz showed the number of airways in 6th to 8th generation visualised on CT correlated with FEV1% in COPD[165]. Another study also showed a significant reduction in airways of 2-2.5mm on CT imaging with increasing severity of COPD[292]. The precise mechanism of this is unknown, however it has been hypothesised that emphysematous change may be responsible for the reduction in airways and some evidence backs this up[165,292,293]. When correcting for the number of airways measured using partial correlation there was no significant association between airway measurement and FEV1. This suggests that the extra airways measured in subjects with PLF represent smaller airways, and this is why it seemed that subjects with COPD had larger airway dimensions. It is therefore vital that any analysis of airway dimensions corrects for the number of airways measured.

Another measurement I used was the square root of the wall area for a hypothetical airway with an internal perimeter of 10mm (Pi10). The advantages of this method over the previous one is that it standardises the measurement for airway size and only includes airways with an internal perimeter of less that 20mm. In addition it gives one value for each subject making it an elegant and simple method that is potentially extremely useful for large clinical trials. When analysing Pi10 in my study there was no significant difference between subjects with COPD and those with PLF. In contrast to my results a previous large study demonstrated significant differences between Pi10 in

COPD subjects and controls[197]. This study used a sub-optimal CT protocol with a slice interval of 2cm, meaning the reconstructions were not contiguous and voxels would not be isometric, potentially introducing errors. I found no significant association between Pi10 and airflow obstruction in the whole cohort, although there was however a significant negative association when analysing the COPD sub-cohort. A number of studies have also shown that Pi10 is inversely associated with FEV1[158,200,201,294], although the strength of these associations were variable. This not a universal finding however, as another study did not find any association between Pi10 and FEV1%[295]. Although this provides some evidence that airway walls may be larger in COPD, it is not overwhelming and the variable nature makes it unlikely this measurement will be a particularly useful tool. A single measurement is unlikely to adequately reflect the complexity of the bronchial tree, as it is unable to account for the regional variations as well as the variability in airway generations. In addition, this measurement is also likely to be influenced by the number of airways measured especially if these represent smaller airways.

The bronchial tree consists of a number of different generations, with potentially different sized airways in each generation and so I assessed airway dimensions in each individual generation. There are two methods by which to label the generations of the airway tree; the first is the standard method with the trachea labelled as 0 and then at each branch point the generation increasing by 1 generation. The second method is by defining generation 3 as the segmental generation bronchi and each branch point increases the generation by one. The problem with the first method is that it means that a generation three airway in the upper lobe is a segmental airway while in the lower lobe it is the RLL bronchus. The second method groups anatomically similar airways together and logically seems a better method. However I performed my analysis on both methods.

When using the standard method for labelling airways, once again I demonstrated that significantly more airways were measured in COPD subjects in generations 6 to 8 and the number of airways measured was associated with FEV1% in generation 5 to 10. All subjects contributed to airways measured up to generation 6, but this was not the case distal to this and so I only performed further analysis up to generation 6 airways. When comparing airway dimensions in these different generations there were very few significant differences between COPD subjects and those with PLF. Airways and airway walls tended to be slightly larger in subjects than in subjects without airflow obstruction but this was mostly not statistically significant. After correcting for the number of airways there were also very few significant associations between airflow obstruction and airway dimensions. Repeating this analysis by labelling the airway by segmental generation yielded broadly similar results with few differences between subjects with COPD and with PLF. There were some associations between FEV1% and airway dimensions, suggesting that airway dimensions were larger in subjects with higher FEV1%. This was especially the case in segmental generation 3. WA%

had a negative association with FEV1% suggesting that although airways get smaller with more airflow obstruction, the proportion of the airway made of the airway wall increases.

To further investigate the variability of these measures I also assessed airway dimensions in five different airway paths at each segmental generation. When using this method there were still few differences in airway dimension between COPD subjects and those with PLF. There were also significant differences in dimensions between airways in different paths. There were some associations between airway dimensions and FEV1%, although these tended to vary in different airway paths and different segmental generations. This all suggests that airway measurements are quite variable and are not uniform throughout the airway tree and therefore cannot be relied upon as helpful measure of explaining airway abnormalities in COPD.

A number of studies have investigated the airway dimensions in COPD and found quite varying results. One study found that there was positive association between airway flow obstruction and luminal area and a negative association with WA% in the 3rd, 4th and 5th generation[166]. However this was only measured in two airway paths (RB1 and RB10 paths) and only one cross-sectional slice for each airway was chosen rather than using three-dimension reconstructions as in my study. Another study showed no association between WA% and FEV1% in either proximal generation (1st and 2nd) or distal generation airways (3rd to 6th)[296]. Another study showed no association between WA% and FEV1% in generation 3 but did show a significant weak negative association between FEV1% and WA%[297]. However this study only measured dimensions in one airway path per subject and so could not account for intra-subject variability. Another study found a positive significant association between airway lumen area and FEV1% and a negative association with WA%. These associations became stronger in the more distal airways. However, this study only measured two airway paths and only one airway per generation. Finally, Smith et al in a large study demonstrated that wall area was reduced in COPD compared to controls in all generations and lumen diameters were smaller in generation 3-6[196]. Once again this study averaged the dimensions in each airway path and did not take into account variability between the different airway paths. It is clear from my study and these previous study that there is significant inconsistencies with results, which questions the value of measuring dimensions of airways in COPD. What is unclear and yet to be establishes is whether this is because CT lacks the sensitivity to measure the airway dimensions or because there are no abnormalities in these dimensions in COPD.

Given these inconsistencies, it makes it difficult to assess the relationship between airway dimensions and biological markers of disease. There were no associations between PI10 measurements and BAL neutrophils, inflammatory cytokines or MMPs. Pi10 was also no different between subjects with and without bacteria was detected in their sputum. When comparing airway

dimensions in generation 3 and 4 there were few significant associations with biological measures. When repeating this analysis in the different airway paths there were once again variable associations, making it very difficult to interpret.

5.4.4 Strengths and limitations

One of the major strengths of this study compared to some of the previous studies assessing CT imaging in COPD was the imaging protocol I used. The protocol in my study used thin slices with minimal distances between each slice, meaning voxels were essentially isometric (the same size in all three dimensions), allowing full contiguous CT reconstructions and therefore accurate measurements. Many older studies only used axial slices to perform measurements and even some of the more recent larger trials have used sub-optimal imaging protocols, possibly introducing errors. By performing expiratory CT scans I was also able to accurately measure small airways disease and by using this was able to show novel associations with airway inflammation and MMPs. Once again one of the major limitations of my study was the small sample size and the multiple comparisons made. As with the previous chapter I also found a number of correlations that were significant in the whole cohort but were not significant in the COPD cohort. This is likely to represent inter-group differences and so have to be treated with caution. This analysis was also performed in a mild cohort due to the need perform bronchoscopy and it is unknown whether the results would be the same in a more severe cohort. The amounts of emphysema in this cohort were limited and it may be that these subjects suffered with mainly small airways disease, giving rise to stronger associations with small airways disease. Finally, although I have shown multiple associations between airway inflammation and MMPs and small airways disease and emphysema this doesn't necessarily imply causation. Further mechanistic work is required to understand the action of these processes.

5.4.5 Summary

In summary I have shown that quantitative analysis of CT imaging can be used to measure emphysema and small airways disease and this could provide important information about the anatomical distribution of these pathological processes. Furthermore, I was also able to demonstrate that novel CT analysis can be used to determine emphysema sub-types. By combining CT analysis and biological profiling of inflammatory markers, and proteases I was able to describe novel relationships between MMPs and small airways disease and emphysema and further understand the role of inflammation in these processes. Measuring dimensions of large and intermediate airways was less successful despite assessing a number of different methods and my results to do not provide any definitive evidence that this is a useful tool in COPD.

Chapter 6: Quantitative CT analysis and relationship with pulmonary physiology, functional and clinical data in the AERIS study

In the previous chapter I demonstrated that emphysema and small airways disease could be measured using quantitative CT image analysis. However, this was in a small cohort of relatively mild COPD patients and I wanted to validate these findings in a larger cohort of more severe COPD subjects. In addition, I wanted to compare these CT parameters with the more detailed pulmonary physiology, functional and clinical data captured in this larger study. This study was also performed over a two-year period, with CTs performed at the beginning and end of the study thereby allowing the longitudinal changes in the CT image analysis to be assessed. In this chapter I present the results of the cross-sectional data from the enrolment scan.

6.1 Demographic data

In total 152 subjects were screened for the study and 127 were enrolled. The consort diagram for the study is shown in Figure 48**.** Of those 105 subjects completed one year of the study. Demographic data for the cohort is shown in Table 72.

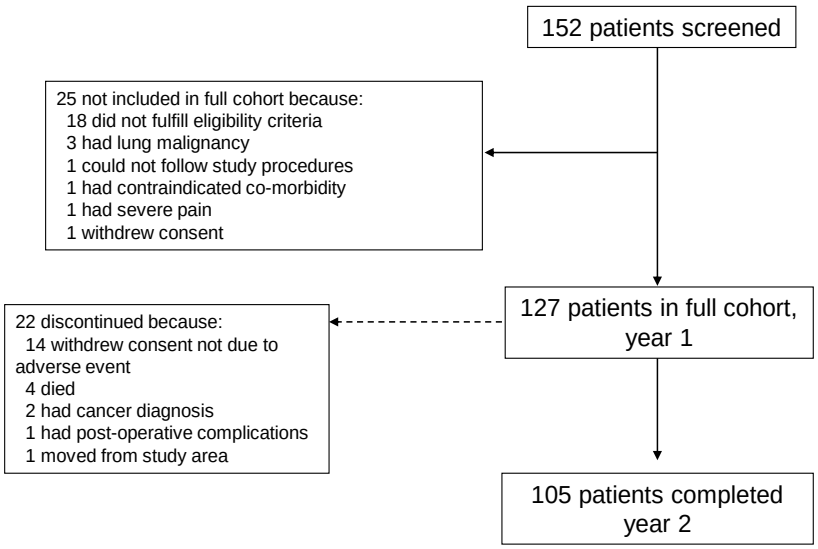


Figure 48. Consort diagram for the study recruitment and 2 year study period.

Table 72. Demographic data for the study cohort

	N=127
Age	67.0 (11.0)
Female sex	59 (46.5)
Pack years	47.0 (26.3)
Current Smoker	49 (38.6)
BMI	27.0 (6.7)
Spirometry	
FEV1%	46.7 (25.3)
FVC%	86.2 (25.2)
FEV1/FVC	0.43 (0.17)
FEF75-25%	13.2 (8.2)
FEV1% reversibility	11.2 (17.0)
GOLD Status	
Mild	0 (0)
Moderate	57 (44.9)
Severe	51 (40.2)
Very Severe	19 (15.0)
Pulmonary Function Tests	
TLCO%	57.9 (29.5)
TLC%	105.0 (22.0)
RV%	144.3 (59.8)
RV/TLC	0.54 (0.14)
Functional Parameters	
6MWD	300.0 (170.0)

Values given as medians (IQR). Females, smoking status and GOLD stage given as number of subjects (% of subjects). N= 127 for whole cohort. For spirometry n = 126 (one reading excluded due to poor quality). For reversibility testing n=105. For gas transfer n=122 and for plethysmography n=116. For 6MWD n = 125 subjects.

6.2 Baseline CT Data for Emphysema, gas trapping and Bronchial wall Dimensions

Each subject had a CT performed at baseline and these were analysed using the same approach as the previous study. For both emphysema and gas trapping a visual score was measured as well as four automated parameters for each. As no adequate visual score exists for measuring dimensions of large and intermediate airways only one automated measure, Pi10 was initially performed. The baseline CT data is shown below in Table 73. Of the 127 enrolment scans, 123 scans were successfully analysed for emphysema and large and intermediate airway dimensions. Four CT scans could not be analysed due to poor quality of the images. For automated analysis of gas trapping 122 subjects scans were successfully analysed as one further expiratory scan was of poor quality and could not be analysed.

Table 73. CT data for enrolment scan.

CT Variable	
Emphysema	
Visual Score	5.00 (7.00)
%LAA$_{<-950}$	12.1 (20.9)
%LAA$_{<-910}$	38.9 (31.6)
PERC$_{15}$	-945.0 (47.4)
Gas Trapping	
Visual Score	5.00 (6.00)
%LAA$_{EXP<-856}$	37.7 (32.6)
%LAA$_{EXP<-856-950}$	31.0 (21.3)
RVC	-27.1 (22.4)
E/I MLD	0.92 (0.07)
Bronchial wall Dimension	
Pi10	3.79 (0.09)

Values given as medians (IQR). N =127, apart from %LAA$_{<-950}$ and Pi10 where n=123 and for E/I MLD where n=122.

As in the previous study there were strong associations between all of the automated emphysema parameters (Table 74). Unlike the previous study visual emphysema score also had strong associations with all of the automated measures of emphysema. The automated measures of gas trapping also had very strong associations with one another (Table 75). All automated scores of gas trapping also had significant associations with visual gas trapping score although these were not as strong as for emphysema.

Table 74. Association between different methods of estimating emphysema on CT.

	%LAA$_{<-950}$	**%LAA$_{<-910}$**	**PERC$_{15}$**
%LAA$_{<-910}$	0.96***		
PERC$_{15}$	-0.99***	-0.96***	
Emphysema Score	0.83***	0.71***	-0.81***

Spearman's correlation. N=123. *** p <0.001.

Table 75. Association between different methods of estimating gas trapping on CT.

	%LAA$_{EXP<-856}$	**%LAA$_{EXP<-856-950}$**	**RVC**	**E/I MLD**
%LAA$_{EXP-856-950}$	0.92***			
RVC	0.81***	0.73***		
E/I MLD	0.83***	0.81***	0.88***	
Gas trapping score	0.65***	0.45***	0.49***	0.38***

Spearman's correlation. N=122. *** p <0.001.

6.2.1 Relationships with Pulmonary function

Next I assessed the association between spirometric markers of disease and CT measures (Table 76). There were strong associations between FEV1% and all measures of emphysema. They all had similarly strong associations. They also all had equally strong associations with FEV1/FVC ratio. There were also significant associations between emphysema measures and FEF75-25%, although these were less strong than for the other spirometric markers. There were no associations between emphysema measures and FVC.

Measures of gas trapping all had significant strong associations with FEV1% and FEV1/FVC ratio. They all also had significant associations with FEF75-25% and these were stronger than for emphysema measures. FVC had a weak significant association with RVC and E/I MLD. Pi10 only had a relatively weak significant association with FVC% and did not have any other associations with other spirometric markers.

Table 76. Association between different measures of emphysema, gas trapping and Pi10 and spirometry.

	FEV1%	FVC%	FEV1/FVC	FEF75-25%	FEV1 Reversibility
Emphysema					
Visual Score	**-0.47*** **	0.03	**-0.63*** **	**-0.42*** **	-0.09
%LAA$_{<-950}$	**-0.50*** **	0.05	**-0.72*** **	**-0.41*** **	-0.04
%LAA$_{<-910}$	**-0.47*** **	0.07	**-0.70*** **	**-0.33*** **	-0.05
PERC$_{15}$	**0.49*** **	-0.06	**0.72*** **	**0.35*** **	0.03
Gas Trapping					
Visual Score	**-0.51*** **	-0.14	**-0.55*** **	**-0.41*** **	-0.12
%LAA$_{EXP<-856}$	**-0.63*** **	-0.06	**-0.80*** **	**-0.55*** **	0.02
%LAA$_{EXP<-856-950}$	**-0.55*** **	-0.04	**-0.71*** **	**-0.49*** **	0.09
RVC	**-0.65*** **	**-0.24** **	**-0.64*** **	**-0.57*** **	-0.04
E/I MLD	**-0.63*** **	**-0.22***	**-0.64*** **	**-0.55*** **	0.08
Airway Dimension					
Pi10	-0.12	**-0.30** **	0.10	-0.13	0.12

Spearman's r values given. N=126 for visual scores. N=122 for %LAA$_{<-950}$ and Pi10 and 121 for E/I MLD. *p<0.05, **p<0.01, *** p <0.001.

To further explore the relationship between CT markers of disease and airflow obstruction I divided the cohort into GOLD groups according to their severity of FEV1% (Table 77). There was significantly increased emphysema in more severe GOLD categories as measured by both %LAA$_{<-950}$ and visual scoring and this was also the case for gas trapping. Pi10 was significantly increased between GOLD 3 and 4 but not between other groups.

Table 77. CT markers of emphysema, gas trapping and Pi10 in different GOLD groups.

	GOLD 2 (n=57)	GOLD 3 (n=51)	GOLD 4 (n=19)	Significance
Emphysema				
Visual Score	3.0 (5.0)	6.0 (8.0)	7.0 (4.0)	**<0.001*** **
%LAA$_{<-950}$	9.6 (13.1)	15.6 (27.0)	24.8 (8.4)	**<0.001*** **
%LAA$_{<-910}$	32.7 (24.7)	38.9 (36.4)	54.2 (12.2)	**<0.001*** **
PERC$_{15}$	-937.6 (34.1)	-952.2 (53.7)	-966.1 (18.2)	**<0.001*** **
Gas Trapping				
Visual Score	3.0 (5.0)	6.0 (7.0)	8.0 (4.0)	**<0.001*** **
%LAA$_{EXP<-856}$	27.6 (26.4)	42.3 (32.0)	65.3 (16.1)	**<0.001*** **
%LAA$_{EXP<-856-950}$	25.5 (23.4)	32.8 (18.2)	49.2 (12.7)	**<0.001*** **
RVC	-36.3 (17.5)	-23.4 (16.1)	-12.7 (9.3)	**<0.001*** **
E/I MLD	0.88 (0.07)	0.93 (0.05)	0.96 (0.03)	**<0.001*** **
Airway Dimension				
Pi10	3.79 (0.13)	3.78 (0.08)	3.84 (0.05)	**0.038***

Values given as medians (IQR). For visual emphysema and gas trapping n=127, for automated emphysema and Pi10 N=123, for automated gas trapping N=122. *p<0.05, **p<0.01 and *** P<0.001 using Independent samples Kruskal-Wallis test.

My previous study did not include any pulmonary function testing apart from simple spirometry, whereas this study includes both gas transfer and plethysmography, allowing further validation of CT markers of disease by comparing them with these more detailed lung function. All of the emphysema parameters had significant associations with TLCO% (Table 78). The strongest association was with the visual score.

Measures of gas trapping also had significant associations with all pulmonary function test markers (Table 78). The associations with TLCO% were less strong than emphysema parameters had exhibited. However the associations with RV% and RV/TLC ratio were stronger than for emphysema. E/I MLD had the strongest association with RV% and especially with RV/TLC. Pi10 only had a relatively weak association with TLC% and had no other significant associations

Table 78. Association between different measures of emphysema, gas trapping and PI10 and gas transfer and static lung volumes.

	TLCO%	TLC%	RV%	RV/TLC
Emphysema				
Visual Score	**-0.65****	**0.32*****	**0.20***	0.10
%LAA$_{<-950}$	**-0.52*****	**0.45*****	**0.34*****	0.08
%LAA$_{<-910}$	**-0.40*****	**0.50*****	**0.40*****	**0.19***
PERC$_{15}$	**0.50*****	**-0.45*****	**-0.36*****	-0.17
Gas Trapping				
Visual Score	**-0.56*****	**0.34*****	**0.33*****	**0.23****
%LAA$_{EXP<-856}$	**-0.44*****	**0.56*****	**0.56*****	**0.44*****
%LAA$_{EXP<-856-950}$	**-0.29*****	**0.53*****	**0.52*****	**0.40*****
RVC	**-0.52*****	**0.43*****	**0.52*****	**0.54*****
E/I MLD	**-0.34*****	**0.52*****	**0.64*****	**0.62*****
Airway Dimension				
Pi10	0.01	**-0.22***	-0.05	0.16

Spearman's r values given. N=122 for relationship between TLCO% and visual scores. N=116 for relationship between visual scores and other lung function parameters. N=118 for relationship between LAA% and Pi10 and TLCO% and n=114 for relationship with all other lung function. N= 117 for relationship between E/I MLD and TLCO% and n=113 for other lung function. *p<0.05, **p<0.01, *** p <0.001.

As all of the emphysema automated scores had very strong associations with one another I chose to use %LAA$_{<-950}$ for the rest of the study. %LAA$_{<-950}$ and PERC$_{15}$ had the strongest associations with both FEV1% and TLCO% and therefore are likely to represent the good measures of emphysema. These two measures also had very strong associations with one another and so it is likely that they are both equally good at estimating emphysema and so to avoid unnecessary multiple comparisons, for the rest of the study I chose to use only %LAA$_{<-950}$.

For gas trapping all measures had equally strong associations with FEF75-25%. However E/I MLD had the strongest association with RV/TLC, which represents the most accurate physiological measure of gas trapping and so for the rest of the study I chose to use this. This measures also has the advantage of being much simpler than RVC.

6.2.1.1 Multivariate Analysis

Univariate analysis demonstrated significant associations between spirometric markers of disease and %LAA$_{<-950}$ and E/I MLD. However, there are a number of potential confounding influences and so I performed multivariate linear regression analysis in an attempt to correct for these. The CT parameters %LAA$_{<-950}$, E/I MLD and PI10 were combined into a regression model along with demographic variables, with FEV1% as the outcome variable (Table 79). Variables that made no significant difference to the model were Pi10, age, gender, current smoking status and smoking

pack years. The three variables that were determined to be statistically useful to the model were $LAA_{<-950}$, E/I MLD and BMI. The r^2 for this model was 0.49.

This was repeated, this time with FEF75-25% as the outcome variable (Table 79). The natural log of this was used to normalise the distribution of data. Variables that made no significant difference to the model were Pi10, BMI, gender and smoking pack years. The variables that significantly predicated ln FEF75-25% were $LAA_{<-950}$, E/I MLD and current smoking status and the r^2 for the model was 0.37.

Table 79. Multiple regression analysis predicting FEV1% and FEF75-25%

	B coefficient	Standardised B coefficient	significance
FEV1%			
%$LAA_{<-950}$	**-0.42**	**-0.34**	<0.001
E/I MLD	**-180.8**	**-0.56**	<0.001
BMI	**-0.56**	**-0.20**	0.012
Ln FEF75-25%			
%$LAA_{<-950}$	**-0.01**	**-0.30**	0.001
E/I MLD	**-0.3.9**	**-0.42**	<0.001
Current smoker	**-0.17**	**-0.19**	0.014

Next, the CT parameters %$LAA_{<-950}$, E/I MLD and PI10 were combined into a regression model along with demographic variables to predict values of TLCO% (Table 80). Variables that made no significant difference to the model were Pi10, E/I MLD, age, current smoking status and smoking pack years. The three variables that were determined to be statistically useful to the model were %$LAA_{<-950}$, FEV1% and gender. The r^2 for this model was 0.34.

Another regression model was performed to predict RV/TLC (Table 80). Variables that made no significant difference to the model were PI10, %$LAA_{<-950}$, BMI, current smoking status and smoking pack years. The variables that significantly predicated RV/TLC were E/I MLD, FEV1% and current smoking status and the r^2 for the model was 0.37.

Table 80. Multiple regression analysis predicting TLCO% and RV/TLC

	B coefficient	Standardised B coefficient	significance
TLCO%			
$LAA_{<-950}$	**-0.61**	**-0.40**	<0.001
FEV1%	**0.50**	**0.37**	<0.001
Gender	**-7.8**	**-0.20**	0.010
RV/TLC			
E/I MLD	**0.66**	**0.25**	0.015
FEV1%	**-0.003**	**-0.34**	0.001
Gender	**0.08**	**0.31**	<0.001

6.2.2 Relationship between CT Parameters and Functional parameters

In the previous section I have demonstrated that CT parameters have a number of associations with lung function. However, lung function does not necessarily reflect symptoms and functional capacity. In this study, the six minute walk test and a number of symptom questionnaires were performed and subjects were also categorised into MRC dyspnoea groups. The medians for these variables are shown in Table 81. This was a severe cohort as the median six-minute walk distance was low and 72% of subjects fell into the most severe MRC dyspnoea score.

Table 81. Baseline functional parameters

	N=127
Six minute walk test	
6MWD	300.0 (170.0)
Resting Oxygen sats	95.0 (2.0)
Minimum oxygen sats	91.0 (5.0)
Desaturation on 6MWT	4.0 (5.0)
mMRC	
1	1 (0.8)
1	28 (22.0)
3	6 (4.7)
4	92 (72.4)
Questionnaires	
CAT score	16.0 (10.0)
EXACT score	37.0 (12.0)

Values given as medians (IQR) apart from mMRC where values represents number of subjects (%). N=125 for 6MWT and 127 for mMRC and questionnaires.

6.2.2.1 6MWT

There were no associations between emphysema, gas trapping and bronchial wall thickening and six minute walk distance (Table 82). There was only a weak negative association between resting oxygen saturations and Pi10 but not with any of the other CT measures. There were however significant associations between minimum saturations and desaturation during the 6MWT and E/I MLD and $\%LAA_{<-950}$.

Table 82. Association between CT parameters and 6MWT parameters.

	6MWD	Resting Oxygen Sats.	Minimum Sats	Desaturation on 6MWT
$LAA_{<-950}$	-0.16	-0.02	**-0.51*** **	**0.56*** **
E/I MLD	-0.10	-0.17	**-0.34*** **	**0.27*** **
Pi10	-0.11	**-0.20***	0.03	-0.11

Spearman's correlation. N=121 for emphysema and Pi10 and N=120 for E/I MLD.

When performing multivariate regression analysis to predict the 6MWD (Table 83), the CT parameters $\%LAA_{<-950}$, E/I MLD and PI10 as well as age, smoking status and pack years made no significant difference to the model. The variables that were determined to be statistically useful to the model were FEV1%, BMI and gender. The r^2 for this model was 0.2. When doing the same for a model predicting desaturation on exertion (Table 83), $\%LAA_{<-950}$, BMI and gender were statistically useful to the model. Pi10, E/I MLD, FEV1%, age and smoking status and pack year history made no significant impact on the model. The r^2 for this model was 0.36.

Table 83. Multiple regression analysis predicting TLCO% and RV/TLC

	B coefficient	**Standardised B coefficient**	**significance**
6MWD			
FEV1%	3.1	0.42	<0.001
BMI	-5.0	-0.24	0.005
Gender	-36.4	-0.16	0.049
Desaturation on Exertion			
$\%LAA_{<-950}$	**0.04**	**0.71**	<0.001
BMI	**0.03**	**0.24**	0.006
Gender	**0.23**	**0.17**	0.039

N=120. For desaturation on exertion, values were logged to normalise residuals.

Previous studies have suggested that COPD subjects who walk less than 350 metres at the 6-minute walk test have poorer outcomes. I therefore used this as cut-off to split the cohort into two groups; those who walked less than 350 metres and those who walked more. There were no significant differences in the CT parameters between the two groups (Table 84). FEV1% was significantly lower in subjects who walked less than 350 meters compared to those who walked further. On multivariate analysis FEV1% was the only variable that significantly predicted the chance of being able to walk further than this distance at six-minute walk test (Table 85). The CT parameters, gender, age, BMI and smoking status showed no significant ability to predict this.

Table 84. CT parameters and FEV1% in patient who could walk more or less than 350 meters at the 6MWT.

	<350 metres (N=80)	**>350 metres (N=45)**	**P Value**
FEV1%	39.8 (25.8)	53. 4 (14.9)	**0.001****
$\%LAA_{<-950}$	16.0 (24.8)	9.7 (13.9)	0.151
E/I MLD	0.92	0.91	0.451
Pi10	3.81 (0.12)	3.79 (0.12)	0.495

Values represent medians and IQR. **P<0.01 using Mann Whitney U test.

Table 85. Logistic regression to predict being able to walk further than 350 meters at the 6MWT.

	Odds ratio	95% Lower CI	95% upper CI	Significance
FEV1%	1.05	10.02	1.07	0.001

Next, I assessed oxygen desaturation during the six-minute walk test in more detail. A desaturation rate of over 5% at the six-minute walk test has been previously linked with a poor prognosis. Therefore I used this figure to split the cohort into two groups. FEV1% was significantly lower and $\%LAA_{<-950}$ and E/I MLD significantly higher in the group that desaturated (Table 86). There was no significant difference in Pi10 between the two groups. Using logistic regression $\%LAA_{<-950}$ was the only variable that significantly predicted whether a subject desaturated on exertion independently of the other variables (Table 87).

Table 86. CT parameters and FEV1% in patient who desaturated or not at 6MWT.

	<5% desaturation (N=79)	>5% desaturation (N=46)	P Value
FEV1%	52.0 (19.8)	36.5 (19.0)	**<0.001*** **
$\%LAA_{<-950}$	8.2 (13.7)	24.9 (19.3)	**<0.001*** **
E/I MLD	0.91 (0.07)	0.94 (0.06)	0.037
Pi10	3.81 (0.12)	3.78 (0.11)	0.210

Values represent medians and IQR. P values calculated using Mann Whitney U test.

Table 87. Logistic regression to predict desaturation at the 6MWT.

	Odds ratio	95% Lower CI	95% upper CI	significance
$\%LAA_{<-950}$	1.05	1.05	1.13	<0.001

6.2.2.2 mMRC

Next, I assessed the CT variables and clinical parameters in the different mMRC dyspnoea scores (Table 88). In this analysis I excluded severity group one as this included only one subject. There were significant differences in FEV1%, RV%, E/I MLD, and resting oxygen saturations between the groups. On pairwise analysis the significant differences for these variables were between mMRC group 2 and 4. There was no significant difference in emphysema and Pi10 between mMRC groups.

Table 88. CT, lung function and functional parameters in different mMRC groups.

	2	3	4	P value
Lung Function				
FEV1%	59.3 (10.9)	44.4 (22.7)	39.6 (18.9)	**<0.001*** **
TLCO%	67.5 (16.0)	48.2 (47.9)	53.4 (28.2)	**0.026***
TLC%	102.6 (15.0)	120.5 (20.9)	105.2 (23.5)	0.430
RV%	133.8 (39.4)	176.4 (38.9)	148.8 (64.9)	**0.021***
CT Parameters				
%LAA$_{<-950}$	7.4 (13.8)	12.6 (30.2)	15.8 (23.4)	0.340
E/I MLD	0.89 (0.08)	0.91 (0.08)	0.92 (0.06)	**0.001***
Pi10	3.78 (0.10)	3.77 (0.18)	3.81 (0.12)	0.413
Six minute walk test				
Resting 02	96.0 (2.0)	95.0 (4.0)	95.0 (2.0)	**0.011***
Desat. on exercise	3.0 (4.0)	4.5 (9.3)	4.0 (6.0)	0.242
6MWD	347.0 (132.0)	400.0 (297.0)	300.0 (184.0)	0.278

N=126 for spirometry. N=124 for 6MWD. N=122 for LAA% and Pi10. N=120 for E/I MLD. P values calculated using independent samples Kruskal-Wallis test.

6.2.2.3 Symptom Questionnaires

In this study two different validated symptom questionnaires were taken at enrolment. These were the CAT and EXACT score. When analysing the relationship between these questionnaire scores and CT parameters there were no significant associations (Table 89). Next, I divided the cohort into four groups according to their CAT score. There were no significant differences in any of the CT parameters between these groups although there was a non-significant trend towards increased %LAA$_{<-956}$ in the more symptomatic groups (Table 90). TLCO%, TLC% and RV% showed a significant difference between the groups. On pairwise analysis subjects with a CAT score between 21-30 had significantly lower TLCO% than those who had a CAT score between 0-10. RV% and TLC% were significantly raised in subjects who had a CAT score of 31-40 compared to those with a score of 0-10. 6MWD also showed a significant difference between these groups and on pairwise analysis subjects walked further when they had a CAT score between 0-10 and 11-20 than 21-30. Resting saturations also showed significant differences between all groups but no specific difference on pairwise analysis.

Table 89. Associations between CT parameters and CAT and EXACT symptom scores.

	CAT score	EXCAT Score
%LAA$_{<-950}$	0.16	0.00
E/I MLD	0.08	0.14
Pi10	-0.10	-0.10

Spearman's correlation. N=123 for LAA and Pi10. N =122 for E/I MLD.

Table 90. CT, lung function and functional parameters in subjects grouped according to CAT score.

	0-10	11-20	21-30	31-40	P value
Lung Function					
FEV1%	50.7 (30.2)	47.5 (23.8)	39.6 (23.9)	36.6 (30.4)	0.062
TLCO%	68.2 (46.4)	60.8 (25.3)	50.4 (22.0)	48.5 (29.3)	**0.011***
TLC%	99.8 (19.7)	106.3 (19.1)	103.7 (32.6)	121.3 (11.7)	**0.044***
RV%	135.1 (52.8)	143.4 (48.8)	140.8 (85.0)	199.0 (122.4)	**0.040***
CT Parameters					
%LAA$_{<-950}$	10.3 (21.8)	9.8 (15.8)	17.0 (21.1)	25.3 (20.8)	0.054
E/I MLD	0.92 (0.08)	0.92 (0.06)	0.91 (0.07)	0.95 (0.07)	0.266
Pi10	3.80 (0.14)	3.81 (0.11)	3.78 (0.11)	3.81 (0.09)	0.481
Six minute walk test					
Resting 02	96.0 (2.0)	95.0 (2.0)	95.0 (1.0)	95.0 (7.3)	**0.038***
Desat on exercise	3.0 (7.5)	4.0 (4.0)	4.0 (6.0)	6.0 (5.0)	0.602
6MWD	383.5 (158.0)	321 (157.0)	233.0 (167.0)	300.0 (169.0)	**0.004***

N=127 for spirometry. N=125 for 6MWD. N=123 for LAA% and Pi10. N=122 for E/I MLD. P values calculated using independent samples Kruskal-Wallis test .

6.2.3 Relationship with sputum inflammatory markers

Although bronchoscopy was not performed in this study sputum analysis and cell differential was performed and could be used as a marker of airway inflammation. The median sputum cell differentials are shown in Table 91. As expected neutrophils were the predominant cells in the sputum, followed by macrophages. There were no associations between any of the automated CT measures and sputum cell differentials (Table 92).

Table 91. Baseline sputum cell differential.

	N=69
Sputum Cell differential	
% Neutrophils	47.5 (71.1)
% macrophages	32.2 (37.9)
% eosinophils	1.9 (5.1)
% Lymphocytes	0.20 (0.60)

Values given as medians and IQR.

Table 92. Associations between CT parameters and sputum cell differential.

	%neutrophils	% Macrophages	%eosinophils	%Lymphocytes
Lung function				
FEV1%	-0.18	**0.29***	-0.03	**-0.27***
CT Parameters				
%LAA$_{<-950}$	0.16	-0.23	0.15	0.11
E/I MLD	0.19	-0.20	0.04	0.18
Pi10	-0.10	0.05	-0.11	-0.13

N= 67 for pi10 and %LAA$_{<-950}$, n=66 for E/I MLD. *p<0.05.

Previous studies have identified that relative sputum neutrophil counts of over 60% represent significant neutrophilic airway inflammation. I therefore split the cohort into two using this figure as a cut-off. When doing this there were no significant difference in CT parameters, lung function or six minute walk test between subjects with relative airway neutrophils over 60% and those with lower neutrophil counts (Table 93). Studies have also suggested that subjects with over 3% relative sputum eosinophil counts represent significant eosinophilic airway inflammation. Once again I divided the cohort into two using this cut-off. There were no significant differences in CT, FEV1% or 6MWT parameters between these two groups (Table 94).

Table 93. Lung function and CT parameters in subjects with and without neutrophilic airway inflammation.

	Neutrophils<61%	Neutrophils >61%	P value
Lung Function			
FEV1%	49.8 (24.4)	43.4 (23.3)	0.302
CT Parameters			
%LAA$_{<-950}$	11.1 (25.1)	14.6 (21.0)	0.857
E/I MLD	0.90 (0.07)	0.91 (0.06)	0.537
Pi10	3.79 (0.13)	3.80 (0.13)	0.634

Values given as medians and IQR. For FEV1% N=42 non-neutrophilic and N=27 neutrophilic. For %LAA$_{<-950}$/Pi10 N= 41 for non-neutrophilic and N=26 for neutrophilic. For E/I MLD N=40 non-neutrophilic and N=26 neutrophilic. P value calculated using Mann-Whitney U test.

Table 94. Lung function and CT parameters in subjects with and without eosinophilic airway inflammation.

	Eosinophils <3%	Eosinophils>3%	P value
Lung Function			
FEV1%	49.5 (24.3)	44.9 (21.6)	0.545
Emphysema			
%LAA$_{<-950}$	11.1 (20.9)	14.6 (25.0)	0.814
E/I MLD	0.90 (0.07)	0.92 (0.05)	0.719
Pi10	3.79 (0.13)	3.79 (0.13)	0.763

Values given as medians and IQR. For FEV1% N=45 non-eosinophilic and N=24 eosinophilic. For %LAA$_{<-950}$/Pi10 N= 43 for non-eosinophilic and N=24 for eosinophilic. For E/I MLD N=42 non-eosinophilic and N=24 eosinophilic. P value calculated using Mann-Whitney U test.

6.2.4 Relationship with blood inflammatory markers

A number of blood inflammatory markers were measured in the study cohort and the results for these are shown in Table 95. There were significant negative correlations between %LAA$_{<-950}$ and lymphocytes and CRP (Table 96). There were also positive associations between Pi10 and CRP and fibrinogen. E/I MLD had significant negative associations with white cell count, lymphocytes and CRP. Logistic regression was used to predict CRP (Table 97) and when doing this %LAA$_{<-950}$ was the

only variable that significantly predicted CRP. E/I MLD, Pi10, FEV1%, gender, age, BMI and smoking status showed no significant ability to predict CRP. The R^2 for this model was relatively weak however at 0.09.

Table 95. Baseline Blood inflammatory markers.

Leukocytes	7.6 (2.2)
Neutrophils	4.8 (1.7)
Eosinophils	0.20 (0.20)
Lymphocytes	1.7 (0.9)
CRP	5.0 (8.0)
Fibrinogen	4.8 (1.0)
PCT	0.059 (0.03)

Values given as medians and IQR. N=126 for white cell counts and fibrinogen, N=127 for CRP, N=114 for PCT.

Table 96. Associations between CT parameters and blood inflammatory markers.

	WCC	**Neutrophils**	**Eosinophils**	**Lymphocytes**	**CRP**	**Fibrinogen**	**PCT**
%LAA$_{<-950}$	-0.13	-0.05	0.16	**-0.24****	**-0.24****	-0.17	0.01
E/I MLD	**-0.25****	-0.11	0.01	**-0.34*****	**-0.28****	-0.04	-0.06
Pi10	0.06	0.14	0.01	-0.07	**0.25****	**0.21***	0.18

Spearman's correlation. For associations with %LAA$_{<-950}$/Pi10, N=122 for leukocytes/PCT, N=123 for CRP and N=110 for fibrinogen. For associations with E/I MLD N=121 for leukocytes/PCT, N=122 for CRP and N=109 for fibrinogen. * p<0.05 **p<0.01 ***p<0.001

Table 97. Multiple regression analysis predicting CRP.

	B coefficient	**Standardised B coefficient**	**significance**
CRP			
LAA$_{<-950}$	-0.02	-0.30	0.001

The natural log of CRP was used for this analysis to normalise the distribution of the residuals. N=122.

6.2.5 Relationship with bacteria

One of the major strengths of this study was the in-depth microbiological sampling performed on sputum from each subjects. Sputum samples were analysed for detection of the five main bacteria known to be pathogenic in lungs (potentially pathogenic microorganisms, PPM); NTHI, MCAT, SP,SA and PA. This was done by both traditional culture techniques and PCR detection. 110 subjects had sufficient sputum to analyse by traditional culture techniques. 57 of these were culture positive for PPMs and the breakdown for each of the organisms is shown in Table 98. As sputum was prioritised

for traditional culture techniques there were fewer samples available for PCR (N=102). 63 subjects had PPMs detected by PCR in their sputum.

Table 98. Bacterial detection by culture and PCR in Sputum

	Culture Positive	PCR Positive
PPM	57	63
NTHI	32	45
MCAT	8	14
SP	19	14
SA	6	7
PA	6	7

Values given as number of subjects. N=110 for culture and N=102 for PCR. PPM - Potentially pathogenic microorganism, SP - streptococcus Pneumoniae, MCAT- Moraxella catarrhalis, NTHI- non-typeable Haemophilus influenzae.

Next I explored CT parameters in subjects with and without PPM their sputum. There were no significant differences in FEV1% and CT parameters in subjects who had PPMs detected in their sputum or not, by either traditional culture or PCR techniques (Table 99). When repeating the analysis for each individual bacteria, there were no significant differences in FEV1% and CT parameters between subjects with or without NTHI (Table 100), SP (Table 101) and MCAT (Table 102) detected in their sputum. There was however increased Pi10 in subjects who didn't have SA detected in their sputum by traditional culture (Table 103). Subjects who had PA detected in their sputum by traditional culture had significantly worse airflow obstruction, higher emphysema score and higher E/I MLD (Table 104). When PCR was used for detection of PA only E/I MLD was significantly different between groups.

Table 99 CT parameters and FEV1% in subjects with and without potentially pathogenic micro-organisms detected in sputum using culture techniques.

	PPM+ve	PPM-ve	P Value
Culture			
FEV1%	47.0 (22.5)	49.5 (26.4)	0.368
%LAA$_{<-950}$	10.9 (17.2)	15.5 (23.9)	0.200
E/I MLD	0.92 (0.07)	0.90 (0.07)	0.152
Pi10	3.79 (0.12)	3.79 (0.09)	0.149
PCR			
FEV1%	45.5 (19.5)	43.6 (22.4)	0.931
%LAA$_{<-950}$	13.3 (22.2)	11.2 (21.2)	0.900
E/I MLD	0.91 (0.08)	0.91 (0.08)	0.531
Pi10	3.79 (0.11)	3.81 (0.13)	0.261

Values given as medians and IQR. For culture results N= 110 for FEV1%, N=107 for %LAA$_{<-950}$/Pi10 and N=106 for E/I MLD. For PCR results N=102 for FEV1%, N= 99 for %LAA$_{<-950}$/Pi10 and N=98 for E/I MLD. P values calculated using Mann-Whitney U-test. PPM - Potentially pathogenic microorganism

Table 100. CT parameters and FEV1% in subjects with and without NTHI detected in sputum using culture techniques.

	NTHI+ve	NTHI-ve	P Value
Culture			
FEV1%	46.3 (15.8)	51.7 (24.4)	0.320
%LAA$_{<-950}$	13.8 (21.9)	11.5 (12.0)	0.321
E/I MLD	0.92 (0.08)	0.91 (0.07)	0.207
Pi10	3.79 (0.11)	3.79 (0.10)	0.199
PCR			
FEV1%	47.0 (20.7)	50.8 (24.5)	0.344
%LAA$_{<-950}$	13.9 (25.4)	11.5 (20.0)	0.913
E/I MLD	0.92 (0.08)	0.91 (0.06)	0.748
Pi10	3.79 (0.09)	3.8 (0.12)	0.639

Values given as medians and IQR. For culture results N= 110 for FEV1%, N=107 for %LAA$_{<-950}$/Pi10 and N=106 for E/I MLD. For PCR results N=102 for FEV1%, N= 99 for %LAA$_{<-950}$/Pi10 and N=98 for E/I MLD. P values calculated using Mann-Whitney U-test.

Table 101. CT parameters and FEV1% in subjects with and without SP detected in sputum using culture techniques.

	SP+ve	SP-ve	P Value
Culture			
FEV1%	49.2 (21.5)	47.6 (24.6)	0.865
%LAA$_{<-950}$	10.2 (9.9)	14.4 (25.2)	0.459
E/I MLD	0.92 (0.05)	0.91 (0.08)	0.626
Pi10	3.83 (0.11)	3.79 (0.09)	0.202
PCR			
FEV1%	55.7 (16.7)	46.7 (23.9)	0.186
%LAA$_{<-950}$	14.3 (10.7)	11.5 (24.9)	0.944
E/I MLD	0.89 (0.08)	0.92 (0.07)	0.141
Pi10	3.78 (0.11)	3.80 (0.12)	0.237

Values given as medians and IQR. For culture results N= 110 for FEV1%, N=107 for %LAA$_{<-950}$/Pi10 and N=106 for E/I MLD. For PCR results N=102 for FEV1%, N= 99 for %LAA$_{<-950}$/Pi10 and N=98 for E/I MLD. P values calculated using Mann-Whitney U-test.

Table 102. CT parameters and FEV1% in subjects with and without MCAT detected in sputum using culture techniques.

	MCAT+ve	MCAT-ve	P Value
Culture			
FEV1%	48.1 (21.3)	47.7 (23.9)	0.936
%LAA$_{<-950}$	12.4 (23.1)	12.8 (20.4)	0.943
E/I MLD	0.93 (0.07)	0.91 (0.07)	0.720
Pi10	3.78 (0.10)	3.79 (0.11)	0.541
PCR			
FEV1%	51.7 (23.1)	47.2 (23.9)	0.176
%LAA$_{<-950}$	10.9 (26.8)	13.3 (19.6)	0.560
E/I MLD	0.89 (0.11)	0.91 (0.06)	0.446
Pi10	3.78 (0.12)	3.80 (0.12)	0.280

Values given as medians and IQR. For culture results N= 110 for FEV1%, N=107 for %LAA$_{<-950}$/Pi10 and N=106 for E/I MLD. For PCR results N=102 for FEV1%, N= 99 for %LAA$_{<-950}$/Pi10 and N=98 for E/I MLD. P values calculated using Mann-Whitney U-test.

Table 103. CT parameters and FEV1% in subjects with and without SA detected in sputum using culture techniques.

	SA+ve	SA-ve	P Value
Culture			
FEV1%	55.8 (25.3)	47.5 (23.9)	0.286
%LAA$_{<-950}$	13.5 (22.4)	12.8 (22.0)	0.640
E/I MLD	0.91 (0.08)	0.91 (0.07)	0.682
Pi10	3.74 (0.05)	3.79 (0.11)	**0.037***
PCR			
FEV1%	53.4 (22.9)	47.5 (24.0)	0.786
%LAA$_{<-950}$	16.7 (19.8)	12.5 (22.7)	0.886
E/I MLD	0.92 (0.11)	0.91 (0.07)	0.799
Pi10	3.76 (0.07)	3.80 (0.12)	0.079

Values given as medians and IQR. For culture results N= 110 for FEV1%, N=107 for %LAA$_{<-950}$/Pi10 and N=106 for E/I MLD. For PCR results N=102 for FEV1%, N= 99 for %LAA$_{<-950}$/Pi10 and N=98 for E/I MLD. P values calculated using Mann-Whitney U-test.

Table 104. CT parameters and FEV1% in subjects with and without PA detected in sputum using culture techniques.

	Pseu+ve	Pseu-ve	P Value
Culture			
FEV1%	32.9 (21.1)	48.8 (23.7)	**0.033***
%LAA$_{<-950}$	21.4 (27.5)	12.1 (20.3)	0.394
E/I MLD	0.95 (0.06)	0.91 (0.07)	**0.034***
Pi10	3.83 (0.16)	3.79 (0.11)	0.494
PCR			
FEV1%	36.2 (24.5)	49.2 (24.3)	0.097
%LAA$_{<-950}$	21.4 (27.5)	12.1 (20.3)	0.387
E/I MLD	0.95 (0.06)	0.91 (0.07)	**0.032***
Pi10	3.83 (0.16)	3.79 (0.11)	0.557

Values given as medians and IQR. For culture results N= 110 for FEV1%, N=107 for %LAA$_{<-950}$/Pi10 and N=106 for E/I MLD. For PCR results N=102 for FEV1%, N= 99 for %LAA$_{<-950}$/Pi10 and N=98 for E/I MLD. P values calculated using Mann-Whitney U-test.

6.2.6 Relationship with exacerbations

Exacerbations were captured prospectively during the first year of follow-up. In total there were 355 exacerbations and the mean annual exacerbation rate was 3.09 (Table 105). Severities of exacerbations were defined by whether a subject needed treatment (moderate) or needed hospital admission (severity), with the vast majority being moderate in this study. None of the automated CT measures had any associations with total exacerbation rate or exacerbation rate when stratified by severity (Table 106). A regression model predicting exacerbation rate was attempted but none of the variables tested, including CT parameters, FEV1%, BMI, gender, smoking status or pack years significantly predicted exacerbations.

Table 105. Number of exacerbations and exacerbation rate in year one

Number of Exacerbations	
All	355
mild	31
moderate	304
Severe	20
Exacerbation Rate	
All	3.09 (2.72)
Mild	0.26 (0.53)
Moderate	2.59 (2.37)
Severe	0.24 (1.08)

Values given number of events or for rate as means and SD. N=127.

Table 106. Associations between CT parameters and exacerbation rate.

	Exacerbation Rate	**Mild**	**Moderate**	**Severe**
%LAA$_{<-950}$	0.13	-0.09	0.14	0.08
E/I MLD	0.09	-0.13	0.13	0.10
Pi10	-0.03	-0.12	-0.01	0.10

Spearman's correlation. N=123 for %LAA$_{<-950}$/Pi10 and N=122 for E/I MLD.

Of the subjects who had CT analysis performed, 19 subjects suffered no exacerbations in year one of the study, whereas 104 subjects suffered at least one exacerbation. I therefore analysed CT parameters in these two groups to ascertain whether there was any significant difference. There were no significant differences in any of the CT parameters between subject who suffered no exacerbations and those who had at least one exacerbation (Table 107). There was a trend for Pi10 being lower in the subjects who suffered exacerbations.

Table 107. CT parameters and FEV1% in subjects with and without exacerbations

	No Exacerbations (N=19)	**Exacerbations (N=104)**	**P value**
FEV1%	51.3 (27.4)	45.8 (24.6)	0.299
%LAA$_{<-950}$	7.36 (17.3)	14.8 (22.8)	0.121
E/I MLD	0.92 (0.06)	0.92 (0.07)	0.986
Pi10	3.82 (0.09)	3.79 (0.11)	0.052

Values given as medians (IQR). N=126 for FEV1%, N=123 for %LAA$_{<-950}$/Pi10 and N=122 for E/I MLD. P values calculated using Mann-Whitney U test.

Frequent exacerbations are by convention defined as patients who suffer with two or more exacerbations in a year. I therefore split the cohort into two groups; subjects who had fewer than two exacerbations and those that had at least two exacerbations. When doing this there were no significant differences in CT parameters in the two groups (Table 108). However there was a trend

towards significantly raised emphysema and gas trapping in the frequent exacerbator group. There was also significantly lower FEV1% in the frequent exacerbator group.

Table 108. CT parameters and FEV1% in subjects with and without frequent exacerbations

	<2 exacerbation	≥2 exacerbations	P value
FEV1%	53.4 (24.6)	40.2 (21.1)	**0.006****
%LAA$_{<-950}$	10.6 (18.1)	15.9 (23.8)	0.073
E/I MLD	0.90 (0.08)	0.92 (0.06)	0.055
Pi10	3.79 (0.10)	3.81 (0.12)	0.661

Values given as medians (IQR). N=126 for FEV1%, N=123 for %LAA$_{<-950}$/Pi10 and N=122 for E/I MLD. P values calculated using Mann-Whitney U test.

There were a number of subjects who suffered very frequent exacerbations. To investigate this group, I divided the cohort around the 75th centile of exacerbation frequency, which was 4.9 exacerbations per year. There were no significant differences in CT parameters or FEV1% between subjects who suffered fewer or greater than 4.9 exacerbations per year (Table 109).

Table 109. CT parameters and FEV1% in subjects with and without very frequent exacerbations

	<4.9 exacerbation	≥4.9 exacerbations	P value
FEV1%	47.5 (24.6)	45.2 (27.3)	0.901
%LAA$_{<-950}$	11.5 (21.1)	14.4 (20.9)	0.954
E/I MLD	0.91 (0.07)	0.92 (0.07)	0.930
Pi10	3.79 (0.10)	3.81 (0.13)	0.862

Values given as medians (IQR). N=126 for FEV1%, N=123 for %LAA$_{<-950}$/Pi10 and N=122 for E/I MLD. P values calculated using Mann-Whitney U test.

6.2.7 Relationship with blood Biomarkers

Five different blood biomarkers were measured in serum in this study. Median values for these are shown in (Table 110). The only one of these biomarkers to show any significant associations with CT measures was high sensitivity troponin, which showed a positive association between LAA and E/I MLD (Table 111). FEV1% had a significant negative association with HS-troponin and IP-10 as well as a positive association with SP-D.

Table 110. Baseline serum biomarker concentrations

hs-troponin	0.012 (0.01)
IL-6	4.01 (2.7)
IP-10	143.0 (92.0)
NT-proBNP	96.4 (167.9)
Sp-D	166.0 (102.0)

Values given as medians (IQR). N=121 for hs-troponin, N=88 for IL-6, N=123 for IP-10/Sp-D and N=122 for NT-proBNP.

Table 111. Associations between CT parameters and serum biomarkers.

	Hs-troponin	**IL-6**	**IP-10**	**NT-proBNP**	**Sp-D**
FEV1%	**-0.30****	-0.12	**-0.21***	-0.15	**0.23***
$LAA_{<-950}$	**0.44*****	-0.06	0.14	-0.04	0.05
E/I MLD	**0.34*****	0.02	0.17	0.09	-0.05
Pi10	0.04	-0.01	-0.13	0.14	-0.15

Spearman's correlation. For FEV1% N=120 for hs-troponin, N=87 for IL-6, N=122 for IP-10/Sp-D and N=121 for NT-proBNP. For $\%LAA_{<-950}$/Pi10 N=118 for hs-troponin, N=86 for IL-6, N=120 for IP-10/Sp-D and N=119 for NT-proBNP. For E/I MLD N=117 for hs-troponin, N=85 for IL-6, N=119 for IP-10/Sp-D and N=118 for NT-proBNP.*p<0.05, **p<0.01, ***p<0.001.

As troponin was significantly associated with a number of CT parameters I performed multivariate analysis to ensure that these associations were independent. Troponin data was highly skewed and therefore linear regression could not be performed and so instead the cohort was dichotomised around the median of 0.012 to form two groups. Both emphysema and gas trapping were significantly higher in the group with raised troponin (Table 112). There was also significantly worse airflow obstruction in the raised troponin group. There was no significant difference in Pi10 between the two groups. Logistic regression was performed in order to identify variables that increase the odds of being in the raised troponin group. None of the CT measures, as well as gender, smoking status and pack years significantly increased the odds of having raised troponin. The measures that significantly predicted raised troponin were FEV1%, age and gender (Table 113).

Table 112. CT parameters and FEV1% in subjects with and without raised troponin

	<0.012	**>0.012**	**Significance**
FEV1%	54.0 (23.3)	37.3 (22.4)	<0.001***
$\%LAA_{<-950}$	7.7 (13.6)	22.2 (20.1)	<0.001***
E/I MLD	0.90 (0.06)	0.94 (0.07)	<0.001***
Pi10	3.79 (0.12)	3.81 (0.10)	0.785

Values given as medians (IQR). For FEV1% N=120, for $\%LAA_{<-950}$/Pi10 N=118 and for E/I MLD N=117. ***p<0.001.

Table 113. Logistic regression analysis to predict raised troponin

	Odds ratio	95% Lower CI	95% upper CI	significance
FEV1%	0.92	0.88	0.95	<0.001
Age	1.1	1.04	1.18	0.002
Sex	0.2	0.08	0.56	0.002

6.3 Emphysema

In the previous parts of this chapter I have investigated the association with the CT measures and a number of physiological clinical and biological markers of disease. In this next section I performed more in-depth analysis of each of the CT parameters.

6.3.1 Regression model to predict emphysema

A regression model was developed in order to discover the variables that significantly predict emphysema, which included FEV1%, BMI and current smoking status (Table 114). Variables that had no influence on $\%LAA_{<-950}$ were age, gender, PPM, exacerbations, sputum neutrophils and CRP.

Table 114. Multiple regression analysis to predict $\%LAA_{<-950}$.

	B coefficient	Standardised B coefficient	significance
$\%LAA_{<-850}$			
FEV1%	**-0.35**	**-0.42**	<0.001
BMI	**-0.90**	**-0.38**	<0.001
Current smoking	**-5.8**	**-0.22**	<0.001

6.3.2 Emphysema Distribution throughout the cohort

Initially I explored the distribution of emphysema throughout the cohort and the distribution histogram is shown in Figure 49. There is no consensus about the quantity of emphysema which is deemed clinically significant. I therefore split the cohort into three tertiles depending on the amount of emphysema; subjects with $\%LAA_{<-950}$ <6.85, subjects with $\%LAA_{<-950}$ between 6.85 and 19.6 and those with $\%LAA_{<-950}$ over 19.6. There were significant differences between these groups for FEV1%, TLCO%, TLC% and RV% (Table 115). There were also significant differences in 6MWD, desaturation during the 6MWT, CRP and troponin. When analysing pairwise difference between groups for these variables there were significant differences between the two lower tertiles of LAA and the top tertile. There was no significant difference for these variables between the lowest and intermediate tertile group of emphysema.

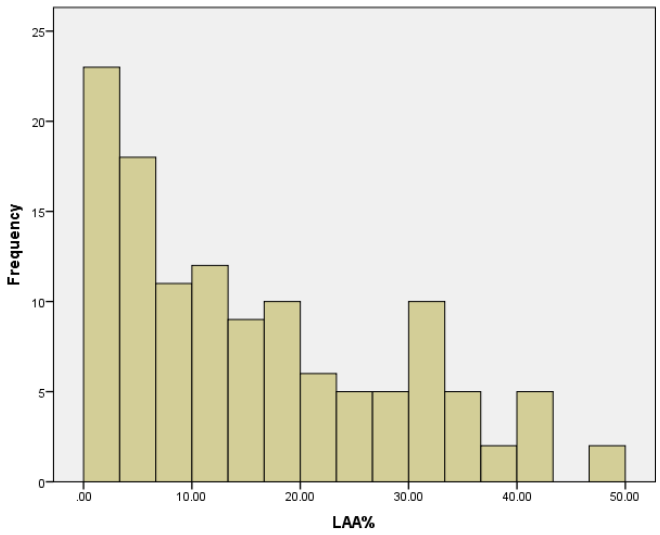


Figure 49. Distribution histogram for %$LAA_{<-950}$ in 123 subjects.

Table 115. Pulmonary physiology, functional capacity, inflammatory markers, biomarkers and exacerbation frequency in tertiles ranked according to LAA severity.

	<6.85	6.85 – 19.6	>19.6	Significance
Pulmonary Function				
FEV1%	54.2 (21.7)	50.0 (18.2)	32.8 (14.2)	**<0.001*** **
TLCO%	69.3 (25.9)	59.6 (27.5)	44.6 (15.1)	**<0.001*** **
TLC%	100.8 (14.0)	103.6 (25.0)	120.0 (23.7)	**<0.001*** **
RV%	139.3 (40.7)	138.3 (58.5)	176.5 (83.7)	**0.001** **
RV/TLC	0.54 (0.13)	0.50 (0.13)	0.58 (0.17)	**0.007** **
Functional Capacity				
6MWD	321 (185)	327.0 (168.0)	253.0 (168.0)	**0.040***
Oxygen desaturation	2.0 (3.0)	3.0 (5.0)	8.0 (7.0)	**<0.001*** **
CAT	16.0 (9.0)	16.0 (10.0)	20.0 (13.0)	0.162
EXCAT	36.0 (10.0)	34.0 (12.0)	37.5 (14.0)	0.776
Sputum Differential				
Neutrophils%	10.9 (61.9)	58.0 (74.1)	50.4 (57.5)	0.543
Macrophages%	46.9 (48.2)	25.2 (40.1)	32.1 (23.1)	0.139
Eosinophils%	2.1 (5.6)	0.7 (4.7)	2.6 (6.5)	0.095
Blood Inflammatory Markers				
CRP	6.0 (11.0)	6.0 (7.0)	3.0 (6.8)	**0.013***
Eosinophils	0.20 (0.20)	0.20 (0.25)	0.20 (0.20)	0.252
Fibrinogen	5.1 (1.0)	4.9 (1.3)	4.8 (1.1)	0.296
Neutrophils	4.9 (1.6)	4.6 (1.4)	4.8 (1.9)	0.633
PCT	0.06 (0.03)	0.06 (0.03)	0.06 (0.03)	0.957
Blood Biomarkers				
hs-troponin	0.010 (0.01)	0.012 (0.01)	0.017 (0.01)	**<0.001*** **
IL-6	4.13 (2.75)	3.95 (2.31)	3.37 (2.29)	0.569
IP-10	140.5 (105.1)	142.0 (141.0)	148.0 (76.0)	0.362
NT-proBNP	115.0 (195.7)	62.7 (133.9)	94.7 (150.2)	0.140
Sp-D	144.5 (100.8)	179.0 (135.0)	172.0 (109.8)	0.226
Exacerbations				
Exacerbation rate	1.9 (4.0)	2.9 (4.0)	3.0 (3.0)	0.345

Values represent medians (IQR). N=122 for FEV1% and PCT, N=118 for TLCO% and hs-troponin, n=114 for all other lung function, N=121 for 6MWT, N=123 for CAT/EXACT scores, N= 67 for sputum differential, N=123 for CRP and exacerbation rate and N=110 for fibrinogen, N=86 for IL-6, N=120 for IP-10/Sp-D and N=119 for NT-proBNP. *p<0.05, **p<0.01, ***p<0.001 using independent samples Kruskal-Wallis test.

6.3.3 Regional Variation of emphysema

Next I investigated the regional distribution of emphysema by analysing the $\%LAA_{<-950}$ in each of the five individual lobes. Figure 50 shows coronal reconstructions from three subjects with different distributions of emphysema. The RUL was most often the lobe with the highest amount of emphysema in each individual (Figure 51Figure 50), closely followed by the RML. The other lobes were less frequently the lobes with greatest $\%LAA_{<-950}$ in each subject, with the RLL being the lobe least often affected by the most emphysema.

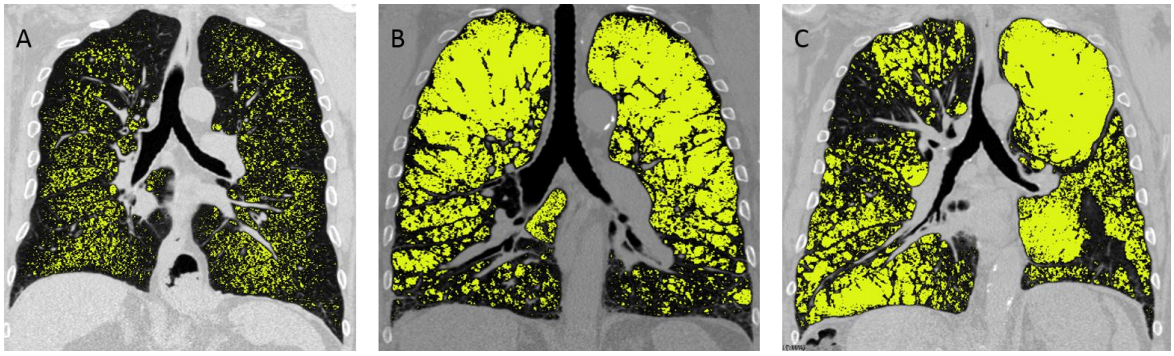


Figure 50. Coronal reconstruction from three subjects showing differing distributions of emphysema (A) moderate homogenous emphysema (B) severe upper lobe emphysema (C) severe left upper lobe and both lower lobe emphysema.

Median values for $\%LAA_{<-950}$ in each lobe for the entire cohort are shown in Figure 52Figure 51. These median lobar values of LAA were remarkably consistent, although there was a significant difference in $\%LAA_{<-950}$ between the RML and RLL. This difference was however minimal at just 1.3%. There were no significant differences in $\%LAA_{<-950}$ between any of the other lobes. When repeating this analysis in the different GOLD groups (moderate, severe and very severe) there was no significant difference in $\%LAA_{<-950}$ between lobes in either severe or very severe subjects (Figure 53). There was however a significant difference in LAA between the RML and RUL as well as the RML and RLL in moderate subjects.

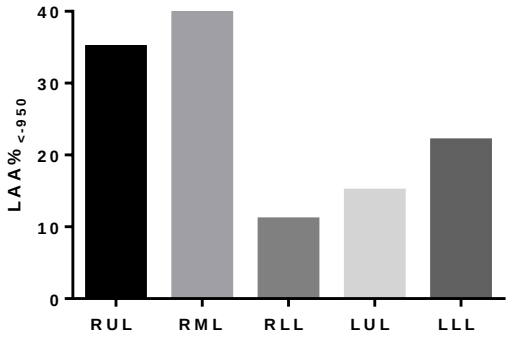


Figure 51. The frequency that each lobe had the greatest amount of emphysema in the cohort. N=123.

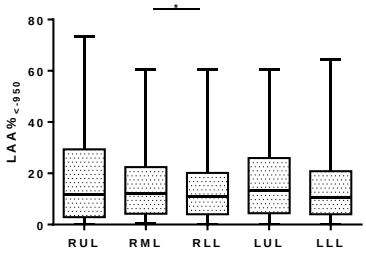


Figure 52. Median values of LAA% in each lobe with IQR and range. N=123. *p<0.05 using related-samples Friedman's two-way analysis of variance.

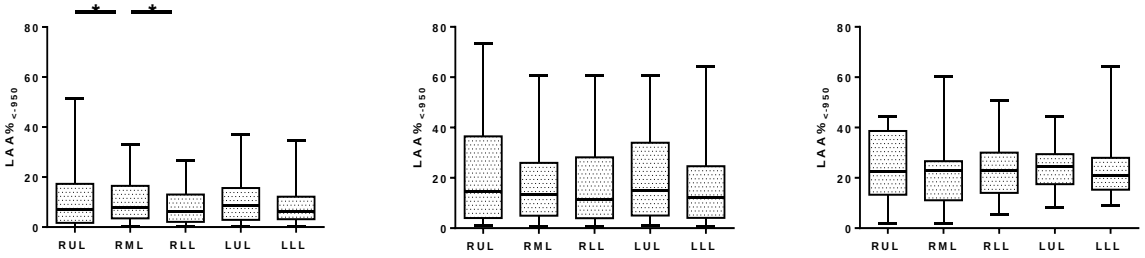


Figure 53. Median values of LAA% in each lobe with IQR and range. (A) Moderate COPD, N=56. (B) Severe COPD, N=50. (C) Very severe COPD, N=17. *p<0.05 using related-samples Friedman's two-way analysis of variance.

There were strong associations between all lobar values of $\%LAA_{<-950}$ and total $\%LAA_{<-950}$ (Table 116). The $\%LAA_{<-950}$ in the middle lobe had a slightly weaker association with overall $\%LAA_{<-950}$ than the other lobes. The $\%LAA_{<-950}$ in each of the lobes had moderate-strong associations with one another. $\%LAA_{<-950}$ in the two upper lobes had very strong associations with one another and likewise the $\%LAA_{<-950}$ in the lower lobes had very strong associations with one another. The associations between the upper lobes and lower lobes were slightly weaker as were the associations with the middle lobe.

Table 116. Association between overall LAA and lobar emphysema

	Overall	RUL	RML	RLL	LUL
RUL	0.90***	-			
RML	0.80***	0.69***	-		
RLL	0.91***	0.75***	0.78***	-	
LUL	0.95***	0.91***	0.73***	0.80***	
LLL	0.87***	0.67***	0.73***	0.90***	0.78***

Spearman's correlation. N=123. ***p<0.001.

Next I assessed the associations between $\%LAA_{<-950}$ in each of the lobes and physiological, functional and biological markers of disease to determine whether one lobe contributes to these more than others. There were similarly strong associations between all lobar values of $\%LAA_{<-950}$ and FEV1%, TLCO%, oxygen desaturation on exertion and CRP (Table 117). There were some mild variations in associations between $\%LAA_{<-950}$ in different lobes and 6MWD, CAT score, sputum neutrophils, blood fibrinogen and exacerbation rate. However, these variations were small.

Table 117. Association between lobar LAA% and pulmonary physiology, functional capacity, inflammatory markers and exacerbation rate

	RUL	RML	RLL	LUL	LLL
Pulmonary Function					
FEV1%	-0.44***	-0.37***	-0.52***	-0.48***	-0.45***
TLCO%	-0.52***	-0.28**	-0.45***	-0.47***	-0.40***
Functional					
6MWD	-0.11	-0.13	-0.20*	-0.12	-0.10
Oxygen Desaturation	0.50***	0.42***	0.52***	0.49***	0.51***
CAT	0.09	0.14	0.24**	0.16	0.18*
EXACT	-0.03	-0.01	0.04	-0.01	0.00
Sputum Differential					
Neutrophils%	0.10	0.13	0.21	0.12	0.28*
Macrophages%	-0.25*	-0.16	-0.22	-0.18	-0.21
Eosinophils%	0.17	0.12	-0.01	0.17	0.08
Blood Inflammatory Markers					
CRP	-0.27**	-0.24**	-0.19*	-0.24**	-0.13
Fibrinogen	-0.22*	-0.12	-0.13	-0.19*	-0.08
Neutrophils	-0.11	-0.06	-0.02	-0.09	-0.01
PCT	0.04	-0.01	-0.04	0.04	0.01
Exacerbations					
Exacerbation rate	0.07	0.08	0.14	0.09	0.20*

Spearman's correlation. N=122 for FEV1% and PCT, N=118 for TLCO%, N=121 for 6MWT, N=123 for CAT/EXACT scores, N= 67 for sputum differential, N=123 for CRP and exacerbation rate and N=110 for fibrinogen. *p<0.05, **p<0.01, ***p<0.001.

The traditional held belief in COPD is that emphysema predominantly affects the upper lobes and so I assessed the proportion of emphysema affecting the upper and lower lobes. For the purposes of this analysis the upper lobes were defined as the RUL, RML and LUL and the lower lobes the LLL and RLL. Median $\%LAA_{<-950}$ in the upper lobes was 12.2 (21.0) and this was significantly higher ($p<0.001$) than the lower lobes where the median value was 11.1 (16.7). The ratio of the upper lobes $\%LAA_{<-950}$ to the lower lobes $\%LAA_{<-950}$ was calculated and result are presented in Table 118. There was no significance difference in this ratio between the different GOLD groups.

Table 118. Upper/lower lobe emphysema ratio in whole cohort and the different GOLD groups.

	Upper lobe LAA/lower lobe $\%LAA_{<-950}$
Whole cohort	1.15 (0.79)
Moderate	1.17 (0.82)
Severe	1.10 (0.98)
V. Severe	1.13 (0.59)

Values given as medians (IQR). N=123.

Next the upper lobe/lower lobe $\%LAA_{<-950}$ was split into four equal groups by using the 25th, 50th and 75th centile. For the first group the 25th centile was 0.85 and this therefore represented lower lobe predominant disease. The group between the 25th centile ratio (0.85) and 50th centile (1.14) represented a relatively even distribution of emphysema. The group between the 50th centile and 75th centile (1.64) represented mild upper lobe predominance and the groups above the 75th centile represented severe upper lobe predominance. A number of physiological, functional, clinical and biological variables were then assessed across the groups (Table 119). There were however, no significant differences in any of these variables between groups.

Table 119. Pulmonary physiology, functional capacity, inflammatory markers and exacerbation frequency in quartiles ranked according to upper/lower lobe $\%LAA_{<-950}$

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P value
Overall $\%LAA_{<-950}$	10.1 (17.2)	11.1 (16.4)	18.3 (25.3)	12.7 (18.1)	0.425
Pulmonary Function					
FEV1%	41.5 (23.8)	48. 3 (23.5)	44.9 (23.2)	49.0 (25.2)	0.691
TLCO%	64.0 (37.6)	60.7 (36.0)	53.0 (27.0)	58.5 (23.9)	0.832
Functional					
6MWD	300.0 (187.0)	321.0 (148.0)	316.0 (196.0)	315.0 (176)	0.788
Oxygen Desaturation					0.305
CAT	16.0 (11.0)	16.0 (9.0)	20.0 (10.0)	13.0 (12.0)	0.086
EXACT	39.0 (9.0)	33.5 (16.0)	38.0 (14.0)	35.0 (10.0)	0.166
Sputum Differential					
Neutrophils%	53.3 (78.8)	33.4 (72.6)	53.2 (63.3)	7.9 (62.3)	0.288
Macrophages%	31.3 (41.8)	36.6 (55.8)	32.1 (31.6)	34.9 (26.1)	0.797
Eosinophils%	1.06 (2.34)	1.17 (5.7)	3.71 (6.5)	2.90 (4.75)	0.228
Blood Inflammatory Markers					
CRP	6.0 (11.3)	5.0 (6.5)	5.0 (5.8)	4.5 (5.0)	0.278
Fibrinogen	5.1 (1.1)	5.0 (1.1)	4.7 (1.0)	4.8 (1.3)	0.398
Neutrophils	5.0 (1.8)	4.9 (1.6)	4.5 (1.7)	4.7 (1.8)	0.200
PCT	0.057 (0.03)	0.061 (0.03)	0.061 (0.04)	0.059 (0.03)	0.949
Exacerbations					
Exacerbation rate	2.98 (3.6)	3.50 (4.0)	2.98 (3.0)	1.37 (4.9)	0.169

Values represent medians and IQR. N=122 for FEV1% and PCT, N=118 for TLCO%, N=121 for 6MWT, N=123 for CAT/EXACT scores, N= 67 for sputum differential, N=123 for CRP and exacerbation rate and N=110 for fibrinogen.

6.4 Gas trapping

6.4.1 Regression model to predicted E/I MLD

A regression model was developed in order to discover the variables that significantly predict gas trapping, which included FEV1%, BMI, age and PPM detection (Table 120). Variables that had no influence on E/I MLD were gender, exacerbations, smoking status, sputum neutrophils and CRP.

Table 120. Multiple regression analysis to predict E/I MLD.

	B coefficient	Standardised B coefficient	significance
E/I MLD			
FEV1%	**-0.002**	**-0.577**	<0.001***
BMI	**-0.003**	**-0.292**	<0.001***
Age	**0.001**	**0.166**	0.014*
PPM detected	**0.014**	**0.149**	0.027*

6.4.2 E/I MLD distribution in the cohort

The distribution histogram for E/I MLD is shown in Figure 54. As there is no consensus about the amount of gas trapping which is deemed significant I divided the cohort into three tertiles depending on the amount of gas trapping; subjects with E/I MLD <0.8941, subjects with E/I MLD between 0.8941 and 0.9424 and those with E/I MLD over 0.9424. FEV1% and RV/TLC ratio were significantly different across all three groups (Table 121). TLCO% was significantly lower in tertile 3 as opposed to tertile 1. Desaturation on exertion was significantly greater in tertile three compared to tertile one. CRP was significantly higher in tertile 3 compared to tertile 1 or 2.

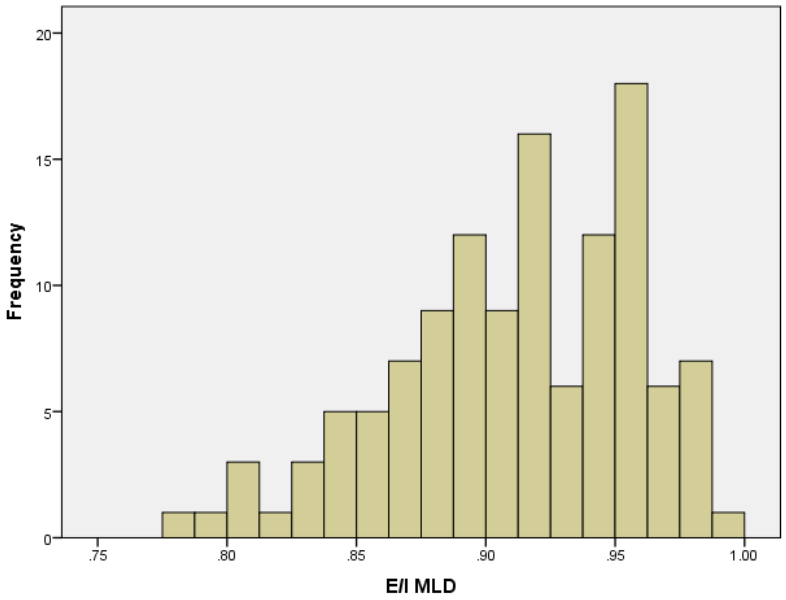


Figure 54. Distribution histogram for E/I MLD for this cohort.

Table 121. Pulmonary physiology, functional capacity, inflammatory markers, biomarkers and exacerbation frequency in tertiles ranked according to E/I MLD severity.

	<0.8941	0.8941- 0.9424	>0.9424	Significance
Spirometry				
FEV1%	58.3 (18.3)	46.5 (19.5)	32.4 (19.2)	**<0.001*** **
TLCO%	67.5 (35.3)	59.6 (23.5)	48.4 (23.1)	**0.003** **
RV/TLC	0.48 (0.13)	0.54 (0.12)	0.61 (0.11)	**<0.001*** **
Functional Capacity				
6MWD	321 (206)	320 (136)	270 (184)	0.338
Oxygen desaturation	3.0 (4.0)	4.0 (5.8)	6.0 (6.0)	**0.012***
CAT	16.0 (12.0)	16.0 (10.0)	16.5 (10.0)	0.576
EXCAT	35.0 (10.0)	37.0 (14.0)	37.0 (13.0)	0.528
Sputum Differential				
Neutrophils%	7.9 (63.3)	48.1 (75.2)	55.7 (61.6)	0.122
Macrophages%	34.9 (52.6)	27.2 (33.6)	34.7 (30.3)	0.100
Eosinophils%	2.0 (4.3)	3.7 (6.5)	1.6 (3.0)	0.452
Blood Inflammatory Markers				
CRP	7.0 (7.0)	4.0 (6.8)	4.0 (6.0)	**0.005** **
Fibrinogen	4.9 (1.1)	4.9 (0.8)	4.8 (1.4)	0.682
Neutrophils	4.9 (1.7)	4.5 (1.9)	4.7 (1.7)	0.188
PCT	0.060 (0.03)	0.062 (0.02)	0.058 (0.03)	0.739
Exacerbations				
Exacerbation rate	1.88 (4.0)	2.99 (4.0)	2.97 (3.6)	0.735

Values represent medians and IQR. N=121 for FEV1%, N= 117 for TLCO%, N=113 for RV/TLC, N=120 for 6MWT, N =122 for CAT/EXACT, CRP and exacerbations, N=66 for sputum differential, N=121 for PCT and N=109 for fibrinogen. * p<0.05 **p<0.01 ***p<0.001

6.4.3 Regional Variation of Gas trapping

Next I assessed the regional variation of gas trapping by calculating E/I MLD in each individual lobe. The RML had the highest amount of E/I MLD in 69 subjects. The RUL had the highest E/I MLD in 23 individuals, while the LUL was most severely affected in 14 subjects. The LLL and RLL had the highest E/I MLD in 9 and 3 subjects respectively. Median values for E/I MLD in each lobe are shown in Figure 55. There were significant differences between all lobes apart from between the RUL and LUL as well as the RLL and LLL. Interestingly 6 lobar values of E/I MLD were above one. Four of these were in the RML, one in the RUL and LUL. This suggests that attenuation values in these lobes decreased in expiration, which would not have been expected.

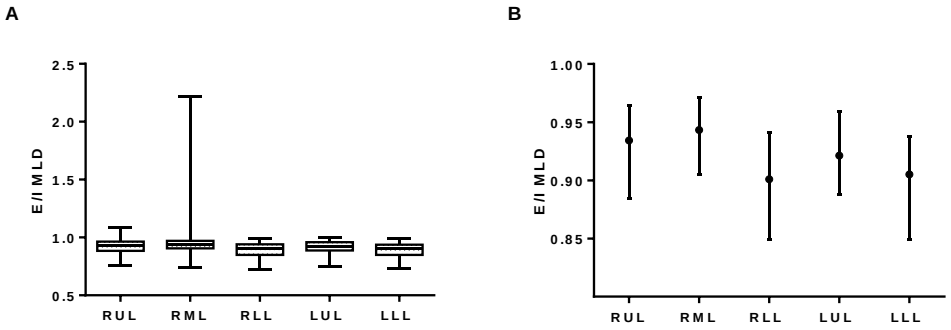


Figure 55. Median values of E/I MLD in each lobe with IQR and range. (A) Whole cohort, N=122 (B) whole cohort minus two outliers, N=120. *p<0.05.

All lobar values of E/I MLD had strong associations with overall E/I MLD, although the upper lobes exhibited the strongest association (Table 122). E/I MLD in all lobes showed strong associations with one another. The strongest association was between the two upper lobes.

Table 122. Association between overall E/I MLD and lobar E/I MLD

	Overall	**RUL**	**RML**	**RLL**	**LUL**
Overall	-				
RUL	0.93***				
RML	0.85***	0.83***			
RLL	0.88***	0.75***	0.79***		
LUL	0.95***	0.92***	0.80***	0.76***	
LLL	0.82***	0.60***	0.64***	0.82***	0.69***

Spearman's correlation. N=122. ***p<0.001.

Next I assessed the association of lobar values of E/I MLD with physiological, functional and biological markers of disease. There were similarly strong associations between lobar values of E/I MLD and FEV1%, TLCO% and RV/TLC ratio (Table 123). E/I MLD in all lobes apart from the LLL had significant negative associations with CRP. E/I MLD in the RUL also had negative associations with sputum macrophages and serum neutrophils.

Table 123. Association between lobar E/I MLD and pulmonary physiology, functional capacity, inflammatory markers and exacerbation rate

	RUL	RML	RLL	LUL	LLL
Pulmonary Function					
FEV1%	**-0.58*****	**-0.52*****	**-0.56*****	**-0.61*****	**-0.52*****
TLCO%	**-0.39*****	**-0.31****	**-0.20***	**-0.36*****	-0.15
RV/TLC	**0.50*****	**0.45*****	**0.65*****	**0.54*****	**0.60*****
Functional					
6MWD desaturation	-0.07	-0.08	-0.10	-0.07	-0.07
CAT	0.05	0.08	0.07	0.10	0.06
EXACT	0.16	0.09	0.10	0.15	0.06
Sputum					
Neutrophils%	0.15	0.14	0.20	0.16	0.18
Macrophages%	**-0.25***	-0.16	-0.04	-0.20	-0.12
Eosinophils%	0.07	-0.12	-0.11	0.13	-0.15
Blood					
CRP	**-0.30****	**-0.26****	**-0.19***	**-0.23***	-0.12
Fibrinogen	-0.13	-0.05	0.01	-0.07	0.07
Neutrophils	**-0.20***	-0.08	-0.08	-0.16	-0.02
PCT	-0.01	-0.04	-0.15	0.02	-0.08
Exacerbations					
Exacerbation rate	0.05	0.09	0.15	0.06	0.11

Spearman's correlation. N=121 for FEV1%, N= 117 for TLCO%, N=113 for RV/TLC, N=120 for 6MWT, N =122 for CAT/EXACT, CRP and exacerbations, N=66 for sputum differential, N=121 for PCT and N=109 for fibrinogen. * p<0.05 **p<0.01 ***p<0.001

As with emphysema I assessed the amount of gas trapping affecting the upper lobes and lower lobes. For the purposes of this analysis the upper lobes were defined as the RUL, RML and LUL and the lower lobes the LLL and RLL. The Median value for E/I MLD in the upper lobes was 0.927 (0.066) and in the lower lobes was 0.904 (0.088) and this difference was statistically significant (p 0.001). The ratio of the upper lobe E/I MLD to the lower lobe E/I MLD was calculated and results are presented in Table 124. There was no significance difference in this ratio between the different GOLD groups (p=0.214).

Table 124. Upper/lower lobe E/I MLD ratio in different GOLD groups

	Upper lobe /lower lobe E/I MLD
Whole	1.03 (0.05)
Moderate	1.04 (0.06)
Severe	1.03 (0.05)
V. Severe	1.02 (0.05)

Next, I divided the cohort the cohort into three equal groups depending on their upper lobe E/I MLD to lower lobe E/I MLD. The first group had a ratio below 1.02 and represented those with either lower lobe predominant disease or no difference between the upper or lower lobes. The second group had a ratio between 1.0202 and 1.0542 and represented mildly upper lobe

predominant gas trapping. The remaining group consisted of those with a ratio above 1.0542, which represented substantial upper lobe predominant gas trapping. There was significantly less overall gas trapping in the third tertile compared to the other two tertiles (Table 125). There was also significantly lower RV/TLC ratio in group three compared to the other two groups. PCT was significantly higher in tertile three compared to tertile 2.

Table 125. Pulmonary physiology, functional capacity, inflammatory markers and exacerbation frequency in tertiles ranked according to upper/lower lobe E/I MLD.

	Tertile 1	Tertile 2	Tertile 3	P value
Overall E/I MLD	0.92 (0.07)	0.93 (0.05)	0.90 (0.07)	**0.005****
Pulmonary Function				
FEV1%	43.6 (26.1)	44.0 (21.5)	53.4 (23.3)	0.111
TLCO%	66.0 (48.3)	58.8 (27.5)	57.6 (26.8)	0.470
RV/TLC	0.56 (0.16)	0.58 (0.14)	0.48 (0.14)	**<0.001*****
Functional				
6MWD	300.0 (185.0)	274.0 (157.0)	362.0 (185.0)	0.286
Oxygen Desaturation	4.0 (4.0)	3.0 (4.0)	4.0 (7.0)	0.479
CAT	15.5 (10.0)	17.0 (9.0)	16.0 (13.0)	0.913
EXACT	36.0 (11.0)	37.0 (14.0)	36.0 (14.0)	0.823
Sputum Differential				
Neutrophils%	51.0 (75.6)	37.5 (75.2)	29.9 (67.2)	0.349
Macrophages%	37.7 (40.0)	29.6 (46.1)	32.2 (54.8)	0.408
Eosinophils%	1.78 (4.0)	1.95 (3.5)	2.6 (7.2)	0.657
Blood Inflammatory Markers				
CRP	5.0 (9.8)	4.0 (4.5)	5.0 (6.0)	0.271
Fibrinogen	5.05 (1.2)	4.85 (1.0)	4.70 (1.0)	0.224
Neutrophils	4.80 (1.7)	4.70 (1.7)	4.90 (1.7)	0.783
PCT	0.056 (0.03)	0.054 (0.03)	0.069 (0.03)	**0.012***
Exacerbations				
Exacerbation rate	2.99 (3.9)	3.0 (3.9)	1.4 (3.9)	0.289

Values represent medians and IQR. N=121 for FEV1%, N= 117 for TLCO%, N=113 for RV/TLC, N=120 for 6MWT, N =122 for CAT/EXACT, CRP and exacerbations, N=66 for sputum differential, N=121 for PCT and N=109 for fibrinogen. * p<0.05 **p<0.01 ***p<0.001

6.5 Bronchial Wall Dimensions

6.5.1 Pi10 Distribution in the cohort

Pi10 distribution is shown Figure 56. To investigate Pi10 further I divided the cohort into three equal tertiles according to Pi10; subjects with Pi10 <3.76, subjects with Pi10 between 3.76 and 3.83 and those with Pi10 over 3.83. When analysing these three groups there were very few differences in physiological, functional, clinical or biological parameters between them (Table 126). FEV1 reversibility and TLC% showed a significant difference between the group with the highest Pi10

compared to the intermediate Pi10 group, but not between other groups. CRP was significantly raised in the group with the highest Pi10 compared to the group with the lowest Pi10.

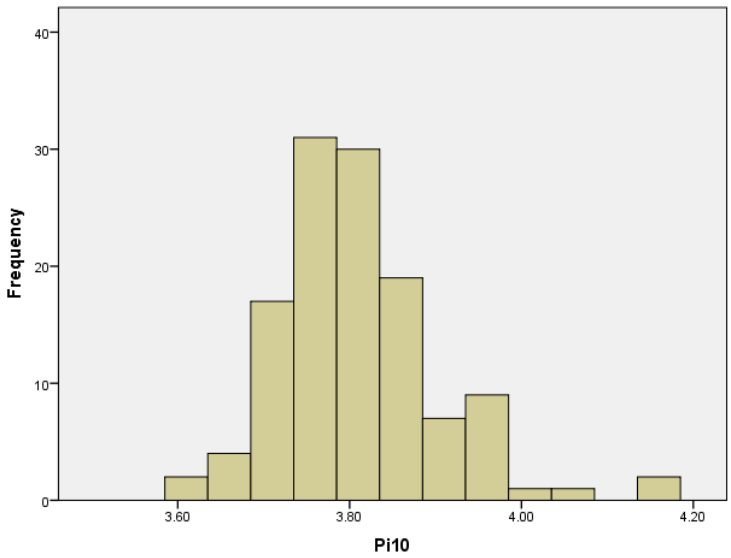


Figure 56. Distribution histogram for Pi10 in this cohort.

Table 126. Pulmonary physiology, functional capacity, inflammatory markers, biomarkers and exacerbation frequency in tertiles ranked according to Pi10 severity.

	<3.76	**3.76-3.83**	**>3.83**	**Significance**
Pulmonary Function				
FEV1%	46.5 (20.9)	46.1 (26.0)	46.7 (30.4)	0.608
FEV1 reversibility	10.4 (12.4)	7.3 (11.5)	15.8 (24.0)	**0.037***
TLCO%	55.9 (26.3)	59.0 (35.8)	60.1 (28.4)	0.615
Functional Capacity				
6MWD	325.5 (173.0)	305.5 (204.0)	274.0 (219.0)	0.384
Oxygen desaturation	4.5 (6.8)	4.0 (4.0)	3.0 (4.0)	0.396
CAT	16.0 (13.0)	17.5 (8.0)	14.0 (11.0)	0.397
EXCAT	36.5 (16.0)	37.0 (10.0)	34.0 (11.0)	0.303
Sputum Differential				
Neutrophils%	61.3 (69.4)	47.0 (68.7)	10.3 (76.6)	0.365
Macrophages%	28.0 (28.6)	35.5 (37.4)	38.3 (67.6)	0.454
Eosinophils%	2.62 (5.1)	1.84 (5.2)	1.64 (3.3)	0.398
Blood Inflammatory Markers				
CRP	4.0 (5.5)	5.0 (7.0)	6.0 (11.0)	**0.014***
Fibrinogen	4.7 (0.83)	5.0 (1.1)	5.0 (1.3)	0.094
Neutrophils	4.4 (1.7)	4.9 (1.5)	4.8 (1.9)	0.237
PCT	0.0545 (0.02)	0.0610 (0.04)	0.0650 (0.02)	0.123
Exacerbations				
Exacerbation rate	2.97 (4.5)	1.97 (3.6)	3.0 (3.9)	0.230

Values represent medians (IQR). N=122 for FEV1% and PCT, N=118 for TLCO% and hs-troponin, n=114 for all other lung function, N=121 for 6MWT, N=123 for CAT/EXACT scores, N= 67 for sputum differential, N=123 for CRP and exacerbation rate and N=110 for fibrinogen, N=86 for IL-6, N=120 for IP-10/Sp-D and N=119 for NT-proBNP. *p<0.05, **p<0.01, ***p<0.001 using independent samples Kruskal-Wallis test.

6.5.2 Segmental generation Analysis

As discussed in the previous chapter, Pi10 may not reflect the complexity of the bronchial tree and in this cohort it has shown few associations with markers of disease severity or activity. I therefore performed further analysis on bronchial wall dimensions by investigating these in different segmental generations. The number of airways measured in each segmental generation and the number of subjects these were taken from are shown in Table 127. All subjects contributed to measurements of segmental generation 3 and 4 airways. Fewer subjects contributed to measurements for airways more distal to this. This is especially the case for segmental generation 7 where only 91 subjects contributed to airway measurements and for this reason I decided to perform the analysis up to segmental generation 6 airways. I demonstrated in the previous chapter that the number of airways measured at each segmental generation was important and could bias results. I therefore tested this again by assessing the association between the number of airways measured and FEV1%. There were weak but significant positive correlations between FEV1% and number of airways measured in segmental generation 4, 5 and 6.

Table 127. Number of airways measured in each segmental generation and the association with FEV1%.

Segmental Generation	Number of Subjects airways measured	Number of airways measured	Association between airways measured and FEV1
3	123	19.0 (0.0)	0.08
4	123	10.0 (2.0)	0.22*
5	122	14.0 (6.0)	0.24**
6	117	11.0 (11.0)	0.21*
7	91	4.0 (8.0)	-
8	49	4.0 (4.0)	-
9	21	3.0 (3.0)	-
10	5	2.0 (1.0)	-
11	2	2.0 (0.0)	-

Number of airways given as median and IQR. Spearman's association between number of airways measured and FEV1%. *p<0.05, **p<0.001.

In the previous section I measured seven different airway dimensions, however I demonstrated this was unnecessary as some of these measurements did not add additional information. In this study I therefore chose to reduce this to five measurements. Median values for airway dimensions in the different segmental generations are shown in Table 128.

Table 128. Airway Dimensions in different segmental generations.

Segmental Generation	3	4	5	6
Wall Thickness	1.50 (0.20)	1.19 (0.18)	1.04 (0.15)	0.99 (015)
Lumen Area	17.1 (7.2)	8.2 (4.1)	5.5 (2.3)	4.9 (1.7)
Wall Area	30.4 (9.3)	17.7 (6.0)	12.7 (3.9)	12.0 (2.9)
Total wall area	47.9 (16.0)	25.8 (10.3)	18.1 (5.7)	17.1 (4.0)
WA%	64.1 (3.4)	67.1 (3.1)	68.6(2.4)	69.2 (3.0)

Values given as medians and IQR. N=123 in generation 3 and 4, N= 122 in generation 5 and N= 117 in generation 6.

Next I assessed the association between airway dimensions in the different segmental generations and FEV1% (Table 129). There were no associations between FEV1% and airway dimensions in segmental generation 3 or 4 airways. In segmental generation 5 airways there were significant but weak negative associations between FEV1% and wall thickness, wall area and total airway area. For segmental generation 6 airways there were also weak significant associations between FEV1% and lumen area and wall area %. When partial correlation was used to correct for the number of airways measured, results did not change substantially for generation 3, 4 and 6 (Table 130). However in segmental generation 5 a weak significant association between FEV1% and lumen area became apparent.

Table 129. Association between Airway Dimensions in different segmental generations and FEV1%

	Gen.3	Gen.4	Gen.5	Gen. 6
Wall Thickness	0.01	-0.08	**-0.23***	0.05
Lumen area	-0.02	-0.02	-0.16	**-0.22***
Wall area	-0.03	-0.04	**-0.25****	-0.09
Total Airway Area	-0.03	-0.04	**-0.21***	-0.16
Wall area %	-0.01	0.07	0.05	**0.24****

Spearman's correlation. N=122 in generation 3 and 4, N= 121 in generation 5 and N= 116 in generation 6. *p<0.05, **p<0.001.

Table 130. Association between Airway Dimensions in different segmental generations and FEV1% when correcting for number of airways measured.

	Gen 3	Gen 4	Gen.5	Gen. 6
Wall Thickness	0.02	-0.09	**-0.25****	0.04
Lumen area	-0.02	-0.05	**-0.18***	**-0.26***
Wall area	-0.02	-0.06	**-0.27****	-0.11
Total Airway Area	-0.03	-0.06	**-0.23***	-0.18
Wall area %	-0.01	0.10	0.10	**0.30****

Spearman's partial correlation correcting for number of airways. N=122 in generation 3 and 4, N= 121 in generation 5 and N= 116 in generation 6. *p<0.05, **p<0.001.

I then went on to assess the association between these airway measurements in the different segmental generations and a number of functional and biological markers of disease. There was no association between airway dimensions and 6MWD in segmental generation 3 and 4 (Table 131). In generation 5 there was a weak association between wall area and 6MWD and in generation 6 there was a weak association between lumen area and 6MWD. I did not perform partial correlation as this made little difference to the results in the FEV1% analysis.

Table 131. Association between Airway Dimensions in different segmental generations and 6MWD.

	Gen.3	**Gen.4**	**Gen.5**	**Gen. 6**
Wall Thickness	-0.00	0.02	-0.13	-0.05
Lumen area	0.01	-0.01	-0.16	**-0.20***
Wall area	0.00	0.03	**-0.18***	-0.12
Total Airway Area	0.01	0.00	-0.17	-0.16
Wall area %	0.00	0.01	0.05	0.18

Spearman's partial correlation correcting for number of airways. N=121 in generation 3 and 4, N= 120 in generation 5 and N= 115 in generation 6. *p<0.05, **p<0.001.

When testing the associations between sputum neutrophils and airway dimensions there were significant positive associations between all of these in segmental generation 5 airways (Table 132). In segmental generation 3 airways there was a significant association between wall area and sputum neutrophils and in generation 6 there was a negative association between sputum neutrophils and WA%. In generation 4 there were significant associations between lumen area and WA%.

Table 132. Association between Airway Dimensions in different segmental generations and sputum neutrophils.

	Gen.3	**Gen.4**	**Gen.5**	**Gen. 6**
Wall Thickness	0.22	0.14	**0.31****	0.04
Lumen area	0.18	**0.27***	**0.30***	0.16
Wall area	**0.23***	0.21	**0.32****	0.15
Total Airway Area	0.21	0.22	**0.29***	0.17
Wall area %	-0.11	**-0.24***	**-0.29***	**-0.26***

Spearman's partial correlation correcting for number of airways. N=67 in generation 3 and 4, N=66 in generation 5 and N= 61 in generation 6. *p<0.05, **p<0.001.

The only association between airway dimensions and CAT score was in generation 5 where there was a negative association with WA% (Table 133). For associations with CRP there was only a weak association with wall thickness in generation 3 and no other significant associations (Table 134). There were no associations between airway wall dimensions and exacerbation rate (Table 135).

Table 133. Association between Airway Dimensions in different segmental generations and CAT score.

	Gen.3	Gen.4	Gen.5	Gen. 6
Wall Thickness	0.05	-0.00	-0.03	0.08
Lumen area	0.08	0.04	0.14	0.15
Wall area	0.05	0.00	0.08	0.06
Total Airway Area	0.07	0.03	0.11	0.12
Wall area %	-0.03	-0.07	**-0.21***	-0.16

Spearman's partial correlation correcting for number of airways. N=123 in generation 3 and 4, N= 122 in generation 5 and N= 117 in generation 6. *p<0.05, **p<0.001.

Table 134 Association between Airway Dimensions in different segmental generations and CRP.

	Gen.3	Gen.4	Gen.5	Gen. 6
Wall Thickness	**0.18***	0.08	0.05	0.09
Lumen area	0.01	0.07	-0.00	0.03
Wall area	0.11	0.09	0.07	0.07
Total Airway Area	0.07	0.07	0.06	0.04
Wall area %	0.12	0.02	0.01	0.03

Spearman's partial correlation correcting for number of airways. N=123 in generation 3 and 4, N= 122 in generation 5 and N= 117 in generation 6. *p<0.05, **p<0.001.

Table 135. Association between Airway Dimensions in different segmental generations and exacerbations.

	Gen.3	Gen.4	Gen.5	Gen. 6
Wall Thickness	0.06	-0.02	-0.01	-0.09
Lumen area	-0.03	0.05	0.07	0.06
Wall area	0.04	0.02	0.05	-0.06
Total Airway Area	0.02	0.03	0.08	-0.03
Wall area %	0.07	-0.11	-0.11	-0.16

Spearman's partial correlation correcting for number of airways. N=123 in generation 3 and 4, N= 122 in generation 5 and N= 117 in generation 6. *p<0.05, **p<0.001.

6.5.3 Regional Variation of Bronchial wall Dimensions

Next I assessed airway dimensions in five different airway paths in the 3rd to 6th segmental generation (Table 136). There were significant differences between all airway dimensions in the different airway paths (p<0.001, apart from for wall thickness in generation 6 where p= 0.001 and WA% in segmental generation 6 where p=0.009) apart from WA% in generation 5 (p=0.082).

Table 136. Number of airways measured and airway wall dimensions in different segmental generations in 5 airway paths.

	RB1	RB4	RB10	LB1	LB10
Segmental airway	N=121	N=119	N=123	N=123	N=121
No. Airways	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
Wall Thickness	1.56 (0.21)	1.36 (0.27)	1.50 (0.27)	1.37 (0.27)	1.53 (0.25)
Lumen area	17.6 (10.4)	13.2 (5.4)	17.2 (11.1)	12.9 (6.2)	20.3 (11.3)
Wall Area	32.3 (12.1)	25.1 (8.8)	31.1 (13.1)	24.4 (10.6)	33.8 (11.2)
Total Airway area	50.4 (22.8)	37.6 (13.9)	48.5 (23.0)	37.7 (16.8)	53.4 (22.7)
WA%	64.0 (5.8)	65.2 (3.9)	64.0 (5.9)	65.5 (4.3)	62.8 (6.0)
Generation 4	N=113	N=114	N=114	N=115	N=114
No. Airways	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)
Wall Thickness	1.25 (0.22)	1.06 (0.22)	1.23 (0.24)	1.14 (0.22)	1.25 (0.27)
Lumen area	9.8 (6.4)	7.4 (3.3)	10.5 (6.2)	7.4 (3.8)	11.7 (6.5)
Wall Area	19.8 (8.1)	15.0 (5.4)	20.2 (8.5)	16.1 (6.5)	21.4 (8.8)
Total Airway area	29.6 (14.2)	22.2 (8.4)	31.1 (13.3)	23.5 (10.5)	33.7 (14.8)
WA%	66.9 (4.4)	67.3 (3.0)	66.1 (5.1)	67.9 (3.9)	65.6 (3.9)
Generation 5	N=112	N=102	N=100	N=95	N=107
No. Airways	4.0 (0.0)	2.5 (2.0)	4.0 (2.0)	3.0 (2.0)	3.0 (2.0)
Wall Thickness	1.0 (0.2)	0.96 (0.23)	1.05 (0.28)	0.98 (0.23)	1.17 (0.26)
Lumen area	5.8 (3.3)	5.1 (1.8)	6.2 (3.3)	5.4 (2.1)	7.7 (4.3)
Wall Area	12.8 (6.0)	11.5 (3.5)	13.3 (6.4)	11.6 (3.8)	16.1 (7.1)
Total Airway area	18.2 (9.7)	16.8 (5.2)	19.7 (9.8)	17.3 (5.8)	23.6 (11.5)
WA%	69.1 (4.1)	68.3 (3.7)	68.0 (3.6)	68.8 (2.4)	67.7 (3.5)
Generation 6	N=88	N=90	N=81	N=59	N=93
No. Airways	4.0 (4.0)	2.0 (2.0)	4.0 (3.0)	3.0 (3.0)	4.0 (2.0)
Wall Thickness	0.96 (0.18)	0.97 (0.20)	1.07 (0.19)	0.99 (0.22)	1.09 (0.24)
Lumen area	4.6 (1.8)	4.8 (1.8)	5.8 (2.6)	5.2 (2.0)	6.6 (3.4)
Wall Area	11.0 (3.0)	11.1 (3.4)	13.4 (4.5)	11.8 (3.0)	13.9 (5.9)
Total Airway area	15.7 (4.4)	16.2 (4.3)	19.2 (6.9)	17.4 (4.7)	20.9 (8.8)
WA%	69.5 (3.6)	69.7 (3.8)	68.8 (3.3)	69.7 (3.7)	68.4 (4.3)

Values given as medians and IQR.

Next I assessed the association between FEV1% and these airway dimensions in the different airway paths in each segmental generation. In segmental generation 3 (Table 137), 4(Table 138) and 5 (Table 139) there were no significant associations between FEV1% and airway dimensions. In generation 6 there were relatively weak associations between FEV1% and wall area in RB1 and WA% in LB1 (Table 140).

Table 137. Association between FEV1% and away dimensions in 5 airway paths in segmental generation 3.

	RB1	RB4	RB10	LB1	LB10
Wall Thickness	0.08	-0.01	-0.02	0.07	-0.00
Lumen area	0.06	-0.04	0.03	0.13	-0.10
Wall area	0.05	-0.02	0.02	0.11	-0.06
Total Airway Area	0.06	-0.03	0.02	0.12	-0.09
Wall area %	-0.07	0.05	-0.08	-0.12	0.11

Spearman's correlation.

Table 138. Association between FEV1% and away dimensions in 5 airway paths in segmental generation 4.

	RB1	RB4	RB10	LB1	LB10
Wall Thickness	0.04	-0.04	-0.08	-0.04	0.01
Lumen area	0.02	-0.17	-0.09	-0.13	-0.02
Wall area	0.02	-0.12	-0.11	-0.13	0.02
Total Airway Area	0.02	-0.14	-0.11	-0.14	0.01
Wall area %	0.02	0.07	0.07	0.10	0.01

Spearman's correlation.

Table 139. Association between FEV1% and away dimensions in different airway paths in segmental generation 5.

	RB1	RB4	RB10	LB1	LB10
Wall Thickness	-0.14	0.07	0.01	0.15	-0.05
Lumen area	0.01	-0.06	-0.07	0.15	-0.14
Wall area	-0.07	0.03	0.00	0.12	-0.11
Total Airway Area	-0.04	-0.01	-0.02	0.13	-0.12
Wall area %	-0.10	0.15	0.12	-0.05	0.16

Spearman's correlation.

Table 140. Association between FEV1% and away dimensions in different airway paths in segmental generation 6.

	RB1	RB4	RB10	LB1	LB10
Wall Thickness	-0.19	0.08	-0.11	0.01	0.07
Lumen area	-0.09	0.03	-0.05	-0.25	0.02
Wall area	**-0.22***	0.00	-0.06	-0.14	0.09
Total Airway Area	-0.19	-0.03	-0.05	-0.15	0.06
Wall area %	0.08	0.01	-0.05	**0.37****	0.11

Spearman's correlation. *p<0.05, **p<0.005.

6.6 Bronchiectasis

In this study, bronchiectasis was identified by a thoracic radiologist and a visual score was used to quantitate it. 10 (7.9%) subjects out of 127 had bronchiectasis present on their enrolment scan.

Subjects with bronchiectasis had significantly higher RV%, sputum relative neutrophil count, blood neutrophils and blood Il-6 than subjects without bronchiectasis (Table 141). In addition subjects with bronchiectasis had lower sputum relative macrophage and eosinophil counts and there was a trend towards lower FEV1% and raised CRP.

Table 141. Differences in pulmonary physiology, functional status, inflammation, CT markers, biomarkers and exacerbation rate between subjects with and without bronchiectasis.

	Bronchiectasis N=10	**No Bronchiectasis N=117**	**P Value**
Pulmonary Function			
FEV1%	39.2 (17.7)	47.7 (24.4)	0.057
TLCO%	62.0 (51.7)	57.7 (28.7)	0.788
TLC%	113.6 (23.0)	104.3 (22.2)	0.102
RV%	183..9 (68.0)	141.3 (3)	**0.025***
6MWT			
6MWD	300.0 (114)	325.0 (175)	0.510
Oxygen desat	4.0 (3.5)	4.0 (6.0)	0.954
CAT	16.5 (10.0)	16.0 (10.0)	0.542
EXACT	38.5 (19.0)	36.0 (12.0)	0.632
Sputum			
% neutrophils	90.9 (22.6)	30.8 (67.2)	**<0.001*****
%macrophages	2.8 (2.0)	35.1 (35.5)	**<0.001*****
%eosinophils	0.2 (0.4)	2.1 (5.1)	**0.006****
%lymphocytes	0.26 (0.75)	0.20 (0.60)	0.387
Blood			
CRP	10.0 (14.8)	5.0 (7.0)	0.059
Neutrophils	5.4 (1.6)	4.8 (1.6)	**0.032***
Eosinophils	0.2 (0.2)	0.2 (0.2)	0.900
Fibrinogen	5.0 (0.8)	4.8 (1.1)	0.183
PCT	0.062 (0.04)	0.059 (0.03)	0.814
CT			
%LAA$_{<-950}$	21.1 (24.0)	11.5 (20.7)	0.126
E/I MLD	0.95 (0.05)	0.92 (0.07)	0.052
Pi10	3.80 (0.08)	3.79 (0.13)	0.743
Biomarkers			
hs-troponin	0.016 (0.01)	0.012 (0.01)	0.205
IL-6	7.8 (21.7)	3.7 (2.5)	**0.005****
IP-10	133.5 (86.7)	145.0 (99.5)	0.259
NT-proBNP	117.0 (237.9)	91.0 (161.7)	0.560
Sp-D	178.5 (138.7)	166.0 (103.0)	0.912
Exacerbations			
Exacerbation Rate	2.48 (3.3)	2.94 (3.95)	0.501

Values given as medians (IQR). For FEV1%, white cell counts and fibrinogen N = 126, for gas transfer, E/I MLD and NT-proBNP n=122, for plethysmography n=116, for 6MWD n = 125 subjects, for exacerbations, CRP and questionnaires N=127, for sputum cell differential N=69, for PCT N=114, N=121 for hs-troponin, N=88 for IL-6, N=123 for %LAA$_{<-950}$, Pi10 and IP-10/Sp-D.

Next I assessed the rates of bacterial detection in sputum from subjects with and without bronchiectasis. When doing this there was no significant difference between detection of PPMs in

either of the groups using traditional culture or PCR techniques (Table 142). There was also no difference in detection of the specific bacteria (NTHI, MCAT, SP, SA or PA) between subjects with and without bronchiectasis.

Table 142. Bacteria detected in sputum using culture or PCR in subjects with and without bronchiectasis.

	Bronchiectasis N=10	**No Bronchiectasis N=100**	**P Value**
Culture			
PPM +ve	7	50	0.324
NTHI +ve	5	27	0.151
SP +ve	1	18	>0.999
MCAT +ve	0	8	>0.999
SA +ve	1	5	0.433
PA +ve	1	5	0.433
PCR	**N=9**	**N=93**	
PPM +ve	6	57	>0.999
NTHI +ve	6	39	0.178
SP +ve	0	14	0.355
MCAT +ve	1	13	>0.999
SA +ve	1	6	0.487
PA +ve	1	6	0.487

Values are number of samples with bacteria detected in sputum. P value calculated using Fishers exact test. PPM - Potentially pathogenic microorganism, SP - streptococcus Pneumoniae, MCAT- Moraxella catarrhalis, NTHI- non-typeable Haemophilus influenzae.

The 9 subjects with bronchiectasis had the severity of bronchiectasis visually graded. 4 of the subjects scored 1, 2 scored 2, 1 scored 3 and 3 scored 8. As there were so few subjects with bronchiectasis, meaningful further analysis on bronchiectasis severity could not be performed.

6.7 Combined analysis

Up to now I have treated the different CT parameters as separate features and assessed their association with disease parameters in isolation. However, these morphological features are not isolated features and are intertwined with one another to contribute to clinical features. I therefore assessed the relationship between these three main morphological features and the scatterplots are shown in Figure 57. There was a significant positive association between $\%LAA_{<-950}$ and E/I MLD (rho 0.47***, p<0.001). There was also an inverse relationship between $\%LAA_{<-950}$ and Pi10 (rho -0.36***, p<0.001) and a much weaker association between E/I MLD and Pi10 (rho 0.18*, p 0.045).

Throughout this study it has been apparent that measures of bronchial wall dimensions have had few associations with physiological, functional and biological parameters. Therefore, when trying to define subtypes based on CT measures I excluded this variable. Instead I focused on emphysema and gas trapping. There is no agreed cut-off for either of these features and so I dichotomised them both around the median. Four groups were made; group 1 had low $\%LAA_{<-950}$ and low E/I MLD, group 2 had high $\%LAA_{<-950}$ and low E/I MLD, group 3 had low $\%LAA_{<-950}$ and high E/I MLD and group 4 had high LAA and high E/I MLD. Next I assessed a multitude of variables in each of these groups (Table 143). FEV1% was significantly lower in group 4 compared to the other three groups. TLCO% was significantly lower in group 4 that group3 or group 1. RV% was significantly higher in group 3 and 4 compared to group1 and 2. TLC% was also significantly higher in group 4 compared to group 1 and 2. There was no difference in sputum cell differential between the four groups. There was however significantly lower CRP in group 4 compared to group 1 and higher troponin in group four compared to group 1 and in group 2 compared to group 1. There were no significant differences between functional variables and exacerbation rate between the groups.

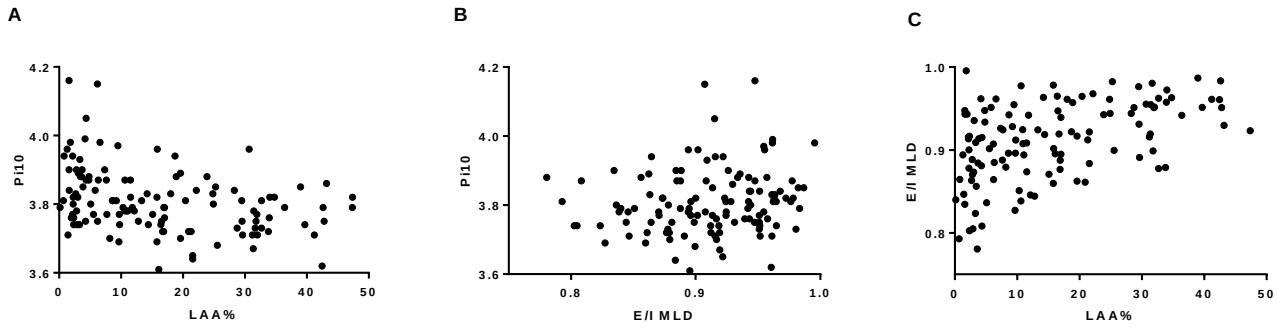


Figure 57. Scatterplots of (A) $\%LAA_{<-950}$ against Pi10 (B) E/I MLD against Pi10 (C) $\%LAA_{<-950}$ against E/I MLD

Table 143. Differences in pulmonary physiology, functional status, inflammation, biomarkers and exacerbation rate between subjects in the four groups.

	Group 1 N=42	Group 2 N=19	Group 3 N=19	Group 4 N=42	P Value
Pulmonary Function					
FEV1%	57.0 (20.4)	53.6 (16.6)	46.4 (14.3)	32.3 (12.7)	**<0.001*** **
TLCO%	68.8 (21.0)	56.0 (22.4)	71.8 (38.9)	47.2 (19.1)	**<0.001*** **
TLC%	99.8 (19.0)	102.6 (25.1)	106.9 (13.0)	120.0 (28.4)	**<0.001*** **
RV%	128.6 (44.7)	126.3 (36.3)	151.2 (33.6)	181.5 (69.3)	**<0.001*** **
6MWT					
6MWD	323.5 (166.0)	324.0 (177.0)	311.0 (165.0)	259.5 (208.0)	0.426
Oxygen desat	3.0 (4.0)	5.0 (6.0)	2.0 (1.0)	6.5 (6.0)	**<0.001*** **
CAT	17.5 (11.0)	16.0 (15.0)	13.5 (7.0)	19.0 (10.0)	0.113
EXACT	36.0 (11.0)	33.0 (10.0)	37.0 (17.0)	38.0 (11.0)	0.083
Sputum					
% neutrophils	10.3 (63.0)	53.8 (69.7)	44.3 (70.6)	55.6 (58.4)	0.253
%macrophages	46.4 (51.9)	32.1 (37.3)	35.3 (50.7)	26.8 (28.8)	0.140
%eosinophils	1.93 (3.3)	2.41 (5.6)	0.75 (6.7)	2.77 (5.0)	0.557
Blood					
CRP	6.0 (7.3)	6.0 (7.0)	4.0 (12.0)	2.5 (5.5)	**0.002****
Neutrophils	4.9 (1.3)	4.8 (1.7)	4.7 (1.4)	4.5 (1.9)	0.569
Eosinophils	0.20 (0.20)	0.20 (0.20)	0.20 (0.20)	0.20 (0.30)	0.845
PCT	0.056 (0.04)	0.066 (0.02)	0.061 (0.02)	0.0566 (0.03)	0.554
Fibrinogen	5.0 (1.1	4.7 (0.9)	5.1 (0.8)	4.7 (1.5)	0.088
Biomarkers					
hs-troponin	0.009 (0.01)	0.013 (0.01)	0.011 (0.01)	0.016 (0.01)	**<0.001*** **
IL-6	4.3 (3.6)	3.1 (1.6)	3.5 (2.3)	4.0 (2.6)	0.330
IP-10	139.0 (92.4)	138.0 (128.0)	129.0 (132.5)	150.5 (87.3)	0.253
NT-proBNP	124.0 (204.0)	61.8 (75.3)	76.3 (153.3)	100.6 (270.2)	0.194
Sp-D	161.0 (96.5)	179.0 (110.0)	166.0 (109.5)	177.0 (119.8)	0.904
Exacerbations					
Exacerbation Rate	1.92 (2.99)	1.99 (4.97)	2.98 (4.99)	2.99 (3.39)	0.508

Values represent medians and IQR. N=121 for FEV1%, N= 117 for TLCO%, N=113 for RV/TLC, N=120 for 6MWT, N =122 for CAT/EXACT, CRP and exacerbations, N=66 for sputum differential, N=121 for PCT and N=109 for fibrinogen. *p<0.05 **p<0.01 ***p<0.001 using independent samples Kruskal-Wallis test.

6.8 Body Composition

CT can also provide significant information about non-pulmonary manifestations of COPD. Using novel software, I analysed pectoralis muscle area (PMA) and anterior chest sub-cutaneous fat (ACF). In this study a number of traditional and more advance body composition markers were also measured. CT analysis of body composition was successfully achieved in 122 subjects with median values for CT and other measures of body composition shown in Table 144. Traditionally body composition has been analysed separately in the different genders and median values for these measures in the two different genders are also shown in Table 144. Of the CT parameters there was significantly increased anterior chest fat in females compared to males. There was significantly increased pectoralis muscle area and lean pectoralis muscle area in males compared to female. Of

the more traditional measures of body composition males were significantly taller, heavier and had a higher waist circumference compared to females. Grip strength and fat free mass index were significantly higher in males. There was no significant difference in BMI between males and females in this cohort. As these results confirm that body composition measures are significantly different between sexes the rest of the analysis will be performed separately for each gender.

Table 144. Traditional and CT measures of body composition in the whole cohort and individual genders.

	Whole cohort	Males	Females	P value
CT Measures				
Anterior subcut. fat	5018.5 (3688.6)	4078.9 (2147.8)	6407.5 (4130.5)	**<0.001*** **
Lean Pectoralis muscle area	2654.0 (1105.8)	3055.1 (1174.2)	2296.1 (713.5)	**<0.001*** **
Pectoralis muscle area	2781.9 (1184.7)	3162.2 (1213.1)	2399.4 (744.3)	**<0.001*** **
Traditional Measures				
Height	167.0 (14.7)	173.5 (11.2)	161.0 (6.3)	**<0.001*** **
Weight	77.5 (25.6)	84.2 (21.3)	67.7 (19.5)	**<0.001*** **
Grip Strength	235.0 (109.5)	276.6 (113.6)	182.9 (83.8)	**<0.001*** **
BMI	27.0 (6.7)	27.5 (5.5)	26.8 (7.4)	0.387
FFMI	48.9 (21.5)	60.7 (11.3)	39.9 (8.4)	**<0.001*** **
Waist circumference	103.6 (19.8)	107.2 (18.0)	97.3 (22.9)	**0.003** **
Triceps skin fold	17.9 (11.8)	13.8 (11.3)	20.6 (10.4)	**0.004** **

Values represent medians and IQR. N=122 for CT measures of body composition, N=127 for height, weight and BMI, N=124 for waist circumference and triceps skin fold, N=125 for FFMI and impedance and N=110 for grip strength. *p<0.05 **p<0.01 ***p<0.001 between gender using Mann-Whitney U test.

The measures were compared with one another. In males there was no association between ACF and lean PMA (rho 0.11, p 0.382) or PMA (rho 0.17, p 0.71), but there was a strong association between lean PMA and PMA (rho 0.98, p<0.001). In females there was an association between ACF and PMA (rho 0.33, p 0.012) but not with lean PMA (rho 0.25, p 0.067). There was a strong association between lean PMA and PMA (rho 0.99, p<0.001). As the relationships were so strong between lean PMA and PMA I decided to use PMA in the further analysis.

Next the CT parameters were compared to the other measures of body composition. In males ACF had significant positive associations with weight, BMI, FFMI, waist circumference and triceps skin fold (Table 145). The associations for ACF in females were very similar (Table 146). In males PMA had significant positive associations with weight, BMI, FFMI and waist circumference. There were no significant associations between PMA and height, grip strength or triceps skin fold. Associations for PMA/lean PMA in females were once again very similar.

Table 145. Association between CT and traditional measures of body composition in males

	Anterior chest fat	Pectoralis muscle
Height	0.13	0.18
Weight	**0.79*** **	**0.42** **
Grip Strength	-0.10	0.07
BMI	**0.84*** **	**0.40** **
FFMI	**0.64*** **	**0.42** **
Waist circumference	**0.78*** **	**0.31***
Triceps skin fold	**0.61*** **	0.14

Spearman's correlation. N=66 for height, weight, BMI, triceps skin fold. N=65 for waist circumference and FFMI. N=59 for grip strength. *p<0.05 **p<0.01 ***p<0.001.

Table 146. Association between CT and traditional measures of body composition in males

	Anterior chest fat	Pectoralis muscle
Height	0.13	0.17
Weight	**0.85*** **	**0.45** **
Fatigability	-0.07	**-0.09**
Grip Strength	0.14	-0.04
BMI	**0.89*** **	**0.42** **
FFMI	**0.63*** **	**0.47*** **
Waist circumference	**0.88*** **	**0.41** **
Impedance value	**-0.41** **	**-0.39** **
Triceps skin fold	**0.58*** **	**0.33***

Spearman's correlation. N=56 for height, weight and BMI. BMI, triceps skin fold. N=55 for waist circumference and FFMI. N=53 for triceps skin fold. N=46 for grip strength. *p<0.05 **p<0.01 ***p<0.001.

Next I assessed these CT measures of body composition against a number of clinical, physiological and biological measures of disease severity. In males there were significant associations between both lean PMA and PMA and FEV1%, TLCO% and LAA% (Table 147). There were no associations between PMA and TLC, RV, gas trapping, sputum differential count, blood inflammatory markers or exacerbation rate. ACF in males had significant associations with TLC%, RV%, $\%LAA_{<-950}$, E /I MLD and CRP. These associations were similar in females with ACF having significant associations with TLCO%, TLC%, RV%, LAA%, E/I MLD and Pi10 (Table 149). PMA had significant associations with TLC%, RV%, LAA% and E/I MLD.

When using a regression model to predict ACF in males, $\%LAA_{<-950}$, E/I MLD and FEV1% all significantly predicted ACF (Table 148). Variables that did not make any significant difference to the model were age, smoking status and smoking history. In females $\%LAA_{<-950}$ and E/I MLD significantly predicted ACF while FEV1% and the other variables listed for males made no difference to the model (Table 150). No variables significantly predicted PMA in either male or females.

Table 147. Association between CT body composition and clinical, physiological and biological measures of disease severity in males.

	Anterior chest wall fat	Pectoralis muscle area
Pulmonary Function		
FEV1%	0.22	**0.33***
TLCO%	0.16	**0.46*****
CT parameters		
LAA	**-0.43*****	**-0.38****
E/I MLD	**-0.54*****	-0.18
Pi10	0.14	0.15
Functional Capacity		
6MWD	-0.13	0.24
Oxygen desaturation	-0.09	-0.17
CAT	-0.05	-0.13
EXCAT	-0.06	-0.18
Sputum Differential		
Neutrophils%	-0.24	-0.03
Macrophages%	0.15	0.11
Eosinophils%	-0.12	-0.09
Blood Inflammatory Markers		
CRP	**0.29***	-0.05
Fibrinogen	0.04	-0.09
Neutrophils	0.15	-0.19
PCT	0.22	-0.16
Exacerbations		
Exacerbation rate	-0.06	-0.11

Spearman's correlation. N=66 for FEV1%, 6MWT, questionnaires, $\%LAA_{<-950}$, Pi10, CRP, PCT and exacerbations. N=65 for TLCO%, E/I MLD and serum neutrophils. N=58 for fibrinogen and N=36 for sputum cell differential. *p<0.05 **p<0.01 ***p<0.001.

Table 148. Multiple regression analysis to predict anterior chest fat in males.

	B coefficient	Standardised B coefficient	significance
ACF			
LAA%	**-62.0**	**-0.39**	0.003
E/I MLD	**-26056.8**	**-0.60**	<0.001
FEV1%	**-58.2**	**-0.43**	0.003

Table 149. Association between CT body composition and clinical, physiological and biological measures of disease severity in females.

	Anterior chest wall fat	Pectoralis muscle area
Pulmonary Function		
FEV1%	0.19	0.24
TLCO%	**0.28***	0.16
CT parameters		
LAA	**-0.52*****	**-0.43****
E/I MLD	**-0.42****	**-0.27***
Pi10	**0.28***	0.16
Functional Capacity		
6MWD	-0.16	0.06
Oxygen desaturation	-0.25	-0.18
CAT	-0.02	0.08
EXCAT	-0.05	-0.12
Sputum Differential		
Neutrophils%	-0.04	-0.34
Macrophages%	-0.05	0.07
Eosinophils%	-0.14	0.05
Blood Inflammatory Markers		
CRP	0.26	0.12
Fibrinogen	0.10	0.12
Neutrophils	-0.00	-0.06
PCT	0.21	0.04
Exacerbations		
Exacerbation rate	-0.14	0.04

Spearman's correlation. N=56 for CRP, neutrophils, PCT and exacerbations. N=55 for FEV1% and questionnaires. N=54 for 6MWT. N=52 for $\%LAA_{<-950}$, Pi10 and E/I MLD. N=51 for fibrinogen. N=49 for TLCO%. N=31 for sputum differential. *p<0.05 **p<0.01 ***p<0.001.

Table 150. Multiple regression analysis to predict anterior chest fat in females.

	B coefficient	Standardised B coefficient	significance
ACF			
LAA%	**-78.5**	**-0.30**	0.043
E/I MLD	**-19437.3**	**-0.30**	0.043

6.8.1 Distribution of CT body composition

To further analyse the CT body composition measures I divided each measure into tertiles. The distribution histogram for ACF for males is shown in Figure 58 and for females in Figure 59. For males and females there were significantly higher emphysema and gas trapping in the tertiles with the lowest ACF compared to higher ACF (Table 151 and Table 152). In females only, Pi10 was raised in the highest tertile for ACF than the other two tertiles. In males only, CRP was significantly lower in the lowest tertile for ACF than the other tertiles.

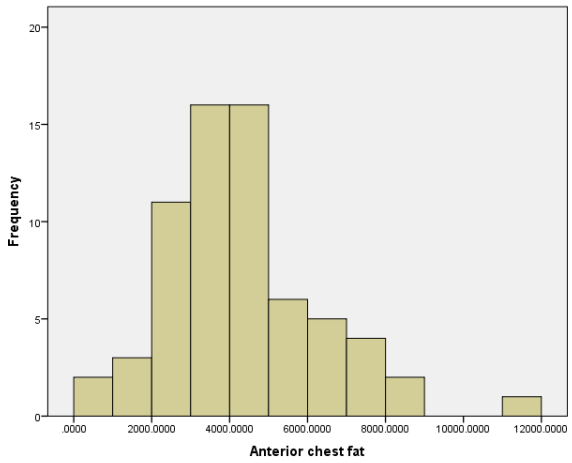


Figure 58. Distribution histogram for anterior chest fat in males.

Table 151. Pulmonary physiology, functional capacity, inflammatory markers, biomarkers and exacerbation frequency in tertiles ranked according to anterior chest fat in males.

	<3348.92	3348.92-4703.78	>4703.78	P value
Pulmonary Function				
FEV1%	42.1 (23.0)	43.0 (28.1)	49.8 (14.3)	0.396
TLCO%	53.4 (28.1)	58.1 (40.2)	59.3 (26.9)	0.820
CT parameters				
LAA	23.4 (18.3)	17.4 (25.0)	11.5 (10.1)	**0.033***
E/I MLD	0.95 (0.05)	0.90 (0.07)	0.88 (0.07)	**0.001****
Pi10	3.76 (0.10)	3.79 (0.08)	3.80 (0.14)	0.702
Functional Capacity				
6MWD	379.5 (210.0)	258.5 (207.0)	310.5 (224.0)	0.058
Oxygen desaturation	4.0 (4.0)	3.0 (3.0)	5.0 (6.3)	0.316
CAT	15.5 (13.0)	16.0 (13.0)	19.0 (14.0)	0.886
EXCAT	35.5 (15.0)	37.0 (8.0)	37.0 (13.0)	0.973
Sputum Differential				
Neutrophils%	48.7 (80.2)	58.9 (74.7)	12.9 (68.5)	0.475
Macrophages%	27.2 (42.9)	28.2 (28.5)	29.1 (24.7)	0.777
Eosinophils%	1.21 (8.7)	2.62 (5.5)	0.85 (4.6)	0.444
Blood Inflammatory Markers				
CRP	3.0 (5.0)	6.0 (9.3)	5.5 (6.3)	**0.036***
Eosinophils	0.20 (0.30)	0.30 (0.23)	0.20 (0.25)	0.181
Fibrinogen	4.8 (0.9)	4.9 (1.5)	4.9 (1.2)	0.810
Neutrophils	4.8 (1.7)	4.8 (1.7)	4.9 (1.1)	0.437
PCT	0.0542 (0.03)	0.0671 (0.04)	0.0661 (0.03)	0.059
Exacerbations				
Exacerbation rate	2.02 (3.5)	3.46 (4.5)	2.48 (3.9)	0.924

N=66 for FEV1%, 6MWT, questionnaires, $\%LAA_{<-950}$, Pi10, CRP, PCT and exacerbations. N=65 for TLCO%, E/I MLD and serum neutrophils. N=58 for fibrinogen and N=36 for sputum cell differential. *p<0.05 **p<0.01 ***p<0.001.

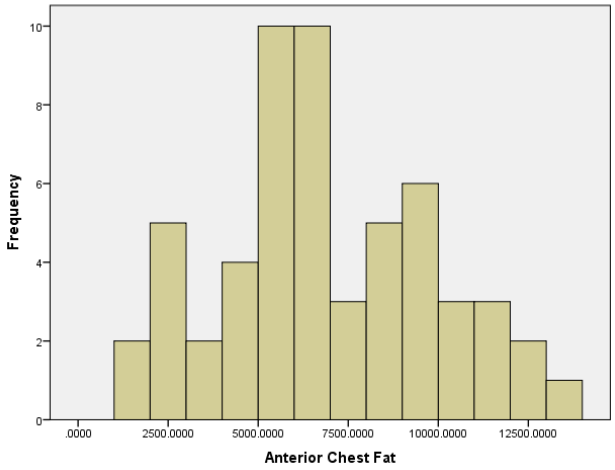


Figure 59. Distribution histogram for anterior chest fat in females.

Table 152. Pulmonary physiology, functional capacity, inflammatory markers, biomarkers and exacerbation frequency in tertiles ranked according to anterior chest fat in females.

	<5573.1	**5573.1-8648.0**	**>8648.0**	**P value**
Pulmonary Function				
FEV1%	39.8 (26.3)	49.8 (24.7)	47.8 (24.4)	0.262
TLCO%	50.0 (24.5)	62.0 (22.1)	65.4 (34.5)	0.061
CT parameters				
LAA	17.0 (24.9)	10.3 (16.9)	2.8 (2.7)	**<0.001*****
E/I MLD	0.95 (0.07)	0.92 (0.04)	0.90 (0.09)	**0.016***
Pi10	3.79 (0.12)	3.78 (0.09	3.87 (0.15)	**0.026***
Functional Capacity				
6MWD	300.0 (110.0)	316.0 (153.0)	272.0 (154.0)	0.280
Oxygen desaturation	7.0 (10.5)	4.0 (5.0)	2.5 (4.3)	0.066
CAT	16.0 (7.0)	17.0 (14.0)	17.5 (9.0)	0.969
EXCAT	38.0 (6.0)	37.0 (23.0)	36.0 (10.0)	0.719
Sputum Differential				
Neutrophils%	44.3 (47.9)	32.5 (76.2)	35.1 (65.4)	0.875
Macrophages%	46.2 (30.9)	14.2 (70.4)	45.4 (41.8)	0.513
Eosinophils%	1.64 (4.0)	1.39 (5.8)	1.74 (3.2)	0.776
Blood Inflammatory Markers				
CRP	3.0 (12.3)	4.5 (13.5)	7.0 (9.3)	0.153
Eosinophils	0.20 (0.20)	0.20 (0.15)	0.20 (0.20)	0.857
Fibrinogen	4.8 (1.4)	4.8 (0.8)	5.2 (1.0)	0.361
Neutrophils	5.15 (2.1)	4.35 (2.0)	4.85 (2.30)	0.313
PCT	0.044 (0.02)	0.0597 (0.04)	0.0560 (0.04)	0.128
Exacerbations				
Exacerbation rate	3.02 (3.3)	1.02 (2.8)	2.99 (4.5)	0.052

Values given as medians and IQR. N=56 for CRP, neutrophils, PCT and exacerbations. N=55 for FEV1% and questionnaires. N=54 for 6MWT. N=52 for $\%LAA_{<-950}$, Pi10 and E/I MLD. N=51 for fibrinogen. N=49 for TLCO%. N=31 for sputum differential. *p<0.05 **p<0.01 ***p<0.001.

The distribution histograms for PMA is shown for males in Figure 60 and for females in Figure 61. Males and females were divided into equal tertiles according to PMA. There was significantly more

emphysema in the lowest tertile compared to the other tertiles in both sexes (Table 153 and Table 154). In males only, there was significantly lower FEV1% in the lowest tertile compared to the other tertiles and there was also significantly higher gas transfer in the highest tertile compared to the other two tertiles.

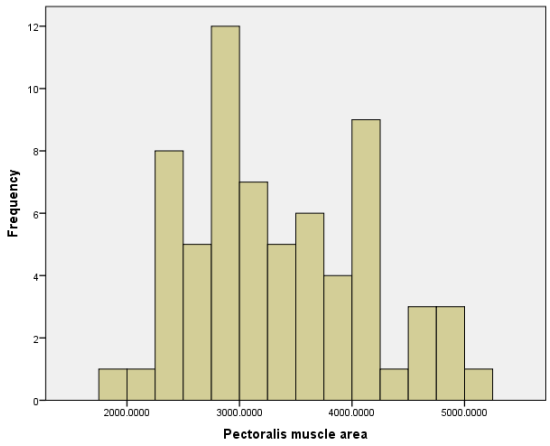


Figure 60. Distribution histogram for pectoralis muscle area in males.

Table 153. Pulmonary physiology, functional capacity, inflammatory markers, biomarkers and exacerbation frequency in tertiles ranked according to pectoralis muscle area in males.

	<2922.32	2922.32-3693.62	>3693.62	P value
Pulmonary Function				
FEV1%	39.0 (18.8)	45.4 (19.4)	53.6 (19.6)	**0.040***
TLCO%	50.4 (23.4)	53.0 (25.3)	75.7 (33.8)	**0.002****
CT parameters				
LAA	30.3 (21.3)	13.8 (20.7)	14.8 (12.5)	**0.013***
E/I MLD	0.92 (0.07)	0.91 (0.09)	0.90 (0.07)	0.398
Pi10	3.76 (0.08)	3.81 (0.14)	3.80 (0.13)	0.115
Functional Capacity				
6MWD	312.0 (233.0)	253.0 (213.0)	379.5 (165.0)	0.106
Oxygen desaturation	5.0 (6.3)	4.5 (4.5)	3.0 (3.3)	0.393
CAT	19.0 (16.0)	17.5 (11.0)	14.5 (12.0)	0.358
EXCAT	37.0 (16.0)	37.0 (14.0)	36.0 (11.0)	0.702
Sputum Differential				
Neutrophils%	39.4 (74.9)	57.3 (73.5)	45.4 (78.0)	0.950
Macrophages%	29.6 (32.2)	25.9 (23.7)	30.2 (31.0)	0.961
Eosinophils%	1.35 (5.2)	3.68 (7.9)	1.86 (8.7)	0.893
CRP	4.5 (7.3)	6.0 (8.3)	4.0 (5.5)	0.378
Eosinophils	0.40 (0.25)	0.20 (0.13)	0.20 (0.20)	0.193
Fibrinogen	4.8 (1.0)	5.2 (0.7)	4.6 (1.3)	0.212
Neutrophils	4.8 (1.9)	5.0 (1.7)	4.7 (0.8)	0.245
PCT	0.0695 (0.04)	0.0650 (0.04)	0.0554 (0.02)	0.403
Exacerbations				
Exacerbation rate	3.09 (3.5)	2.0 (3.2)	2.48 (4.5)	0.550

N=66 for FEV1%, 6MWT, questionnaires, $\%LAA_{<-950}$, Pi10, CRP, PCT and exacerbations. N=65 for TLCO%, E/I MLD and serum neutrophils. N=58 for fibrinogen and N=36 for sputum cell differential. *p<0.05 **p<0.01 ***p<0.001.

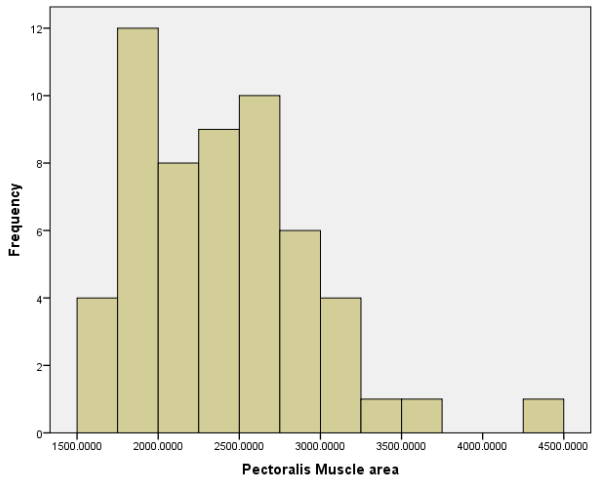


Figure 61. Distribution histogram for pectoralis muscle area in females.

Table 154. Pulmonary physiology, functional capacity, inflammatory markers, biomarkers and exacerbation frequency in tertiles ranked according to pectoralis muscle area in females.

	<2080.3	2080.3-2630.6	>2630.6	P value
Pulmonary Function				
FEV1%	40.4 (31.8)	42.6 (23.6)	58.3 (23.4)	0.058
TLCO%	51.6 (36.2)	56.1 (20.1)	66.4 (25.4)	0.210
CT parameters				
LAA	18.0 (21.6)	5.3 (16.5)	3.7 (7.8)	**0.003****
E/I MLD	0.92 (0.06)	0.92 (0.04)	0.90 (0.07)	0.090
Pi10	3.79 (0.10)	3.81 (0.13)	3.83 (0.12)	0.650
Functional Capacity				
6MWD	268.0 (170.0)	300.0 (99.0)	313.5 (167.0)	0.724
Oxygen desaturation	5.5 (9.8)	4.0 (5.0)	2.5 (4.3)	0.231
CAT	15.0 (15.0)	16.0 (9.0)	18.0 (8.0)	0.776
EXCAT	39.0 (17.0)	37.0 (15.0)	34.0 (10.0)	0.583
Sputum Differential				
Neutrophils%	48.7 (85.5)	62.3 (45.2)	10.3 (52.9)	0.199
Macrophages%	38.3 (69.1)	35.3 (34.6)	55.5 (44.3)	0.715
Eosinophils%	1.06 (2.5)	2.08 (6.1)	2.02 (2.8)	0.859
Blood Inflammatory markers				
CRP	3.5 (8.5)	6.0 (11.3)	5.0 (11.0)	0.478
Eosinophils	0.20 (0.20)	0.20 (0.18)	0.20 (0.13)	0.559
Fibrinogen	4.8 (0.9)	5.0 (1.2)	5.1 (1.3)	0.540
Neutrophils	5.15 (1.8)	5.00 (2.5)	4.8 (2.7)	0.932
PCT	0.0561 (0.03)	0.0555 (0.04)	0.0557 (0.04)	0.959
Exacerbations				
Exacerbation rate	1.49 (2.7)	3.50 (2.8)	1.21 (2.3)	0.061

Values given as medians and IQR. N=56 for CRP, neutrophils, PCT and exacerbations. N=55 for FEV1% and questionnaires. N=54 for 6MWT. N=52 for $\%LAA_{<-950}$, Pi10 and E/I MLD. N=51 for fibrinogen. N=49 for TLCO%. N=31 for sputum differential. *p<0.05 **p<0.01 ***p<0.001.

6.9 Discussion

In this larger study, subjects were characterised with more in-depth pulmonary functional tests, functional measures and clinical parameters with which to validate my quantitative CT analysis. This study also included a much more severe cohort in order to ascertain whether the quantitative CT analysis shows similar properties in different severity cohorts. I have once again demonstrated that emphysema and gas trapping/small airways disease can be measured using CT imaging. However, measurements for bronchial wall dimensions were less convincing.

6.9.1 Emphysema

As with the previous study I used three different automated measures and a visual score to estimate emphysema. I once again showed that all of these automated measures had very strong associations with one another and unlike the last study they all had relatively strong associations with the visual emphysema score. This possibly suggests that visual emphysema score is more accurate when more emphysema is present. All measures of emphysema had significant inverse associations with FEV1%, FEV1/FVC ratio and FEF75-25%. $\%LAA_{<-950}$ had the strongest association with these spirometric measures closely followed by $PERC_{15}$ and the visual score.

One of the limitations of the previous study was that only spirometry was used to characterise pulmonary physiology. In addition to spirometry, this study measured static lung volume using plethysmography and carbon monoxide diffusion capacity (TLCO). TLCO, or gas transfer has traditionally been thought off as good proxy-measure of emphysema in COPD as it measures transfer of carbon monoxide across the alveolar capillary membrane from the lungs into the capillaries. Anything that destroys the alveoli such as the parenchymal destruction seen in emphysema will cause a decrease in gas transfer. It is unsurprisingly then that all CT measures of emphysema had strong associations with TLCO. This is consistent with previous studies that have also demonstrated an association between CT measures of emphysema and gas transfer[152,160–162,168,210,298,299] and have shown significantly reduced gas transfer in subjects with significant emphysema[162,165]. Surprisingly the strongest of these associations were with the visual score and of the automated scores $\%LAA_{<-950}$ and $PERC_{15}$. $\%LAA_{<-856}$ and to a lesser extent $\%LAA_{<-910}$ had a much weaker association with TLCO than the other CT measures of emphysema.

As with my previous study I have been unable to definitively demonstrate which of the CT measures of emphysema was the most accurate. However, it is clear that both $\%LAA_{<-950}$ and $PERC_{15}$ have stronger associations with FEV1% and TLCO% than $\%LAA_{<-910}$. There seems to be little difference between $\%LAA_{<-950}$ and $PERC_{15}$ and my work suggest they are both equally good at measuring

emphysema. However, as $\%LAA_{<-950}$ gives a more direct measure of emphysema percentage I chose to use this for the remainder of the study.

Multivariate analysis showed that $\%LAA_{<-950}$ significantly predicted FEV1% independently of other CT measures, airflow obstruction, BMI, age, smoking status and gender. I also found that $\%LAA_{<-950}$ increased throughout GOLD stages, although this did not reach statistical significance between stage 3 and 4, perhaps due to the smaller numbers in group four. When splitting the cohort into tertiles based on the amount of emphysema the group with the highest amount of emphysema had significantly lower airflow obstruction than the groups with less emphysema. Taken together these provide significant evidence that $\%LAA_{<-950}$ has significant associations with airflow obstruction and providing further evidence that emphysema directly contributes to airflow obstruction. On regression analysis $\%LAA_{<-950}$ also significantly predicted reduced gas transfer independently of airflow obstruction and other CT parameters and demographic variables and when the cohort was divided into tertiles there was significantly reduced gas transfer in the tertile with the most emphysema compared to the other groups. This all confirms $\%LAA_{<-950}$ as a good measure of the reduction in TLCO seen in COPD.

Plethysmography allows the measurement of static lung volumes including TLC, RV and the RV/TLC ratio which have all been shown to be increased in COPD[300]. TLC is the total volume of gas in the lungs after maximum inspiration and in COPD is felt to represent hyperinflation, which is likely to be caused by a combination of emphysema and small airways disease. RV and especially RV/TLC ratio are felt to represent pulmonary gas trapping, most likely due to small airways disease. My data supports this theory by showing $\%LAA_{<-950}$ had a significant association with TLC% and a slightly weaker one with RV%, suggesting that subjects with increased emphysema have increased TLC and RV, which is consistent with other studies[299,301]. Emphysema is likely to contribute towards hyperinflation, by causing loss of elastic recoil within the lung architecture. The results were less suggestive of a link between emphysema and RV/TLC ratio as I found no significant association between this measure and $\%LAA_{<-950}$. RV/TLC was only significantly higher in the group with the highest $\%LAA_{<-950}$ tertile compared to the groups with intermediate amount of emphysema, but not between other groups. Other studies have however, shown an association between LAA and RV/TLC ratio[162,167,210,299], although many of these associations were rather weak and some of the studies used a sub-optimal CT protocol.

When analysing functional parameters of disease there were no associations between $\%LAA_{<-950}$ and six minute walk distance. On multivariate regression analysis only FEV1%, BMI and gender significantly explained the variability of 6MWD, although the r^2 with this model was only 0.2. Previous studies have suggested that COPD subjects who walk under 350 metres have a poorer

prognosis[123,124] and so I split the cohort into two around this threshold. Subjects who walked less than 350 metres had significantly lower FEV1%, but had no significant difference in emphysema, gas trapping or Pi10. Using logistic regression FEV1% was the only variable that significantly independently predicted the risk of belonging to the group that walked less than 350 metres at the six-minute walk test. This is inconsistent with previous work which have suggested that subjects with increased emphysema walk shorter distances at the six minute walk test[167,168,180,208]. However, similarly to my results another study found no association with emphysema[302]. It is unsurprising there are variable results with the six-minute walk distance as there is significant heterogeneity within COPD and a number of factors may influence this. My results would seem to support this with no single variable influencing the six-minute walk distance substantially. However, 6MWD is an important test as lower exercise capacity has been linked with increased mortality[120,122–124,126], with studies suggesting various distances from 340 to 395 metres as a prognostic markers for increased mortality[120,123–126].

As six-minute walk distance did not show any significant associations with CT variables I also assessed the association with desaturation during the 6MWD. This variable is important as it has been linked with increased mortality in COPD subjects[125–127]. $\%LAA_{<-950}$ had significant strong inverse association with desaturation during the 6MWT. When performing multivariate regression analysis only $\%LAA_{<-950}$, BMI and gender significantly predicted exertional desaturation. In addition subjects in the highest tertile of $\%LAA_{<-950}$ had significantly increased desaturation compared to subjects in the lowest tertile. A number of different cut-offs have been used to define significant desaturation during the 6MWT. Waatevik recently suggested desaturation of over 4% increased risk of death[127], while other studies have suggested over 5%[125] and over 3%[126]. I chose to use the stricter cut-off of >5% to define significant desaturation and when doing this found that those who desaturated had significantly lower FEV1% and higher $\%LAA_{<-950}$. On logistic regression analysis the only variable that significantly independently increased the odds of belonging to the group that desaturated was $\%LAA_{<-950}$. My results therefore suggest that emphysema is directly involved in oxygen desaturation during exertion in COPD and to the best of my knowledge no other studies have investigated this link. This study does not provide any mechanistic explanations for this although a plausible suggestion would be the ventilation/perfusion mismatch that is caused by alveolar destruction seen in emphysema.

I did not find any further associations between emphysema and functional measures of disease. Although $\%LAA_{<-950}$ was increased in more severe mMRC groups this was not statistically significant, which is agreement with other studies[172,296,302]. One study however did show a significant association between mMRC dyspnoea score and $LAA\%_{<-950}$[168]. I also found no association between CAT or EXACT scores and $\%LAA_{<-950}$. There was also no difference in $\%LAA_{<-950}$ between different

severity groups of the CAT score. To the best of my knowledge no previous studies have analysed the association between emphysema and these symptom scores. However, CAT score is similar to the St George's Respiratory Questionnaire (SGRQ) and a number of studies have demonstrated an association between emphysema and this symptom score[168,169,296,303,304]. This is far from universal though with other studies showing no associations[172,302193]. I suspect the variable results in functional markers of disease are caused by the significant heterogeneity that exists within COPD and the fact that multiple complex factors interact to cause symptoms and functional limitation.

I found very few associations between emphysema severity and biological markers of disease. There was no association between emphysema and sputum cell differential. When dividing the cohort into those with and without significant neutrophilic inflammation based on a cut-off of 60% sputum neutrophils there was no significant difference in $\%LAA_{<-950}$. This provides doubt about the role of neutrophils in emphysema and is consistent with multiple previous studies that also show no link between emphysema and airway neutrophils[163,164,191,193,283], although one study has shown an association[190]. There has been recent significant interest in the role of eosinophils in COPD. I found no association between emphysema and sputum eosinophil counts and there was also no difference in $\%LAA_{<-950}$ when dividing the cohort into those with high and low sputum eosinophils based on a threshold of 3%. This suggests that eosinophils may not have a role to play in the development of emphysema. Previous work provides inconsistent evidence with one study finding no association between sputum eosinophils and emphysema[193] and another other study showing associations between the two[164].

When assessing the association between systematic inflammatory markers and emphysema there were only relatively weak negative associations between $\%LAA_{<-950}$ and CRP and lymphocytes. $\%LAA_{<-950}$ was also the only variable that significantly predicted CRP although the R^2 was quite low. When dividing the cohort into three based on the amount of emphysema, CRP was significantly reduced in the tertile with the highest amount of emphysema compared to the group with the lowest amount of emphysema. There was no significant difference in other inflammatory markers between groups. As previously discussed, surprisingly few studies have assessed the association between systemic inflammatory markers and emphysema and none have suggested a definitive association. In my previous study there was no association between blood inflammatory markers and emphysema. However this present study suggests those with the highest amount of emphysema have a lower CRP and presumed lower systemic inflammation, although the mechanism behind this is not entirely clear.

As with my previous study there was no association between $\%LAA_{<-950}$ and sputum microbiology either detected with culture or PCR. There were also no association between emphysema and the

exacerbation rate in the first year of the study. There remained no association with $\%LAA_{<-950}$ when exacerbation were divided based upon their severity. However, the numbers of mild and severe exacerbations were low in this cohort. When dividing the cohort into three based on severity of emphysema there was no difference in exacerbation rate between the groups. There was also no difference in emphysema severity between subjects who had exacerbations and those that did not and between subjects who had frequent exacerbations and those that did not. A number of previous studies have investigated the link between emphysema and exacerbations. One study suggested that in subjects with severe emphysema, increasing LAA was independently associated with increased exacerbations[180]. As opposed to my study this was based on retrospective recall of exacerbations by the patient. Another study showed that subjects with incidental emphysema, identified by visual inspection had significantly increased risk of severe exacerbations[182]. Another small study found no difference in emphysema between subjects with frequent exacerbations and those without[296], although once again this was based on patient recall. Like my study most other work has found no association between emphysema and exacerbations[168,172,187,193,200,302], although most of these also measured exacerbations by patient recall. This study is the first to carefully and prospectively capture COPD exacerbations in a cohort a compare these to CT findings.

Once again, CT analysis has allowed me to investigate the distribution of emphysema throughout the lungs. As with my previous study the RML and RUL were most frequently the lobes with the most emphysema, while the lower lobes were less often the most severely affected. Median values of LAA throughout the lobes were relatively consistent with only a small but statistically significant difference between the quantities in the RML and RLL. In the severe and very severe subjects there was no difference between $\%LAA_{<-9500}$ in each of the lobes while in the moderate subjects there was a statistically significant difference in $LAA\%_{<-950}$ between lobes. Associations between lobar $\%LAA_{<-950}$ and overall $\%LAA_{<-950}$ were once again very strong and relatively consistent across the lobes. If anything, the amount of $\%LAA_{<-950}$ in the RML had the weakest association with overall emphysema. Associations between lung function parameters and lobar LAA were very similar throughout, suggesting all the lobes equally contribute to airflow obstruction. The exception to this was once again was the RML, which had the weakest associations with airflow obstruction, and gas transfer. This may be explained by the relative smaller size of this lobe compared to the other lobes meaning it doesn't contribute as much to lung function. When combining the upper lobes and lower lobes together there was significantly higher $\%LAA_{<-950}$ in the upper lobes versus the lower lobes and the median ratio of upper lobe $\%LAA_{<-950}$/lower lobe $\%LAA_{<-950}$ was 1.15. This confirms that the upper lobes are more severely affected by emphysematous change in COPD, although often this difference was minor. This upper lobe LAA/lower lobe LAA ratio was no significantly difference between severity stages of COPD. When dividing the cohort into four equal groups according to this

ratio there was no significant difference in physiological, functional or biological parameters between these groups. This suggests the distribution of emphysema does not affect pulmonary physiology. Concurring with my results, Bhatt also found that the RML was also the most severely affected lobe by emphysema and also demonstrated that $\%LAA_{<-950}$ in all lobes correlated with FEV1%[177]. Unlike my results, a large study showed that upper lobe predominant disease was associated with worse lung function[175]. However these subjects either did not have airflow obstruction or were on the mild spectrum of COPD. Other studies have also shown upper lobe predominance in COPD[158,205,302], while one of these studies found that those with upper zone predominant emphysema had higher symptom scores[302]. This study however, split upper to lower zone based on the middle point of the lung by volume rather than by lobes. Other studies have shown that lower zone emphysema has stronger associations with defects in pulmonary physiology[205] and another showed greater lower/upper zone emphysema predicted mortality in the medical wing of the NETT trial[115]. My study was not able to explain why some individuals developed a more heterogeneous distribution than others and obviously more work is required to identify biological mechanism for this.

6.9.2 Small airways disease

As in my previous study I initially assessed four automated measures of gas trapping and a visual score. Once again all automated measures had strong associations with one another, although there were weaker associations with the visual score. All CT measures of gas trapping had significant association with FEV1%, FEV1/FVC ratio and FEF75-25%. E/I MLD, RVC and $\%LAA_{EXP<-856}$ had equally strong associations with spirometry while visual score and $\%LAA_{EXP<-856-950}$ had slightly weaker associations. I also compared these CT measures to the plethysmography measures of TLC, RV and RV/TLC ratio and found all had significant associations with one another. This is consistent with previous studies, which have also shown strong associations between RV/TLC ratio and CT measures of gas trapping[163,206,210,211]. Visual gas trapping score had the weakest association with these static lung volume measures, which suggests it is the worst CT parameter with which to measure gas trapping. E/I MLD had the strongest association with both RV and RV/TLC ratio and this suggests this is the best CT measure of gas trapping. This is consistent with my previous study and I therefore chose to use this measure for the remainder of the study.

On multivariate analysis E/I MLD significantly predicted FEV1%, suggesting small airways disease contributes to airflow obstruction. This is in agreement with my work on the previous study and with other published studies[161,208,210]. In addition when stratifying the cohort into three tertiles according to E/I MLD, FEV1% was significantly lower in tertiles with higher E/I MLD. There was also a strong association between FEF75-25% and E/I MLD and on multivariate analysis independently

predicted FEF75-25%. Other studies have also demonstrated this finding for E/I MLD [208] and other CT markers of small airways disease[206,208,209]. E/I MLD also had a significant univariate association with TLCO% and there was a significant difference between the tertile with the highest E/I MLD compared to the lowest tertile. Unlike emphysema, E/I MLD was not significantly predictive of TLCO% on multivariate analysis. One previous study showed a weak association between E/I MLD and KCO[210], while other studies have demonstrated no significant association[161,163]. It is unsurprising that small airways disease is not significantly associated with gas transfer as it does not specifically cause interruption of the alveolar-capillary membrane.

There was no significant association between E/I MLD and 6MWD as well as there being no difference in E/I MLD between subjects who could walk further than 350 meters or not. On splitting the cohort into tertiles according to E/I MLD, there was also no significant difference in 6MWD between groups. A small study showed significant associations between CT markers of gas trapping and the 6MWD[161]. Another larger study demonstrated significant associations between CT markers of gas trapping and 6MWD at higher levels of gas trapping but not lower levels MLD[157]. Furthermore, $\%LAA_{Exp<-856}$ was used as a marker for gas trapping, which I have shown is less reliable than E/I MLD and the association disappeared on multivariate analysis. Hersh et al also demonstrated a moderate negative association between E/I MLD and 6MWD in the COPDgene cohort[208]. The reasons for these apparent discrepancies are unknown but in my cohort there was significant variability in 6MWD and it is most likely to be affected by a complex interaction of features. I did not find any significant evidence that gas trapping was associated with exertional desaturation. To the best of my knowledge this is the first study assessing the effects of gas trapping on exertional desaturation. As previously described, the exertional desaturation is likely to be caused by a V/Q mismatch that airways disease would not cause and so it is perhaps unsurprising no association was found. Subjects with a more severe mMRC dyspnoea score had significantly more E/I MLD. There was however was no association between E/I MLD and the symptom scores (CAT and EXACT scores). Although no studies have assessed the relationship between E/I MLD and these symptom scores, one study used SGRQ, which showed a mild but significant independent association between this and E/I MLD.

In this study I found no association between E/I MLD and sputum cell differential and this was further supported by the fact that there was no significant difference in E/I MLD for subjects who had significant neutrophilic and eosinophilic inflammation and those that did not. This is inconsistent with the results from my previous study although the most obvious difference between these two studies was the different sampling methods (BAL vs sputum). Also this study consisted of a more severe cohort. Only one study has investigate the link between CT parameters of gas trapping and airway neutrophil counts and this found a significant relationship[163], although this

study also utilised BAL sampling. Histological studies have shown equivocal results regarding neutrophilia within the distal airways[73,289,290]. With regards to systemic inflammatory markers there was only a weak negative association between E/I MLD and CRP and lymphocytes, although on multivariate analysis this lost significance. There were also no associations between the serum biomarkers and E/I MLD. I also did not find any association between exacerbations and E/I MLD and there were no difference in E/I MLD between subjects who suffered with exacerbations and those that did not and also those that suffered frequent exacerbations and those that did not. No previous studies have assessed the effect that small airways disease has on exacerbations. My study however suggests it has no effect on exacerbations and it may be that structural lung disease in COPD does not influence exacerbation frequency instead it may be inflammatory cell infiltration or immune deficiencies that cannot be identified on CT.

When assessing the regional distribution of gas trapping there was significant differences between all lobes apart from between the RUL and LUL as well as the RLL and RUL. Median levels of E/I MLD were highest in the RML and this lobe was also the lobe that was most frequently the most severely affected. This is in agreement with a study by Bhatt et al, who also showed that the RML was most commonly the most severely affected by CT measured gas trapping in COPD[177]. The significant difference between this and my study was they utilised $\%LAA_{EXP<-856}$ to measure gas trapping and not E/I MLD. They also found that gas trapping in the right middle lobe had a weaker association with airflow obstruction than the entire lung or other lobes. In my study the association between E/I MLD and airflow obstruction was relatively consistent throughout the lobes. The amount of E/I MLD in each lobe also have equally high correlations with overall E/I MLD suggesting they all contribute relatively evenly to this marker and overall small airways disease. On further assessment of these results there were six lobar ratios that were above one, suggesting that attenuation values had decreased in expiration. Four of these were from the RML, one from the RUL and one from the LUL. For three of these lobes the mean lung density was very low at inspiration, which may partially explain this result. The causes for the other lobes are not absolutely apparent and this study cannot answer this. Possible explanations may be abnormalities within each individual lobe such as fibrosis, consolidation, causing increased attenuation. Presently when assessing lobar values there has been assumption that lobes are independent of each other. However this is unlikely to be true as they may affect and interact one another with expansion in one lobe causing changes to another and therefore some caution is needed when interpreting these results.

There was significantly higher gas trapping in the combined upper lobes (RUL, RML and LUL) than the combined lower lobes (RLL and LLL). The median upper lobe to lower lobe ratio of E/I MLD was 1.02 and this did not change significantly throughout the different GOLD groups. Only 17 subjects had a ratio under one, suggesting lower lobe predominance for gas trapping, compared to 96 who

had upper lobe predominance. When dividing the cohort into three based on the upper lobe/lower lobe E/I MLD ratio, subjects in the upper tertile with the most upper lobe gas trapping had significantly lower overall E/I MLD and lower RV/TLC. This suggests that those with upper lobe predominant gas trapping have overall less gas trapping and possibly less overall small airways disease. No significant biological mechanisms were determined to explain why some subjects have a heterogeneous distribution of small airways disease. Compared to emphysema, few studies have assessed the regional anatomical distribution of gas trapping and this is poorly understood. A small study using visual gas trapping as a marker, found that in healthy smokers and never smokers when gas trapping was present it was usually lower lobe predominant[305]. In COPD subjects, Bhatt et al assessed lobar gas trapping using $\%LAA_{EXP<-856}$, but did not specifically analyse the difference between upper and lower lobes[177], although it is clear from their data that upper lobe predominance was also found. Bommart also demonstrated that healthy subjects had significant differences in E/I MLD between four slices throughout the lungs with the uppermost slice having the highest value[207]. Schroeder et al found gas trapping was significantly raised in the upper lobes compared to the lower lobes[158], although they only used $\%LAA_{EXP<-856}$. Also as subjects in increased in severity through GOLD grouping this upper lobe predominance became reduced. My data is in agreement with this, as subjects with more upper lobe predominance seemed to have milder disease, suggesting that there are perhaps regional differences in small airways disease in early disease.

6.9.3 Large and Intermediate Airway Morphology

As already discussed there are a number of different methods with which to measure dimensions of the large and intermediate airways and in the previous chapter I have assessed a number of these. In this study I chose to use two of these; pi10 the one most commonly used in most large studies and airway dimensions in different airway paths at different segmental generations. As with the previous study there were few significant associations between Pi10 and pulmonary physiology. No association was found between Pi10 and FEV1%, FEV1/FVC ratio and FEF75-25, although there was a weak association with FVC. This provides further evidence that Pi10 is not a good marker of airway dimensions in COPD. Pi10 also had no associations with TLCO%, RV%, and RV/TLC ratio, but had a weak negative association with TLC%.

Pi10 had no association with 6MWD, mMRC group or CAT and EXACT scores. PI10 also had no associations with sputum cell differential, blood biomarkers, and bacterial presence in sputum or exacerbation frequency. It did have a very weak association with blood CRP and fibrinogen although these disappeared on multivariate analysis. All of this confirms my findings from the previous study that Pi10 is either not an accurate or useful marker of airway dimensions in COPD.

When analysing airway dimensions in different segmental generations I found very weak negative associations between these and FEV1%, which if anything is the opposite of what I found in the previous study. As I have already discussed previous evidence is also equivocal and so this further questions the usefulness of this measure in COPD. There were few significant associations between these dimensions and exacerbation rate, serum CRP, symptom scores and 6MWD. There were a number of positive associations between these dimensions and sputum neutrophil count although these were relatively weak and therefore of questionable significance.

Like my previous study I found significant variation between airway dimensions in the five different airway paths that I measured. This further demonstrates the error of trying to use global measures of airway dimensions in studies and once again may explain why there have been a number of differing results. There were few significant associations between dimensions in these different airway paths at different generations and FEV1%.

6.9.4 Bronchiectasis

Previous studies have demonstrated high prevalence of bronchiectasis in COPD populations[133–135]. This was not the case in my cohort where only 7.9% were deemed to have bronchiectasis. The reasons for this are not clear however subjects from these studies were from East London and Madrid, two large centres with likely high rates of previous TB. Also most of my patients were recruited from specialist COPD clinics and many subjects would have previously had a CT scan and subjects with significant bronchiectasis would not have been considered for the study.

Subjects who had bronchiectasis present trended towards having worse airflow obstruction, which is consistent with previous work[135]. I also found that subjects with bronchiectasis had significantly raised sputum neutrophils and IL-6 as well as raised blood neutrophils. This suggests that concomitant bronchiectasis is associated with both airway and systemic neutrophilic inflammation. Previous work has also demonstrated increased airway inflammation in COPD subjects with bronchiectsis[134], while other studies have also shown increased systemic inflammation[133,135]. There was no difference in exacerbation rate between subjects who had bronchiectasis present and those that did not. A number of other studies have found higher rates of exacerbations in COPD subjects with bronchiectasis[133,135], although it has to be considered those studies had much larger numbers of subjects with bronchiectasis. I also found no difference between bacterial detection rates in sputum between subjects with bronchiectasis and without. Both groups, however had very high rates of bacterial detection and this along with the low number of bronchiectatic subjects may explain differing results from other studies[133–135].

6.9.5 Body Composition

Cachexia and low body mass are frequently found in COPD[105,107,108,306–310] with multiple studies demonstrating that patients with a low BMI have higher mortality [105–108,306–308]. However, the precise mechanism of this is uncertain and it has been hypothesised that skeletal muscle wasting is the most important process. BMI is a composite and non-specific measure and therefore an imprecise tool with which to assess body composition and especially skeletal muscle mass. A number of other techniques have therefore been used to assess body composition including full anthropometric measurements (triceps skin-fold, waist measurements), dual-energy X-ray absorptiometry (DEXA) and bioelectrical impedance analysis. These measures can provide estimates of muscle mass often referred to as fat free mass index (FFMI) and fat mass. FFMI has been shown to be an independent predictor of mortality irrespective of fat mass[107] and has been shown to be associated with mortality independently of BMI[108,311].

CT imaging can provide significant information about body composition. One previous study used CT to image the mid-thigh muscle and found cross-sectional area of this muscle was a better predictor of mortality in COPD than BMI[312]. I chose to assess body composition using chest CT by measuring the cross-sectional area of the pectoralis muscle and anterior chest fat as previously described[222,313]. Males had significantly higher PMA but lower ACF than females, indicating there is a significant difference in body composition between the genders. This was consistent with a previous study which also showed males had higher PMA and lower ACF[313]. This was also reflected by the results from the traditional measures of body composition, meaning the genders needed analysing separately. Interestingly, there was no significant difference in BMI between the two genders, providing further evidence that this does not reflect the complexity of body composition. In females there was only a weak association between ACF and PMA, while in males there was no significant association. This suggests that body fat and muscle bulk are independent of one another and therefore to accurately assess body composition both of these need to be taken into account. When comparing CT measured body composition with the other techniques, I found strong positive associations between anterior chest fat area and weight, BMI, FFMI, waist circumference and triceps skin fold in both men and women. There were also significant associations between PMA and these traditional measures of body composition in both genders (apart from triceps skin fold in females), although the associations were a little weaker. Diaz et al, also found that PMA and ACF both had significant associations with BMI and FFMI[313], while another study found that PMA also had significant association with BMI and FFMI[222]. There was no association between hand grip strength and CT measures of body composition. To the best of my knowledge this was the first study to assess this association and also with waist circumference and triceps skin fold.

When assessing the association between CT body composition and clinical, physiological and biological measures of disease severity only $\%LAA_{<-950}$, E/I MLD and FEV1% significantly predicted ACF in males and $\%LAA_{<-950}$ and E/I MLD in females. This seems to suggest a strong negative relationship between morphological features of disease within the lungs and body fat in COPD. The only association between biological markers of disease and ACF was a rather weak association with CRP, although this did not persist on multivariate analysis. The only other study to assess relationship between ACF and biomarkers showed an association with CRP and fibrinogen in females only[313]. For PMA there were a number of weak associations on univariate analysis but on multivariate analysis none of the variables significantly predicted PMA. This is in contrast to a previous study which showed that lower PMA was associated with lower FEV1%, higher emphysema and lower 6MWD[222]. Another study agreed that there was no association between PMA and CRP or fibrinogen[313]. My results were broadly similar when the cohort was divided into tertile based on ACF or PMA.

Although there are limited studies assessing CT markers of body composition and these clinical, physiological and biological measures of disease severity, there are a number of studies which have assessed them using traditional measures of body composition. These have found associations between FFMI and FEV1%[314,315] and 6MWD[314,315]. Multiple studies have also shown that lower BMI and/or lower FFMI is associated with greater amounts of emphysema[162,164,171,172,316]. A number of studies have shown higher BMI is associated with higher CRP in COPD[317–319], although one other study showed the opposite[320]. Association between CRP and fat mass have also been demonstarted[319,321], although the evidence for an association between CRP and FFMI is equivocal as two studies showed weak positive association[321,322], while another did not show an association[317]. One prior study also showed no association between BMI/FFMI and blood neutrophils[315]. Lower BMI has been associated with increased exacerbation frequancy[309].

Taken together with my results it seems that lower body fat and to a lesser extent muscle mass are associated with emphysema, gas trapping and FEV1%, although the mechanisms behind this are unclear. There is also no clear signal that blood or sputum inflammatory markers have an effect on body composition.

6.9.6 Strengths and limitations

Once again a significant strength of this study was the optimal CT protocol used that allowed accurate automated analysis to be performed. This was also a much larger cohort than my previous study with a broad range of subjects with more severe disease. A wide-range of investigations were performed including in-depth pulmonary function testing, allowing further validation of the CT

analysis than was possible in the previous study. A number of different biological measurements were also captured in order to attempt to understand disease activity and mechanisms.

In contrast to my previous study, sputum sampling was performed instead of bronchoscopic sampling, meaning a direct comparison for airway inflammation between the two studies could not be made. It is also possible that sputum inflammatory markers are a less accurate marker of airway inflammation than BAL. However, due to the size and severe nature of the cohort it would not have been possible to perform bronchoscopy. Another potential limitation was that much of the statistical analysis, used association which does not necessarily imply causation. Also multiple comparisons were often performed which increases the chance of finding significant associations by chance. However many of the results were similar to what I found in the previous study.

Although this cohort was well characterised with multiple pulmonary function testing, clinical follow-up and biological sampling there was still significant heterogeneity. This undoubtedly limited the statistical power of the study and one that is commonly encountered when performing clinical studies in COPD.

6.9.7 Summary

In summary, I have validated the results from my previous study and have once again successfully demonstrated that quantitative analysis of CT imaging can be used to measure emphysema and small airways disease, this time in a more severe cohort. I was also able to show that this technology could be used to describe the anatomical distribution of these pathological processes, although I was unable to determine any of the biological causes for this. As with my previous study, I was unable to demonstrate the usefulness of measuring dimensions of large and intermediate airways in COPD.

Chapter 7: Longitudinal Change in Quantitative CT data in the AERIS study

In the previous chapters I have presented cross-sectional results from my studies, however it is vital studies are performed to track CT changes over time to get an accurate picture of the progression of the disease and how effective CT imaging is at capturing this. In this chapter I present the longitudinal results from the AERIS cohort where CT imaging was performed two years apart.

123 subjects had CT at enrolment and 79 subjects had scan at month 24. 77 of these had scans at both enrolment and month 24. To ensure there was no systematic bias I compared these two groups (Table 155). There was significantly lower FEV1%, 6MWD and higher CAT score in the group that did not have a scans at month 24. This suggests that subjects who dropped out of the study had more severe COPD.

Table 155. Demographic, physiological, functional and CT data for subjects with only an enrolment scan and both enrolment and month 24 scans

	Both Scans available N=77	**Only enrolment N=123**	**P value**
Age	67.0 (12.0)	67.0 (11.0)	0.611
Male Gender	49	19	0.005
BMI	26.9 (5.9)	27.6 (8.6)	0.515
Smoking pack years	46.0 (25.1)	50.0 (30.6)	0.330
Current smoker	26	23	0.166
Spirometry			
FEV1%	49.2 (22.4)	40.1 (26.6)	**0.046***
TLCO%	58.5 (31.1)	56.1 (29.4)	0.234
TLC%	104.6 (23.7)	106.9 (20.4)	0.810
RV%	144.9 (58.7)	138.6 (65.5)	0.961
Functional Capacity			
6MWD	328.0 (144.0)	229.5 (174.0)	**<0.001*****
CAT	15.0 (12.0)	18.0 (9.0)	**0.021***
EXCAT	36.0 (14.0)	37.0 (10.0)	0.245
CT Parameters			
LAA	15.1 (22.3)	10.5 (19.7)	0.245
E/I MLD	0.92 (0.07)	0.92 (0.08)	0.561
Pi10	3.79 (0.13)	3.82 (0.09)	0.223
Exacerbations			
Exacerbation rate	2.94 (3.99)	2.16 (3.68)	0.478

Values given as medians and IQR, apart from males and current smokers which are given as number of subjects. *p<0.05, ***p<0.001 using

7.1 Emphysema Progression

7.1.1 Overall %LAA$_{<-950}$ Progression

I chose to use %LAA$_{<-950}$ to track the progression of emphysema over the two-year period. Median %LAA$_{<-950}$ at month 24 was 15.6% and was significantly higher than the median %LAA$_{<-950}$ at enrolment which was 15.1% ($p<0.001$).

The median %LAA$_{<-950}$ progression over the two year period was 1.14% (4.25) and the histogram for LAA progression is shown in Figure 62. One striking finding is that 24 subjects had lower levels of %LAA$_{<-950}$ at the month 24 scan than at enrolment. This was unexpected as it would be anticipated that emphysema does not improve over time. It is therefore likely that this represents an inaccuracy of %LAA$_{<-950}$ as a measure of emphysema and this needs to be considered especially when evaluating longitudinal change.

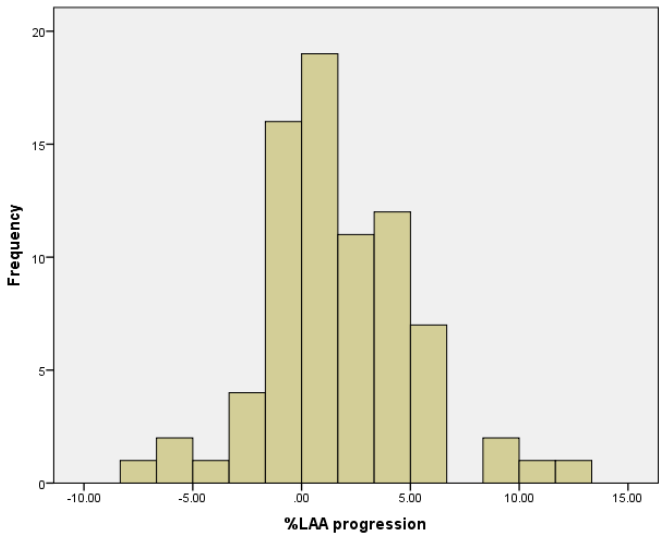


Figure 62. Distribution histogram for %LAA$_{<-950}$ progression. Positive represents increasing %LAA$_{<-950}$ over the two year period.

To understand common factors for predicting emphysema progression, the association between %LAA$_{<-950}$ progression and a number of clinical, physiological and biological parameters at enrolment were assessed. There were significant negative associations between %LAA$_{<-950}$ progression and FEV1% and TLCO% at enrolment (Table 156). There were significant positive associations with TLC% and RV% and oxygen desaturation at baseline. There were also significant positive associations between %LAA$_{<-950}$ progression and %LAA$_{<-950}$ and E/I MLD at baseline. There

were no significant associations between sputum cell differential, blood inflammatory markers, novel serum biomarkers or exacerbation rate and progression of emphysema.

Table 156. Association between $\%LAA_{<-950}$ progression and physiological, functional, clinical and biological markers at enrolment.

	$\%LAA_{<-950}$ progression
Pulmonary Function	
FEV1%	**-0.38****
TLCO%	**-0.41*****
TLC%	**0.35****
RV%	**0.36****
Functional Capacity	
6MWD	-0.06
Oxygen desaturation	**0.24***
CAT	0.18
EXCAT	0.09
CT Parameters	
LAA	**0.34****
E/I MLD	**0.38****
Pi10	-0.02
Sputum Differential	
Neutrophils%	-0.05
Macrophages%	-0.04
Eosinophils%	-0.07
Blood Inflammatory Markers	
CRP	-0.06
Eosinophils	0.04
Fibrinogen	0.09
Neutrophils	-0.06
PCT	0.04
Hs-cTNT	0.13
IL-6	0.03
IP-10	0.14
NT-proBNP	0.06
SP-D	0.14
Exacerbations	
Exacerbation rate	0.07

Spearman's correlation. N=77 for FEV1%, CT parameters, exacerbations, CRP, questionnaires, 6MWD. N=74 for TLCO%, hs-troponin and NT-proBNP. N=73 for pulmonary function. N=76 for leukocytes and PCT. N=69 for fibrinogen. N=47 for sputum cell differential. N=53 for IL-6. N=75 for IP-10 and Sp-D. *p<0.05, **p<0.01, ***p<0.001.

Next, I performed linear regression analysis to correct for baseline $\%LAA_{<-950}$ and FEV1%. On their own each one of these variables had significant associations with $\%LAA_{<-950}$ progression, but when combined into a regression model only FEV1% at baseline remained significant (B coefficient -0.065, p 0.046). There were no significant associations with clinical, physiological and biological parameters at enrolment when correcting for $\%LAA_{<-950}$ and FEV1% (Table 157).

Table 157. Multiple regression analysis to predict $\%LAA_{<-950}$ progression

	B coefficient	P value
Age	0.002	0.971
Gender	-0.09	0.914
BMI	-0.03	0.724
Current smoker	0.38	0.645
Pack years	-0.01	0.456
Pulmonary Function		
TLCO%	-0.04	0.133
TLC%	0.03	0.247
RV%	0.007	0.414
Functional Capacity		
6MWD	0.001	0.866
Oxygen desaturation	0.015	0.884
CAT	0.008	0.876
EXCAT	-0.01	0.798
CT Parameters		
E/I MLD	12.9	0.245
Pi10	-2.69	0.576
Sputum Differential		
Neutrophils%	-0.01	0.228
Macrophages%	0.019	0.230
Eosinophils%	-0.022	0.548
Blood Inflammatory Markers		
CRP	-0.03	0.402
Eosinophils	-0.64	0.754
Fibrinogen	0.54	0.288
Neutrophils	-0.02	0.946
PCT	1.41	0.928
Serum Biomarkers		
Hs-cTNT	36.1	0.354
IL-6	-0.13	0.216
IP-10	0.03	0.365
NT-proBNP	-0.00	0.941
SP-D	0.007	0.125
Exacerbations		
Exacerbation rate	0.153	0.335
Sputum Bacterial detection		
PPM	0.253	0.734

To understand the relationship between emphysema progression and features at baseline in more detail I divided the cohort into tertiles based on the degree of emphysema progression. The lowest tertile had less than 0.37% $\%LAA_{<-950}$ progression and this represented subjects who did not progress over the two year period. The highest tertile had more than 2.92% $\%LAA_{<-950}$ progression and represented subjects who definitely progressed. The middle tertile progressed by 0.37-2.92% over the two years and due the possible inaccuracies of the test represents an intermediate groups who did not progress or who had minimal progression. FEV1% and TLCO% were significantly higher in subjects in tertile 1 compared to subjects in tertile 2 and 3 (Table 158). E/I MLD was significantly lower in tertile one compared to tertile two and three. $\%LAA_{<-950}$, TLC and RV there was significantly higher in tertile 3 than tertile 1. There were no other significant differences between the groups.

Table 158. Physiological, functional, clinical and biological markers at enrolment in the tertiles stratified according to $\%LAA_{<-950}$ progression.

	<0.37	0.37-2.92	>2.92	Significance
Pulmonary Function				
FEV1%	58.2 (15.4)	45.1 (23.2)	41.3 (21.5)	**0.003****
TLCO%	73.3 (30.4)	57.6 (29.6)	50.7 (18.0)	**0.003****
TLC%	99.8 (17.5)	104.0 (22.1)	120.7 (27.1)	**0.002****
RV%	126.6 (51.9)	143.9 (53.6)	172.5 (72.8)	**0.003****
Functional Capacity				
6MWD	374.0 (220.0)	326.0 (134.0)	328.0 (125.0)	0.625
Oxygen desaturation	3.0 (5.0)	5.0 (6.5)	6.0 (7.0)	0.067
CAT	12.0 (7.0)	18.5 (11.0)	16.0 (10.0)	0.108
EXCAT	34.0 (12.0)	35.0 (17.0)	37.0 (13.0)	0.850
CT Parameters				
$\%LAA_{<-950}$	4.80 (13.2)	18.2 (23.1)	18.7 (20.4)	**0.003****
E/I MLD	0.88 (0.07)	0.92 (0.05)	0.94 (0.07)	**0.001****
Pi10	3.79 (0.13)	3.76 (0.18)	3.80 (0.06)	0.633
Sputum Differential				
Neutrophils%	45.4 (70.5)	61.3 (78.50)	47.5 (59.7)	0.669
Macrophages%	33.1 (56.2)	23.8 (23.8)	46.0 (28.1)	0.061
Eosinophils%	1.97 (5.3)	2.62 (7.0)	2.62 (3.6)	0.604
Blood Inflammatory Markers				
CRP	4.0 (8.5)	5.0 (6.5)	5.0 (8.5)	0.984
Eosinophils	0.20 (0.28)	0.20 (0.20)	0.20 (0.20)	0.571
Fibrinogen	4.70 (1.08)	4.90 (0.70)	4.80 (0.96)	0.474
Neutrophils	4.85 (1.58)	4.20 (1.83)	4.10 (1.40)	0.488
PCT	0.057 (0.02)	0.061 (0.03)	0.064 (0.04)	0.681
Hs-cTNT	0.012 (0.01)	0.012 (0.01)	0.014 (0.01)	0.776
IL-6	3.79 (2.51)	4.43 (2.77)	4.48 (2.15)	0.861
IP-10	133.0 (60.0)	152.5 (167.0)	151.0 (87.5)	0.426
NT-proBNP	89.8 (213.7)	62.7 (98.3)	103.0 (142.8)	0.581
SP-D	176.0 (84.0)	142.5 (118.6)	191.0 (144.0)	0.250
Exacerbations				
Exacerbation rate	1.98 (4.0)	2.95 (3.9)	3.0 (4.4)	0.811

N=77 for FEV1%, CT parameters, exacerbations, CRP, questionnaires, 6MWD. N=74 for TLCO%, hs-troponin and NT-proBNP. N=73 for pulmonary function. N=76 for leukocytes and PCT. N=69 for fibrinogen. N=47 for sputum cell differential. N=53 for IL-6. N=75 for IP-10 and Sp-D. *p<0.05, **p<0.01, ***p<0.001 using independent samples Kruskal-Wallis test.

7.1.2 Lobar Progression of emphysema

Next I investigated the progression of emphysema in each of the individual lobes to discover whether there is anatomical variability in this process. Median $\%LAA_{<-950}$ was significantly higher in month 24 than at enrolment in each individual lobe (Table 159). Median progression in the RUL was 1.30% (4.20), the RML was 1.47% (4.99), the RLL% was 0.90 (4.29), LUL% 0.94 (3.78) and LLL 1.23% (4.54). The only lobes to show a significant difference between the progressions of $\%LAA_{<-950}$ was

between the LLL and the RUL (p 0.005). There was no significant difference in progression of $\%LAA_{<-950}$ between any of the other lobes.

There were very strong associations between lobar progressions of $\%LAA_{<-950}$ and overall progression of $\%LAA_{<-950}$ (Table 160). There was also strong associations in progression of $\%LAA_{<-950}$ between all of the individual lobes.

Table 159. Median $\%LAA_{<-950}$ at enrolment and month 24 in each lobe.

	Enrolment	Month 24	P Value
RUL	12.1 (25.9)	14.9 (28.3)	**<0.001*** **
RML	14.0 (19.5)	16.2 (20.6)	**0.002** **
RLL	11.7 (15.9)	12.6 (18.1)	**0.002** **
LUL	13.4 (22.4)	15.4 (25.5)	**<0.001*** **
LLL	12.2 (17.0)	12.7 (18.1)	**0.003** **

Values represent medians and IQR. N=77. *p<0.05, **p<0.01, ***p<0.001.

Table 160. Associations between lobar and overall $\%LAA_{<-950}$.

	RUL	RML	RLL	LUL	LLL
RML	0.77***				
RLL	0.71***	0.77***			
LUL	0.81***	0.76***	0.77***		
LLL	0.68***	0.70***	0.70***	0.73***	
Overall LAA	0.88***	0.81***	0.84***	0.88***	0.88***

Spearman's correlation. N=77. *p<0.05, **p<0.01, ***p<0.001.

7.2 Bronchial Wall progression

7.2.1 Change in Pi10

To investigate the longitudinal change in airway dimensions I initially used the CT parameter Pi10. In the 77 subjects who had CT at enrolment and month 24, median Pi10 at enrolment was 3.79 (0.13) and median Pi10 at month 24 was 3.80 (0.12). There was no significant difference between Pi10 at enrolment or month 24 (p 0.225). The distribution histogram for progression of Pi10 over the two year period is shown in Figure 63. The median change in Pi10 over this time was 0.01 (0.11).

Next I assessed the association between change in Pi10 and physiological, functional, clinical and biological markers at enrolment. There was a moderate negative association between Pi10 at enrolment and Pi10 change over the two-year period. There was a significant association between airway neutrophils at enrolment and change in Pi10. There was also a weak association between Pi10 change and exacerbation rate in the first year.

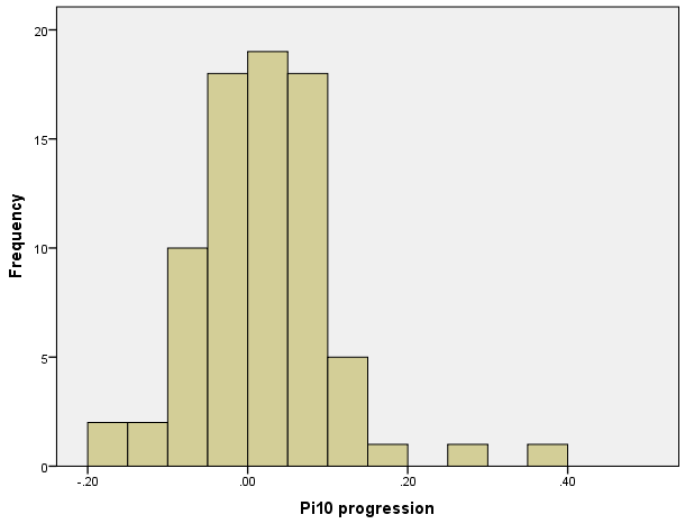


Figure 63. Distribution histogram for Pi10 progression. Positive represents increasing Pi10 over the two year period.

Table 161. Association between Pi10 progression and physiological, functional, clinical and biological markers at enrolment.

	Pi10 progression
Pulmonary Function	
FEV1%	-0.10
TLCO%	-0.22
TLC%	-0.04
RV%	-0.04
Functional Capacity	
6MWD	-0.19
Oxygen desaturation	-0.03
CAT	0.18
EXCAT	0.25
CT Parameters	
LAA	0.18
E/I MLD	-0.11
Pi10	**-0.39****
Sputum Differential	
Neutrophils%	**0.32***
Macrophages%	-0.19
Eosinophils%	0.22
Blood Inflammatory Markers	
CRP	0.08
Eosinophils	0.11
Fibrinogen	0.08
Neutrophils	0.13
PCT	-0.15
Hs-cTNT	-0.04
IL-6	-0.05
IP-10	0.18
NT-proBNP	-0.19
SP-D	0.13
Exacerbations	
Exacerbation rate	**0.24***

Spearman's correlation. N=77 for FEV1%, CT parameters, exacerbations, CRP, questionnaires, 6MWD. N=74 for TLCO%, hs-troponin and NT-proBNP. N=73 for pulmonary function. N=76 for leukocytes and PCT. N=69 for fibrinogen. N=47 for sputum cell differential. N=53 for IL-6. N=75 for IP-10 and Sp-D. *p<0.05, **p<0.01, ***p<0.001.

Of the 77 subjects who had Pi10 measured at enrolment and at month 24, 35 subjects had an increase in Pi10, while 42 either remained static or decreased. To determine if there was any differences between these two groups I analysed physiological, functional, clinical and biological markers at enrolment in them (Table 162). Baseline Pi10, TLCO% and PCT were all significantly higher in the group where Pi10 did not increase compared to the group where Pi10 did increase. There were no other significant differences.

Table 162. Physiological, functional, clinical and biological markers at enrolment in the tertiles stratified according to $\%LAA_{<-950}$ progression.

	No Increase pi10	Increase Pi10	Significance
Pulmonary Function			
FEV1%	52.7 (28.7)	48.3 (21.1)	0.287
TLCO%	63.0 (33.8)	52.4 (26.4)	**0.034***
TLC%	106.4 (22.7)	104.0 (26.5)	0.938
RV%	142.8 (47.1)	145.8 (63.1)	0.956
Functional Capacity			
6MWD	364.0 (164.0)	325.0 (147.0)	0.146
Oxygen desaturation	4.0 (8.0)	5.0 (6.0)	0.766
CAT	14.0 (10.0)	16.5 (13.0)	0.098
EXCAT	31.5 (17.0)	37.0 (9.0)	0.058
CT Parameters			
LAA	11.8 (21.0)	16.6 (26.5)	0.248
E/I MLD	0.92 (0.07)	0.92 (0.06)	0.402
Pi10	3.81 (0.13)	3.76 (0.09)	**0.005****
Sputum Differential			
Neutrophils%	9.77 (75.0)	61.3 (66.3)	0.182
Macrophages%	33.8 (62.5)	31.3 (26.8)	0.406
Eosinophils%	1.34 (4.1)	2.13 (5.0)	0.417
Blood Inflammatory Markers			
CRP	4.0 (5.0)	5.0 (8.3)	0.423
Eosinophils	0.20 (0.20)	0.25 (0.23)	0.259
Fibrinogen	4.7 (1.2)	5.0 (0.7)	0.142
Neutrophils	4.15 (1.9)	4.75 (1.5)	0.240
PCT	0.069 (0.03)	0.057 (0.02)	**0.008****
Hs-cTNT	0.013	0.012	0.556
IL-6	3.98 (2.1)	4.32 (2.7)	0.920
IP-10	133.5 (83.5)	154.0 (112.5)	0.203
NT-proBNP	108.0 (172.4)	72.1 (120.8)	0.088
SP-D	171.5 (90.0)	179.0 (145.0)	0.443
Exacerbations			
Exacerbation rate	1.98 (3.99)	2.99 (3.99)	0.303

Spearman's correlation. N=77 for FEV1%, CT parameters, exacerbations, CRP, questionnaires, 6MWD. N=74 for TLCO%, hs-troponin and NT-proBNP. N=73 for pulmonary function. N=76 for leukocytes and PCT. N=69 for fibrinogen. N=47 for sputum cell differential. N=53 for IL-6. N=75 for IP-10 and Sp-D. *p<0.05, **p<0.01, ***p<0.001.

7.2.2 Change in Airway dimensions in specific airway paths

As I have previously demonstrated that Pi10 is of questionable use when measuring airways and there is significant anatomical variability in airway dimensions I also compared the differences between airway dimensions in 5 different airway paths and each segmental level (Table 163). When doing this there were a number of significant differences in airway dimensions between enrolment and month 24. However there was significant variability, with no discernible pattern elicited.

Table 163. Airway wall dimensions in 5 airway paths at different segmental generations and enrolment and month 24.

	RB1		RB4		RB10		LB1		LB10	
	M0	M24	M0	M24	M0	M24	M0	M24	M0	M24
Generation 3										
No. Airways	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
Wall Thickness	1.55 (0.29)	1.37*** (0.24)	1.34 (0.26)	1.37* (0.24)	1.49 (0.25)	1.52 (0.27)	1.37 (0.3)	1.39 (0.2)	1.57 (0.3)	1.58 (0.3)
Lumen area	19.0	14.2 ***	12.7 (5.2)	14.2 (6.0)	17.5 (13.0)	20.0 (11.5)	12.7 (5.9)	13.8 (7.5)	21.1 (11.4)	24.8 (13.0)*
Wall Area	32.7 (15.3)	25.1*** (8.6)	24.2 (9.3)	25.1** (8.7)	31.3 (13.9)	32.1 (12.9)	24.2 (10.8)	25.2 (9.3)	35.2 (14.0)	36.6 (13.9)
Total Airway area	51.8 (26.9)	38.7*** (15.7)	37.3 (13.9)	38.8 *(15.5)	48.6 (28.1)	52.1 (23.0)	38.2 (16.4)	38.1 (16.7)	57.1 (25.5)	59.9 (27.4)
WA%	63.3 (6.2)	65.4* (4.7)	65.0 (3.7)	65.3 (4.6)	63.3 (6.1)	62.4 (5.4)	65.3 (4.7)	65.4 (4.9)	62.5 (5.8)	61.2 (6.2)
Generation 4										
No. Airways	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)
Wall Thickness	1.3 (0.3)	1.0 (0.2)***	1.1 (0.3)	1.0 (0.2)	1.2 (0.2)	1.3 (0.2)	1.1 (0.3)	1.2** (0.2)	1.3 (0.3)	1.3 (0.3)
Lumen area	10.1 (7.0)	7.0 (3.5)***	6.9 (3.3)	7.0 (3.4)	11.1 (7.2)	11.7 (5.9)	7.4 (3.7)	8.1* (4.5)	12.7 (7.4)	12.8 (7.5)*
Wall Area	20.6 (9.0)	14.3 (5.4)***	14.9 (5.7)	14.3 (5.4)	21.0 (8.6)	21.6 (8.4)	15.8 (5.6)	17.8 (5.9)*	22.1 (11.0)	22.2 (10.7)
Total Airway area	31.0 (15.0)	21.6 (8.8)***	21.7 (8.5)	21.6 (8.8)	32.2 (15.2)	33.0 (14.1)	23.3 (9.4)	25.7 (10.7)*	34.9 (18.3)	34.5 (18.5)*
WA%	66.7 (4.1)	67.1 (4.2)	67.5 (3.6)	66.9 (4.1)	65.6 (5.3)	65.6 (5.5)	67.8 (3.7)	68.1 (3.9)	65.2 (4.6)	65.6 (5.5)*
Generation 5										
No. Airways	4.0 (0.0)	3.0 (2.0)	3.0 (2.0)	3.0 (2.0)	4.0 (2.0)	4.0 (2.0)	4.0 (2.0)	4.0 (2.0)	4.0 (2.0)	3.0 (2.0)
Wall Thickness	1.03 (0.2)	0.97 (0.2)	0.95 (0.2)	0.95 (0.2)	1.1 (0.3)	1.1 (0.2)	0.95 (0.2)	0.98 (0.2)	1.2 (0.3)	1.2 (0.3)
Lumen area	6.3 (4.5)	5.4 (1.6)	5.0 (1.5)	5.4 (1.5)	6.0 (3.6)	6.8 (4.1)	5.0 (1.5)	5.4 (2.3)	8.0 (4.7)	8.2 (5.3)
Wall Area	14.2 (6.7)	11.6 (3.7)	11.5 (3.2)	11.5 (3.6)	13.2 (7.1)	14.4 (7.4)	11.1 (2.9)	12.2 (4.8)	16.1 (9.0)	16.6 (7.5)
Total Airway area	20.5 (11.2)	17.2 (4.3)	16.7 (3.9)	17.0 (4.4)	19.0 (9.8)	21.2 (11.2)	16.3 (4.7)	17.9 (7.4)	23.8 (13.2)	25.2 (12.1)
WA%	68.5 (3.8)	68.6 (3.1)	68.9 (3.7)	68.7 (3.1)	68.0 (3.2)	68.3 (5.0)	68.7 (2.5)	69.2 (3.2)	67.3 (4.2)	67.4 (5.2)
Generation 6										
No. Airways	6.0 (3.0)	2.0 (2.0)***	3.0 (3.0)	3.0 (2.0)	4.0 (3.0)	4.0 (4.0)	3.0 (4.0)	3.0 (4.0)	4.0 (2.0)	4.0 (2.0)
Wall Thickness	0.95 (0.15)	1.00 (0.3)	0.97 (0.2)	1.00 (0.3)	1.07 (0.2)	1.07 (0.2)	1.00 (0.3)	1.00 (0.24)	1.08 (0.3)	1.1 (0.2)
Lumen area	4.8 (1.4)	5.4 (2.4)	5.2 (1.9)	5.4 (2.1)	5.9 (2.8)	6.4 (2.5)	5.2 (1.9)	5.0 (1.9)	6.9 (3.5)	6.8 (3.2)
Wall Area	11.1 (2.5)	12.0 (4.4)	11.2 (3.2)	11.8 (4.7)	14.1 (5.0)	13.6 (4.3)	12.8 (3.5)	11.9 (3.8)	14.0 (6.2)	14.7 (5.8)
Total Airway area	15.9 (3.4)	16.9 (6.0)	16.5 (5.1)	11.8 (4.7)	19.7 (7.0)	20.0 (6.4)	18.0 (5.0)	16.1 (5.4)	21.1 (9.4)	21.6 (8.2)
WA%	69.1 (3.7)	68.5 (2.9)	68.7 (4.2)	68.5 (3.0)	67.8 (2.6)	68.0 (3.7)	69.7 (4.1)	69.5 (2.6)	67.9 (4.4)	67.9 (3.8)

Data represents medians and IQR. N=74 for generation 3. N=68 for generation 4. N=55 for generation 5. N=30 for generation 6. *p<0.05, **p<0.01, ***p<0.001 using related samples Wilcoxon signed rank test.

7.3 Body Composition progression

Body composition was successfully measured on the enrolment CT and at month 24 in 76 subjects. As I have previously demonstrated that gender is important in body composition analysis I chose to perform further analysis on the individual sexes.

7.3.1 Body composition in males

45 males had CT body composition data available at enrolment and month 24. At enrolment the median ACF for these 45 subjects was 3763.3 (2107.2) while at month 24 it was 3800.2 (2270.0). There was no significant differences between these two values (p 0.608). The histogram for change in ACF in males is shown in Figure 64. Median change in ACF was -13.0 (723.4).

PMA at enrolment was 3317.2 (1183.4) and at month 24 was 3284.8 (1291.8). Although relatively small this difference was statistically significant (p<0.001). The histogram for change in PMA in males is shown in Figure 65. Median change in PMA was -210.2 (463.1).

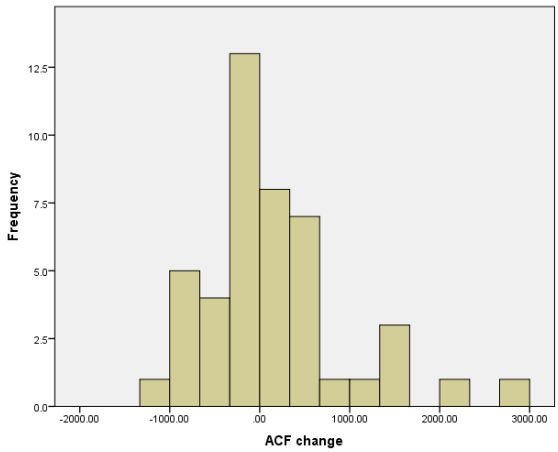


Figure 64. Distribution histogram for change in anterior chest fat over two years in males. Positive results, represents increasing ACF over the two year period.

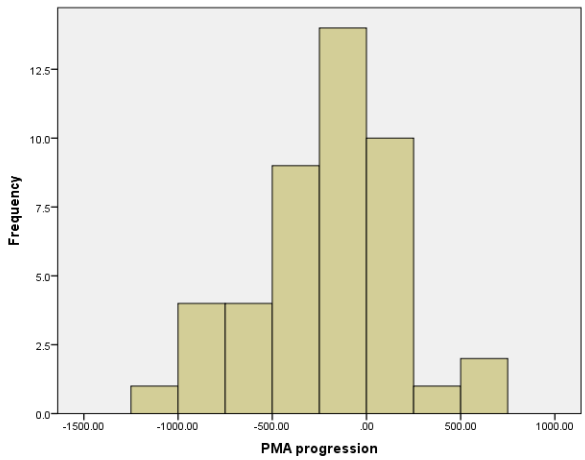


Figure 65 Distribution histogram for change in pectoralis muscle area over two years in males. Positive results, represents increasing PMA over the two year period.

Table 164. Association between ACF and PMA progression and physiological, functional, clinical and biological markers at enrolment in males.

	ACF Progression	PMA Progression
Pulmonary Function		
FEV1%	0.11	0.11
TLCO%	-0.08	0.06
TLC%	-0.23	0.00
RV%	-0.28	0.02
Functional Capacity		
6MWD	-0.09	0.11
Oxygen desaturation	0.17	-0.06
CAT	-0.06	**-0.32***
EXCAT	**-0.39***	-0.13
CT Parameters		
%LAA$_{<-950}$	0.02	0.05
E/I MLD	-0.08	-0.21
Pi10	0.14	-0.10
Sputum Differential		
Neutrophils%	0.01	-0.08
Macrophages%	-0.13	0.21
Eosinophils%	0.03	-0.05
Blood Inflammatory Markers		
CRP	0.28	-0.04
Eosinophils	0.17	0.11
Fibrinogen	0.25	-0.19
Neutrophils	0.12	0.05
PCT	0.29	-0.14
Serum Biomarkers		
Hs-cTNT	0.00	-0.18
IL-6	-0.01	-0.25
IP-10	0.19	0.00
NT-proBNP	0.11	-0.09
SP-D	-0.05	-0.17
Exacerbations		
Exacerbation rate	-0.09	-0.07

Spearman's correlation. N=45 for FEV1%, 6MWT, questionnaires, CT measures, CRP, PCT, exacerbations. N=44 for pulmonary function, blood white cells, hs-troponin, IP-10, Sp-D. N=27 for sputum cell differential. N=40 for fibrinogen. N=35 for Il-6 and N=43 for NT-proBNP.

There were few associations between change in CT derived body composition markers and physiological, functional, clinical and biological markers of disease in males (Table 164). When dividing change in ACF into tertiles the lowest tertile (those who lost fat) had a significantly higher exact score and lower PCT (Table 165). The middle tertile had the highest exacerbation rate. When dividing change in PMA into tertiles the only significant difference between the groups that the middle tertile had significantly higher IP-10 and pro-BNP (Table 166).

Table 165. Physiological, functional, clinical and biological markers at enrolment in the tertiles stratified according to ACF progression in males.

	<-233.0	-233.0-314.9	>314.9	Significance
Pulmonary Function				
FEV1%	44.9 (24.9)	47.5 (20.7)	49.5 (18.3)	0.613
TLCO%	55.8 (33.8)	49.6 (28.0)	59.0 (45.3)	0.284
TLC%	116.8 (23.2)	106.9 (33.2)	97.8 (11.3)	0.266
RV%	150.6 (89.4)	138.2 (80.7)	127.7 (45.2)	0.084
Functional Capacity				
6MWD	364.0 (210.0)	326.0 (164.0)	388.0 (158.0)	0.967
Oxygen desaturation	3.0 (4.0)	6.0 (7.0)	5.0 (7.0)	0.456
CAT	21.0 (13.0)	15.0 (15.0)	15.0 (12.0)	0.783
EXCAT	43.0 (11.0)	36.5 (18.0)	33.0 (14.0)	**0.029***
CT Parameters				
%LAA$_{<-950}$	16.7 (30.1)	16.1 (22.0)	16.9 (15.3)	0.970
E/I MLD	0.92 (0.08)	0.92 (0.09)	0.90 (0.05)	0.756
Pi10	3.74 (0.13)	3.75 (0.10)	3.79 (0.10)	0.313
Sputum Differential				
Neutrophils%	50.6 (82.7)	37.1 (72.3)	68.5 (84.4)	0.590
Macrophages%	28.8 (50.1)	27.9 (46.5)	26.1 (25.0)	0.779
Eosinophils%	2.21 (8.3)	1.27 (4.2)	2.20 (6.2)	0.723
Blood Inflammatory Markers				
CRP	2.0 (5.0)	6.0 (6.0)	5.5 (13.3)	0.078
Eosinophils	0.20 (0.3)	0.20 (0.1)	0.30 (0.2)	0.420
Fibrinogen	4.6 (1.7)	5.1 (0.6)	5.0 (0.6)	0.478
Neutrophils	4.7 (1.7)	4.7 (1.3)	5.1 (2.7)	0.478
PCT	0.055 (0.03)	0.055 (0.02)	0.070 (0.04)	**0.046***
Serum Biomarkers				
Hs-cTNT	0.018 (0.02)	0.013 (0.01)	0.017 (0.01)	0.977
IL-6	3.3 (3.3)	2.9 (1.5)	4.1 (2.1)	0.417
IP-10	133.0 (83.3)	216.5 (131.3)	178.5 (133.8)	0.113
NT-proBNP	112.0 (342.1)	88.7 (119.8)	90.6 (325.1)	0.798
SP-D	234.0 (143.5)	239.0 (163.3)	178.0 (146.0)	0.566
Exacerbations				
Exacerbation rate	1.99 (2.1)	4.91 (3.9)	0.99 (4.0)	**0.024***

N=45 for FEV1%, 6MWT, questionnaires, CT measures, CRP, PCT, exacerbations. N=44 for pulmonary function, blood white cells, hs-troponin, IP-10, Sp-D. N=27 for sputum cell differential. N=40 for fibrinogen. N=35 for Il-6 and N=43 for NT-proBNP.

Table 166. Physiological, functional, clinical and biological markers at enrolment in the tertiles stratified according to PMA progression in males.

	<-316.5	-316.5—32.2	>-32.2	Significance
Pulmonary Function				
FEV1%	44.9 (21.7)	48.3 (13.2)	46.8 (25.1)	0.533
TLCO%	58.7 (38.5)	53.4 (29.6)	62.1 (33.3)	0.716
TLC%	103.4 (11.7)	102.9 (28.0)	107.9 (25.2)	0.851
RV%	143.9 (22.2)	125.3 (50.9)	144.5 (77.4)	0.347
Functional Capacity				
6MWD	364.0 (132.0)	321.0 (141.0)	410.0 (221.0)	0.210
Oxygen desaturation	5.0 (4.0)	4.0 (5.0)	4.0 (7.0)	0.848
CAT	21.0 (11.0)	18.0 (15.0)	14.5 (9.0)	0.071
EXCAT	38.0 (14.0)	37.0 (16.0)	36.0 (10.0)	0.735
CT Parameters				
%LAA$_{<-950}$	15.8 (15.2)	16.1 (21.7)	18.7 (30.4)	0.886
E/I MLD	0.92 (0.06)	0.90 (0.06)	0.90 (0.09)	0.320
Pi10	3.81 (0.16)	3.76 (0.10)	3.75 (0.09)	0.338
Sputum Differential				
Neutrophils%	58.9 (78.6)	65.4 (52.9)	40.2 (76.7)	0.721
Macrophages%	26.5 (31.3)	23.0 (21.0)	35.7 (60.0)	0.626
Eosinophils%	0.20 (8.3)	3.7 (5.7)	1.27 (3.2)	0.465
Blood Inflammatory Markers				
CRP	4.0 (6.0)	6.0 (11.5)	4.0 (8.5)	0.276
Eosinophils	0.20 (0.3)	0.30 (0.2)	0.20 (0.2)	0.726
Fibrinogen	5.0 (0.8)	5.1 (0.6)	4.8 (1.3)	0.400
Neutrophils	4.5 (2.)	4.8 (0.7)	4.8 (1.8)	0.684
PCT	0.063 (0.02)	0.069 (0.04)	0.055 (0.01)	0.602
Serum Biomarkers				
Hs-cTNT	0.018 (0.01)	0.016 (0.01)	0.015 (0.01)	0.565
IL-6	3.5 (2.8)	5.1 (2.5)	3.0 (0.8)	0.061
IP-10	130.0 (67.0)	219.5 (245.5)	144.0 (162.6)	**0.044***
NT-proBNP	68.5 (180.0)	223.0 (334.4)	75.0 (83.4)	**0.049***
SP-D	175.5 (167.3)	284.5 (128.5)	190.0 (98.5)	0.415
Exacerbations				
Exacerbation rate	1.99 (4.0)	4.87 (5.0)	2.00 (3.0)	0.364

N=45 for FEV1%, 6MWT, questionnaires, CT measures, CRP, PCT, exacerbations. N=44 for pulmonary function, blood white cells, hs-troponin, IP-10, Sp-D. N=27 for sputum cell differential. N=40 for fibrinogen. N=35 for Il-6 and N=43 for NT-proBNP.

7.3.2 Body composition in Females

31 females had CT body composition data available at enrolment and month 24. At enrolment the median ACF for these subjects was 6290.2 (3630.0) while at month 24 it was 5551.1 (4648.9). There was no significant differences between these two values (p 0.096). The histogram for change in ACF in females is shown in Figure 66. Median change in ACF was 252.0 (1660.7).

PMA at enrolment was 2237.1 (772.0) and at month 24 was 2139.6 (738.8). There was no significant difference between these two values (p 0.724). The histogram for change in PMA in females is shown in Figure 67. Median change in PMA was 18.5 (363.9).

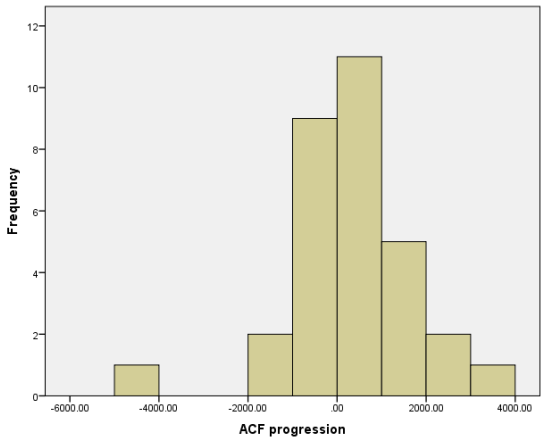


Figure 66. Distribution histogram for change in anterior chest fat over two years in females.

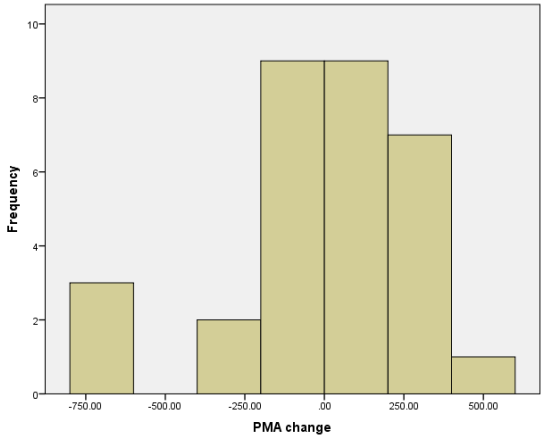


Figure 67 Distribution histogram for change in pectoralis muscle area over two years in females

There were few associations between change in CT derived body composition markers and physiological, functional, clinical and biological markers of disease in females (Table 167). When dividing change in ACF and change in PMA into tertiles there were no significant differences between groups (Table 168 and Table 169).

Table 167. Association between ACF and PMA progression and physiological, functional, clinical and biological markers at enrolment in females.

	ACF Progression	PMA Progression
Pulmonary Function		
FEV1%	0.26	-0.04
TLCO%	**0.40***	-0.18
TLC%	0.15	-0.26
RV%	0.24	-0.12
Functional Capacity		
6MWD	0.33	-0.03
Oxygen desaturation	-0.12	-0.03
CAT	0.02	0.05
EXCAT	0.18	0.17
CT Parameters		
%LAA$_{<-950}$	0.05	-0.29
E/I MLD	-0.06	-0.05
Pi10	-0.33	0.17
Sputum Differential		
Neutrophils%	0.10	0.28
Macrophages%	0.16	-0.24
Eosinophils%	0.11	-0.19
Blood Inflammatory Markers		
CRP	-0.09	0.06
Eosinophils	0.09	-0.19
Fibrinogen	-0.28	0.02
Neutrophils	-0.15	0.07
PCT	0.18	-0.15
Serum Biomarkers		
Hs-cTNT	0.10	0.10
IL-6	-0.15	0.00
IP-10	0.05	0.10
NT-proBNP	0.33	0.14
SP-D	0.10	-0.03
Exacerbations		
Exacerbation rate	-0.09	-0.00

N-31 for FEV1%, questionnaires, blood white cells, PCT, exacerbations. N=30 for TLCO%, 6MWT. N=27 for plethysmography, CT measures. N=20 for sputum differential. N=28 for fibrinogen, hs-troponin. N=29 for IP-10, NT-proBNP and Sp-D. N=16 for IL-6.

Table 168. Physiological, functional, clinical and biological markers at enrolment in the tertiles stratified according to ACF progression in females.

	<-155.4	-155.4-872.1	>872.1	Significance
Pulmonary Function				
FEV1%	56.8 (28.3)	47.0 (22.2)	51.5 (22.2)	0.618
TLCO%	58.8 (36.1)	57.6 (16.6)	73.5 (24.6)	0.071
TLC%	111.9 (35.2)	107.9 (23.2)	118.6 (35.2)	0.624
RV%	141.2 (99.2)	151.3 (42.5)	157.6 (59.6)	0.647
Functional Capacity				
6MWD	272.0 (93.0)	316.0 (166.0)	345.0 (134.0)	0.316
Oxygen desaturation	4.5 (14.3)	4.0 (6.0)	4.0 (5.5)	0.860
CAT	15.0 (11.0)	19.0 (10.0)	14.0 (10.0)	0.202
EXCAT	34.0 (20.0)	38.0 (9.0)	37.0 (20.0)	0.178
CT Parameters				
%LAA$_{<-950}$	9.1 (25.1)	4.6 (26.7)	12.1 (12.8)	0.834
E/I MLD	0.92 (0.06)	0.90 (0.10)	0.92 (0.06)	0.282
Pi10	3.8 (0.16)	3.8 (0.14)	3.76 (0.1)	0.328
Sputum Differential				
Neutrophils%	17.7 (42.7)	48.1 (53.4)	6.6 (90.3)	0.725
Macrophages%	35.5 (45.0)	46.3 (29.7)	56.1 (69.1)	0.844
Eosinophils%	2.02 (12.1)	0.47 (1.5)	2.9 (5.3)	0.320
Blood Inflammatory Markers				
CRP	4.0 (7.3)	4.0 (15.0)	4.5 (18.8)	0.962
Eosinophils	0.20 (0.13)	0.20 (0.20)	0.20 (0.20)	0.538
Fibrinogen	5.0 (2.2)	5.1 (1.3)	4.7 (1.1)	0.403
Neutrophils	4.5 (1.7)	5.2 (1.5)	3.9 (2.3)	0.095
PCT	0.048 (0.03)	0.054 (0.03)	0.060 (0.03)	0.290
Serum Biomarkers				
Hs-cTNT	0.008 (0.01)	0.009 (0.01)	0.009 (0.00)	0.675
IL-6	4.9 (2.0)	7.1 (5.0)	5.0 (2.7)	0.747
IP-10	150.0 (104.4)	125.5 (54.1)	146.0 (271.3)	0.364
NT-proBNP	48.7 (63.7)	75.9 (90.8)	85.1 (96.8)	0.254
SP-D	137.0 (63.0)	132.5 (174.5)	148.0 (96.8)	0.984
Exacerbations				
Exacerbation rate	1.98 (2.3)	3.97 (3.0)	0.99 (3.0)	0.085

N-31 for FEV1%, questionnaires, blood white cells, PCT, exacerbations. N=30 for TLCO%, 6MWT. N=27 for plethysmography, CT measures. N=20 for sputum differential. N=28 for fibrinogen, hs-troponin. N=29 for IP-10, NT-proBNP and Sp-D. N=16 for IL-6.

Table 169. Physiological, functional, clinical and biological markers at enrolment in the tertiles stratified according to PMA progression in females.

	<-98.9	146.0-98.9	>146.0	Significance
Pulmonary Function				
FEV1%	56.9 (27.8)	56.7 (25.7)	42.5 (20.8)	0.434
TLCO%	65.8 (23.1)	69.3 (44.7)	60.2 (22.1)	0.498
TLC%	114.5 (28.6)	130.6 (40.3)	104.3 (20.6)	0.409
RV%	144.2 (80.9)	151.2 (87.0)	154.5 (37.5)	0.900
Functional Capacity				
6MWD	313.5 (140.0)	321.0 (147.0)	312.5 (135.0)	0.761
Oxygen desaturation	3.5 (7.0)	4.5 (12.0)	4.5 (6.5)	0.804
CAT	15.5 (10.0)	16.0 (5.0)	17.5 (16.0)	0.730
EXCAT	34.0 (16.0)	33.5 (21.0)	39.0 (12.0)	0.094
CT Parameters				
%LAA$_{<-950}$	10.9 (29.9)	14.3 (18.1)	5.4 (15.1)	0.462
E/I MLD	0.91 (0.08)	0.93 (0.06)	0.91 (0.07)	0.346
Pi10	3.79 (0.1)	3.80 (0.2)	3.78 (0.2)	0.823
Sputum Differential				
Neutrophils%	14.0 (55.1)	6.6 (61.6)	48.7 (72.7)	0.563
Macrophages%	41.0 (50.2)	56.1 (32.6)	25.2 (39.8)	0.487
Eosinophils%	1.35 (3.7)	0.63 (3.2)	0.75 (4.0)	0.841
Blood Inflammatory Markers				
CRP	2.0 (18.0)	7.0 (11.0)	4.0 (16.3)	0.659
Eosinophils	0.20 (0.23)	0.20 (0.10)	0.20 (0.13)	0.508
Fibrinogen	4.8 (1.0)	4.9 (1.0)	4.7 (2.0)	0.787
Neutrophils	4.5 (2.6)	4.8 (2.9)	5.1 (1.7)	0.953
PCT	0.054 (0.03)	0.061 (0.04)	0.047 (0.03)	0.539
Serum Biomarkers				
Hs-cTNT	0.008 (0.00)	0.01 (0.01)	0.01 (0.01)	0.769
IL-6	4.8 (4.5)	4.7 (2.6)	7.0 (2.7)	0.400
IP-10	118.5 (75.1)	138.0 (184.5)	140.5 (153.2)	0.724
NT-proBNP	53.8 (69.7)	84.1 (62.3)	89.2 (146.1)	0.477
SP-D	138.0 (98.3)	147.0 (107.8)	128.0 (138.7)	0.879
Exacerbations				
Exacerbation rate	2.48 (3.3)	3.00 (4.9)	1.99 (2.3)	0.656

N-31 for FEV1%, questionnaires, blood white cells, PCT, exacerbations. N=30 for TLCO%, 6MWT. N=27 for plethysmography, CT measures. N=20 for sputum differential. N=28 for fibrinogen, hs-troponin. N=29 for IP-10, NT-proBNP and Sp-D. N=16 for IL-6.

7.4 Discussion

My previous chapters have focused on CT imaging in cross-sectional studies. However, it is vital studies are performed to track CT changes over time to get an accurate picture of the progression of the disease and how effective CT imaging is at capturing this. To date, there have been relatively few longitudinal studies utilising CT imaging and these have shown variable results.

7.4.1 Emphysema Progression

To understand progression of emphysema I used the CT measure $\%LAA_{<-950}$, as I have previously demonstrated that this had the best association with physiological markers of disease. My studies were not conceived to answer which was the best marker of emphysema and it could be argued that $PERC_{15}$ is an equally good measure with which to measure longitudinal change. A number of studies have used PERC15 to study change over time[217], while other studies have used $\%LAA_{<-950}$[204,323,324]. Without linking these changes to pathological changes of emphysema over time it will be impossible to say with confidence which of these markers is the most accurate and in all likelihood both CT markers of emphysema will give similar results. However, as I have previously stated, $\%LAA_{<-950}$, holds a number of advantages as it has the benefit of being a direct measure of emphysema. In addition to this $PERC_{15}$ can also be affected by areas of higher attenuation. It is for these reasons that I chose to use $\%LAA_{<-950}$ in my longitudinal study.

The only factor at enrolment which significantly predicted progression of emphysema was FEV1%. On univariate analysis FEV1%, TLCO%, TLC%, RV%, LAA and E/I MLD all had significant associations with decline in $\%LAA_{<-950}$, however when correcting for FEV1% and LAA% at baseline only FEV1% remained significant. This therefore suggests that subjects with worse airflow obstruction have accelerated emphysema progression. Previous studies have also found that airflow obstruction was associated with significantly greater decline in emphysema over time over time[189,325]. Unlike my study, other studies have however shown other factors which predict increased decline in emphysema. One study showed that females had greater decline and current smokers had increased rate of decline in emphsyema[325]. However, this was a lung cancer screening trial and as such had a much milder cohort and the CT protocol was sub-optimal with rather thick CT slices, possibly decreasing resolution and accuracy. Another study showed increased progression in emphysema in smokers and increasing age[323], although once again this was a lung cancer screening trial with mostly non-obstructed subjects. Lower BMI has also been shown to be associated with increase rate of decline of PD15, another measure of emphsyema[189]. Accelerated decline was also seen in subjects with exacerbations[187].

Unfortunately, in my study I was unable to investigate the association between longitudinal change in emphysema and lung function and physiological parameters, due to the data not being made available. Previous studies have investigated this link, with two studies finding a significant but rather weak associations between FEV1% decline and emphysema decline[189,326]. There were differing results when using data from the Danish Lung cancer screening trial with one study finding a weak association between FEV1% decline and PD15 decline[327] while another showed no association between FEV1% decline and LAA% decline[323]. This may be explained by the different techniques with which to estimate emphysema. However further studies are needed to investigate these changes with optimal CT protocols and are more severe cohort than lung cancer screening trails provide.

Few previous studies has investigated the link between emphysema progression and biological markers of disease. One of these used CT data from the ECLIPSE cohort and investigated the association between emphysema progression and a number of serum biomarkers. They found that raised SPD and CRP were associated with increase rate of decline of PD15, whereas raised sRAGE, fibrinogen, IL-6 associated with less decline of PD15[189]. This is inconsistent with my results, as I did not find any associations between serum or sputum biomarkers and $\%LAA_{<-950}$ progression. Specifically, I did not find any association between CRP, fibrinogen, IL-6 or SP-D concentrations and emphysema progression. There were a number of differences between my study and the ECLIPSE study, which involved far more study subjects which may have helped in exhibiting significant associations. Secondly CT protocols were different in the ECLPISE cohort, which used thicker CT slices and therefore may have been less accurate than the protocol used in my study. Finally they also used a different measure of emphysema which may have influenced the results. Another study assessed T-cell responses to elastin fragments and emphysema progression. In smokers there was an association between T-cell production of IFN-γ and IL-6 in response to elastin fragments and emphysema progression. This suggests a possible autoimmune process in emphysema development. Interestingly I found no association between exacerbation frequency and emphysema progression. A previous study has also demonstrated this finding[324], although this is in contrast to another study which showed an accelerated decline in LAA% in subjects with exacerbations[187].

As already mentioned, some subjects had lower $\%LAA_{<-950}$ at the month 24 scan than at enrolment, suggesting inaccuracies with the quantification of emphysema. To ensure this did not unduly influence my results I divided the cohort into three tertiles. The lowest tertile represented patients whose emphysema did not progress while the highest tertile represented subjects whose emphysema did progress. The middle tertile was an intermediate group who may have progressed a little or who may have been relatively static. When analysing these results there was significantly

lower FEV1%, TLCO% and higher E/I MLD in tertile two and three compared to tertile one and LAA%, TLC and RV was significantly higher in tertile 3 than tertile 1. This all agrees with my previous findings that subjects whose emphysema progressed over the two year period had worse airflow obstruction, with more emphysema and gas trapping on CT imaging and evidence of hyperinflation on CT imaging. Once again there remained no difference between any of the blood or sputum biomarkers and progression of emphysema.

In this study I was also able to investigate the progression of emphysema in each individual lobe. I demonstrated a significant increase in $\%LAA_{<-950}$ in each lobe between the enrolment scan and month 24 scan. Median progression in each lobe was relatively consistent and was very similar to overall progression and varied from 0.9% to 1.3% over the two-year period. The only lobes that showed a significant difference between progressions of emphysema was between the LLL and the RUL, although the difference was small. This all suggests that emphysema progresses relatively evenly throughout the lungs. No other studies have investigated this progression of emphysema in different lobes. One small study looked at emphysema progression in three different slices throughout the lung fields and found that there was significant progression of LAA in the middle and lower zone but not in the upper zone[204]. This is obviously different to my results but measuring just emphysema in individual slices is unlikely to be as accurate as using volumetric reconstructions.

7.4.2 Longitudinal Change in Airway Dimensions

In my studies so far I have shown that measuring large and intermediate airway dimensions Is not particularly helpful in COPD. However, how airway dimensions change over time is not particularly well understood in COPD and so I elected to investigate this is in my cohort by comparing the enrolment and month 24 scan. I found no significant difference between PI10 at enrolment and at month 24. The only variables at enrolment that had significant associations with PI10 change was a negative association with baseline Pi10, a moderate positive association with sputum neutrophils and a weak association with exacerbation rate. When dividing the cohort into two based on whether Pi10 increased or not over the two-year study period, there was no significant differences in FEV1% between those who's Pi10 increased and those whose did not. There was no difference in sputum neutrophils and exacerbation rate between the two groups. Subjects whose Pi10 increased had significantly lower TLCO%, lower Pi10 and lower serum PCT at enrolment than those whose Pi10 did not increase. I also have previously shown there is significant variability in airway dimensions in different airway paths, which is not reflected in the Pi10 parameter. I therefore elected to compare the changes in these airway dimensions throughout the five different airway

paths between the enrolment scans and the CT at month 24. When doing this there were rather haphazard results with some airway paths in some segmental generation showing worsening airway wall thickening and larger airways while other showing thinning of airway walls and smaller airways. There is little sense to be made of these results as there does not really seem to be any significant pattern to them.

Only one previous study has analysed the change in airway wall dimensions over time in a relatively small cohort of 38 subjects over a four-year period. The researchers measured wall area percentage in one of the basal segmental bronchi on one axial slice. They also found no significant change in WA% over the time period and also no association between bassline lung function and change in WA%. They did however find a weak association between annual change in WA% and change in FEV1%[204]. However, I have already demonstrated the problem with only measuring one airway and so I think these results are flawed. My results did not really provide any conclusive results regarding longitudinal change in airway dimensions in COPD patients and continues with the idea from previous chapters that measuring airway wall dimensions in COPD are of questionable use.

7.4.3 Longitudinal Change in Body composition measurements

In the previous chapter I demonstrated that ACF and PMA could be measured on chest CT to provide an assessment of body composition in COPD. In this chapter I have once again used these measures and analysed how they changed over the two-year study period. When doing this I found that there was a small but significant decrease in PMA in male subjects but not in females. There was also no significant difference in ACF between the two sets of CT scans in either males or females. This suggests that over a two-year period, body composition as measured by chest CT doesn't change significantly in COPD. There were also few associations between change in either PMA or ACF and physiological, functional, clinical and biological markers at enrolment. There was however significant variation within the cohort and so I divided the cohort into three based on PMA and ACF. When doing this there were also few differences in physiological, functional, clinical and biological markers at enrolment between groups. This suggests that underlying disease severity or biological activity does not have an influence on change in fat or muscle mass in COPD.

To the best of my knowledge no previous work has investigated the longitudinal change in these CT parameters over time. In general, surprisingly few studies have assessed the longitudinal change in body composition in COPD. A study evaluating 5 year change in BMI in a large cohort of COPD subjects showed that in severe subject's average weight loss was relatively modest at 0.2 BMI in both males and females[328]. However, in the same study moderate COPD patients gained weight. Up

to 35% of subjects lost over 1 BMI unit of weight, suggesting there was significant heterogeneity with weight loss. This study also showed this was important as subjects who lost weight had significantly increased mortality. Another study analysed the change in fat mass and FFMI over a three year period and also found on average minimal change in these parameters[322]. Similarly to my study they also found quite significant heterogeneity in this progression. They found that blood CRP and IL-6 and IL-1B did not have much of an effect on loss of FFMI, although TNF-a did cause increased rate of decline.

7.4.4 Strengths and Limitations

The most obvious limitation in this study was the lack of longitudinal data, other than the CT variables. This data has been captured but due to the nature of working with a commercial sponsor I have been unable to have access to it. Therefore, I was only able to analyse which variables at enrolment predicted change in CT measurements and I could not compare the change in physiological, functional and biological markers over time with the change in CT parameters. There is a risk when measuring a variable at only one-time point that this does not accurately reflect the status of the subject. For example, many biomarkers have natural variation over time and tracking its change over period of time may be necessary. Another limitation in this study was the relatively small number of subjects who had both an enrolment and month 24 CT scan. A number of subjects dropped out during the two-year study and this in itself could have introduced bias into the results as I showed that these subjects had more severe COPD. This also lessoned the statistical power of my study and this was especially the case for body composition where the genders had to be analysed separately. Another limitation of my study was the fact that a full expiratory scan was not performed at month 24 and only limited slices were taken in order to limit radiation exposure. This meant I was unable to assess gas trapping on the follow-up scan and was therefore unable to assess the longitudinal change in this.

Another limitation of this longitudinal study was that I do not know the variability of the measurements of CT parameters, making it difficult to interpret the longitudinal results. This is less of an issue with emphysema measurements as more complete and longitudinal studies have already been performed. However, for airway wall dimensions this is unknown and the results need to be interpreted with caution. In any case my results throughout the entire study suggests that using CT to measure airway wall dimensions is unreliable.

A significant strength of my study was that this was a single centre study with all CT scans being performed on the same machine with the same imaging protocol. This is especially important when

evaluating longitudinal change and is in contrast to many other larger studies, which could therefore cause errors in these studies.

7.4.5 Summary

In summary I have shown that in a cohort of moderate to severe COPD subjects, emphysema measured by CT imaging significantly worsens over a two-year period. There is however, significant heterogeneity within this progression and the only variable at enrolment that was a significant predictor of progression was FEV1%. Markers of airways dimensions were unable to demonstrate significant change in the cohort over a two-year period and the only body composition CT measure that showed significant change was a decrease in pectoralis muscle are in males.

Chapter 8: Summary Discussion

The primary goal of my PhD thesis was to further understand the role of CT imaging in COPD by investigating its relationship with disease activity and to assess longitudinal changes in COPD. Many of my findings were novel and have been discussed in detail in the relevant chapters. The main findings were as follows:-

- Emphysema can be quantitated on CT imaging using threshold-based analysis software and this was equally successful for both cohorts of patients. $\%LAA_{<-950}$ was the only automated marker of emphysema that showed a significant difference between subjects with COPD and those with preserved lung function and also generally had the strongest associations with lung function in both studies.
- Small airways disease can be measured using a surrogate marker of gas trapping on inspiratory and expiratory CT images. E/I MLD had the strongest associations with lung function markers of small airways dysfunction/gas trapping.
- Emphysema sub-types can also be quantitated using a novel local histogram-based emphysema algorithm to analyse CT images.
- Body composition could also be assessed in COPD using measurements of pectoralis muscle and anterior chest fat on CT images and these had strong associations with traditional markers of body composition.
- The optimum method for determining dimensions of large and intermediate dimensions could not be determined as none of the methods assessed showed significant differences between subjects with and without COPD and there were few associations with lung function parameters. There were also significant variation in the dimensions of large and intermediate airways measured in different airway paths.
- Multiple MMPs were raised in subjects with COPD compared to those with preserved lung function. They also had significant associations with spirometry, emphysema and small airways disease, suggesting a key role in the abnormal tissue remodelling seen in COPD.
- Unlike previous cohorts bronchiectasis was not particularly common in the AERIS study. However, when it was present it was associated with worse airflow obstruction and higher neutrophilic systemic and airway inflammation.
- In the MICA study there were significant associations between airway Inflammation and CT-measured small airways disease. This however could not be replicated in the larger study, although fewer and less specific markers of airways inflammation were measured in this study.

- There were no significant associations between exacerbation frequency or microbiology and CT parameters.
- Emphysema severity was associated with desaturation on exertion in COPD.

Next I discuss my results in consideration of each of my specific aims outlined in chapter 2.

8.1 To characterise the systemic and airway inflammatory profile, airway bacteria presence and airway proteases in a cohort of COPD subjects undergoing bronchoscopy.

Using a novel, targeted bronchoscopy technique I have I demonstrated that a range of MMPs are raised in COPD subjects as opposed to subjects with preserved lung function. Unlike most studies I measured a broad range of MMPs and have found many had associations with airflow obstruction, suggesting they may play an important role in COPD. I also found further evidence for neutrophilic airway inflammation by finding higher concentrations of neutrophils, IL-6 and IL-8 in COPD subjects compared to those without airflow obstruction. Neutrophils and both of these cytokine also had an inverse association with airflow obstruction.

As opposed to airway inflammation, I did not find any significant evidence for systemic inflammation on this relatively mild cohort. I also did not find any difference in detection rate of bacteria in sputum between subjects with COPD and PLF.

8.2 To demonstrate that quantitative CT analysis can be used to adequately measure the main morphological features of COPD and determine the optimum methods for measuring these.

I successfully used quantitative analysis of CT imaging to measure emphysema and small airways disease in two different cohorts of COPD subjects. Furthermore I also used novel CT analysis to measure different emphysema sub-types and markers of body composition. I was less successful in measuring dimensions of large and intermediate airways and my results have led me to question the utility of these measures.

For emphysema measurements I assessed three different automated methods and a visual emphysema score. Of the automated scores I demonstrated that $\%LAA_{<-950}$ was the only measure

that demonstrated significantly higher values in COPD subjects compared to subjects without airflow obstruction and it also had the strongest associations with airflow obstruction and gas transfer. It has to be noted that results with $PERC_{15}$ were very similar and it is likely that both are good measures of emphysema. However, $\%LAA_{<-950}$ has a couple of advantages over $PERC_{15}$. Firstly it gives a direct measure of emphysema in a percentage form while $PERC_{15}$ gives a result in Hounsfield units, which feels less accessible and therefore less easy to use. Secondly, $PERC_{15}$ has the potential to be influenced by higher attenuation areas within the lung parenchyma, giving inaccurate results, although admittedly I was unable to demonstrate this in my studies. I was also unable to show that automated analysis was superior to visual score for measuring emphysema. In the larger cohort the visual score had the strongest association with gas transfer and also had as strong relationship with FEV1% as $\%LAA_{<-950}$. There were, however, suggestions that it was not as good in the smaller cohort, which could be due to this score being less accurate in milder cohorts and is unable to differentiate subtle disease from normal tissue. Automated scores have a number of potential advantages over visual scores. Automated scores are much quicker to perform than manual reading of the scans, while manual scores may also be affected by inter-observer differences. Automated measures also give information about anatomical distribution of emphysema and are likely to have greater sensitivity to detect longitudinal change although I was unable to test this due to not having visual scores available for the month 24 scan. I also successfully used novel analysis based on local-emphysema histograms to determine emphysema sub-types in my smaller study. I demonstrated that all tissue sub-types were present in COPD and the most common tissue sub-types in COPD was non-emphysematous tissue and mild CLE. What was particularly striking was that subjects without airflow obstruction also had high quantities of mild CLE.

To measure small airways disease using CT imaging I used gas trapping as a surrogate marker. Four different automated methods and a visual score were used to measure gas trapping and I showed that E/I MLD was the best of these methods. Like all the automated measures it demonstrated significantly more gas trapping in COPD subjects than those with preserved lung function. It also had the strongest association with spirometric markers in both cohorts as well as the strongest association with RV/TLC in the larger cohort. The automated estimate, RVC also had strong associations with these parameters, but it has the significant disadvantage of being quite complex and complicated. Unlike in emphysema, visual scoring of gas trapping was clearly inferior to automated analysis and was not an accurate measure. In the smaller cohort it did not show any associations with spirometry and actually showed more gas trapping in subjects with PLF as opposed to those with COPD. It also had only a weak association with RV/TLC in the larger cohort.

Measuring dimensions of intermediate and large airways on CT was the most challenging of the morphological features and ultimately the most disappointing. I used a number of different methods to measure airway dimensions and did not find any of them particularly useful in either cohort of patients. Many larger studies use PI10 as a measure of airway wall thickening, however I found few meaningful associations with it. I also showed there was significant variation in airway dimensions in different generations of the bronchial tree and in different airway paths. This demonstrates that using a single measure of airway wall dimensions is a deeply flawed strategy, as it does not take into account any of this variability in individuals. My study could not really provide any definitive proof that COPD or worse airway obstruction was associated with airway wall thickening. What I could not determine from this was whether there are no substantial differences between airway dimensions in COPD or whether measuring it with CT is not an accurate enough. It does however lead me to conclude that measuring dimensions in large and intermediate airways in COPD is unlikely to be particularly useful.

One of the major limitations of my study when trying to determine the best methods of assessing each morphological feature is not having a gold–standard with which to compare my imaging findings. For emphysema this would be histological specimens, but instead I had to use spirometry and gas transfer as surrogate markers of emphysema. Without having histological specimens, I was also unable to prove that CT measures of emphysema are superior to lung function measures. It is even more difficult with small airways and airway dimensions as there is no gold-standard investigations.

8.3 To show that quantitative CT imaging can be used to understand disease activity by comparing the morphological features of COPD with airway and inflammatory status, airway bacterial presence, airway proteases and longitudinal change of clinical and physiological measures of disease.

One of the major aims of my study was to demonstrate that quantitative CT analysis could be used to understand underlying disease mechanisms and disease activity. I successfully achieved this by demonstrating a novel association between MMPS and small airways disease and by building on previous work to show an association between MMPS and emphysema. Using CT analysis allowed me to explore the roles of these in more detailed than had been done previously. Interestingly many of the MMPs associated with these CT features of disease were not elastases, but collagenases and stromelysins, suggesting the degradation of collagen and proteoglycans and subsequent abnormal

remodelling of the pulmonary ECM is an important feature in emphysema and small airways disease.

Furthermore I used novel CT analysis to explore the association between emphysema sub-types and MMPs and found that all sub-types apart from mild CLE had significant associations with a number of MMPs, suggesting they are involved in the lung destruction occurring in more established disease. However, the patterns of expression of MMPs was relatively consistent throughout the different emphysema sub-types, suggesting they are not responsible for why some individuals develop these different sub-types of emphysema. Clearly more work is required to understand this in more detail. Mild CLB did not have any significant associations with MMPs, apart from a negative one with MMP-1, suggesting that early disease may be caused by an alternative mechanism and it would be very interesting to explore how mild CLB progresses over time.

I also demonstrated an association between CT derived small airways disease and neutrophilic airway inflammation, by showing a significant correlation between E/I MLD and neutrophils and IL-8. Admittedly, this result was not replicated within the larger cohort, but this may have been down to using sputum sampling rather than BAL. In emphysema there was no conclusive association with airway inflammation in either of the studies. This supports the notion that airway inflammation seems to be an important process in small airways disease but not so much in emphysema.

Although many clinical studies have previously investigated a number of these biological mechanisms of disease, many have just described them as being raised in COPD. Using quantitative CT image analysis has helped further define some of the above biological mechanisms of disease. Whereas previously these various biological processes have been shown to be raised in COPD, I have gone further and suggested their association with specific morphological features, providing further information about where in the lungs these are active. My results seemed to be more convincing with the bronchoscopy study, which may indicate that bronchoscopic sampling is a better method of identifying airway abnormalities than sputum sampling. Alternatively, I measured different biological markers in the bronchoscopic study compared to the larger cohort. In the larger study I used differential cell counts as a marker of airway inflammation while in the bronchoscopy study I also measured inflammatory cytokines, which may be a more specific marker of inflammation. I also measured MMPs and TIMPs in the smaller cohort in an attempt to characterise their function in COPD, while I did not in the larger cohort. This suggests that smaller studies utilising bronchoscopic sampling and characterising specific biological mechanisms may be more effective than larger studies where multiple different general samples are taken. It is obviously important that mechanistic studies are performed in order to explore these associations in more detail.

8.4 To explore the anatomical regional variation of disease, both in terms of disease mechanisms and imaging abnormalities and understand how these correspond to disease severity and clinical features.

The way emphysema is distributed throughout the lungs has long been identified as an important clinical factor. However the causative factors for different patterns and distributions are poorly understood as are the regional distribution of small airways disease and bronchial wall dimensions. Using quantitative CT image analysis allowed me to study the regional distribution of these morphological features of COPD.

I found that emphysema was distributed relatively evenly throughout the five lobes and more severe COPD subjects had less variability. Lobar values of $\%LAA_{<-950}$ all had strong associations with one another and with overall emphysema and had similar associations with pulmonary physiology, suggesting the emphysema in each lobe contributes to airflow obstruction relatively evenly. I did however find that emphysema was more likely to be upper lobe predominant in COPD subjects although there was no difference in pulmonary physiology or functional status between subjects with upper lobe predominant disease or more homogenous disease.

Lobar distribution was much more heterogeneous for small airways disease than for emphysema with significantly higher E/I MLD in the upper and middle lobes than the lower lobes. Subjects with more upper lobe predominant gas trapping had less overall gas trapping as measured by E/I MLD and RV/TLC, although the mechanisms for this are unclear. What does have to be acknowledged though is that in some subjects some of the lobes increased in attenuation in expiration, which would not have been expected. The reason for this is unclear, although it may be that the lobes interact with one another and therefore cannot be considered as truly independent of one another.

I also found significant differences in airway dimensions between the five different airway paths, although no obvious pattern was elicited. It is therefore not possible to give any sort of explanation as to the distribution of abnormal large and intermediate airways in COPD. What is clear is that using a combined or composite measure of airway wall dimensions is unlikely to give an accurate measurement.

I was unable to demonstrate any biological or underlying causes for the differing distribution of emphysema and gas trapping throughout the lungs. None of the biological samples showed significant difference between subjects with upper lobe predominant disease and those with more homogenous disease. Furthermore when sampling two lobes in each individual subjects in the

smaller study there were few significant differences between lobes in concentrations of cytokines, MMPs and TIMPs. The reasons for this are unclear, although one thought may be that these biological mechanisms are relatively even throughout the lung and other factors are responsible for disease variability within the lung. Another reason for this may also be that quantities of emphysema and gas trapping were low within this cohort and therefore there were limited differences in between lobes. The approach I used, however demonstrates how these important mechanisms could be elicited.

8.5 Use CT imaging to investigate how structural features of COPD change over time by analysing CT scans taken two years apart in a COPD cohort and identifying other disease components which can predict progressions of these.

Although many studies have examined the progression of airflow obstruction relatively few have investigated the progression and change in emphysema and bronchial wall dimensions. I have demonstrated that on average emphysema progressed over a two-year period, albeit at a slow rate. There was however, significant heterogeneity in the rate of decline and on multivariate analysis the only variable at baseline that predicted increased progression was a lower FEV1%. Some subjects had lower levels of $\%LAA_{<-950}$ at month 24 than at enrolment, suggesting that there may be an amount of error in the measurements of $\%LAA_{<-950}$. I was unsuccessful in determining any biological markers that was associated with progression of emphysema. I also investigated the progression of emphysema in each individual lobe and found that like overall emphysema there was a small amount of deterioration over the two-year period. Median progression in each lobe was relatively uniform with only some minor variation.

There was no significant difference in Pi10 between enrolment scans and month 24 scan. When measuring the progression in different airway paths at different generations there seemed to be very variable changes over the two-year period, with some variables increasing in some paths and not others. Given this variation it is unlikely to be of any significance and the usefulness of these measures has to be questioned. Unfortunately I was unable to investigate longitudinal change in gas trapping/small airways disease as full a full expiratory scan was not performed at month 24. I was also unable to compare longitudinal change in emphysema with longitudinal change in other variables such as lung function as this data was not available to me.

8.6 Strengths of Study

In order to understand the role of quantitative CT image analysis in COPD it is vital that optimal CT protocols are used. The protocol in my study used thin slices with minimal distances between each slice, allowing full contiguous CT reconstructions and therefore accurate measurements. Many previous studies have used sub-optimal CT protocols, meaning their results are less robust and reliable. Previous studies, especially the large multi-centre studies such as ECLIPSE, COPDgene and lung cancer screening trials use a number of different scanners to perform their scans. Each CT scanner has minor variations in settings, meaning this has the potential to introduce errors if using multiple scanners. My study did not have this problem as all CT scans were performed on the same scanner using the same protocol.

I used a number of different techniques to systematically assess each of the morphological features of disease. This was especially important for small airways disease where I compared using expiratory scans alone to using paired analysis of inspiratory and expiratory scans. It was also important when assessing CT measured large and intermediate airway dimensions. Most previous studies have used composite measures of these airway dimensions such as PI10 or measured only one airway, giving variable results. My results clearly showed that there was significant variability of airway dimensions throughout the bronchial tree, therefore questioning the use of this as a useful measure in COPD. Unlike most other studies I also investigated the anatomical distribution of emphysema and gas trapping to further understand how this affects disease in more detail. I also used a number of novel techniques to successfully measure emphysema sub-types and body composition in order to extract the maximum amount of information from the CT imaging.

I used two different studies to investigate the use of quantitative CT imaging in COPD. One of the main strengths of the bronchoscopy study was the sampling techniques I employed. By utilising CT imaging to guide sampling from two different lobes I was able to ensure that results were not affected by random sampling. This also allowed me to investigate the differences in inflammatory cells, proteases and bacteria in different regions of the lungs. Unfortunately I did not find many intra-subject differences, although my approach is one which may be used in future to further understand regional differences within the lungs in COPD. One of the main focuses of this study was understanding the role of MMPs in COPD. I chose to use a multiplex assay to measure a broad range of theses proteases, while previous studies have only focused on individual MMPs. By combining this with CT image analysis I was able to suggest a novel role for MMPs in small airways disease and provide further evidence for their role in emphysema. In the larger cohort, a wide-range of investigations were performed, allowing careful characterisation of moderate to very severe COPD subjects. Although in comparison to some of the large cohort studies this was still

quite a small study, it benefitted from these in-depth and complete investigations with which to compare my CT analysis.

8.7 Limitations of Study

One of the main limitations of this study was the small sample size. This was especially the case for the smaller bronchoscopy study, which was a proof of concept study and therefore pragmatically powered. This meant that it was perhaps not sufficiently powered to detect all associations and I was unable to correct for certain aetiological factors such as smoking. Despite this I found remarkable results for MMPs, suggesting their importance in the development of small airways disease and emphysema. Even the larger study of 127 subjects was small compared to some of the large cohort studies such as COPDgene, which had over 9000 subjects. There were disappointing relationships between CT parameters and many of the biological and clinical parameters in this study and one of the reasons for this may have been the heterogeneity of disease. It may be that large numbers are needed to elicit significant relationships and as such my study was underpowered.

Another limitation of the study was the lack of any reproducibility of the CT measurements. Previous work using Apollo software in phantom studies and different cohorts have however shown the results are reproducible. However, due to funding and time constraints I was unable to repeat the analysis in my cohorts to confirm this.

When comparing the two studies there were a number of differences that meant making a direct comparison difficult. Firstly, both cohorts were of different severities and therefore differing results may have been down to this. The difference in airways sampling may also have been an issue. In the first study the airways were sampled using bronchoscopy while in the second study the airways were sampled using sputum induction. It is unknown whether these different techniques show the same results and it may be that the disappointing results in the larger study were down to using the less accurate sampling technique. It would not however have been possible to perform bronchoscopy on the more severe cohort of patients due to safety concerns. Due to the nature of funding and sponsorship of the second study the biological analyses performed were different than bronchoscopy study. Ideally I would have measured airway MMPs and inflammatory cytokines in both studies.

Another limitation was the multiple comparisons made in this study. This was done due to the nature of the study and the sheer number of variables analysed. I chose not to use a post hoc

multiple comparison test such as the Bonferroni correction as the cut-point for statistical significance would be prohibitively, and unrealistically low. However, throughout the study I have been sceptical about isolated significances and have discussed these analyses fully. In many cases it was clear when there were genuine associations present as I found many more significant associations than would have been expected to be significant just by chance at the 5% level. For example, with the MMP analysis.

Another limitation in the larger study was the incomplete data capture for year two. This was beyond my control and meant I was unable to fully analyse the longitudinal changes of CT parameters. Another limitation of my study was the fact that a full expiratory scan was not performed at month 24 and only limited slices were taken in order to limit radiation exposure. This meant I was unable to assess gas trapping on the follow-up scan and was therefore unable to assess the longitudinal change in this.

Chapter 9: Future Work

There is still a significant amount of work that I plan to perform in the future to further understand the role of CT image analysis in understanding disease endotypes and mechanisms of disease. One of the main parts of this will be analysing the longitudinal data in AERIS study and specifically how changes in pulmonary physiology, functional capacity and biological mechanisms compare to changes in CT parameters. I also plan to perform further MMP work on sputum samples for this larger cohort to identify whether the findings in this more severe cohort are the same as for my milder cohort. It will also be very interesting to see how the concentrations of MMPs in the airways change over time and whether they could be used as a biomarker of disease progression and also how they change in exacerbations.

I also plan to apply the emphysema sub-types to the larger study and analyse the different distributions in a more severe cohort. This can also be applied to the month 24 scans to assess the longitudinal change of these emphysema sub-types. Of particular interest will be how the mild CLE sub-type progresses of the two year period. I also plan to work with the imaging team in Harvard to refine the sub-type analysis so that it can be used to calculate the different emphysema sub-types in each individual lobe. I also plan to perform further novel CT analysis on these scans which include co-registering inspiratory and expiratory scans in order to try and further improve the quantitation of small airways.

I also plan to perform another bronchoscopy study with larger numbers and more severe CT abnormalities in an attempt to further understand the causes for the anatomical regional differences in disease. One of the weaknesses of more bronchoscopy study was that in many subjects there was only minor disease heterogeneity within the lungs and this may have led to the inconclusive results. Furthermore, diseased lobes were chosen by subjective radiology reporting, while further studies should utilise automated analysis.

Finally, I would like to include the use of other imaging modalities in order to understand disease mechanisms in COPD. One such modality which has considerable potential in this respect is PET-CT, which potentially combines structural and functional imaging.

Appendices

Appendix A

Publications

Ostridge K, Wilkinson TMA. Present and future utility of computed tomography scanning in the assessment and management of COPD. *Eur Respir J* 2016;ERJ – 00041–2016.

Ostridge K, Williams N, Kim V, *et al.* Distinct emphysema subtypes defined by quantitative CT analysis are associated with specific pulmonary matrix metalloproteinases. *Respir Res* 2016;17:92.

Ostridge K, Williams N, Kim V, Bennett M, Harden S, Welch L, Bourne S, Coombs NA, Elkington PT, Staples KJ, Wilkinson TMA: Relationship between pulmonary matrix metalloproteinases and quantitative CT markers of small airways disease and emphysema in COPD. *Thorax* 2015:1–7.

Abstracts

K Ostridge, S Harden, S Bourne, N Coombs, R Estepar, G Washko, P Elkington, K Staples, T Wilkinson. Relationship between Pulmonary Matrix Metalloproteinases and Emphysema Sub-types. (2016*) American Thoracic Society Meeting*, San Francisco.

Kristoffer Ostridge, Nicholas Williams, Victoria Kim, Michael Bennett, Stephen Harden, Lindsay Welch, Simon Bourne, Ngaire A Coombs, Paul T Elkington, Karl J. Staples, Tom MA Wilkinson. Pulmonary matrix metalloproteinases and small airways disease in COPD – the origins of airflow obstruction. (2015) *British Thoracic Society Winter Meeting*, London, UK

K Ostridge, N Williams, V Kim, MM Wojtas, S Harden, E Aris, M Peeters, JM Devaster, S Bourne, T Wilkinson. Bronchiectasis Prevalence and relationship with clinical features in COPD. (2015) *European Respiratory Society Meeting*, Amsterdam

K Ostridge, N Williams, V Kim, MM Wojtas, S Harden, M Bennett, E Aris, M Peeters, JM Devaster, S Bourne, T Wilkinson. Impact of lobar variation in emphysema on lung function and functional capacity. (2015) *European Respiratory Society Meeting*, Amsterdam

K Ostridge, N Williams, V Kim, A Barton, S Harden, E Aris, M Peeters, JM Devaster, S Bourne, T Wilkinson. Incidental lung cancer and pulmonary nodules identified by research CT in a population with moderate to very severe COPD (2015*) American Thoracic Society Meeting*, Denver

K Ostridge, N Williams, V Kim, A Barton, MM Wojtas, S Harden, E Aris, M Peeters, JM Devaster, S Bourne, T Wilkinson. Correlation of quantitative chest CT measures with lung function and functional parameters in a cohort of moderate to very severe COPD patients. (2014) *British Thoracic Society Winter Meeting*, London, UK

K Ostridge, S Harden, P Elkington, KJ Staples, T Wilkinson. Assessment of regional variability in matrix metalloproteinase concentrations by CT informed bronchoalveolar lavage in patients with COPD. (2014*) British Thoracic Society Winter Meeting*, London, UK

Appendix B

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Respiratory Research

RESEARCH Open Access

Distinct emphysema subtypes defined by quantitative CT analysis are associated with specific pulmonary matrix metalloproteinases

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Abstract

Background: Emphysema is characterised by distinct pathological sub-types, but little is known about the divergent underlying aetiology. Matrix-metalloproteinases (MMPs) are proteolytic enzymes that can degrade the extracellular matrix and have been identified as potentially important in the development of emphysema. However, the relationship between MMPs and emphysema sub-type is unknown. We investigated the role of MMPs and their inhibitors in the development of emphysema sub-types by quantifying levels and determining relationships with these sub-types in mild-moderate COPD patients and ex/current smokers with preserved lung function.

Methods: Twenty-four mild-moderate COPD and 8 ex/current smokers with preserved lung function underwent high resolution CT and distinct emphysema sub-types were quantified using novel local histogram-based assessment of lung density. We analysed levels of MMPs and tissue inhibitors of MMPs (TIMPs) in bronchoalveolar lavage (BAL) and assessed their relationship with these emphysema sub-types.

Results: The most prevalent emphysema subtypes in COPD subjects were mild and moderate centrilobular (CLE) emphysema, while only small amounts of severe centrilobular emphysema, paraseptal emphysema (PSE) and panlobular emphysema (PLE) were present. MMP-3, and -10 associated with all emphysema sub-types other than mild CLE, while MMP-7 and -8 had associations with moderate and severe CLE and PSE. MMP-9 also had associations with moderate CLE and paraseptal emphysema. Mild CLE occurred in substantial quantities irrespective of whether airflow obstruction was present and did not show any associations with MMPs.

Conclusion: Multiple MMPs are directly associated with emphysema sub-types identified by CT imaging, apart from mild CLE. This suggests that MMPs play a significant role in the tissue destruction seen in the more severe sub-types of emphysema, whereas early emphysematous change may be driven by a different mechanism.

Trial registration: Trial registration number NCT01701869.

Keyword: COPD, Emphysema, CT, Imaging, MMPs

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Appendix B

Background

Emphysema is a key feature of chronic obstructive pulmonary disease (COPD) and contributes directly to airflow obstruction. It is defined as an abnormal permanent enlargement of air spaces distal to the terminal bronchioles, accompanied by destruction of alveolar walls [1]. Three distinct pathological sub-types of emphysema are classified according to the distribution around the secondary pulmonary lobule and are termed centrilobular (CLE), panlobular (PLE) and paraseptal (PSE) emphysema [1]. All sub-types are found in COPD patients [2, 3] although how they are distributed in individuals and populations and hence contribute to the disease is uncertain. CLE is the commonest form [3, 4] and is associated with older age [4], smoking history [4, 5] and lower FEV1 [3, 5]. PLE is common in younger age [4] and is associated with lower BMI [3, 5] and more severe GOLD stage [3]. PSE is the least common form and is associated with male sex [3], older age, worse respiratory symptoms and interstitial abnormalities [6].

Efforts have been made to study emphysema in more objective detail using CT image analysis and threshold-based quantification methods, such as $\%LAA_{<-950}$ are the current standard [7]. These techniques do not provide any information about emphysema sub-types, whereas local histogram-based emphysema (LHE) quantification is a novel method, which uses regional histogram data to quantify different emphysema sub-types on CT images [2]. This analysis has shown stronger associations with physiological and functional measures of disease than $\%LAA_{<-950}$ [2]. However, limited work has assessed the relationship between emphysema sub-types and underlying mechanisms of disease and consequently our understanding of the aetiology of these sub-types is poor. PLE in the context of alpha-1-antitrypsin deficiency (A1ATD), is perhaps the most well understood as it is the predominant sub-type in this condition where it is encoded by the Serpina 1 gene, resulting in unopposed neutrophil elastase activity [8]. In subjects without A1ATD, PLE has been linked with polymorphisms of the Serpina 2 gene [9], whilst CLE has been associated with matrix metalloproteinase-9 (MMP-9) and Transforming growth factor-β (TGF- β) polymorphisms [10] and PSE with tissue inhibitor of MMP-2 (TIMP-2) and tumour necrosis factor (TNF) polymorphisms [10].

There is growing evidence that proteases such as matrix metalloproteinases (MMPs) are important in the aetiology of emphysema. Animal models suggest a role for MMP-1 [11], -9 and -12 [12] in emphysema development while human studies demonstrate increased expression of MMP-1 [13–15], - 2 [15], -3 [15], -8 [14–16], -9 [13–19], -10 [15] and -12 [13, 20, 21] in the airways of COPD subjects. We have previously reported MMP-3, -7 and -10 were associated with overall quantitative measures of emphysema [15] while Chaudhuri found MMP-9 and -12 were associated with this measure [17, 20]. We have also previously shown that MMP-3, -7, -8, -9, -10 and -12 were associated with CT markers of small airways disease, but MMPs were not associated with bronchial wall thickening of the larger airways [15]. Despite the evidence linking MMPs to COPD globally, the specific role of MMPs in the different emphysema subtypes have not been investigated. A further complicating factor in understanding the role of MMPs in emphysema is the role of proteinase inhibitors. MMPs are tightly regulated by endogenous inhibitors, the four Tissue inhibitors of MMPs (TIMPs) [22]. Sputum MMP-9/TIMP-1 ratio has been found to be significantly raised in COPD [19], although other studies do not show increased airway ratios of MMPs/TIMPs [13, 20, 23].

In this study we used novel CT analysis of LHE patterns to systematically characterise emphysema subtypes in COPD subjects and ex/current smokers with preserved lung function. We combined this analysis with multiplex profiling of MMPs and TIMPs in bronchoalveolar lavage (BAL) fluid to further understand the complex relationship between these enzymes and inhibitors in the initiation and development of emphysema. Understanding specific mechanisms driving emphysema is a key step in the stratification of fundamental COPD pathology and provides a route into developing new therapies targeting particular disease endotypes.

Methods

Subjects

Subjects gave written informed consent and the study (ClinicalTrials.gov:NCT01701869) was approved by the South Central-Southampton B NRES Committee (12/SC/0304).

The methods have been described in detail previously [15]. Twenty-four subjects with mild-moderate COPD as defined by GOLD guidelines [24] were recruited. Post-bronchodilator spirometry was used to assess airflow obstruction with a FEV1/FVC ratio of <0.7 and an FEV1 of ≥50 % predicted value required. Eight current or ex-smokers with preserved lung function were also recruited. Subjects had at least a 10 pack year smoking history and exclusion criteria included a history of other pulmonary disease, long-term antibiotics/steroids or an exacerbation within the month prior to recruitment.

CT scanning and quantitative image analysis

Subjects underwent volumetric CT scans of the chest in full inspiration using a Siemens Sensation 64 scanner. The imaging protocol consisted of; slice thickness 0.75 mm, slice separation 0.5 mm, tube voltage 120KV, effective mAs 90mAs (using dose modulation), collimation 0.6 mm and a pitch of 1. Images reconstructed with

the B30 kernel were used for image analysis by custom software to characterise the emphysema pattern using local histogram information as previously described [2]. Briefly, regions of interest of size 24.18×24.18 mm^2 were detected and automatically classified into one of six categories; non-emphysematous (NE), centrilobular emphysema by increasing severity (mild CLE, moderate CLE and severe CLE), panlobular emphysema (PLE) and paraseptal emphysema (PSE). This was applied to each CT scan, generating six continuous measures representing the lung volume percentage that was classified into each of the six patterns.

Bronchoscopy

In each subject two lobes were targeted at bronchoscopy. These lobes were determined prior to the procedure by a thoracic radiologist reporting the lobes most and least affected by disease. BAL was performed by instilling 100 ml of 0.9 % saline into each lobe and recovered by aspiration. BAL fluid was filtered and centrifuged and the resultant supernatant was stored at −80 °C prior to analysis.

MMP and TIMP analysis

MMP and TIMP concentrations in BAL were quantified using a microparticle based multiplex immunoassay (R&D systems, Abingdon, UK) and analysed on the Luminex 200 platform (Biorad Bioplex 200, Hemel Hempstead, UK). We analysed MMP-3, -7, -8, -9, -10 and -12 as we had previously linked these to CT parameters of disease [15] as well as TIMP 1–4 (Additional file 1).

Statistical analysis

Analyses were performed using SPSS version 22. Mann-Whitney U and Fishers Exact tests compared data between groups. Associations between MMPs, spirometry and CT parameters were analysed using Spearman's Correlation. Each subject had two lobes sampled and the mean concentrations between the lobes were used. For the purpose of statistical analysis, values that were below the lower limit of detection were given the value of half the concentration of detection. A p-value of <0.05 was considered statistically significant.

Results

Groups were well matched for age, gender and smoking status (Table 1).

Emphysema sub-types

CT analysis defining specific emphysema sub-types was successfully achieved in 31 subjects. Figure 1 shows a typical LHE reconstruction of a subject with COPD and preserved lung function. One scan from a subject with preserved lung function could not be analysed for technical reasons.

Table 1 Characteristics of participants included in the study

	COPD ($n = 24$)	Preserved lung function ($n = 8$)	P value
Age	66.0 (12.0)	56.0 (18.0)	0.064
Male	16	6	>0.999
Current smoker	11	5	0.685
Pack Years	39 (39.8)	32.25 (23.8)	0.268
FEV1 % predicted	69.00 (21.0)	108.00 (20.3)	<0.001*
FEV1/FVC	54.50 (10.8)	78.00 (9.5)	<0.001*
Mild CLE	33.8 (8.3)	31.9 (9.5)	0.44
Moderate CLE	21.3 (15.3)	7.1 (9.2)	0.008*
Severe CLE	0.9 (2.0)	0.0 (0.0)	0.008*
Panlobular	2.7 (1.1)	0.0 (0.0)	0.048*
Paraseptal	6.9 (3.9)	3.7 (3.0)	0.014*
Non-emphysema	34.6 (20.6)	53.5 (21.4)	0.011*

Values are given as medians (IQR). Tissue sub-types given as median % of CT lung volume classified as particular sub-type
Male and current smokers given as number of subjects
Tissue sub-types given for 31 subjects
*$p < 0.05$. Fisher's exact test for male and current smoker. Mann-Whitney U-test for all other variables

All subjects (COPD and preserved lung function) had non-emphysematous tissue, mild CLE, moderate CLE and PSE. Only eight subjects had >1 % of PLB. 10 subjects had >1 % of severe CLE. Non-emphysematous tissue was the most common in 15 subjects. Mild CLE was the most common in 12 subjects and moderate CLE the most common in 4 subjects.

The median percentage of each LHE pattern is shown in Table 1. Moderate CLE, severe CLE, PLE and PSE were all significantly raised in COPD subjects compared to those with preserved lung function. Non-emphysematous tissue was significantly higher in those with preserved lung function. Median percentage for mild CLE was over 30 % for subjects with and without airflow obstruction with no significant difference between the two.

There were significant associations between FEV1 % predicted and moderate CLE (rho −0.39, p0.032) and severe CLE (rho −0.37, p0.044). There were no associations between FEV1 % predicted and mild CLE (rho 0.07, p0.695), panlobular (rho −0.30, p0.097), paraseptal (−0.31, p0.094) or NE (rho 0.32, p0.075).

MMP and TIMP concentrations

We previously reported significantly raised concentrations of MMP-3, -8, -9 and -10 in COPD [15]. TIMP-1 and -2 were found in abundant concentrations within BAL (Fig. 2), while TIMP-4 was found in lower concentrations. TIMP-2 and -4 were significantly increased in COPD subjects while there was no significant difference between groups for TIMP-1 and TIMP-3.

Appendix B

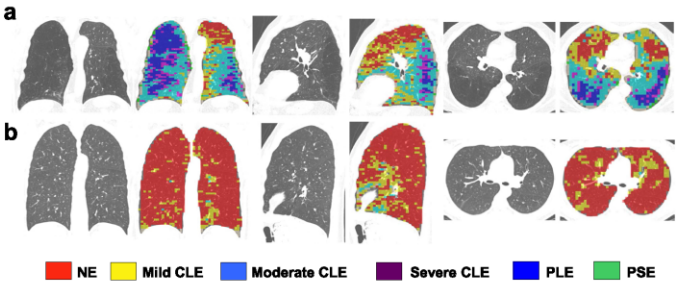

Fig. 1 Local histogram emphysema (LHE) classification results for a coronal, sagittal and axial slice corresponding to (**a**) COPD subject and (**b**) subject with preserved lung function

Emphysema sub-types and MMPs and TIMPs

We performed a systematic analysis of associations between emphysema sub-types and MMP and TIMP BAL concentrations in the whole cohort. MMP-3 and -10 had significant associations with all emphysema sub-types apart from mild CLE. MMP -7 and -8 had significant associations with all emphysema sub-types apart from mild CLE and PLE (Table 2). MMP-9 had significant associations with moderate CLE and PSE. MMP-12 did not exhibit any significant associations with emphysema sub-types. TIMP-4 had significant associations with moderate and severe CLE and PSE. No other TIMPs had associations with emphysema sub-types (Table 3).

MMPs/TIMPs ratios and emphysema sub-types

To understand the role of a proteinase/antiproteinase imbalance we investigated the MMP/TIMP ratios. Ratios were significantly increased in COPD subjects for MMP-8/TIMP-1, -2, -3, -4, MMP-9/TIMP-1 and MMP-10/TIMP-1 and -2 (Additional file 1).

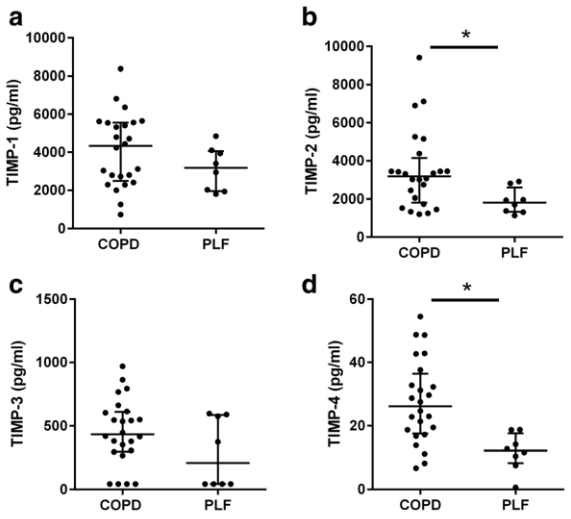

Fig. 2 BAL expression of TIMPs in subjects with COPD and preserved lung function. **a** TIMP-1 (**b**) TIMP-2 (**c**) TIMP-3 (**d**) TIMP-4. Data represents median upper and lower quartiles. Each dot represents BAL concentration of individual MMP in a specific patient, $n = 24$ for COPD and 8 for preserved lung function. * $p < 0.05$ using Mann-Whitney *U* test

Table 2 Spearman's correlation analysis between MMPs and emphysema sub-types

	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12
Mild CLE	−0.09	−0.11	−0.10	−0.8	0.05	−0.30
Moderate CLE	0.45*	0.43*	0.49*	0.42*	0.41*	0.15
Severe CLE	0.52**	0.39*	0.40*	0.33	0.38*	0.11
Panlobular	0.56**	0.34	0.33	0.29	0.43*	0.22
Paraseptal	0.50**	0.49**	0.46**	0.42*	0.44*	0.12
Non-emphysema	−0.45*	−0.39*	−0.41*	−0.36*	−0.45*	−0.44

Spearman's rho values given
$N = 31$. *$p < 0.05$; **$p < 0.01$

Table 4 Spearman's correlation analysis between MMPs/TIMP1 ratios and emphysema sub-types

	MMP-3 /TIMP-1	MMP-7 /TIMP-1	MMP-8 /TIMP-1	MMP-9 /TIMP-1	MMP-10 /TIMP-1	MMP-12 /TIMP-1
Mild CLE	−0.08	−0.06	−0.05	−0.03	0.01	−0.14
Moderate CLE	0.39*	0.39*	0.44*	0.34	0.43*	0.12
Severe CLE	0.44*	0.36*	0.36*	0.28	0.40*	0.08
Panlobular	0.59***	0.43*	0.36*	0.31	0.55**	0.33
Paraseptal	0.47**	0.50**	0.42*	0.37*	0.47**	0.15
Normal	−0.37*	−0.35	−0.36*	−0.29	−0.44*	−0.04

Spearman's rho values given
$N = 31$. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

We also investigated associations between emphysema sub-types and MMP/TIMP ratios (Tables 4, 5, 6 and 7). Mild CLE did not have any significant associations with MMP/TIMP ratios. Multiple MMP/TIMP ratios had associations with all the other tissue sub-types.

Discussion

Using LHE patterns analysed on HRCT images, we successfully measured emphysema sub-types in mild/moderate COPD subjects and ex/current smokers with preserved lung function. The most prevalent tissue sub-types in COPD subjects were mild and moderate CLE and non-emphysematous tissue, whilst severe CLE, PSE and PLE were less frequently present. Furthermore, all emphysema sub-types, apart from mild CLE, had associations with multiple MMPs, particularly the stromelysins MMP-3 and MMP-10, implicating these proteases in the tissue destruction that occurs in these sub-types of emphysema. Interestingly, mild CLE was found in substantial quantities in subjects with and without airflow obstruction and exhibited different properties from the other sub-types of emphysema showing no associations with MMPs.

Emphysema is an important pathological feature of COPD, contributing directly to airflow obstruction and is associated with mortality and worse outcomes [25–27]. LHE CT analysis determines the distribution of the three main emphysema sub-types throughout the lungs and shows stronger associations with physiological and functional measures than current CT emphysema estimation (%$LAA_{<-950}$) [2]. In our mild-moderate COPD subjects, the predominant emphysema sub-types were mild and moderate CLE, with only small quantities of severe CLE present, which is in keeping with previous work [2, 3]. CLE is an abnormal enlargement of air-spaces centred on the respiratory bronchiole and is the classical form associated with smoking [3, 5]. Only small amounts of PLE and PSE were present in COPD subjects which is consistent with other studies [2, 3, 6]. Panlobular emphysema is an abnormal enlargement of airspaces distributed throughout the pulmonary lobule and has been associated with A1ATD and more severe disease [3, 5]. It is therefore unsurprising that PLE was found in such low quantities in our cohort. Paraseptal emphysema refers to emphysematous change adjacent to a pleural surface and is the least well understood form of emphysema and shows no relationship with COPD symptoms [3] or smoking history [4].

The underlying mechanisms driving the evolution of emphysema sub-types are poorly understood. MMPs are proteolytic enzymes implicated in the destruction of the pulmonary extra-cellular matrix (ECM). We have

Table 3 Spearman's correlation analysis between TIMPs and emphysema sub-types

	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Mild CLE	−0.04	−0.21	−0.23	−0.18
Moderate CLE	0.03	0.31	0.20	0.37*
Severe CLE	0.05	0.29	0.18	0.36*
Panlobular	−0.15	0.22	0.21	0.28
Paraseptal	−0.01	0.31	0.24	0.37*
Non-emphysema	−0.07	−0.31	−0.19	−0.36*

Spearman's rho values given
$N = 31$. *$p < 0.05$

Table 5 Spearman's correlation analysis between MMPs/TIMP2 ratios and emphysema sub-types

	MMP-3 /TIMP-2	MMP-7 /TIMP-2	MMP-8 /TIMP-2	MMP-9 /TIMP-2	MMP-10 /TIMP-2	MMP-12 /TIMP-2
Mild CLE	−0.07	−0.09	−0.06	−0.02	0.10	−0.01
Moderate CLE	0.35	0.22	0.42*	0.27	0.37*	−0.05
Severe CLE	0.44*	0.25	0.35	0.23	0.33	−0.10
Panlobular	0.56**	0.32	0.32	0.26	0.46**	0.08
Paraseptal	0.44*	0.34	0.40*	0.31	0.38*	−0.04
Normal	−0.35	−0.18	−0.34	−0.23	−0.40*	0.13

Spearman's rho values given
$N = 31$. *$p < 0.05$; **$p < 0.01$

Appendix B

Table 6 Spearman's correlation analysis between MMPs/TIMP3 ratios and emphysema sub-types

	MMP-3 /TIMP-3	MMP-7 /TIMP-3	MMP-8 /TIMP-3	MMP-9 /TIMP-3	MMP-10 /TIMP-3	MMP-12 /TIMP-3
Mild CLE	−0.04	0.02	−0.09	0.01	0.14	0.04
Moderate CLE	0.36*	0.27	0.44*	0.21	0.32	−0.05
Severe CLE	0.40*	0.22	0.32	0.12	0.23	−0.12
Panlobular	0.44*	0.16	0.30	0.13	0.24	−0.04
Paraseptal	0.32	0.27	0.39*	0.17	0.27	−0.13
Normal	−0.30	−0.20	−0.33	−0.13	−0.32	0.15

Spearman's rho values given
$N = 31$. *$p < 0.05$

previously reported that overall emphysema had associations with MMP-3, -7 and -10 and CT markers of small airways disease had associations with MMP-3, -7, -8, -9, -10 and -12 [15]. MMP-9 polymorphisms have been associated with CLE [10], but ours is the first study evaluating the contribution of MMPs to different emphysema sub-types in detail. MMP-3 and -10 had significant associations with all forms of emphysema (apart from mild CLE) while MMP-7, -8 and -9 had associations with multiple sub-types. MMPs are inhibited by four endogenous inhibitors, the TIMPs which bind with MMPs in a 1:1 manner [28]. TIMP-3 null mice develop emphysema [29] while human studies show TIMP-1 and -2 are raised in the airways of COPD subjects [13, 14, 19, 20] and TIMP-2 polymorphisms are associated with CLE [10]. We found significantly increased TIMP-2 and TIMP-4 in COPD subjects and also found that TIMP-4 had positive associations with several emphysema sub-types. This suggests that TIMPs are raised in COPD and emphysema although their activity and specificity are poorly understood. An imbalance between MMPs and TIMPs may be more important than absolute concentrations although previous studies have not demonstrated this [20, 23], or alternatively MMPs may be acting in the immediate pericellular environment and therefore not inhibited by

Table 7 Spearman's correlation analysis between MMPs/TIMP4 ratios and emphysema sub-types

	MMP-3 /TIMP-4	MMP-7 /TIMP-4	MMP-8 /TIMP-4	MMP-9 /TIMP-4	MMP-10 /TIMP-4	MMP-12 /TIMP-4
Mild CLE	0.03	−0.02	−0.06	0.03	0.16	0.01
Moderate CLE	0.35	0.25	0.43*	0.15	0.30	−0.12
Severe CLE	0.41*	0.20	0.32	0.07	0.22	−0.21
Panlobular	0.54**	0.24	0.29	0.11	0.36*	0.01
Paraseptal	0.42*	0.32	0.38*	0.16	0.32	−0.14
Normal	−0.35	−0.20	−0.32	−0.09	−0.34	0.21

Spearman's rho values given
$N = 31$. *$p < 0.05$; **$p < 0.01$

TIMPs. In contrast to this previous data, we found a number of ratios were increased in COPD and a number had significant associations with emphysema sub-types.

Perhaps the most interesting results from this study were those relating to mild CLE. All subjects, irrespective of whether they had airflow obstruction, had evidence of mild CLE with median values above 30 %. This is consistent with other studies which have demonstrated emphysematous change in smokers without airflow obstruction [3, 4]. Using LHE CT analysis, Castaldi also described high rates of mild CLE in healthy smokers, linking it with degree of airflow obstruction and functional capacity [2]. This suggests that mild CLE is a genuine tissue abnormality and occurs prior to the development of airflow obstruction and a key question is whether this progresses into more significant disease. Unlike more severe forms of emphysema we found no associations between MMPs and mild CLE. When correcting for the other tissue sub-types, using partial correlation (data not shown) no associations between mild CLE and MMPs were found, suggesting mild CLE genuinely does not have any associations with MMPs. We propose a possible explanation for this may be that mild emphysema is ubiquitous in smokers and ex-smokers and occurs via a non-MMP derived mechanism, such as oxidative stress secondary to cigarette smoke exposure. It may be that only subjects with significant MMP activity develop more advanced emphysema and our results suggest MMP-3 and -10 are the most important in this process. These MMPs are stromelysins, mainly degrading collagen and proteoglycans, important constituents of the pulmonary ECM [22]. In addition MMP-7, -8 and -9 had associations with moderate CLE and PSE and MMP-7 and -8 also had associations with severe CLE. MMP-7 is an elastase, MMP-8 a collagenase and MMP-9 a gelatinase and together can degrade all components of the pulmonary ECM [22]. Given that individual MMPs have different substrate specificity, it may be proposed that particular MMPs are responsible for each sub-type of emphysema. However, our results do not support this as the profile of MMP expression is broadly similar across all emphysema sub-types, apart from mild CLE. Further mechanistic work is required to understand this is in more detail and longitudinal studies are needed to understand how mild CLE and the other emphysema sub-types progress and whether this can predicted by MMP concentrations.

The main limitation of this study was the small sample size and associated limited statistical power. Despite this we found strong evidence of associations between MMPs and emphysema sub-types. Another limitation is the multiple comparisons made in this study. Excluding

Table 1, 70 out of 238 comparisons were significant, far more than the 12 associations expected to be significant by chance, suggesting genuine associations. Additionally, all significant results were in the same, expected direction for each MMP/TIMP and emphysema subtype comparison. Due to the need to perform bronchoscopy, our study consisted of patients with mild and moderate COPD, with only limited amounts of emphysema. It is unknown whether these results would be similar in a more severe cohort. Furthermore, the lack of a validation population limits the generalizability of the findings. Using LHE CT analysis we could only determine emphysema sub-types throughout the entire lungs rather than on a lobar basis and further work will involve refining this analysis to include lobar measurements. However we have previously shown that comparing BAL analysis to whole lung CT parameters is a valid technique [15]. Finally quantitative measures of MMPs and ratios with TIMPs do not necessarily equate to enzymatic activity. We are developing techniques to generate in-situ zymography data which will be the next step in understanding the aetiology of emphysema.

Conclusion

In conclusion, mild and moderate CLE were the predominant forms of tissue sub-types measured in a cohort of mild/moderate COPD subjects. Multiple MMPs were associated with moderate and severe CLE, paraseptal and panlobular emphysema. MMP-3 and -10 had associations with all of these sub-types, suggesting they play an important part in the tissue destruction seen in these sub-types of emphysema. Mild CLE was common in both subjects with COPD and those with preserved lung function and unlike other tissue sub-types did not have any associations with MMPs. MMP activity may explain why some subjects progress to more severe forms of emphysema and increased understanding of these specific mechanisms may help inform our knowledge of disease progression and treatment options.

Additional file

Additional file 1: Sensitivity data for MMP and TIMP luminex assays. MMP/TIMP ratios in BAL in COPD and PLF subjects. (DOCX 171 kb)

Abbreviations

A1ATD, alpha-1-antitrypsin deficiency; BAL, bronchoalveolar lavage; CLE, centrilobular emphysema; COPD, chronic obstructive pulmonary disease; ECM, extra-cellular matrix; FEV1, Forced expiratory volume in 1 s; FVC, Forced vital capacity; LHE, local histogram-based emphysema patterns; MMP, matrix metalloproteinase; PLE, panlobular emphysema; PSE, paraseptal emphysema; TIMP, Tissue inhibitor of MMPs

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Availability of data and materials

Not applicable.

Authors' contributions

Conception & design – KO, PTE, KJS & TMAW; Data acquisition, analysis and interpretation – all authors; Drafting of manuscript for important intellectual content – KO, KJS & TMAW. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Patient consent was obtained and the study was approved by the NRES Committee South Central – Southampton B (12/SC/0304).

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REVIEW
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Present and future utility of computed tomography scanning in the assessment and management of COPD

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ABSTRACT Computed tomography (CT) is the modality of choice for imaging the thorax and lung structure. In chronic obstructive pulmonary disease (COPD), it used to recognise the key morphological features of emphysema, bronchial wall thickening and gas trapping. Despite this, its place in the investigation and management of COPD is yet to be determined, and it is not routinely recommended. However, lung CT already has important clinical applications where it can be used to diagnose concomitant pathology and determine which patients with severe emphysema are appropriate for lung volume reduction procedures. Furthermore, novel quantitative analysis techniques permit objective measurements of pulmonary and extrapulmonary manifestations of the disease. These techniques can give important insights into COPD, and help explore the heterogeneity and underlying mechanisms of the condition. In time, it is hoped that these techniques can be used in clinical trials to help develop disease-specific therapy and, ultimately, as a clinical tool in identifying patients who would benefit most from new and existing treatments. This review discusses the current clinical applications for CT imaging in COPD and quantification techniques, and its potential future role in stratifying disease for optimal outcome.

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Appendix B

Introduction

Computed tomography (CT) has revolutionised medical imaging since it was developed in the 1970s. Its ability to provide detailed images of the lungs and thorax has made it an imaging modality of choice in respiratory medicine, where it has become an essential tool in the assessment and management of patients with many lung diseases.

In chronic obstructive pulmonary disease (COPD), CT can image the pathological changes that directly contribute to the airflow limitation that defines the condition [1]. Reliance on using spirometric markers to diagnose and characterise the disease has limited both our understanding of COPD and the individualised clinical management of the condition. COPD is a complex, heterogeneous condition with varying clinical features, consisting of a number of different phenotypes. The origins of clinical phenotypes and the associations of disease processes with endotypes along with the drivers to heterogeneity between individuals have not been fully explored, and are likely to have important implications for understanding disease progression and the future management of this condition [2]. There are currently no significant disease-modifying medications for COPD and there have not been the improvements in outcome seen in other chronic diseases. Other tools are required in addition to spirometry to help explain the heterogeneity, and provide further insights into approaches to study and treat COPD. CT has current practical applications in the routine management and investigation of the disease, and with the development of novel analysis techniques, has the potential to provide further details about the pulmonary and extrapulmonary manifestations of COPD.

CT in current clinical practise

Prior to the 1970s, histological and *post mortem* studies were required to study structural changes of the lung. The introduction of CT made it possible to visualise the thorax and assess lung structure noninvasively. In COPD, CT can identify key morphological features, including emphysema, bronchial wall thickening and gas trapping. These pathologies contribute directly to airflow obstruction and, therefore, CT has the potential to provide vital insights into the underlying pathophysiological changes of COPD. Despite this, routine CT imaging has not necessarily been widely adopted in clinical practise, and its place in the management and investigation of COPD has not been firmly established. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy does not routinely recommend CT scanning in COPD and only advises that it may be helpful in differential diagnosis or when surgical options are being considered [3]. This general ambivalence is highlighted by a study of attitudes in respiratory physicians and surgeons in the UK where only 32% thought it necessary for patients with severe COPD to have a CT scan [4].

There are a number of reasons why the use of CT has not become routine in COPD. One of the most important is the perceived notion that using CT does not alter management, although this is not strictly true. NETT (the National Emphysema Treatment Trial) demonstrated that patients with advanced upper lobe emphysema and limited exercise ability improved after lung volume reduction surgery [5]. There has also been considerable progress and experience in using endobronchial treatments for severe emphysema including coils, valves, sealant and airway bypass. In the correct patients with severe heterogeneous emphysema, these treatments can lead to significant improvement in pulmonary physiology and functional capacity [6, 7]. CT imaging can also identify bullous disease that may be amenable to surgery and bullectomy can lead to improved health status [8]. It therefore seems clear that patients with severe COPD require CT imaging to determine which patients are appropriate for these techniques.

CT imaging can also detect concomitant pulmonary pathology in COPD. Bronchiectasis is particularly prevalent, with studies demonstrating 50% of COPD patients having CT evidence of the disease [9–11]. When present, bronchiectasis is associated with worse airflow obstruction [11], increased exacerbations [9, 11], higher airway and systemic inflammation [9–11], and higher mortality [9]. COPD is also an independent risk factor for lung cancer [12], and higher cancer rates have been associated with worsening airflow obstruction [13, 14] and emphysema [15, 16]. Fibrotic change has also been shown to be common in COPD and seems to confer a poor prognosis [17]. In addition, it can be difficult to diagnose due to the fact that pulmonary physiology may be normalised by the opposing effects of hyperinflation and fibrosis. As these concomitant pathologies are common and often associated with poorer outcomes, and would require a change management, it is vital that CT imaging is used to detect them.

A major limitation to the routine use of CT in COPD has been subjective reporting, making it difficult to interpret the results and guide management plans or give insights into the disease. Objective methods have been targeted to provide numerical data on key disease facets. Initially, simple visual scoring systems were developed, which showed early promise at quantifying the disease. These techniques were unable to detect subtle disease, and were liable to intra- and interobserver errors, as well as being insensitive to longitudinal change. To address these issues, automated quantitative analysis techniques have been developed, which

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are able to segment the lung parenchyma and airways from the chest wall and surrounding structures. Early software analysed two-dimensional axial slices but, coupled with advances in CT technology, a range of different analysis software exists that allows volumetric reconstructions (figure 1). This raises the prospect that quantitative CT analysis can be used to understand the disease and phenotypes in more detail, which the remainder of this review discusses.

Emphysema

Emphysema is traditionally a histological diagnosis, defined as an abnormal permanent enlargement of air spaces distal to the terminal bronchioles, accompanied by the destruction of alveolar walls. Emphysematous changes can be identified on CT and, in 1978, Rosenblum *et al.* [18] described areas of lower lung attenuation and mean lung density in patients with a clinical diagnosis of COPD.

To provide numerical data on emphysema, Goddard *et al.* [19] developed a visual scoring system for emphysema by estimating the amount on axial CT slices, which showed a strong association with airflow obstruction and with histological specimens [20–22]. Automated techniques were developed to segment the lung parenchyma and quantitate emphysema. The two main techniques both use the principle that emphysematous regions are represented by low-attenuation areas on CT. The first, termed CT densitometry, involves applying a density mask to the lung parenchyma and setting a threshold below which all voxels are assumed to be emphysema, giving a low-attenuation percentage (%LAA) (figure 2). Initially, a threshold of −910 HU was used, which showed a strong correlation with pathological measures of emphysema [23]. Subsequently, %LAA under −950 HU ($\%LAA_{<-950}$) was shown to have the strongest associations with both microscopic and macroscopic emphysema [24–27], leading to the common adoption of this threshold. The second method, termed percentile densitometry, involves choosing a threshold percentile in the attenuation distribution curve, which provides the density value in Hounsfield units under which a percentage of the voxels are distributed. A number of different thresholds between 1% and 18% have been used, and correlate strongly with microscopic emphysema on histological specimens [27]. The most commonly used threshold is 15% based on a number of studies in α_1-antitrypsin deficiency (α_1-ATD) [28–30]. There is still no definite consensus about which of these methods is best and, even now, different studies use different thresholds. Given the fact that emphysema is an all-or-nothing phenomenon, it seems more intuitive to use CT densitometry, which uses an absolute cut-off to quantify it.

Studies have confirmed that CT densitometry demonstrates higher levels of emphysema in individuals with COPD than in healthy smokers [31–34]. There is also convincing evidence that CT densitometry has strong associations with airflow obstruction [10, 32–41], and analysis of over 4000 CT scans from the COPDgene study showed a strong negative correlation between $\%LAA_{<-950}$ and forced expiratory volume in 1 s (FEV_1), and demonstrated increased emphysema with worsening GOLD severity [33]. CT densitometry also has strong associations with gas transfer (transfer factor of the lung for carbon monoxide) [10, 35–40], 6-min walk distance (6MWD) [32, 36, 40, 42], BODE (body mass index, obstruction, dyspnoea, exercise capacity) score [36, 39, 43–45] and body composition [37, 39, 46, 47].

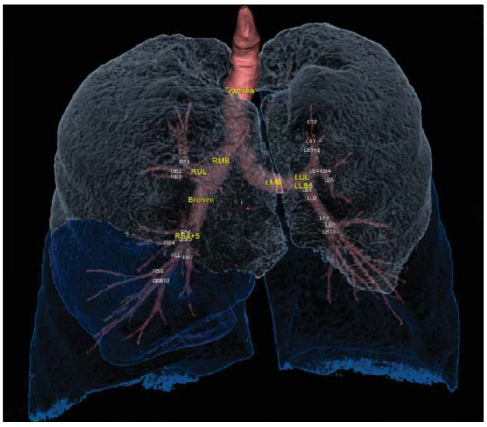

FIGURE 1 Volumetric computed tomography reconstruction of bronchial tree and lung parenchyma using Apollo software (VIDA Diagnostics Inc., Coralville, IA, USA).

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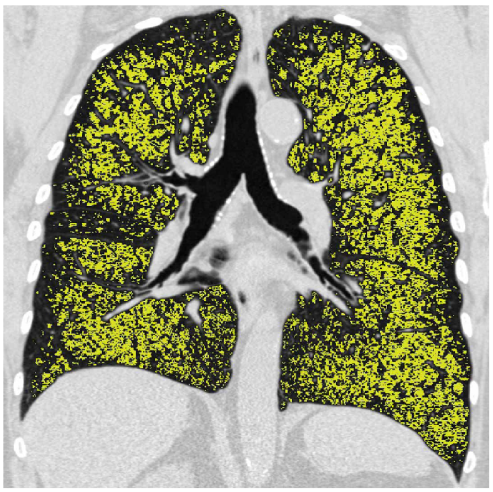

FIGURE 2 Coronal reconstruction of chest computed tomography showing low-attenuation areas below −950 HU.

These densitometry techniques do not take into account the distribution or different subtypes of the disease, which may have important implications. Emphysema does not affect the lung in a uniform way and it is unclear how this affects the clinical presentation of the disease. As previously discussed, it has been shown to be clinically important in determining which patients benefit from lung volume reduction surgery [5]. A small study found that %LAA was higher in central and upper regions within the lung [48]. Interestingly, airflow limitation correlated best with %LAA in the central and lower regions, and also found that more homogenous emphysema was associated with worse airflow obstruction. Other studies have shown accelerated decline in lung function in those with homogenous emphysema [49] and those with more upper-zone emphysema [50]. With advances in quantitative CT analysis, lobar variation of disease can be assessed and significant differences in emphysema quantity between the lobes has been found [51, 52].

Three distinct patterns of emphysema have been described in histological studies, based on the distribution within the secondary pulmonary lobule: centrilobular, panlobular and paraseptal emphysema. CT imaging can visualise these different subtypes. One study using a visual scoring system found that centrilobular emphysema was the most common subtype but over half of patients with emphysema had multiple subtypes [53]. An automated method, called local histogram-based emphysema patterns, involves dividing the lung into small cubes and analysing the subtype of emphysema present in each cube. This was found to be more predictive of lung function than $\%LAA_{<-950}$ [54].

These data point to CT densitometry and other methods being able to accurately quantify emphysema and its association with physiological markers of disease. To validate CT imaging as a biomarker of disease, it is necessary to compare it to underlying disease mechanisms and outcome measures, and track longitudinal change. CT densitometry has been found to correlate with mortality in patients with COPD [55] and analysis from the GenKOLS trial [56] found that %LAA had a better predictive value for respiratory and cardiac mortality than GOLD staging. Emphysematous change on CT has also been associated with higher exacerbation rate [57–59] and increased mortality from exacerbations [59].

A potentially important application of CT imaging is to track the changes in emphysema over time and see how it contributes to functional decline. Until recently, the majority of these studies were conducted in patients with α_1-ATD and have shown that CT densitometry can accurately detect progression of emphysema [60–63] and correlates with decline in lung function [29, 60]. Furthermore, CT lung density parameters are more sensitive in detecting progression of disease than traditional lung function [60] and have therefore been accepted as the primary end-point in prospective studies on treatment effects for α_1-ATD. In COPD, it has been demonstrated that patients with higher quantities of emphysema on CT have an increased rate of decline of FEV1 [34, 50]. CT emphysema progression is also associated with frequent exacerbations [64] and current smoking status [65]. The largest longitudinal study to date assessed CT scans from the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints

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(ECLIPSE) cohort [66], in which it was found that lung density and low-attenuation areas progressed on an annual basis. There were, however, significant variations between subjects and this did not convincingly correlate with lung function.

When comparing CT measures of emphysema with underlying markers of disease, it has to be considered that our knowledge of these mechanisms is imprecise. Emphysematous change has been associated with various matrix metalloproteinases (MMPs) [31, 67–69] as well as raised sputum neutrophils [70] and eosinophils [39, 71]. A number of airway inflammatory cytokines, such as interleukin-8, leukotriene-B_4 and myeloperoxidase, has been associated with emphysema progression [39, 72, 73] and emphysema has also been associated with raised serum fibrinogen [74]. Some work has been performed associating novel biomarkers with emphysema and in the ECLIPSE cohort, surfactant protein D and soluble RAGE (receptor of advanced glycation end-products) were both associated with decline in lung densitometry [66]. Little is known about bacterial colonisation and emphysema; however, Bafadhel *et al.* [75] did not find any difference in bacteria culture between patients who had emphysema on CT and those who did not.

In summary, emphysema quantitation is relatively simple and can be performed with minimal user intervention. CT densitometry is associated with a number of physiological and clinical parameters. However, further work is required to evaluate emphysema distribution and subtypes, and to assess the longitudinal changes in emphysema, and the association with outcome markers of disease and underlying disease mechanisms.

Bronchial wall measurements

Airway wall remodelling is an important feature in COPD and histological specimens confirm airway wall thickening throughout the bronchial tree [76, 77]. The limited resolution of CT means only large and intermediate-sized airways can be visualised directly. Early measurements of the airway relied on manual tracing [78]; however, a number of automated methods have been developed. One of these involves using the "full width at half maximum" principle (figure 3), which uses CT attenuation values to identify the inner and outer airway wall by projecting rays from the centre of the airway. These and other algorithms have been refined, and using region-growing approaches, it is now possible to generate three-dimensional reconstructions of the bronchial tree down to the fifth or sixth generation airway. Multiple airway dimensions can be measured from these reconstructions including bronchial wall thickness, wall area, lumen area, total airway area, wall area percentage (%WA) and internal perimeter (P_i) (figure 4). A standardised parameter called $P_{i_{10}}$ has been developed that predicts the square root of the wall area for a hypothetical airway with an internal perimeter of 10 mm.

Early studies found that markers of bronchial wall thickening on CT were increased in COPD [46, 79–81]. However, more recent studies have shown that both airway wall and lumen size are reduced in COPD, although proportionally the lumen more so, resulting in a larger %WA [33, 79, 82]. The reasons for the discrepancies are unclear although the later studies tended to make more complete measurements of the bronchial tree and spatially matched the airways. By not doing this, there is a chance of introducing bias into results by inadvertently selecting more proximal airways in COPD, which have thicker walls. This highlights the current difficulties in measuring the airways, with the sheer number of variables presenting significant challenges, and it is clear that further research is needed.

Taking the above limitations into account, measures of increased bronchial wall thickness are associated with FEV1 [41, 81, 83, 84] and this is stronger with more distal airways [41]. Bronchial wall markers also

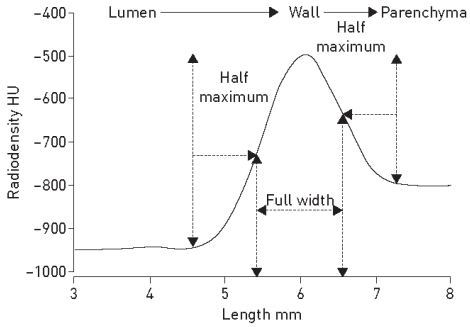

FIGURE 3 Full width at half maximum method for determining airway wall dimensions.

Appendix B

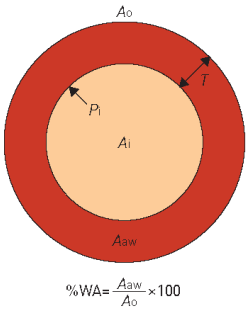

FIGURE 4 Airway wall dimensions. A_{aw}: airway wall area; T: wall thickness; A_o: total airway area; A_i: inner area; P_i: internal perimeter; %WA: percentage wall area.

correlate with functional markers in the form of the BODE index [44, 45], exercise capacity [32, 36] and body composition [46]. Although statistically significant many of these associations tended to be quite weak. In the GenKOLS study, there was generally no increase in mortality with increased Pi_{10} but in a subgroup with severe emphysema, Pi_{10} was associated with mortality [56]. Another study demonstrated that increased bronchial wall dimensions were associated with mortality from exacerbations [59]. It has been hypothesised that increased bronchial wall thickness is a marker of airway inflammation and therefore would be associated with symptoms of chronic bronchitis or frequent exacerbations. HAN *et al.* [57] confirmed this by showing that markers of airway wall thickness were increased in frequent exacerbators, although another study failed to find this association [58]. These measures also show associations with symptoms of chronic bronchitis [84, 85]. Few studies have investigated the link between CT markers of bronchial wall thickening and sputum inflammatory markers or bacterial culture, although BAFADHEL *et al.* [75] did not find any association.

Simply measuring markers of bronchial wall thickness may be insufficient to describe the airway remodelling that occurs in COPD. Other methods have therefore been developed and one of these uses peak wall attenuation, which is a marker likely to reflect thickening and density within the airway wall. This has been shown to have a negative correlation with FEV1 [41, 86].

Minimal work has assessed the change of airway dimensions over time. A small study looked at lower lobe segmental airway changes between scans a year apart and did not find a significant difference, although did find a correlation between annual change in %WA and FEV1 decline [87]. Regional variability in bronchial wall thickness has not been systematically analysed. OHARA *et al.* [88] did not find any difference between the apical upper lobe segmental bronchus and lower lobe segmental bronchus.

In theory, CT should be a useful tool at assessing the morphology of the large and intermediate-sized airways. However, there are still many uncertainties regarding this technology and measurements, and no definite evidence has shown that this is a particularly useful tool in COPD.

Small-airway disease

The small airways are the main contributor to the airflow obstruction seen in COPD [77], and histological studies confirm epithelial metaplasia, goblet cell hyperplasia and peri-bronchial fibrosis. There is currently no gold standard for measuring this small-airway disease and so better methods are required, and considerable research is ongoing in this area. CT cannot image the small airways directly as they are beyond the maximum resolution. The indirect sign of gas trapping can be used as a surrogate marker, which is defined as lack of volume reduction after expiration and is seen as low-attenuation areas on expiratory CT.

A number of quantification methods have been developed to measure gas trapping and the simplest of these involve calculating the number of voxels below −856 HU or the mean lung density (MLD) on expiratory CT. These methods do not compensate for the amount of emphysema and so, in effect, will be a combined score for gas trapping and emphysema. To correct for this, techniques using paired inspiratory and expiratory scans have been developed, which include using the ratio of MLD in expiration to inspiration. MATSUOKA *et al.* [89] developed a more complex technique using the relative volume change between −856 and −950 HU between inspiratory and expiratory scans. This was validated in a study of 36 patients, and found strong associations with pulmonary function test markers of gas trapping and was superior to the other methods described above. When comparing these techniques to single-breath

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nitrogen washout, expiratory to inspiratory MLD ratio exhibited the best performance [90]. A large trial investigating gas trapping in COPDgene subjects found that paired (inspiratory and expiratory) samples were more predictive of pulmonary function in those with severe emphysema than just using the expiratory scan [91]. It therefore seems logical that any CT marker investigating gas trapping excludes emphysema by using paired inspiratory/expiratory scans.

Most studies investigating gas trapping have simply associated it to physiological parameters of disease where it has been found to correlate with FEV1 and other lung function parameters [31–33, 36, 38, 92–94], and with worsening GOLD status [32, 92]. Few studies have investigated the link between gas trapping on CT and underlying mechanisms of disease. Our work has shown that CT markers of small-airway disease are associated with a number of MMPs (MMP-3, MMP-7, MMP-8, MMP-9, MMP-10 and MMP-12), suggesting these play a key role in small airways remodelling in COPD [31]. The longitudinal changes and the distribution of gas trapping throughout the lungs are unknown. More recently, novel CT methods of investigating small-airway disease have been developed, termed parametric response mapping (PRM), which involves co-registering inspiratory and expiratory scans so that voxel-by-voxel comparison can be performed (figure 5) [95]. This allows determination of emphysema and functional small-airway disease, and has been shown to subgroup patients effectively, which correlated with pulmonary function testing. Functional small-airway disease measured in the COPDgene cohort was independently associated with decline in FEV1 and this was especially the case with milder patients [96]. This and other similar techniques offer the opportunity to characterise disease phenotypes, and has the potential to monitor disease status, so considerable research is ongoing.

In summary, using paired inspiratory and expiratory scans, CT imaging can effectively measure gas trapping, although the optimum method has yet to be defined. Valid questions exist about whether gas trapping is truly a surrogate marker of small-airway disease, although it does show strong associations with pulmonary function measures of small-airway disease. Further work is needed to see how underlying mechanisms of disease associate with gas trapping and whether this parameter reflects changes in lung function over time.

Other pulmonary morphological features

There have been efforts to develop quantitative analysis for pulmonary features other than those discussed above. An automated method for bronchiectasis has been developed where the ratio of airway inner diameter to adjacent vessel diameter can be quantified [97]. This has shown the burden of bronchiectasis is higher with increasing airflow obstruction and is associated with increased exacerbations. Further work is required to refine and automate this technique.

There has also been interest in using CT-measured lung density as a marker of pulmonary inflammation. Increased lung density has been found in current smokers as opposed to never/ex-smokers [98–100], and has been associated with greater smoking history [99, 100] and inflammatory cells in bronchoalveolar lavage [98]. Interestingly, in a longitudinal study, lung density decreased after smoking cessation [100], suggesting smoking causes increased lung density, possibly through pulmonary inflammation. This may prove to be a potential tool in evaluating early lung disease prior to structural changes occurring in COPD.

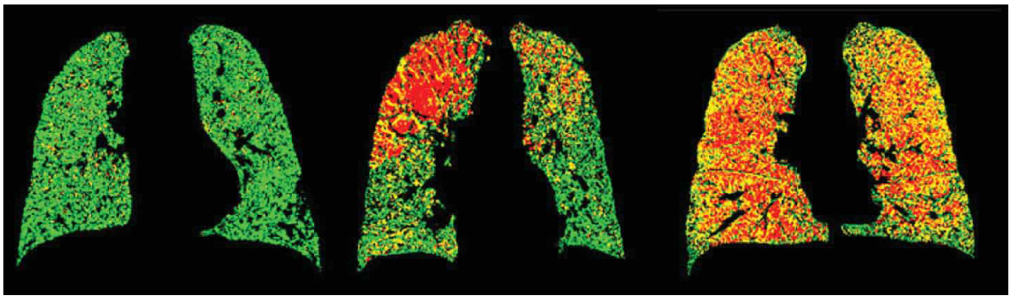

FIGURE 5 Parametric response mapping from computed tomography scans in three individuals. Reproduced and modified from [95] with permission from the publisher.

Pulmonary vascular imaging is an active area of research, as pulmonary vascular disease is well described in COPD and is a significant predictor of mortality [101–103]. The pulmonary artery diameter and ratio of pulmonary artery diameter to aorta diameter is associated with direct measures of pulmonary artery pressures [104] and higher exacerbation rates [105]. Cross-sectional area of small pulmonary vessels on CT is correlated strongly with invasive measures [106]. A technique to automatically segment and create a three-dimensional model of the pulmonary vasculature, allowing blood volume to be calculated, has been developed [107] and subjects with more emphysema had less blood volume. Further work is required to demonstrate the clinical utility of this.

Extrapulmonary manifestations

COPD has multiple systemic manifestations and CT of the chest is able to capture some of this information. Cachexia and skeletal muscle wasting are a significant problem in COPD, and body mass index (BMI) is associated with airflow obstruction, disease severity and mortality [108, 109]. There is growing recognition that other markers of body composition, such as fat-free mass index (FFMI), may be more important, as they are a better marker of skeletal muscle dysfunction. FFMI has been shown to be a predictor of mortality independently of fat mass or BMI [110, 111]. A technique to assess pectoralis muscle area on CT showed significantly reduced muscle area in COPD *versus* healthy controls and was associated with GOLD staging [112]. This was also associated with functional markers of disease including the BODE index, MRC (Medical Research Council) dyspnoea score and 6MWD [112]. Various fat compartments can also be assessed including anterior chest wall subcutaneous fat and intra-abdominal fat, which have shown some clinical correlations. These methods are all relatively crude and still require significant user input.

Osteoporosis is also common in COPD [113] and bone mineral density has been identified as being independently associated with airflow obstruction [114, 115]. Vertebral body attenuation values can be measured by CT and is associated with bone mineral density on dual-energy X-ray absorptiometry [116], and has also show associations with airflow obstruction [117] and exacerbation rate [118].

Using CT to define phenotypes in COPD

A main aim of COPD research is to define clinically meaningful phenotypes, and measuring morphological changes in isolation does not really help. More complex analysis that combines all of these morphological features is needed. One study separated patients into three subgroups: A phenotype had minimal emphysema, E phenotype had emphysema and no bronchial wall thickening, and M phenotype had both. A phenotype was associated with higher BMI, older age of onset of symptoms of breathlessness, fewer pack-years of smoking exposure and better lung function. Both A and M had more evidence of reversibility and higher sputum eosinophils. In another study, patients were split into emphysema-predominant and airway-predominant groups [57], and showed that emphysema patients had lower FEV1, and were more functionally limited, with higher BODE scores and lower BMI. Castaldi *et al.* [119] performed cluster analysis on the COPDgene cohort incorporating quantitative CT analysis. They successfully grouped subjects into four clusters that had their own individual attributes. Other studies have also clustered subjects based on emphysematous change and bronchial wall thickening on CT scans [120]. Clinical applications of these phenotyping methods have thus far not been demonstrated.

Other imaging modalities

Although CT is the imaging modality of choice for providing structural images of the lung, one of the major drawbacks is its inability to provide functional imaging. CT can, however, be combined with other imaging modalities to provide functional data in COPD. Positron emission tomography (PET)-CT and single-photon computed tomography (SPECT) are nuclear medicine scans that combine radioisotopes with CT imaging, allowing both functional and molecular imaging studies with anatomical co-registration. Small molecules, peptides or proteins are labelled with radioactive atoms that emit high-energy photons, which can be detected by PET or SPECT. For PET-CT, ^{18}F-fluorodexoxyglucose (FDG) is the most commonly used radiolabel; it is taken up by metabolically active tissues and can be used as a marker of pulmonary neutrophilic inflammation. Uptake is significantly increased in COPD [121], and is associated with FEV1 and emphysema severity [121, 122]. With SPECT, perfusion and ventilation imaging can be performed with either a vascular or inhaled tracer.

Magnetic resonance imaging (MRI) is another modality that can provide functional images of the lungs in COPD [123, 124]. There are a number of challenges in using magnetic resonance to image the lungs, including the low concentrations of protons, multiple air–tissue interfaces and relatively poor spatial resolution. Until recently, the main pulmonary application was in evaluating the pulmonary vasculature using contrast-enhanced imaging. Advances in technology, especially using oxygen-enhanced or hyperpolarised noble gases to increase the signal, allow functional imaging of ventilation and have shown ventilation defects in COPD [125]. Some studies have combined functional MRI with the structural images

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seen on CT. One such study assessed pulmonary microvascular blood flow, and found it was reduced in COPD and was associated with degree of emphysematous change [126]. Another study compared ventilation and perfusion defects measured on MRI with PRM measurements of emphysema and small-airway disease on CT. Ventilation defects were associated with emphysema and small-airway disease, and diffusion measurements were significantly raised in areas of gas trapping [127]. Magnetic resonance technology is still in early development but does provide an advantage over CT by allowing functional imaging and, importantly, being radiation free. Despite this, magnetic resonance is limited by availability, cost, the complexity of image analysis required and relatively poor spatial resolution.

Future use of CT in research and management

Further work is required to confirm quantitative CT analysis as an imaging biomarker of disease that can be used practically to manage patients with COPD and as a useful research tool to give insights into the disease. From a technical point of view, CT protocols need standardising as radiation dose, slice thickness and reconstruction algorithms can all effect analysis. Methods for quantifying the various morphological features need optimising, and this is especially the case for airway measurements and gas trapping. More sophisticated tools may be needed that do not just simply quantify results but also take into account other features, including emphysema subtypes and airway wall attenuation. Measures for detecting extrapulmonary manifestations are also in early development and further research is required to optimise these.

CT analysis needs to be assessed against outcome measures and markers of disease activity. To date, there have been relatively few longitudinal studies and these have shown variable results. It is vital studies are performed to track CT changes over time to get an accurate picture of the progression of the disease and how effective CT imaging is at capturing this. There have also been relatively few studies assessing quantifiable CT markers of disease and markers of underlying biological disease activity, which includes inflammatory markers, microbiology, proteases and other biomarkers. The approach taken in our study highlights how this can be achieved by combining bronchoscopic sampling and CT imaging to demonstrate a potentially important association between proteases and small-airway disease in COPD [31]. Similar approaches can be used to further our understanding of the active disease processes and endotypes occurring in different compartments and regions of the lung, hopefully allowing the development of novel targeted therapies.

Enhancing CT technology will also increase its application in COPD. A major limiting factor is the radiation dose and associated cancer risks. Dose reduction strategies are vital if longitudinal scans are to be considered. Dual-energy CT technology is being developed that allows functional imaging of the pulmonary vasculature and will have further applications. CT imaging could also be augmented with other imaging tools to provide more information about disease activity. PET-CT and SPECT are current examples, but these could be enhanced with development of novel molecular probes targeting key features of COPD. CT could also be co-registered with MRI to combine structural and functional imaging.

One of the most important questions is how to utilise CT analysis to define clinically important phenotypes. It is unlikely that simply subgrouping patients into emphysema and airway-predominant disease will be sufficiently helpful to guide treatment options. Cluster analysis and other statistical modelling techniques may provide interesting results but are more likely to be research tools with few practical clinical applications. Emphysema and bronchial wall thickening on CT have already been associated with increased mortality [55, 56, 59], and refining this information and combining it with clinical parameters may help enhance multidimensional prognostic tools such as the BODE index.

This technology may have a significant impact in clinical trial and drug discovery work in COPD. Quantitative CT has already been used as an outcome marker for emphysema progression in α_1-ATD, and this could be extended for drugs targeting airway inflammation and small-airway disease. The heterogeneity of the disease causes significant challenges in clinical trial design. Most trials include unselected populations of patients. and therefore have to enrol large numbers and last considerable time to identify sufficient responders. Improving knowledge of patient heterogeneity is vital for optimising drug trials and quantitative CT may play an important role. Potentially, CT could be used to stratify study subjects into phenotypic groups that would be most likely to respond to the investigational drug. For example, roflumilast targets airway inflammation but study results have been underwhelming. CT could be used to define which patients have evidence of airway thickening/inflammation and this subject group could be used in any interventional study. Improving stratification of study subjects may increase treatment effects and reduce variability in response, thereby increasing the chances of novel drugs being developed. This will, in turn, lead to further clinical applications for CT imaging in determining which patients may benefit from novel pharmaceutical agents.

Conclusion

CT imaging has an important and rapidly developing role to play in the investigation of COPD, where it can aid diagnosis and provide information on significant concomitant disease, and can be used to plan

interventional strategies for those with severe disease. It is also able to image the key pulmonary and extrapulmonary manifestations of the disease but the challenge has been how to utilise this information. Quantitative analysis shows promise in objectively measuring these disease features, and many have associations with traditional clinical and physiological markers of disease. These techniques are likely to be used initially as an important research tool with which to understand disease heterogeneity and aid our understanding of the key biological mechanisms in COPD. This understanding will aid optimal patient selection into clinical trials and translation of these approaches into clinical practise may help guide individualised management strategies and improve outcomes.

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Appendix B

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ORIGINAL ARTICLE

Relationship between pulmonary matrix metalloproteinases and quantitative CT markers of small airways disease and emphysema in COPD

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ABSTRACT

Background Matrix metalloproteinases (MMPs) are proteolytic enzymes that can degrade the extracellular matrix and drive tissue remodelling, key processes in the pathogenesis of COPD. The development of small airway disease has been identified as a critical mechanism in the early development of airflow obstruction but the contribution of MMPs in human disease is poorly characterised.

Objectives We investigated the role of MMPs and inflammatory cytokines in the lung by quantifying levels and determining relationships with the key pathological components of COPD in patients and healthy controls.

Methods We analysed levels of MMPs and inflammatory cytokines in bronchoalveolar lavage from 24 COPD and 8 control subjects. Each subject underwent spirometry and high-resolution CT. Image analysis quantitatively assessed emphysema, bronchial wall thickening and small airways disease.

Results Multiple MMPs (MMP-1, -2, -3, -8, -9 and -10) and cytokines (interleukin (IL) 6 and IL-8) were elevated in lungs of subjects with COPD. MMP-3, -7, -8, -9, -10 and -12 concentrations closely associated with CT markers of small airways disease. Emphysema severity was also associated with MMP-3, -7 and -10. However, there were no strong relationships between MMPs and bronchial wall thickness of the larger airways.

Conclusions Pulmonary MMP concentrations are directly associated with the extent of gas trapping and small airways disease identified on CT scan. This study suggests that MMPs play a significant role in small airways remodelling, a key feature in the pathogenesis of COPD.

Trial registration number NCT01701869

Key messages

What is the key question?
- Are matrix metalloproteinases (MMPs) involved in the development of pathological changes in the lung of patients with COPD and what is their association with emphysema formation, bronchial wall thickening and small airways remodelling?

What is the bottom line?
- Bronchoalveolar lavage concentrations of multiple MMPs are increased in patients with COPD, and these are associated with the degree of small airways disease and emphysema measured by CT analysis.

Why read on?
- This study combines quantitative CT analysis with multiplex profiling of MMPs and inflammatory mediators to identify a new role of proteases in COPD.

INTRODUCTION

COPD is a heterogeneous disease characterised by the progressive development of airflow limitation which leads to functional impairment and associated symptoms.[1] The underlying mechanisms of disease are poorly understood, which has limited the development of new therapeutic and diagnostic approaches.[2]

Tissue destruction, inflammation and airway remodelling are important features in COPD, and a number of pathways and mediators have been implicated in these processes.[3] A proteinase/antiproteinase imbalance has been postulated to be a key contributor to emphysematous changes.[4] Matrix metalloproteinases (MMPs), a large family of zinc-dependent proteolytic enzymes, have the ability to degrade the pulmonary extracellular matrix (ECM) and have been implicated in COPD.[4] They are broadly grouped depending on their substrate specificity, including collagenases (MMP-1, -8 and -13), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11) and elastases (MMP-7 and -12),[5] although there is considerable substrate overlap. MMPs are not normally expressed in healthy tissue but in disease can be produced by alveolar macrophages, neutrophils and bronchial epithelial cells.[5] They are tightly regulated by specific endogenous inhibitors, the four Tissue inhibitors of MMPs (TIMP).[5]

Transgenic mice over expressing MMP-1 develop emphysema at an accelerated rate.[6] Selective inhibition of MMP-9 and -12 in guinea pigs reduced the extent of emphysema following exposure to smoke.[7] Human studies demonstrate increased expression of MMP-1,[8 9] -8,[9 10] -9[8–13] and

-12[8 14 15] in the sputum or bronchoalveolar lavage (BAL) of subjects with COPD. However, detailed analysis of the MMP profile has not been integrated with systematic characterisation of lung pathology by high-resolution CT and lung function.

MMPs have complex biology with multistep activation and numerous molecular interactions,[16] and the most important MMPs have yet to be determined in COPD. In addition, MMPs have multiple other functions that are independent of their ability to degrade the ECM, including facilitating cell migration and activating growth factors and cytokines.[5] Most studies investigating MMPs in COPD have only investigated a subset of MMPs. Given their biological complexity, novel multiplex-based arrays provide the opportunity to profile a broad spectrum of MMPs in a carefully characterised clinical cohort.

The key sites of MMP activity in the lung are unknown and it is likely that matrix turnover and remodelling differs between regions. CT imaging provides the opportunity to study key morphological features of COPD including emphysema, bronchial wall morphology and small airways disease. Quantitative analysis software allows numerical, objective estimation of these disease facets.[17] MMP-9 and -12 have been shown to have associations with quantitative measures of emphysematous change on CT.[11 14] Previous studies have not investigated the association between MMP concentrations and bronchial wall measures or small airways disease identified by CT. In this study, we combine CT analysis of lung pathology with multiplex profiling of MMPs and inflammatory mediators, and identify a novel role for MMPs in COPD.

METHODS

Subjects

Subjects gave written informed consent and the study (ClinicalTrials.gov:NCT01701869) was approved by the South Central—Southampton B NRES Committee (12/SC/0304).

Twenty-four subjects with stable mild and moderate COPD as defined by GOLD guidelines[1] were recruited into the study. Postbronchodilator spirometry was used to assess airflow obstruction with an FEV1:FVC ratio of <0.7 and an FEV1 of ≥50% predicted value required for enrolment. Spirometry was conducted in accordance with American Thoracic Society standards.[18] Subjects had at least a smoking history of 10 pack years. Exclusion criteria included a history of other pulmonary disease, α-1-antitrypsin deficiency, long-term antibiotics/steroids or an exacerbation within the month prior to recruitment. A control group of eight current or ex-smokers, with at least a 10 pack year history but preserved lung function were also recruited.

CT scanning and quantitative image analysis

Subjects underwent volumetric CT scans of the chest using a Siemens Sensation 64 CT scanner. The imaging protocol consisted of; slice thickness 0.75 mm, slice separation 0.5 mm, tube voltage 120 KV, effective mAs 90 mAs (using dose modulation), collimation 0.6 mm and a pitch of 1. Subjects were scanned at full inspiration. A subcohort of 22 subjects was scanned at end-tidal volume. The remaining did not have an expiratory scan due to concerns over cumulative radiation exposure having had recent CT scans.

Images reconstructed with the B35 kernel were used for image analysis using Apollo Software (VIDA Diagnostics). Emphysema was quantified by the per cent of lung voxels on the inspiratory scan with attenuation values below −950 HU (% LAA). Bronchial wall thickening was quantified using the standardised parameter Pi10, which is the square root of the wall area of a hypothetical airway with a 10 mm internal perimeter. A surrogate marker for small airways disease was measured using the ratio of mean lung attenuation on expiratory and inspiratory scans (E/I MLD), which has been previously validated.[19]

Bronchoscopy and sample acquisition

Fibre optic bronchoscopy was performed on an outpatient basis. In each subject, two lobes were targeted, and BAL was performed by instilling 100 mL of 0.9% saline into each lobe and recovered by aspiration. BAL fluid was poured through 100 μm filters and cells removed by 400 g centrifugation for 10 min at 4°C. The supernatant was aliquoted and stored at −80°C prior to analysis. The resulting cell pellets were prepared for cytospin analysis as previously described.[20]

MMP and cytokine analysis

MMP and cytokine concentrations in BAL were quantitated using a microparticle-based multiplex immunoassay (R&D systems, Abingdon, UK) developed by Luminex. Samples were analysed on the Luminex 200 platform (Biorad Bioplex 200, Hemel Hempstead, UK), as per manufacturer's instructions. The following MMPs were analysed: MMP-1, -2, -3, -7, -8, -9, -10, -12, -13 and ECM metalloproteinase inducer (EMMPRIN), a cell surface molecule that can be shed. TIMP 1–4 were analysed and cytokine analysis was performed for interleukin (IL) 1β, IL-2, IL-6, IL-8, IL-10, GM-CSF, IFNγ and TNFα (see online supplementary data for assay sensitivities).

Statistical analysis

Statistical analyses were performed using SPSS V.21. Mann–Whitney U test and Fisher's exact tests compared data between COPD and control groups. In the subjects with COPD, associations between MMPs, spirometry, CT parameters and cytokines were assessed using Spearman's correlation with r and p values presented. Partial Spearman's correlation was used to conduct multivariate analysis. Each subject had two lobes sampled, and the mean concentrations between the lobes were used. For the purpose of statistical analysis, values that were below the lower limit of detection were given the value of half the concentration of detection. A p value of <0.05 was considered statistically significant.

Table 1 Characteristics of participants included in the study

	COPD (n=24)	Controls (n=8)	p Value
Age	66.0 (12.0)	56.0 (18.0)	0.064
Male	16	6	>0.999
Current smoker	11	5	0.685
FEV1%	69.00 (21.00)	108.00 (20.25)	<0.001*
FEV1/FVC	54.50 (10.75)	78.00 (9.50)	<0.001*
FEF25%–75%	21.50 (16.0)	77.50 (45.25)	<0.001*
Emphysema % (%LAA)	8.24 (7.17)	2.55 (3.35)	0.029*
Bronchial wall area (Pi10)	3.74 (0.15)	3.74 (0.04)	0.502
Small airways disease (E/I MLD)	0.86 (0.08)	0.76 (0.10)	0.002*

Values are given as medians (IQR).
Male and current smokers given as number of subjects.
%LAA and Pi10 given for 31 subjects. The ratio of mean lung density in expiration to inspiration (E/I MLD) is given for a subcohort of 22 subjects who had expiratory CT scans.
Fisher's exact test for male and current smoker. Mann–Whitney U test for all other variables.
*p<0.05.

Appendix B

RESULTS

Subject characteristics

COPD and control groups were well matched for age, sex and current smoking status (table 1). There were more males in both groups. FEV1% and FEF25%–75% were significantly lower in the COPD group.

MMP concentrations in COPD and controls

Median MMP-1, -2, -3, -8, -9 and -10 were significantly higher in the BAL of subjects with COPD compared with controls (figure 1). There was no significant difference in MMP-7, -12, -13 and EMMPRIN between the groups.

Neutrophil and cytokine concentrations in COPD and controls

BAL neutrophil count, IL-6 and IL-8 concentrations were significantly higher in subjects with COPD than controls (figure 2). There were no significant differences in IL-1β and GM-CSF between groups. IL-2, IL-10, IFNγ and TNF-α were only detected at very low concentration in all samples and so further analysis was not performed.

Associations between MMPs, cytokines, lung function and CT parameters

We investigated the relationships between MMPs, cytokines and physiological and CT parameters in the subjects with COPD.

Relationships of MMPs with cytokines and neutrophils

We performed a systematic analysis of associations between cytokines, neutrophils and tissue-destructive MMPs, using Spearman's correlation. There were significant associations between the cytokines IL-6 and IL-8 and MMP-1, -7, -8, -9, -12 and EMMPRIN (table 2). GM-CSF was associated with MMP-8, -9 and EMMPRIN. IL-1β had a negative correlation

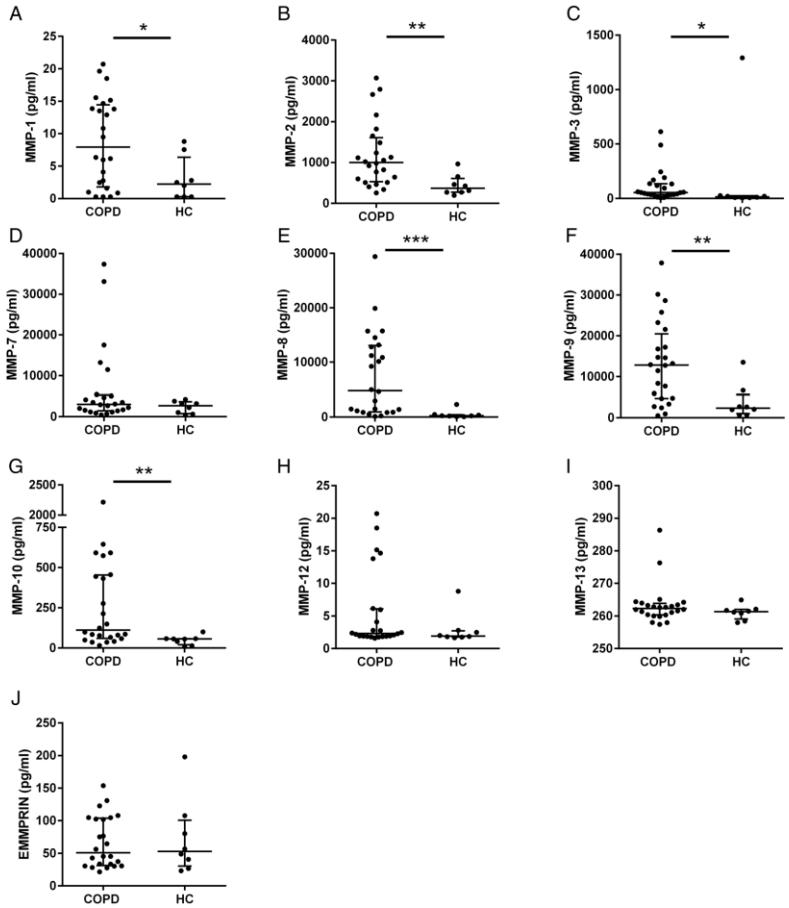

Figure 1 Bronchoalveolar lavage (BAL) expression of matrix metalloproteinases (MMPs) in COPD and controls (HC). (A) MMP-1, (B) MMP-2, (C) MMP-3, (D) MMP-7, (E) MMP-8, (F) MMP-9, (G) MMP-10, (H) MMP-12, (I) MMP-13, (J) extracellular matrix metalloproteinase inducer (EMMPRIN). Data represents median with IQR. Each dot represents BAL concentration of individual MMP in a specific patient, n=24 for COPD and 8 for controls. *p<0.05, **p<0.01, ***p<0.001 using Mann–Whitney U test.

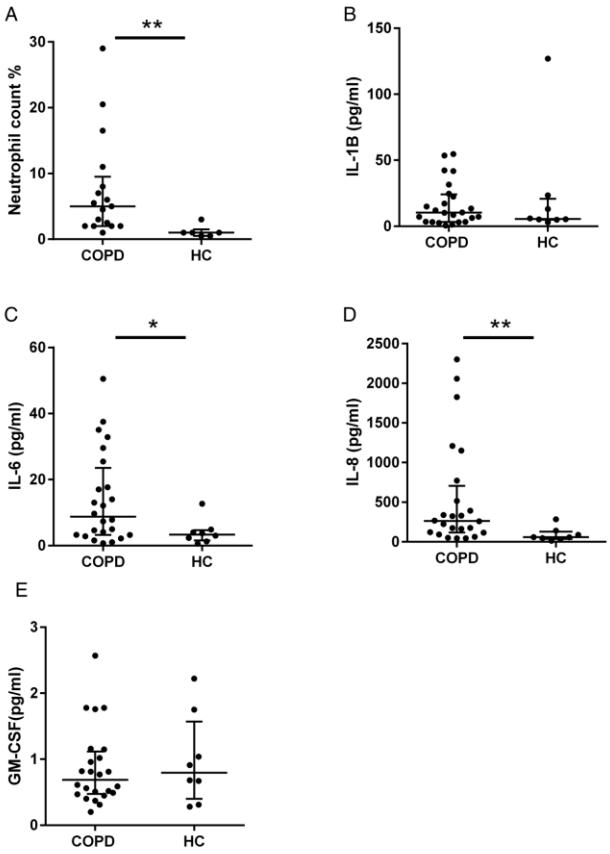

Figure 2 Bronchoalveolar lavage (BAL) neutrophils and cytokines in COPD and controls (HC). (A) differential BAL neutrophil count (%), (B) IL-1β, (C) IL-6, (D) IL-8, (E) GM-CSF. Data represent median with IQR. Each dot represents BAL concentration of individual value in a specific patient, n=24 for COPD and 8 for controls. *p<0.05, **p<0.01 using Mann–Whitney U test. IL, interleukin.

with MMP-3 while neutrophil counts were not associated with any MMPs.

Relationships of MMPs, cytokines, neutrophils with lung function

We compared MMP and cytokine concentrations with lung function markers of disease severity using Spearman's correlation. There were significant associations between airflow obstruction (FEV1%) and MMP-8, -9 and -12 and between FEF25%–75% and MMP-1, -8, -9 and -12 (table 2). MMP-8 had the strongest association with both FEV1% and FEF25%–75% (figure 3) and using partial correlation this remained significant when adjusting for each of the other MMPs in turn apart from MMP-9 (see online supplementary table S1).

FEV1% and FEF25%–75% were significantly associated with IL-8. FEF25%–75% was also associated with IL-1β. There was no association between these spirometric markers and BAL neutrophils, IL-6 and GM-CSF (see online supplementary table S2).

CT analysis

Quantitative CT analysis was performed to study emphysema, bronchial wall morphology and small airways disease and assess the relationships with MMPs and cytokines. Segmentation and quantitative analysis was successfully achieved in 31 subjects. One of the control CT scans could not be analysed for technical reasons.

Emphysema

Subjects with COPD had significantly more emphysema (higher %LAA) than controls (table 1), although there was no significant association between emphysema and FEV1% in the subjects with COPD (r −0.10, p=0.63).

There were significant associations between emphysema (% LAA) and MMP-3, -7 and -10 (table 2). MMP-10 had the strongest association with emphysema (figure 3) and using partial correlation this remained significant when adjusting for each of the other MMPs apart from MMP-3 and -7 (see online supplementary table S1). Emphysema was not associated with neutrophils or cytokines (see online supplementary table S2).

Small airways disease

Small airways disease (E/I MLD) was greater in the COPD group than controls (table 1). Strong associations with FEV1% (r −0.72, p=0.002) and FEF25%–75% (r −0.54, p=0.03) were seen in subjects with COPD.

Appendix B

Table 2 Spearman's correlation analysis between matrix metalloproteinases (MMPs), spirometry, CT measures of disease and cytokines and neutrophils in subjects with COPD

	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12	MMP-13	EMMPRIN
Cytokines and neutrophils										
IL-1β	0.26	−0.20	−0.44*	0.08	0.30	0.21	−0.21	−0.00	0.18	0.22
IL-6	0.51*	−0.18	0.28	0.55**	0.69***	0.55**	0.21	0.52**	−0.22	0.54**
IL-8	0.44*	−0.26	0.06	0.53**	0.90***	0.78***	0.16	0.56**	−0.01	0.46*
GM-CSF	−0.02	−0.16	−0.25	0.15	0.41*	0.41*	0.13	0.20	−0.31	0.68***
Neutrophils	−0.12	0.07	0.44	0.40	0.41	0.28	0.25	0.29	−0.26	0.36
Spirometry										
FEV1%	−0.35	0.28	−0.34	−0.35	−0.60**	−0.59**	−0.19	−0.51*	0.02	−0.20
FEF25%–75%	−0.47*	0.39	−0.03	−0.18	−0.61**	−0.58**	−0.11	−0.44*	0.22	−0.22
CT parameters										
Emphysema % (LAA%)	−0.03	−0.05	0.49*	0.52**	0.35	0.33	0.52*	0.09	−0.24	0.14
Small airways disease (E/I MLD)	0.16	−0.14	0.52*	0.52*	0.60*	0.56*	0.55*	0.50*	−0.03	0.32
Bronchial wall area (Pi10)	0.12	0.08	0.04	0.11	0.23	0.21	0.13	0.17	0.20	0.15

Spearman's r values given.
N=24, apart from associations with E/I MLD (n=16).
*p<0.05, **p<0.01, ***p<0.001.
IL, interleukin.

There were associations between small airways disease (E/I MLD) and MMP-3, -7, -8, -9, -10 and -12 (table 2). MMP-8 had the strongest association with small airways disease (figure 3) and this remained significant after adjusting for MMP-1, -2 and -13 in turn but not the other MMPs (see online supplementary table S1). The only cytokine that correlated with small airways disease was IL-8 (see online supplementary table S2).

Bronchial wall thickening

There was no significant difference in bronchial wall thickening (Pi10) between COPD and controls, but there was an association between Pi10 and FEV1% in subjects with COPD (r −0.59, p=0.003). There were no associations between airway wall area and MMPs (table 2). Neutrophils and cytokines did not show any association with bronchial wall area (see online supplementary table S2).

DISCUSSION

This study has, for the first time, demonstrated an association between small airways disease measured by CT imaging and the lung concentrations of MMPs and inflammatory cytokines in COPD. MMPs and cytokines are also associated with other markers of disease severity, including FEV1% and emphysema severity. The analysis suggests that associations were stronger between MMPs and small airways disease rather than emphysema.

MMPs are structurally similar proteolytic enzymes that have been implicated in the tissue remodelling, matrix destruction

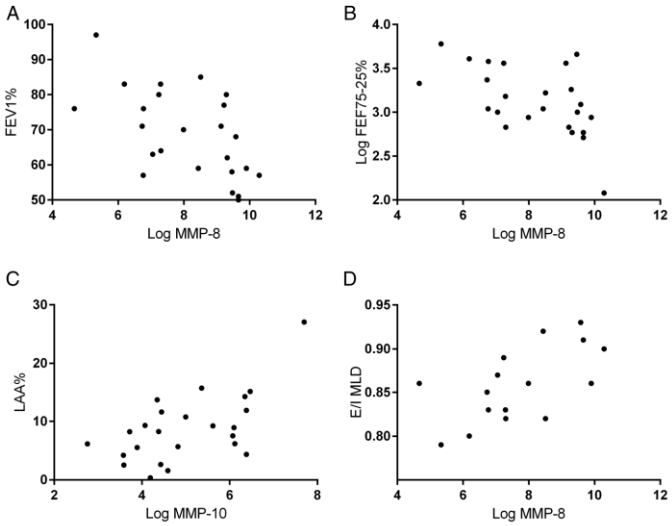

Figure 3 Scatter plots of subjects with COPD for (A) log matrix metalloproteinase (MMP)-8 against FEV1%, (B) log MMP-8 against log FEF25%–75%, (C) log MMP-10 against LAA%, (D) log MMP-8 against E/I MLD. These plots are visualised as they represent the MMPs with the strongest associations with the accompanying outcome variable. N=24, apart from E/I MLD (n=16).

and inflammation seen in COPD.[4] We found that MMP-1, -2, -3, -8, -9 and -10 were elevated in the airways of subjects with COPD. This is in keeping with previous studies which demonstrated that MMP-1,[8 9] -8[9 10] and -9[8–13] are increased in subjects with COPD. Furthermore, we have also shown that MMP-2, -3 and -10 were increased. A histological study of lung resection subjects also found that MMP-2 was upregulated in the lung periphery in COPD.[21] However, Culpitt *et al*[9] found no difference between MMP-2 and -3 in sputum between COPD and controls. Our results may diverge from these due to the difference in sampling locations between sputum and BAL. Previous studies also found that MMP-12 is increased in COPD,[8 14 15] although we did not replicate this observation in our cohort. We analysed the ratio of MMPs: TIMPs and found they were significantly increased in subjects with COPD (data not shown) and this was especially the case for MMP-8 and -10. This supports the idea of a proteinase to antiproteinase imbalance; however, further work is required to understand this fully. We also found the concentrations of neutrophils, IL-6 and IL-8 were increased in COPD, which is consistent with previous work.[22] This provides further evidence for the role of neutrophilic airway inflammation in COPD.

Most work to date has focused on assessing the role of MMPs in emphysema. Transgenic mice overexpressing MMP-1 develop emphysema at an accelerated rate.[6] MMP-12 knockout mice are protected from cigarette induced lung damage.[23] In the SMAD3 mouse model of emphysema, genetic blockade of MMP-9 reduced the amount of emphysema.[24] Selective inhibition of MMP-9 and -12 in guinea pigs reduced the extent of emphysema when exposed to smoke.[7] In human genetic studies, polymorphisms of MMP-9 genes have been linked to emphysematous change[25 26] while polymorphisms of MMP-12 have been linked to airflow obstruction.[27] Previous work using lavage fluid found that MMP-1, -9 and -12 were significantly raised in patients with emphysema when compared with healthy non-smokers but not current smokers.[8] In two studies using quantitative CT analysis, the LAA% correlated with sputum MMP-9 and -12.[11 14] Unlike these two studies, we did not find any associations between CT-measured emphysema and MMP-9 or -12, which may be due to the differences between sampling sputum or BAL. However, we found significant associations between quantified emphysema and MMP-3, -7 and -10, which have not been previously investigated. MMP-10 had the strongest association with emphysema, and interestingly, this and the other significantly associated MMPs have limited elastin degradation properties and mainly degrade collagens and proteoglycans. Studies show that there is actually increased collagen around emphysematous lesions,[28] and therefore a key mechanism may be MMP-induced collagen breakdown and subsequent abnormal remodelling and aberrant deposition of collagen.

We measured bronchial wall thickening of larger airways using the standardised marker Pi10 which has been shown to correlate with FEV1%[29] and is elevated in COPD.[30] Our analysis found no significant difference in Pi10 between COPD and controls but did show an association with airflow obstruction. There were no significant associations between Pi10 and MMPs or inflammatory cytokines. The reasons for this are unknown, but it may be that Pi10 is not the best measure at assessing airway wall morphology. It is also feasible that bronchial wall thickening is not directly linked to luminal inflammatory indices or protease activity and the remodelling response in the proximal airway may be more related to inflammatory infiltration of the submucosa[31] and biopsy studies may be required to elicit these mechanisms.

Small airways disease is a key feature of COPD and has been identified as the main site of the airflow obstruction seen in the disease.[32] Histopathological studies have identified that the small airways are thickened and morphologically abnormal with a combination of squamous cell metaplasia, goblet cell hyperplasia and peribronchial fibrosis apparent.[33] There is currently no gold standard for investigating and assessing small airways disease in COPD. CT lacks the resolution to image the small airways directly; however, the indirect sign of gas trapping can be used.[17] A number of quantitative CT methods exist to measure this, and we chose to use the ratio of mean lung density in expiration against inspiration. Hersh *et al*[34] confirmed that E/I MLD had a good correlation with lung function markers of small airways disease. Bommart also demonstrated that E/I MLD was the best CT marker of gas trapping as measured by nitrogen washout.[19] We found E/I MLD was significantly raised in COPD and had strong associations with FEV1% and FEF25%–75%, supporting its use as a marker of small airway disease.

Few studies have investigated the role of MMPs in small airways disease. In guinea pigs, inhibition of MMP-9 and -12 protected the animals from small airways fibrosis in response to cigarette smoke exposure.[7] A study of symptomatic smokers with normal lung function found that MMP-8, -9 and -12 in induced sputum correlated with FEF25%–75%.[35] Our results found that MMP-1, -8, -9 and -12 had associations with FEF25%–75%. However this is an insensitive and non-specific marker of small airways disease. When using the CT marker E/I MLD we found strong associations with the concentrations of MMP-3, -7, -8, -9, -10 and -12. These associations tended to be stronger than those seen with emphysema, suggesting that MMPs may play a significant role in the development of small airway remodelling and the associated airflow obstruction. MMP-8 had the strongest association with small airways disease and along with the other associated MMPs is mainly produced by neutrophils and macrophages and between them are able to degrade all components of the ECM. In the small airways, it has been demonstrated that elastin and collagen are reduced in patients with COPD,[36] and we propose that this degradation is driven by MMPs leading to deranged remodelling and fibrosis.

We recognise that the main limitation of this study was the small sample size and associated limited statistical power, meaning that we were unable to analyse certain aetiological factors such as smoking. However, every effort was made to phenotype our cohort with CT imaging and invasive bronchoscopy techniques. Despite this, we found strong evidence of the associations between MMPs and clinical features of disease. Another limitation is the multiple comparisons made in this study. We tested 129 associations between parameters of physiology, CT indices and laboratory markers. At the 5% level, around seven associations would be expected to be significant just by chance. We found 38 significant associations, far higher than the number expected by chance, suggesting the presence of genuine associations. Due to the need to perform bronchoscopy, our study consisted of patients with mild and moderate COPD, with only limited amounts of emphysema. It is unknown whether these results would be the same in more severe patients. In this study, we measured concentrations of MMPs rather than MMP activity. Lowrey demonstrated that although MMP-9 concentrations were increased in the sputum of subjects with COPD, MMP-9 activity was not.[12] However, due to the site and nature of action of MMPs, there is considerable debate as to whether concentrations or activity assays best reflect the action of MMPs.[37] Another limitation was that only a subcohort

of 16 subjects with COPD had both an inspiratory and expiratory CT scan, allowing assessment of small airways disease. Repeating emphysema and airflow analysis on this subsample yielded broadly similar results, indicating the smaller sample size did not influence the results.

In conclusion, multiple MMPs are increased in the airways of subjects with COPD and are associated with the severity of airflow obstruction and quantitative CT measures of emphysema and small airways disease. This suggests that MMPs may play a significant role in the pathogenesis of COPD by causing breakdown of the pulmonary ECM leading to abnormal remodelling in both the small airways and lung parenchyma. Further work is required to investigate these important mechanisms and to understand the heterogeneity of the disease within different compartments of the lung. While most previous work has focused on MMPs and emphysema, this study suggests the strongest associations were with small airways disease. Interventions directed at inhibiting MMPs may have a role in preventing small airways remodelling, and any trial investigating modulation of MMPs should use CT analysis as a marker of small airways disease.

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