USING QUANTITATIVE COMPUTED TOMOGRAPHY TO PROVIDE IMPORTANT AND NOVEL INSIGHTS INTO AIRWAY REMODELLING IN ASTHMA AND COPD.

Thesis submitted for the degree of

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by

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MAIN TABLE OF CONTENTS

ABSTRACT

Over the past 20 years, the technology behind Computed Tomography (CT) scan acquisition and image analysis has improved dramatically. The potential for CT to be a non-invasive method to probe the lungs has long been recognised, but there remain large gaps in our knowledge of how changes in airway structure influences airway physiology and clinical outcomes.

In this thesis I examine quantitative CT (QCT) measures of airway remodelling between asthma, COPD and healthy controls, its relationship with immunohistology and its application in stratified medicine intervention studies.

First I present one of the largest studies to date comparing QCT parameters in asthma, COPD and healthy controls. It confirms the heterogeneity within both diseases. However there are still distinct structural differences observed within each cohort, with striking differences seen within and between the cohorts when grouped by airflow limitation.

I then present one of the largest studies to date looking at QCT measures and bronchial biopsies. This shows that changes seen on QCT correlate with typical remodelling parameters such as percentage airway smooth muscle, but not markers of inflammation. It also shows that the QCT marker of air trapping is associated with increased vascularity.

Finally I present a study looking at the use of QCT in assessing the effects of a new drug, fevipiprant, aimed at reducing sputum eosinophilia, over a 12 week course. This study shows that fevipiprant, improves some clinical outcomes such as spirometry and reduces sputum eosinophilia, but no structural changes are seen on QCT.

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STATEMENT DETAILING WORK PERSONALLY PERFORMED.

Study 3.1: Relationship Between Lung Function and Quantitative Computed Tomography Parameters of Airway Remodelling, Air-trapping and Emphysema in Asthma and COPD: A Single Center Study.

I supervised all the COPD CT scans, and all the healthy and asthma CT scans done from September 2011 onwards. I was part of the clinical team involved in recruiting and characterising the COPD patients, and the asthmatic patients who were seen after September 2011. I analysed all the scans, irrespective of acquisition date and did all the statistical analysis, with the exception of the cluster analysis which was performed by our biostatistician Dr Chris Newby. The scans used for the inter user variability analysis was done by Dr Sumit Gupta (SG). I did quality control checks on pre September 2011 clinical data and filled in gaps where possible, and recorded any new clinical data. I wrote the manuscript for this study.

Study 3.2: Associations in asthma between quantitative computed tomography and bronchial biopsy-derived airway remodelling

I was part of the research team involved in the design of this study. I supervised scans done after September 2011 and analysed all the scans included in this study. Biopsy analysis was done by Dr Rachid Berair (RB). I did quality control checks on pre

September 2011 clinical data and filled in gaps where possible, and helped with recording new clinical data. I co-wrote, with Rachid Beriar, the manuscript for this study.

Study 3.3: Randomised controlled trial of the prostaglandin D2 receptor 2 antagonist fevipiprant in persistent eosinophilic asthma

I was part of the clinical team involved in recruiting and characterising patients. I supervised all the CT scans and performed the analysis on all the CT scans. I performed the statistical analysis on the CT data. I was part of the team involved in writing the manuscript.

PUBLICATIONS

Original Articles:

Berair R*, **Hartley R***, Mistry V, Sheshadri A, Gupta S, Singapuri A, et al. Associations in asthma between quantitative computed tomography and bronchial biopsy-derived airway remodelling. Eur Respir J 2017 May 1;49(5):10.1183/13993003.01507-2016. Print 2017 May.

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CONTENTS

LIST OF TABLES

TABLE OF FIGURES

LIST OF ABBREVIATIONS

%WA	Percentage Wall Area
ACQ 6	Asthma Control Questionnaire (first 6 questions)
ACQ 7	Asthma Control Questionnaire (all 7 questions)
AHR	Airway hyperresponsiveness
ASM	Airway smooth muscle
ATS	American Thoracic Society
AQLQ	Asthma Quality of Life Questionnaire
BAL	Bronchoalveolar lavage
BD response	Bronchodilator response
BDP	Beclomethasone dipropionate
Blood eos	Blood eosinophil count
Blood neut	Blood neutrophil count
BMI	Body mass index
BODE	Body mass index, airflow Obstruction, Dyspnea and Exercise capacity
BSA	Body Surface Area (m2)
BT	Bronchial thermoplasty
CRTH2	chemoattractant receptor-homologous molecule expressed on Th2 cells, (also known as Prostaglandin D2 receptor, DP2)
COPD	Chronic obstructive pulmonary disease
CT	Computed Tomography
$CTLV_{E/I}$	CT Lung Volume Expiratory to Inspiratory ratio
DALYs	Disability-adjusted life years
DAMP	Damage-Associated Molecular Patterns
DNA	Deoxyribonucleic acid
D-PBS	Dulbecco's phosphate buffered saline

DP1	Prostaglandin D1 receptor
DP2	Prostaglandin D2 receptor, also known as CRTH2
DC	Dendritic cells
ECG	Electrocardiogram
EC	Epithelial cells
ED	Electron Dense
ERS	European Respiratory Society
Exp VI-856	Expiratory Voxel Index below -856 HU
FC	Fuzzy Connectivity
FDA	Food and Drug Administration
FE_{NO}	Fractional exhaled nitric oxide
FEV_1	Forced Expiratory Volume in 1 second
FEV_1/FVC	Forced Expiratory Volume in 1 second/Forced Vital Capacity
FRC	Functional Residual Capacity
GOLD	Global initiative for chronic Obstructive Lung Disease
GINA	Global Initiative for Asthma
H&E	Haematoxylin & Eosin
HU	Hounsfield Unit
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-13	Interleukin 13
Insp VI -950 (-950%VI)	Inspiratory Voxel Index below -950 HU
IQR	Interquartile range,
JACQ/ACQ	(Juniper) Asthma Control Questionnaire
kVp	Peak kilovoltage
KCO%	Transfer Coefficient

LA	Lumen Area (mm2)
LABA	Long-acting Beta-2 adrenergic agonists
LAMA	Long-acting anticholinergic agents
LB1	Left upper lobe apical
LB2	Left upper lobe posterior
LB3	Left upper lobe anterior
LB4	Left lingular superior
LB5	Left lingular inferior
LB6	Left lower lobe apical
LB1+2	Left upper lobe apicoposterior
LB8	Left lower lobe anteriomedial
LB9	Left lower lobe lateral
LB10	Left lower lobe posterior
LAC-D -950	Low Attenuation Clusters below -950 HU Fractal Dimension value
mAb	Monoclonal antibody
mAs	Milliamperage second
MCH	Major-histocompatibility-complex
$MLD_{E/I}$	Mean Lung Density Expiratory to Inspiratory ratio
mSv	millisieverts
NK	Natural Killer
PC20	Methacholine challenge test
PAMP	Pathogen-Associated Molecular Patterns
PAS	Periodic acid-Schiff
Perc15	The Hounsfield Unit value at which 15% of the voxels lie below
PGD2	Prostaglandin-D2
Pi10	The wall area of theoretical airway with an internal perimeter of 10mm

Po20 (%WA)	The percentage wall area of a theoretical airway with an external perimeter of 20mm
Post-BD	Post Bronchodilator
Pre-BD	Pre Bronchodilator
PRR(s)	Pattern Recognition Receptor(s)
QCT	Quantitative Computed Tomography
RB1	Right upper apical
RB2	Right upper posterior
RB3	Right upper anterior
RB4	Right middle lateral
RB5	Right middle medial
RB6	Right lower apical
RB7	Right lower medial
RB8	Right lower anterior
RB9	Right lower lateral
RB10	Right lower posterior
RBM	Reticular Basement Membrane
ROI	Region of interest
RVC	Relative Voxel Change
RV/TLC	Residual Volume/Total Lung Capacity
SD	Standard deviation
SGRQ-C	St George's Respiratory Questionnaire for COPD patients
SMA	anti-human smooth muscle actin
SNPs	Single nucleotide polymorphisms
Sputum eos (%)	Percentage of sputum eosinophils
Sputum neut (%)	Percentage of sputum neutrophils
TA	Total Area (mm2)
Tc1	Cytotoxic T cell-type 1
Th1	T helper lymphocytes, type 1

Th2	T helper lymphocytes, type 2
Th17	T helper lymphocytes, type 17
TGF-β	Transforming growth factor - beta
TLC	Total Lung Capacity
VC	Vital capacity
VEGF	Vascular endothelial growth factor
VI	Voxel Index
WA	Wall Area (mm2)
WHO	World Health Organisation
WDP	Warwick Density Phantom

1 INTRODUCTION

1.1 Asthma

The word “Asthma”, (from the Greek word “wind” or “to blow”), was first used by Hippocrates around 400 BC, to describe respiratory distress. The Romans were also aware of the condition, with Pliny the elder noting that pollen was a common trigger for respiratory difficulty. It was only in the latter half of the 19th century that the term asthma was refined by Henry Hyde Salter, an asthma sufferer himself, in his treatise “On Asthma and its Treatment”. He described a disease where smooth muscle contraction in the airways causes them to narrow (1). In 1892 Sir William Osler described asthma in the textbook “Principles and Practice of Medicine”, and treating asthma focused mainly on treating bronchospasm.

Despite the availability of a selection of bronchodilators by 1960s, and the knowledge that asthma was associated with airway inflammation, there was little understanding as to why it occurred, and little thought given to long term treatment/control (2). The bulk of the working looking into the pathogenesis of asthma has mostly taken place from the 1970s and onwards (2).

The World Health Organisation (WHO), has defined asthma (3)

“Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment”

1.1.1 Disease burden of asthma

Asthma affects an estimated 300 million worldwide and is increasing in prevalence, with an additional 100 million suffers anticipated by 2025 (4). Asthma is on par with diseases such as diabetes, schizophrenia and liver cirrhosis for the number of disability-adjusted life years (DALYs) lost per year (4). In 2011-2012 it was estimated that 5.4 million people were being treated for asthma in the UK with over 65,000 hospital admissions for asthma (5).

Approximately 10% of sufferers have severe asthma (6). Morbidity and mortality is highest in severe asthma consuming over 50% of the healthcare costs attributed to asthma (7).

1.1.2 Severe asthma

Severe asthma requires treatment with high dose inhaled corticosteroids (ICS) plus a second controller and/or systemic corticosteroids to prevent it from becoming "uncontrolled" or remains "uncontrolled" despite this therapy. Uncontrolled disease is determined by one or more of the following: poor symptom control, frequent severe exacerbations requiring high dose corticosteroid therapy or resulting in hospital admissions, and/or persistent airflow limitation.

Prior to confirming a diagnosis of severe asthma, it is critical to confirm adherence to therapy and optimise treatment of co-morbidities. Whether treatment of co-morbidities modulates asthma severity directly or indirectly, through improving asthma control, remains controversial. Intriguingly, recent evidence has suggested that in obese asthmatics with severe disease there is increased eosinophilic infiltration of the airway

wall, perhaps suggesting in this example a direct effect upon the underlying pathogenesis (7).

Less controversial is the increasing recognition that asthma, particularly severe asthma, is a complex heterogeneous condition encompassing several underlying pathologies that develop as a consequence of a variety of gene-environment interactions that give rise to a clinical phenotype.

1.1.3 Pathogenesis of severe asthma

1.1.3.1 Spatial Scales

1.1.3.1.1 Genes to cells: functional 'omics'

Heterogeneity within asthma is most obviously seen at the genetic level. A number of genes have been implicated in modulating the response of epithelial repair in response to damage in genome wide association studies (8,9). Single nucleotide polymorphisms (SNPs) have been associated with airflow obstruction and lung function impairment (10) and SNPs in the IL-4 receptor are associated with persistent airway inflammation and severe asthma exacerbations (11).

1.1.3.1.2 Cell to tissue: airway inflammation

At the cellular level the numerous permeations of heterogeneity are restricted somewhat as there are a limited number of cells for the genetic/protein differences to exert an influence upon. Nonetheless there are still many permeations which can occur. For example, persistent airway inflammation, despite full treatment is one of the hallmarks of severe asthma. However, critically, there is no clear pathological definition of severe

asthma (12). In allergic asthma airway inflammation is orchestrated by dendritic cell-Th2 cell interactions mediated by epithelial derived-thymic stromal lymphopoietin leading to mast cell activation and eosinophil recruitment. Evidence is emerging, particularly in severe asthma, that Th1/Tc1 and Th17 pathways with activation of neutrophils may play a role (13). Importantly, although these inflammatory profiles may co-exist to varying degrees within an individual, they do not necessarily occur independently. Cellular interactions considered to play important roles in airway inflammation and remodelling are summarised in figure 1.1. It is unclear if these inflammatory profiles are a consequence of environmental exposure to pollutants, smoking and infection, or primary abnormalities. Indeed, persistent bacterial colonisation, which is traditionally associated with chronic obstructive pulmonary disease (COPD), is also evident in some subjects with asthma (14). Fungal colonisation and sensitisation is also observed in severe disease (15).

1.1.3.1.3 Cell to tissue: airway remodelling

A consequence of this inflammation is epithelial damage and ciliary dysfunction (16). Impaired ciliary function, goblet cell hyperplasia and mucus gland enlargement all lead to increased mucus production, which is likely to perpetuate exacerbations and lead to further epithelial damage. Activated epithelium releases various growth factors including transforming growth factor-*beta* (TGF-β) and pro-angiogenic factors e.g. vascular endothelial growth factor (VEGF). In concert with pro-inflammatory cells TGF-β activates sub-epithelial mesenchymal cells to release matrix and proliferate (17). Fibrocytes, which are blood-borne mesenchymal progenitors, are recruited to the airway in response to the 'chronic wound', and differentiation of these cells together with local proliferation of resident mesenchymal stem cells promotes an increase in airway

smooth muscle (ASM) mass (18). ASM mass is the strongest predictor of airflow obstruction. Once activated, ASM in asthma recruits mast cells by releasing chemotactic factors. Mast cells interact with the ASM to promote airway hyperresponsiveness (AHR) (19,20), whilst mast cells and neutrophils localize to glands and are associated with increased mucus plugging (21). In addition to the pathogenesis of persistent disease, recurrent exacerbations are an important component of severe disease and are often associated with pathogens, suggesting abnormalities in innate/adaptive immunity. In asthma the secretion of *beta* and *lambda* interferons from the airway epithelium is impaired in response to rhinovirus (22). This leads to decreased viral clearance and is associated with worsening symptoms at exacerbation.

1.1.3.1.4 Tissue to organ: The roles of large and small airways

Both large and small airway disease leads to airflow obstruction and airway hyperresponsiveness (AHR), in asthma. Large airways account for the majority of airflow resistance behaving effectively like resistors in series. Small airways provide parallel resistance pathways and contribute <10% of total airway resistance. It was only from the 1960s onwards that the role of small airways was investigated and appreciated, prior to this it was thought to be mainly due to large airway disease. A review by Kraft (23) nicely outlines the timeline of the then emerging physiological and pathological evidence which showed the vital role the small airways plays in asthma.

Traditionally, histopathology has been used to study the structural and inflammatory changes seen in the large and small airways in asthma. This requires ex vivo tissue samples to be obtained. The most commonly used techniques are sputum samples, biopsies and bronchoalveolar lavages taken during bronchoscopy. Direct examination of small airways is only really feasible if transbronchial biopsies are obtained or if

resected lung tissue is available. Direct histopathological examination of small airways in asthma is therefore difficult.

1.1.3.1.5 Tissue to organ: The role of Quantitative Computed Tomography (QCT)

As discussed above, there is thought to be great variation between asthmatics in their inflammatory and remodelling responses, these abnormal responses often lead to structural changes. However, irrespective of the exact histopathological cause behind the altered structure, abnormal structure leads to generalised symptoms at the clinical level; such as breathlessness, wheeze, cough. It is at this point it is possible to appreciate how a disease like asthma, despite the histopathological heterogeneity, can be hard to phenotype at a clinical level. As yet there is not an accepted single "best tool" which probes the lungs at organ level, examining abnormal structure. This is where QCT would fit in.

Quantitative Computed Tomography,(QCT) is able to examine lung structure, proximal and distal, in in vivo, and when done responsibly, repeatedly. It is therefore uniquely placed to run alongside the more traditional methods to probe the lungs at the tissue/organ level.

QCT, its background and parameters is discussed in Introduction section 1.4.

1.1.3.2 Temporal scales of severe asthma

Interactions across the spatial scales occur over different timescales. Airway inflammation is diverse, but results in a common pathway of airway wall remodelling, alterations in geometry and biomechanical properties, airway obstruction with mucus plugging and small airway closure. Together these processes result in impaired airflow

and gas exchange, increased susceptibility to exacerbations and “pruning” of airways as seen on CT. Traditionally, these events are considered to occur sequentially over years, but this is inconsistent with some observations related to severe asthma. For example, whether severe asthma represents a distinct disease entity or part of the asthma spectrum remains controversial. The basis of this controversy is largely our lack of understanding of whether severe asthma develops over time in sufferers with initially mild disease or whether severe disease presents *de novo*. The natural history of the disease is poorly understood and severe disease can occur very early in life 'early onset' or later in life 'late onset'. Both hospital admission and need for intensive care can be the first presentation of asthma without any apparent history of mild disease. Remodelling might occur largely in parallel with inflammation or the development of remodelling might occur over shorter timescales than previously considered. To fully understand the dynamics of the interactions between the spatial scales described above we need to focus future attention to the natural history of disease. In order to do this a repeatable and reliable tool is needed. QCT, when done responsibly and with careful monitoring can be used repeatedly over time. Another way to probe natural history is by observing response to therapy particularly if emerging therapies are able to modify disease.

1.1.4 Treating severe asthma

ICS and long-acting bronchodilators have remained the mainstay of therapy in asthma for the past 20 years despite an increase in the understanding of asthma pathogenesis. Current therapies and treatments in late phase development predominately target specific severe asthma domains. The greatest focus has been upon Th2-mediated

eosinophilic airway inflammation and ASM dysfunction. However, new targets are emerging as it has become apparent that there is a complex role for inflammation beyond Th2. Critically, in addition to persistent symptoms and exacerbations, severe asthma is also characterised by progressive decline in lung function and development of persistent airflow obstruction, as a consequence of remodelling. To date this is largely refractory to current therapy. Therefore, targeting airway remodelling remains a major challenge of severe asthma. These domains and the role of current and future therapies approaching the clinic in targeting these domains is summarised in Figure 1.2.

1.1.4.1 Th2-directed therapies –current therapies

1.1.4.1.1 Corticosteroids

ICS are well studied and convincingly demonstrate a reduction in exacerbation frequency across the spectrum of severity. However, studies consistently show that the major benefit occurs in patients with eosinophilic airway inflammation (26). A meta-analysis of randomised controlled trials looking at titrating corticosteroid dose according to sputum eosinophilia concluded that sputum-based strategies were effective in reducing exacerbations in adults with asthma without a net increase in mean inhaled corticosteroid dose (27).

1.1.4.1.2 Anti-leukotriene drugs

Anti-leukotriene drugs are an adjunctive in the management of chronic asthma. They are primarily used in patients who are not controlled on ICS. Evidence suggests that anti-leukotriene drugs may be particularly effective in exercise-induced bronchoconstriction and aspirin-intolerant asthma (28).

1.1.4.1.3 Anti-IgE

Omalizumab is a humanised anti-IgE monoclonal antibody. A systematic review of placebo-controlled trials of omalizumab in moderate or severe allergic asthma showed that it reduced exacerbation frequency and facilitated corticosteroid withdrawal (29). Currently omalizumab is only used in severe uncontrolled allergic asthmatics that have raised IgE levels and a positive skin prick test to a perennial allergen.

1.1.4.2 Th2-directed therapies –future therapies

1.1.4.2.1 Chemoattractant Receptor-homologous molecule expressed on Th2 cells (CRTh2) antagonism

Prostaglandin-D2 (PGD2) is primarily released by IgE-activated mast cells. PDG2 recruits Th2 helper cells and recruits and activates eosinophils through its action on CRTH2, (also known as Prostaglandin D2 receptor DP2), G-protein-coupled receptor expressed on these cells (30,31). DP2 has also been identified on airway epithelial cells (32), and epithelial cells are known to be involved with airway remodelling (33), it is therefore possible, that a drug targeting DP2 receptor may also influence remodelling. A 12 week study showed reduced eosinophilic inflammation in bronchial biopsy and sputum as well as improvements in lung function and health status (study 3.3).

1.1.4.2.2 Anti-IL5, Anti-IL4 and Anti-IL13

Interleukin-5 (IL-5) is vital for eosinophil survival, maturation and activation.

Mepolizumab, an IL-5 monoclonal antibody, has been shown to significantly reduce exacerbation frequency, improve Asthma Quality of Life Questionnaire (AQLQ) scores and allow oral prednisolone dose reduction (34,35), in subjects with refractory

eosinophilic asthma despite high dose corticosteroids. Following cessation of therapy benefits were lost within 3 months (34). Reslizumab, another IL-5 monoclonal antibody, has demonstrated encouraging results when used by eosinophilic asthmatics with uncontrolled asthma despite medium-high dose ICS. It has been found to significantly reduce exacerbation frequency, increase time to first exacerbation and there were statistically significantly improvements in FEV_1, Asthma questionnaires and blood eosinophil levels (36).

Benralizumab, targets the IL-5 receptor, is effective in reducing blood, sputum and tissue eosinophilic inflammation (34,37). It has also been shown to reduce asthma exacerbations in higher doses (38) and in the acute setting, improved rate of recovery when given at the onset of an exacerbation (39). Results from further phase III studies are awaited (40).

Tralokinumab, a humanized IL-13 antibody improved lung function in a trial of 219 poorly controlled asthmatics (41,42). The effects were more pronounced in patients who had high serum periostin, an extracellular protein produced by epithelial cells in response to IL-13 activation. Phase IIb study subgroup analysis, showed a trend of exacerbation reduction in asthmatics not on oral corticosteroids. Phase III trials evaluating the targeted treatment of this sub group with Tralokinumab are currently on going (40). Lebrikizumab, also an IL-13 monoclonal antibody currently being evaluated at phase III stage (40). It too appears to have the greatest effect when targeted to asthmatics with high periostin levels and blood eosinophilia (43).

Dupilumab is an IL-4 receptor (α–subunit) antibody, with the ability to block both the IL-4 and IL-13 pathways is also at phase III trial stage (40). Phase II studies suggested that Dupliumab reduced asthma exacerbations when compared to placebo (44).

1.1.4.3 Airway Smooth Muscle Dysfunction – current therapies

1.1.4.3.1 Long-acting Beta-2 adrenergic agonists (LABAs)

The addition of LABAs to ICS improves symptoms, lung function and reduces exacerbations. Evidence suggests that clinical response to LABAs may be affected by polymorphisms in the β_2-adrenoceptor gene. Most commonly seen polymorphisms are at codons 16 (Arg16Gly) and 27 (Gln27Glu) (45). Patients who are Arg/Arg homozygous at codon 16 may be at increased risk of exacerbations, particularly when treated with LABAs.

1.1.4.3.2 Methylxanthines

Methylxanthines exert both bronchodilator and anti-inflammatory effects on the airways and improve AHR and lung function. It is thought to inhibit phosphodiesterase type VI isoenzyme, which has been shown to relax human ASM and also to have a direct anti-inflammatory effect (46). Methylxanthines also increase corticosteroid responsiveness (47). However, the use of methylxanthines in asthma has always been limited by their significant adverse event profile and a narrow therapeutic index.

1.1.4.4 Airway Smooth Muscle Dysfunction – future therapies

1.1.4.4.1 Long-acting anticholinergic agents (LAMAs)

There is growing evidence that LAMAs may have a role in achieving control in cases of refractory asthma. Peters *et al*. looked at 210 asthmatics requiring ICS and showed that the addition of tiotropium bromide, a LAMA widely used in the treatment of COPD, to asthma treatment was superior to doubling ICS dose, and non-inferior to adding salmeterol, a LABA commonly used in asthma, when measuring morning peak flows (48). It has also been found to increase in FEV_1 which was positively correlated to the proportion of neutrophils in induced sputum (49), and reduce exacerbations (50).

1.1.4.5 Airway remodelling – current Therapies

1.1.4.5.1 Mechanotransduction and breathing exercises

Mechanotransduction refers to the effects mechanical forces have on cellular function. In asthma this particularly refers to the mechanical distortion of airway muscosa during bronchoconstriction. Grainge *et al*. found that irrespective of the stimulus of bronchoconstriction, airway remodelling was evident in mild atopic asthmatics, and it was independent of eosinophil recruitment (51). Another study, which focused more on clinic measures, found that attending personal breathing training improved AQLQ scores more than attending non personalised asthma teaching sessions (52).

1.1.4.5.2 Bronchial Thermoplasty

The therapeutic use of radiofrequency is well established in cardiology for treating arrhythmias. Bronchial thermoplasty (BT), a novel technique that uses radiofrequency to heat the airways, is the only FDA-approved asthma therapy that directly targets

airway remodelling. BT is directed to the proximal conducting airways and aims to reduce the airway smooth muscle mass as demonstrated in earlier dos studies (53). The first human trial was in 9 cancer patients who had BT applied to lung segments that were due for resection. This showed a 50% reduction in airway smooth muscle mass (54). The AIR trial showed improvements, following BT, in asthma control and AQLQ scores in mild-to-moderate asthmatics, but not lung function (55). Similar benefits were confirmed in patients with severe asthma (56). The AIR2 trial studied 288 asthmatics and showed beneficial effects in the BT group when compared to the sham treatment, including health status and reduced exacerbations (57). Initial follow up studies suggest that the improvement in symptoms seem to last at least 5 years. All studies have shown a small increase in short term adverse events in patients undergoing bronchial thermoplasty, including higher rates of pneumonia, hospitalisation and lobar collapse. Therefore predictors of benefit and risk are required.

1.2 Chronic Obstructive Airways Disease (COPD)

Emphysema was described as "voluminous lung" by Bonet in 1679. Other descriptions of emphysema in the lungs came from Morgagni in 1769 and Baille in 1789 (58). It was not until 1814 that Badham identified chronic bronchitis, but he did not identify the connection to emphysema. It was Laennec, the inventor of the stethoscope, a pathologist and clinician who first described emphysema and chronic bronchitis together (58) in 1837. Although the spirometer was invented in 1846, it was not until the mid-1900s that the concept of FEV_1 and FVC were introduced, and only in 1962 that the foundations for our current definitions of COPD were laid.

WHO, has defined COPD (59):

*"*Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease with some significant extrapulmonary effects that may contribute to the severity in individual patients. Its pulmonary component is characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases"

1.2.1 Disease burden of COPD

Globally, the leading causes of death are ischaemic heart disease, cerebrovascular disease, lower respiratory tract infections and COPD, (currently 4th). It is projected to overtake lower respiratory tract infections and become third by 2030 (60). In the UK, in 2004, over 27,000 people died of COPD (61). It was also the leading respiratory related reason for emergency hospital admission and accounted for over 20% of all respiratory

related hospital bed days (61), with an estimated healthcare cost of >£800 million a year.

1.2.2 COPD exacerbations

Exacerbations are associated with increased severity of disease (62), poorer quality of life and increased inflammation, both systemic and in the airways (63). An exacerbation is defined as an event that results in hospitalisation or the prescription of a course of antibiotics or corticosteroids (64). There are a subgroup of COPD sufferers who are considered frequent exacerbators, >2 exacerbations per year. Prolonged exacerbations in COPD patients are also associated with faster lung function decline (63).

1.2.3 Phenotyping COPD

COPD is thought to be mainly driven by two processes in particular, small airway remodelling/obstruction and loss of lung elastic recoil leading to emphysema (65,66). Emphysema was defined in the 1960s as "a condition of the lung characterized by abnormal, permanent enlargement of the air spaces distal to the terminal bronchiole, accompanied by destruction of their walls" (67).

Increased susceptibility to developing COPD is thought to be due to genetic factors, but also the degree of oxidative stress the patient experiences (68). Imbalance between oxidants and antioxidants not only increases the level of inflammation, but it disrupts the balance between cell death and replenishment. This is thought to contribute to the development of emphysema (68). Figure 1.3 outlines COPD pathogenesis.

1.2.3.1 Cells to Tissue

1.2.3.1.1 Cigarette smoke

Cigarette smoking accounts for more than 90 per cent of COPD cases in developed countries (69). Smoking also appears to be a driving force behind the accelerated lung function decline seen in COPD patients. Smoking cessation usually results in a return to normal, or near normal lung function decline, whereas intermittent stopping confers less benefit (70). However the inflammatory process may stay present for years after smoking cessation in those with COPD (71).

All smokers have an inflammatory process occurring in the lung (72-79), but not all will develop COPD. It is not yet possible to predict who will develop an amplified response and who will never suffer from any symptoms (80). One hypothesis is that those who develop COPD have a shift away from innate immunity response to an adaptive autoimmune response (80,81). Exposure to cigarette smoke will activate the innate immune system directly as well as indirectly through causing damage to the epithelial cells (68).

As well as inducing chronic inflammation in the lung, chronic cigarette smoke causes damage to epithelial cells and their tight junctions; this breaks the natural protective physical barrier between the lung and the outside world, thereby promoting acute inflammation (82-84). In addition, chronic cigarette exposure is known to supress the mucocillary clearance, allowing bacterial pathogens are able to establish a foothold (85). And cigarette smoke is thought to increase the expression of NK receptors on epithelial cells, leading to NK cells attacking epithelial cells and perpetuating lung damage (86). In mice, the chronic activation of this system leads to emphysema (87).

1.2.3.1.2 Neutrophils and eosinophils

Neutrophils are strongly associated with COPD and are found with increased frequency in BAL, ASM and sputum (88). Increased levels of neutrophils in sputum are associated with air flow obstruction (89). However levels in airways and lung parenchyma are not increased (90). Neutrophils are thought to perpetuate the damage in the airways and alveoli by increasing the release of, and directly secreting, proteolytic enzymes, proteinases and oxidants.

The role of eosinophils in COPD is not well known. However increased numbers have been seen in COPD sputum samples as well increased levels of IL-5 (91). It has been thought they might represent a subgroup of COPD as, like in asthma, those with an eosinophilia tend to have a better response to corticosteroids (89).

1.2.3.1.3 Bacterial infections

The role of bacterial induced inflammation in the airways in stable COPD is unclear, but, infections are a cause of exacerbations. When stable, 25% of COPD patients will be colonised by bacteria, most commonly H.Influenzae, S.Pneumonia, Moraxella catarrhalis, however this doubles to 50% during exacerbations (92). Even in stable COPD, H.Influenzae has been associated with neutrophilic airway inflammation and poorer clinical outcomes (93).

1.2.3.1.4 Viral Infections

Viruses are seen in the sputum of 10-15% of stable COPD sufferers, and again this goes up to 30-60% in exacerbations (94). Most commonly seen are rhinoviruses, influenza and respiratory syncytial virus (95). One study inoculated a small number of COPD and healthy smokers with experimental rhinovirus. COPD subjects recorded more severe

symptoms, worsening lung function, and longer duration of symptoms than healthy smokers. In addition sputum viral load, sputum neutrophils and neutrophil elastase were all higher in COPD subjects than the control group (94). Inoculation with rhinovirus has also been associated with an increased bacterial load, H. Influenzae in particular (62).

1.2.3.1.5 Adaptive immune response

Adaptive immune response can be triggered by the DNA released by apoptotic cells, including the body's own cells and DNA, a potential trigger for an autoimmune reaction. The main players in COPD's adaptive immune response are CD8+ T cells, CD4+ T cells, T helper 1 and Th helper 17 (90).

The innate and adaptive immune systems can enhance the other's reactions to produce excessive inflammation (81). It has been suggested that if, at an early stage, the innate response does not dampen down, it may over stimulate the adaptive response leading to lung damage and the development of COPD (81).

COPD sufferers have reduced levels of T-regulatory cells. They supress inflammation and inhibit autoimmunity by producing anti-inflammatory cytokines like IL-10 and TGF-β as well as supressed DCs (90).

1.2.3.2 Tissue – Organ

With advances in medical imaging, especially CT, it has become increasingly important in the diagnosis and management of COPD. As well as providing insight into the heterogeneity of the disease, clinical CT scans are able to demonstrate co-morbidities such as interstitial lung disease and bronchiectasis, which may impact upon the management of COPD (96), and emphysema is now essentially a radiological diagnosis..

The extent to which multiple different pathologies may be seen on CT, in COPD, was highlighted by a study by Bafadhel et al. They looked at 75 subjects with a diagnosis of COPD whose CT scans underwent semi quantitative analysis by trained respiratory radiologists. They found that emphysema, bronchiectasis and bronchial wall thickening was seen in 67%, 27% and 27% of scans respectively, with almost 20% of subjects having a combination of findings (96).

1.2.3.2.1 Emphysema

The lung is split into units, divided by septa. These represent areas of the lung supplied by a single terminal bronchiole; this is made up of several acini. Centrilobular emphysema is due to the destruction of these terminal bronchioles and is the most common form of emphysema seen in smokers and predominates in the upper lobes. Panacinar emphysema is seen more commonly in those with α-1 anti-trypsin deficiency and in the lower lobes. Paraseptal emphysema occurs when the damage is near the septa of the lung units (97).

It has been suggested that the distribution of emphysema influences lung function, as those who have predominantly basal emphysema often have a larger reduction in FEV_1, but less impairment of gas exchange (98). An increase in emphysematous changes as seen on CT have also been associated with worsening spirometry (99-102), gas exchange (99), an increase in exacerbations (102), quality of life scores and worse BODE index, (Body-mass index, airflow Obstruction, Dyspnoea and Exercise. The BODE index uses these parameters to form a scoring system in COPD to try to predict long term outcomes) (103).

1.2.3.2.2 Airway remodelling

Changes seen in the airways with COPD include smooth muscle hypertrophy, airway fibrosis, inflammatory cell infiltration, mucus hypersecretion and disruption to the epithelial layer (104).

Direct analysis by QCT can be made of the larger airways, to about the 6^{th} or 7^{th} generation (105). Studies have found that increase airway wall thickness has been associated with worsening FEV_1 (100), patients with worse chronic bronchitis symptoms have higher percentage wall areas (106) and increased airway thickness is associated with COPD exacerbations (102,107).

Terminal and respiratory bronchioles are beyond the resolution of CT scanners, and can only be assessed indirectly. However it is thought to be in these small airways where the earliest signs of COPD can be found, proceeding even emphysema (108,109). One hypothesis for this is that the size of toxic particles is such that they reach, and remain in, the transitional region of the airways (110) and are then able to stimulate an inflammatory response.

Measurements of air trapping are the most commonly used indirect measure of small airways on CT scans.

1.2.3.2.3 The role of QCT

When assessing the impact of inflammation and remodelling in COPD has on the lung, QCT is uniquely placed to provide a global overview of the structural changes at the organ level. As with asthma, QCT can run alongside and compliment histopathological examination and clinical assessment.

1.3 Asthma and COPD; the clinical picture

Asthma and COPD, although different in many ways have a lot of clinical similarities (111). In 1961, at the first Bronchitis Symposium, it was debated if asthma and COPD were different disease entities or different expressions of the same disease (112). Now it is generally accepted that they are different disease entities, with similarities, however there is no hard evidence for this and debate still remains around this issue (112,113).

1.3.1 Comparing symptoms

Clinically, it can be difficult to distinguish between COPD and asthma. Clinical differences are most apparent when comparing young non-smoking asthmatics to older (>60years), COPD patients with an extensive smoking history (114-116). However it is within the elderly population that it is hardest to distinguish between asthma and COPD, with the incidence of both diseases increasing with age (117). A study done in the UK showed that in males aged 40-49 COPD was present in 4.9%, where the incidence for males aged 60-69 was 12.3%. The incidence of asthma also increased with age; 4% of males aged 40-49 being affected compared to 9.6% in the 60-69 age group (117).

Traditionally symptoms are used to distinguish between the diseases. Table 1.1 highlights the most common clinical differences between COPD and asthma. However there can be a lot of cross over, and typical symptoms such as wheeze, cough, dyspnoea can also be due to non-respiratory pathology, such as heart disease, reflux. Nonetheless NICE still recommends using symptoms as the primary method to suspect and diagnose COPD with spirometry (pre and post bronchodilator), to confirm it (118). History of

exposure to a possible aetiological agent, such as cigarette smoke, is also taken into account when diagnosing COPD. Table 1.2 outlines the NICE guidelines for diagnosing COPD.

Asthma and COPD are thought to share risk factors. It has been suggested that asthma itself increases the risk of developing COPD (119).

Other risk factors include increased age, tobacco smoke exposure, bronchial hyper-responsiveness and lower respiratory tract infections (119). COPD is strongly associated with smoking whereas asthma is not. The majority of COPD sufferers are current or ex-smokers, however only about 20% of smokers develop COPD (120).

1.3.2 Comparing physiology

Physiologically it can also be difficult to tell the diseases apart. As per the definitions in sections 1.1 and 1.2, airflow obstruction in asthma is "often" reversible, whereas COPD is "characterised" by non-reversible airflow obstruction. Again allowing overlap between the two.

In 2010 NICE stated that reversibility is not necessary for diagnosing either disease, and may at times be misleading (118).

Chronic asthma can lead to a fixed airflow obstruction picture, thought in part, to be due to chronic bronchial hyper-responsiveness and remodelling (121). Vonk et al followed up 228 asthmatics after 21-33 years (aged 13-44 at baseline) and found that 16% of them had developed irreversible airflow obstruction. These patients also had

more COPD like symptoms, whereas those who retained reversibility still had asthma-like symptoms (122).

However there are some trends that do differentiate between COPD and asthma for the majority of sufferers. For example, on the whole, those with COPD experience a steadier decline in health. For most COPD sufferers, the rate of decline of FEV_1 exceeds that seen in healthy individuals. Decline of FEV1 in COPD subjects is highly variable (123), with published means of 50ml and 33ml, compared to ~20ml seen in healthy subjects (69,123,124). In asthma, the annual decline in lung function has been estimated to be around 38ml/yr, (124).

The other major difference between the diseases is that asthmatics rarely, if ever, get emphysema, whereas in COPD this is a major aspect of the disease, often linked to increased morbidity and mortality (125). McDonough et al (109) have shown that in COPD small airway destruction precedes emphysema onset. Although similar work has not yet been carried out in asthmatics, currently knowledge does suggest small airways are also affected in asthma, however emphysema does not ensue.

Both diseases feature airway remodelling, thought to be due to airway inflammation. However on the whole, mechanisms are different, as discussed above.

1.4 The discovery of x-rays, and origins of CT imaging

In the latter half of the 19th century the discoveries were made which formed the basic principles for developing medical imaging with radiation.
Rontgen is considered to be the founding father of radiology as he discovered X-rays. Within a few years of this Becquerel had discovered radioactivity and the Curies isolated various radioactive isotopes.

1.4.1 Wilhem Roentgen (27th March 1845 – 10th February 1923)

Philipp Lenard had adapted a Crookes tube in order to have a window to allow the cathode rays (electrons) out. These windows were covered with aluminium, thin enough to allow "cathode rays" through, but also strong enough to maintain the vacuum in the tube (126).

Roentgen, a German physicist, was working with the Lenard tubes confirming the findings of Lenard, that cathode rays could penetrate the aluminium. He was wrapping up the tube in tinfoil and cardboard to ensure no visible light was emanating from the tube, and placing a screen, (a piece of cardboard painted with barium platinocyanide), close to the window and observing the fluorescence.
He then repeated the experiment with the Hittorf-Crookes tube (no window present). He wrapped this tube up and turned off the lights. He again observed the fluorescence of the screen in front of the tube. As he was about to turn the lights back on, he noticed out of the corner of his eye a faint light further away. It became apparent that this

fluorescence was also due to the cathode ray tube as the light flickered in time with the discharges of the coil energising the tube.

This finding was unexpected as “cathode rays” were not known to travel those distances. Over the next few weeks he investigated this phenomenon in order to determine if this was simply unobserved properties of cathode rays or in fact an undiscovered ray. He interrupted the rays with objects that were known to block cathode rays. Whilst holding an object he noticed the outline of his finger bones could be seen. He also investigated the effect magnetism had on these new rays, as it was known that the direction of cathode rays would change in response to a magnetic field. The final step in establishing that these were new rays as opposed to unobserved effects of cathode rays, was to place a photographic plate in place of the screens (127).

On the 27th December 1895 he took the first radiograph; an exposure of his wife’s hand.
He then concluded that this was a new type of ray, a highly penetrating one. Due to the lack of knowledge about these new rays, he called them X-rays.

There are conflicting accounts of when he first made his discovery public knowledge (126,127), however his paper, called “On a New Kind of Rays” was definitely known to the rest of the world by 6th January 1896. The news of the discovery circulated fast and the importance recognised immediately. His work was easily reproducible and the scientific world was gripped by this discovery. This accumulated in Roentgen receiving the first Nobel prize for physics in 1901.

Both the Lancet and the British Medical Journal hailed the discovery as remarkable with far reaching consequences for medicine. The first x-rays taken in the UK were by Mr AA Campbell Swinton and Mr G Stanton in the second week of January.

1.4.2 CT scan development – Sir Godfrey Hounsfield and Allan Cormack

The invention of the CT scanner is attributed to Sir Godfrey Hounsfield (29th August 1919 – 12th August 2004), a British engineer in the 1960s, he first thought of the theoretical principles of CT scanning image reconstruction while working for EMI Ldt.
He was inspired by work Cormack had done in the early 1960s. He had started to think about the possibility of measuring X-rays through an object numerous times, "along parallel lines". Each line would then undergo mathematical analysis to establish the linear attenuation coefficient of each line (128). Despite the papers being published, they generated little interest until Hounsfield took it the next step and found a practical application for it.

Hounsfield's work was key in establishing the image reconstruction aspect of CT scanning. He proved his theory, that it was possible to create accurate images using X-rays, and by 1968 and began to do the initial tests.
His very first practical test was to use a gamma radiation source, Americium 95 to x-ray the object in 160 slices. Each slice was x-rayed through at every degree through 180^{o}. A photon counter acted as the detector. It took him 9 days to complete this scan and 2.5 hours to reconstruction the data on an ICL 1905 mainframe computer. Hounsfield and his team then moved onto to scanning animal body parts obtained from abattoirs. However, this tended to produce artefact as the specimens would often decay due to the long scanning time.

Hounsfield began to contact radiologists about his work, and in 1969 met Dr James Ambrose, who arranged for Hounsfield to scan a bottled brain specimen (129). This generated enough interest in the UK for the Department of Health to part fund the building of the first clinical prototype. This was installed in Akinson Morley's Hospital in South London, where in 1971 the first patient (with a frontal lobe tumour) was scanned.

Initially it was just heads that were scanned as the part of the body being scanned had to be surrounded by water to reduce the dynamic range of the X-rays and hence improve the attenuation. At this stage the images were being taken back to EMI to be processed, (often taking about 20 minutes) and were brought back the following day to the hospital (129).

By 1975 the first body scanner had been installed in Northwick Park Hospital. This scanner model, known as the CT5005 became the prototype for the commercial scanners, and by the following year 17 different companies were selling CT scanners.

1.4.3 CT scan development - Technology

Sir Godfrey's initial CT scans (1st generation scanners,Figure 1.2 A), were simple pencil beam radiation sources with a single detector (tube-detector assembly). The movement of the radiation source and detector in a linear manner across the subject was called translation.

After a translation is completed, the tube-detector assembly rotated by 1^{o} and repeated the translation process. In order to acquire the information for a single slice, the tube-detector assembly had to rotate through 180^{o}. These scans (only head scans at this stage), would typically take 5-6 minutes.

In second generation scanners (Figure 1.2 B) the introduction of multiple X-ray beams (form one source) and multiple detectors allowed scanning time to be reduced dramatically.

Third generation scanners (Figure 1.4 C) kept the theory of multiple detectors, but the main difference was the use of a fan beam. A fan beam took away the need for the translation movement of the CT scanner. In place of separate detectors picking up a single beam, an array of continuous detectors was placed. Third generation scanners are also known as "single slice scanners", and form the basis for the design of modern day multi-slice scanners.

The next big step came over a decade later in the late 80s when slip-ring technology removed the need for numerous wires in the gantry. With the slip ring speeding up rotation times, the next issue to address to improve scanning time was to remove the

time taken to move the table. Up until now, the scanners were scanning, stopping, moving the patient and then scanning again. ith the advent of the slip ring gantry, it was now possible to continuously move and scan the patient. This gave rise to the development of helical/spiral scanner. Finally it was possible to have continuous data collection.

The last big leap in the structure of CT scanners came with the development of Multi-Slice CT (MSCT) scanning (Figure 1.4. D) which is the basis for current scanners.

1.4.4 CT and image analysis

1.5.4.1 Development of Densitometry – emphysema markers

Densitometry is well established in quantifying emphysema. CT was shown to be a more accurate method of diagnosing and quantifying emphysema (130,131) in the 1980s. As well as finding it more accurate than pulmonary function tests, Bergin et al concluded that it also better distinguished moderate emphysema from healthy (130). All these studies compared CT and pathological assessment and diagnosis of emphysema.

1.4.4.1.1 Voxel Index and Density masks

Density masks and the cut off of -950 Hounsfield Units (HU) were first investigated in the 1980s, and was one of the first respiratory applications of QCT.

Hayhurst et al (132) studied eleven patients who had a diagnosis of bronchial carcinoma, who had had CT prior to lung resection. All patients had similar lung function, but six had mild centri-acinar emphysema.

They found that when those with emphysema had significantly more pixels with attenuation values of -900 to -1000 HU.

Muller et al (133) took the next step to further define CT values for emphysema. The General Electric scanner they were using had an inbuilt programme that highlighted voxels within a given density range, “density masks”. Twenty-eight patients who were undergoing lung resection for tumours were recruited. They compared density masks (-920, -910, -900 HU) on a representative CT slice to the corresponding pathological slice. All three had good correlation with the pathology score of emphysema, but -910 HU was the best. Kinsella et al later showed that increased emphysema (as defined by

amount of voxels under -910 HU), correlated well with various markers of decreased lung function (e.g. FEV/FVC per cent predicted) (134).

The current use of -950 HU as the cut off for emphysema is due to some work done by Gevenois et al (135). They looked at total of 63 patients who had been referred for thoracic surgery over the course of one year. Patients were undergoing lobectomy or pulmonectomy, however 4 were having transplants. Emyphsema was quantified pathologically, and then compared to density masks at various levels (-900 to -970 HU in increments of -10 HU). They found that the only level that produced the same quantification as the pathological method was -950 HU.

1.4.4.1.2 Lung volume and densitometry

More recently studies have begun to look into quantifying the effects of lung volume on density measures. In a study by Madani et al (136) 20 healthy subjects and 16 COPD patients were scanned. Three 1mm thick slices, (level of the aortic arch, 2cm below the carina, 3cm above the top of the diaphragm), were taken from each subject. Respiratory effort was measured by patients breathing into a mouth piece which was attached to a hand held spirometer. Vital Capacity (VC) was measured. Each anatomical level was scanned at 100%, 90%, 80%, 70% and 50% of VC.

They found that per cent below -950HU in inspiration, (-950%VI) was significantly different between the all levels of VC. Of interest, they did note that -950%VI for 70%VC-100%VC was relatively linear in both COPD and health. At 90% VC the under estimation of -950I% was 3% in the control group and 2% in the COPD group. Total Lung Capacity (TLC) is a highly reproducible measure. Taking these two factors into consideration, they concluded that in practice, spirometric gating is too time consuming

and technically awkward to be worth doing, if patients are actively encouraged to reach TLC when being scanned.

1.4.4.1.3 Percentile 15

The HU at which 15% of the voxels lie below, also known as Percentile 15 (Perc15), is another marker of emphysema that was developed after -950%VI, but is widely used in QCT COPD papers. Figure 1. 5 shows how both Perc15 and -950%VI are calculated.

There is no direct evidence suggesting one method is superior to the other; however there are suggestions that Perc15 is a more consistent measure, not threshold dependent and less dependent on inspiratory volume (137,138). Parr et al found good correlation between Perc15 and -950%VI, and in particular noted that it was a curvilinear relationship. This means that Perc15 is a more sensitive measure in early disease, whereas -950%I is more sensitive to small changes in severe disease (139,140). Perc15 has also been shown to be a reliable method to follow emphysema changes over time (139,141). An expert review also found Perc15 to be a more reliable measure than -950%VI (138).

Density measures have been, to a very large extent, been disregarded in asthma, and certainly have not undergone the same pathological validation. Therefore, we only have the measures provided in COPD to use in asthma. In our own cohort, we plotted asthmatic Perc15 against -950%VI, Figure 1.6. This showed the same pattern that Parr et al described, however it appears to be more linear. As asthmatics are generally considered not to have emphysema, therefore should have low levels of low density, it adds weight to the argument to use Perc15 when comparing asthma and COPD as Perc15 is better at distinguishing between low levels of low attenuation areas (139,142).

One study looked at 70 healthy young men's lung density measures and found that the upper limit of normal for -950%VI was 2.73% and -936 HU for Perc15 (143).

1.4.4.2 Development of Densitometry – Air trapping markers

Various methods have been developed to look at air trapping. Unlike emphysema, it is not possible to conduct pathological validation of air trapping markers as this is a physiological state. Air trapping refers to a dysfunction of the lung that leads to air not being fully expelled in expiration, therefore reducing the efficiency of the lung.

An early attempt at quantifying air trapping in asthmatics was done by Newman et al, who scanned part of the lower lobes in 18 asthmatics and 22 healthy controls. They found the asthmatics had a significantly lower mean pixel index than the healthy controls (144). This finding, assumed to be due to air trapping, correlated with worsening lung function.

However it was noted that just looking at the Mean Lung Density (MLD), or Mean Pixel Index, in expiration, did not fully remove the influence of emphysema.

In 1997, the ratio between Mean Lung Density in Expiration over Inspiration ($MLD_{E/I}$,) was reported by Eda et al (145). Another group found that $MLD_{E/I}$ reflected small airway dysfunction irrespective of levels of emphysema (146). Since the late 1990s, $MLD_{E/I}$ has been increasingly used and is now a well-established, and frequently quoted surrogate measure of air trapping/small airway disease.

Another method developed to try to remove the influence of emphysematous voxels on measures of small airway disease was Relative Volume Change. This is defined as "the difference between expiratory and inspiratory values for relative lung volumes, which is the limited lung volume with attenuation between -856 to -950HU divided by the lung volume without emphysema"(147). Matsuoka et al, one of the first to describe this

method found the best range to look at to assess small airway disease was -860HU to -950HU (148). However since then -856 HU and -850 HU have been established as the upper end of normal lung attenuation in expiration.

A study was done to look at which of the two methods, (-856%E and $MLD_{E/I}$), were the most robust. This study included 45 smokers who were in lung cancer screening programmes and had had 2 CT scans in a space of 3 months. They found that $MLD_{E/I}$ was more reliable (149). Hersh et al (147) however looked at 3 surrogates of small airway disease, -856%E, $MLD_{E/I}$ and Relative Volume Change. They found that both $MLD_{E/I}$ and Relative Volume Change were good markers of small airway disease and were unable to claim one was superior to the other, but noted that $MLD_{E/I}$ was a simpler calculation.

1.4.4.3 Development of airway analysis

Airway remodelling is an area of research in both asthma and COPD. Post mortem studies in asthmatics have shown that patients who die of asthma attacks have thickened airways. This is partly due to acute changes such as inflammatory cell infiltration and into the airways and oedema. But structural changes have also been noted, involving all layers, especially ASM (150,151). The latter is considered a product of chronic inflammation (152-154), so in theory should be possible to study in a non-acute setting.

Initially, geometry measures were mostly done based on subjective methods. In 1993 Lynch et al did a study looking at bronchial dilatation. Dilatation was defined as the airway lumen circumference greater than the outer circumference of the accompanying

artery (155). Boulet et al (156) added a little more objectivity to their study looking at airway wall thickness by measuring the smallest internal diameter of the right intermediary bronchus, and the adjacent airway wall, with electronic callipers. Another early method used to better quantify airway dimensions was manually tracing the lumen and outer perimeters (157-159). However this was still dependent on subjective analysis of the oblique-ness of the airway being measured and the manual tracing of areas. It was from these early days, when the orientation of the branch was important, that RB1 became the standard branch to measure as it was most often perpendicular to the slice of the scan. Wood et al (160) became one the first to computationally segment the airways and used the reconstruction to orientate the airway obliquely so that it was a true cross section that was being measured.

One of the first quantitative methods introduced was determining airway wall boundaries using the "Full Width Half Maximum" principle (159,161), Explained in Figure 1.7. However this method has been shown to systematically under estimate lumen area, overestimate total area and therefore over estimate wall thickness (161) in smaller airways.

Currently there are many methods for airway segmentation available, further discussion can be found the methods section 2.3.2.

1.5 Hypothesis

I hypothesise that quantitative computed tomography measures of airway wall remodelling, gas trapping and emphysema can be applied to provide important insights into the phenotypic heterogeneity of asthma and COPD; the association with lung function and immunohistology and its application in stratified medicine intervention studies.

Sub-hypotheses

1. I hypothesise that QCT morphometry and densitometry measures of proximal airway remodelling, air-trapping and emphysema are different between asthma, COPD and healthy subjects.
2. I hypothesise that in asthma and COPD, the association between lung function impairment (post-bronchodilator FEV_1 % predicted) and QCT morphometry and densitometry measures are distinct.
3. I hypothesis that airway remodelling, determined in bronchial biopsies, is associated with QCT morphometry and densitometry measures of proximal airway remodelling and air-trapping.
4. I hypothesis QCT can detect changes in the airway and lungs of patients who receive targeted treatment of eosinophilic asthma.

1.6 Aims

Relationship Between Lung Function and Quantitative Computed Tomography Parameters of Airway Remodelling, Air-trapping and Emphysema in Asthma and COPD: A Single Center Study.

Sub-hypotheses 1&2

Aim 1. To assess the differences of QCT derived measures of proximal (segmental) airways, between obstructive airway diseases (asthma and COPD), as well as comparing them to healthy airways.

Aim 2. To assess the differences of QCT derived measures of emphysema and air trapping, between obstructive airway diseases (asthma and COPD), as well as comparing them to healthy airways.

Aim 3. To demonstrate distinct QCT groups within, and between, asthma and COPD according to airflow limitation.

Aim 4. To identify links between clinical measures of disease and health status, and QCT parameters.

Associations in asthma between quantitative computed tomography and bronchial biopsy-derived airway remodelling

Sub-hypothesis 3

Aim 5. To demonstrate a link between biopsy assessment of airway remodelling and QCT changes.

Aim 6. To investigate and develop novel interpretations of densitometry findings in asthma.

Randomised controlled trial of the prostaglandin D2 receptor 2 antagonist fevipiprant in persistent eosinophilic asthma

Sub-hypothesis 4

Aim 7. To assess the effects of 12 weeks of therapy with Fevipiprant, a CRTH2 receptor antagonist, on sputum eosinophilia, spirometry and QCT assessed morphometry, densitometry and lung volumes.

1.7 Tables and Figures

Table 1.1 Clinical features differentiating COPD and asthma (118)

	COPD	**Asthma**
Smoker or ex-smoker	Nearly all	Possibly
Symptoms under age 35	Rare	Often
Chronic productive cough	Common	Uncommon
Breathlessness	Persistent and progressive	Variable
Night waking with breathlessness and/or wheeze	Uncommon	Common
Significant diurnal or day to day variability	Uncommon	Common

Table 1.2 Guidelines to diagnosis COPD based on clinical history/symptoms (118)

History (≥2 features)	Risk Factors	Uncommon features
>35 years	Weight loss	Chest pain
Smoking history	Effort intolerance	Haemoptysis
Dyspnoea*	Waking at night	
Chronic cough	Occupational hazards	
Regular sputum production	Fatigue	
Frequent winter bronchitis		
Wheeze		

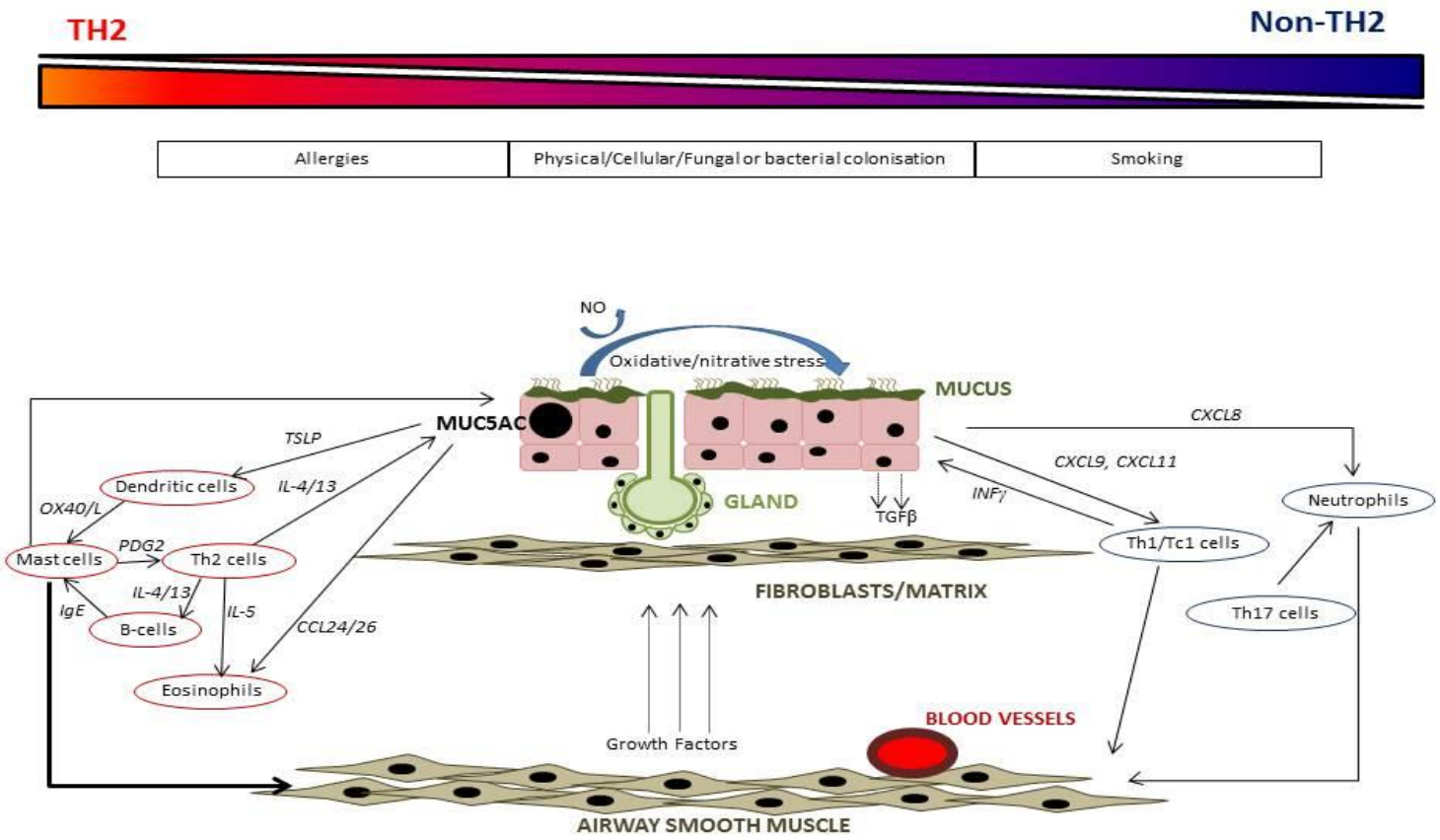


Figure 1.1

Schematic diagram of the pathogenesis of asthma illustrating the role of Th2 and non-Th2 pathways driving interactions between inflammatory cells and the structural components of the airway and the consequent development of airway remodelling

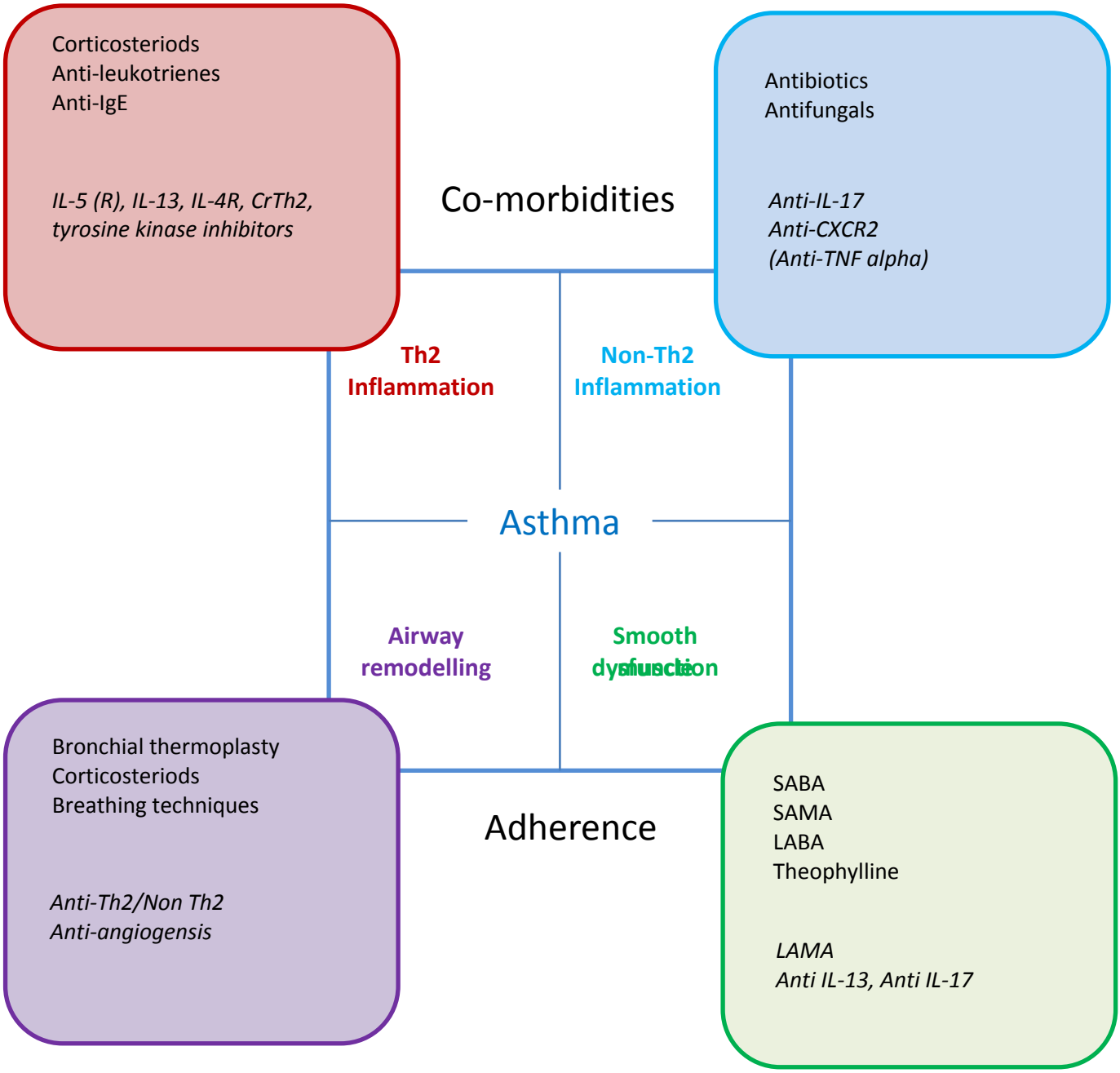


Figure 1.2 Asthma pathological domains, current and future (italicised) treatments

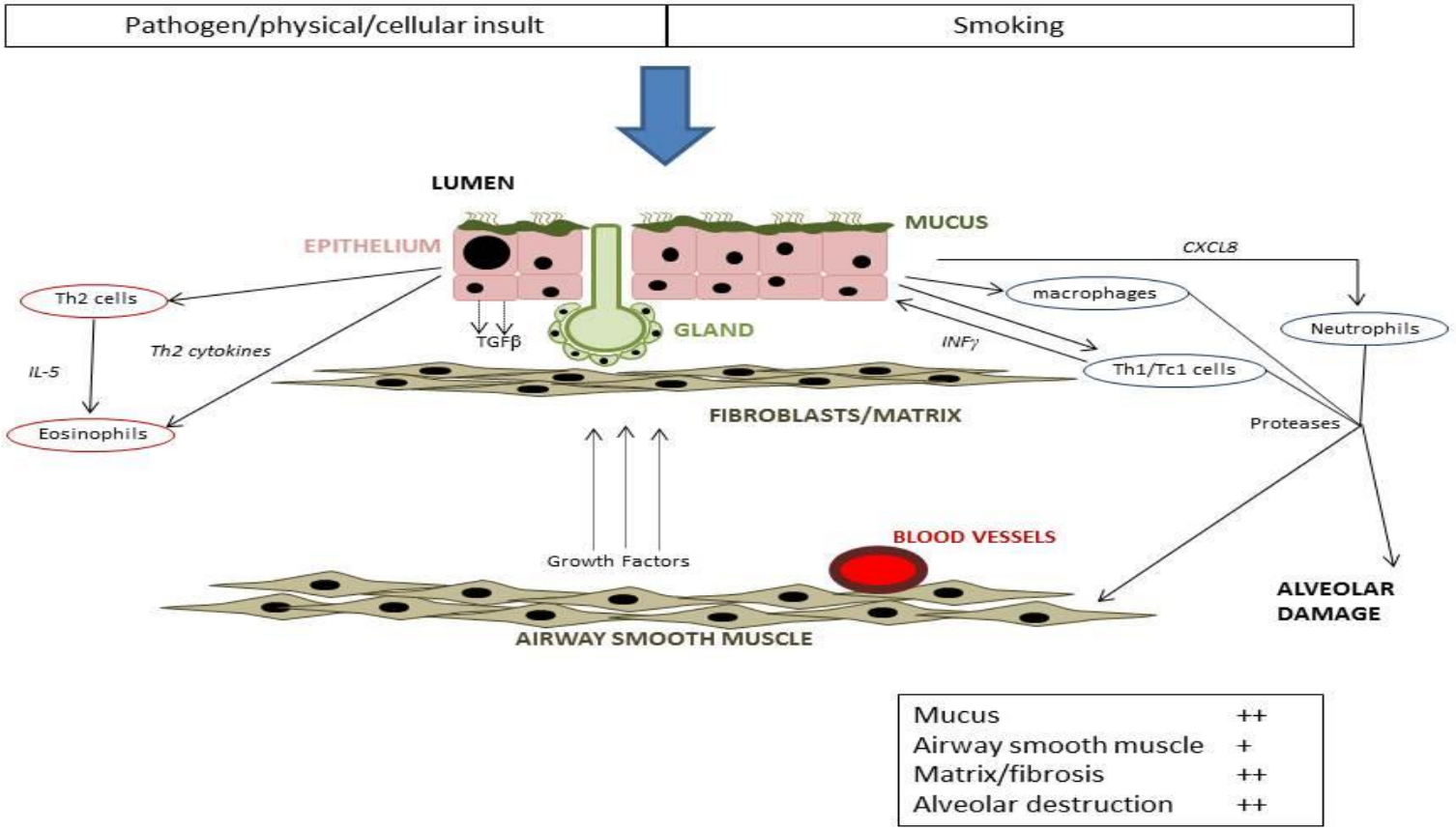


Figure 1.3 Schematic diagram of the pathogenesis of COPD

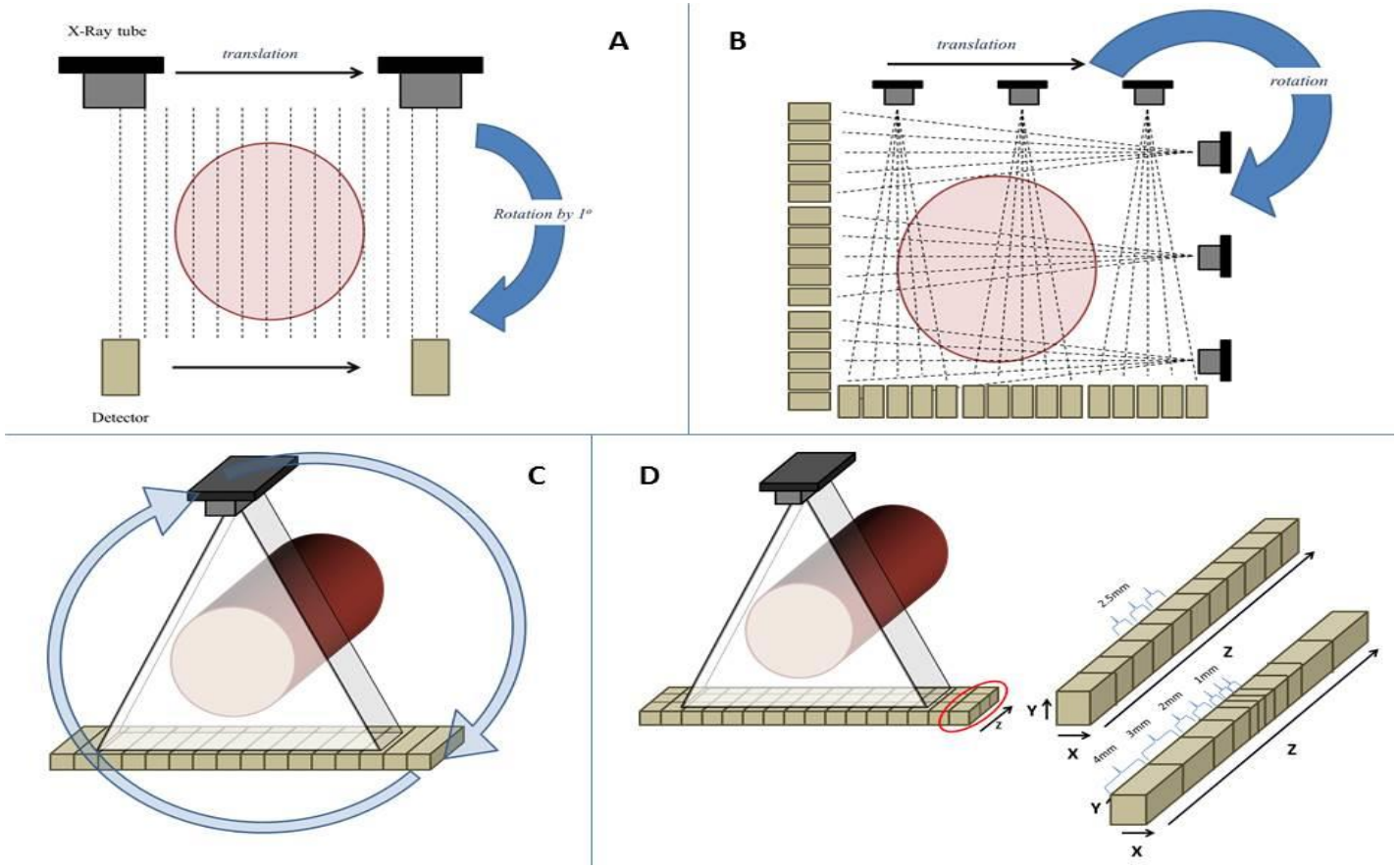


Figure 1.4 CT scanner evolution

A – first generation scanner, B – second generation scanner, C – third generation scanner, D – multi slice CT scanner

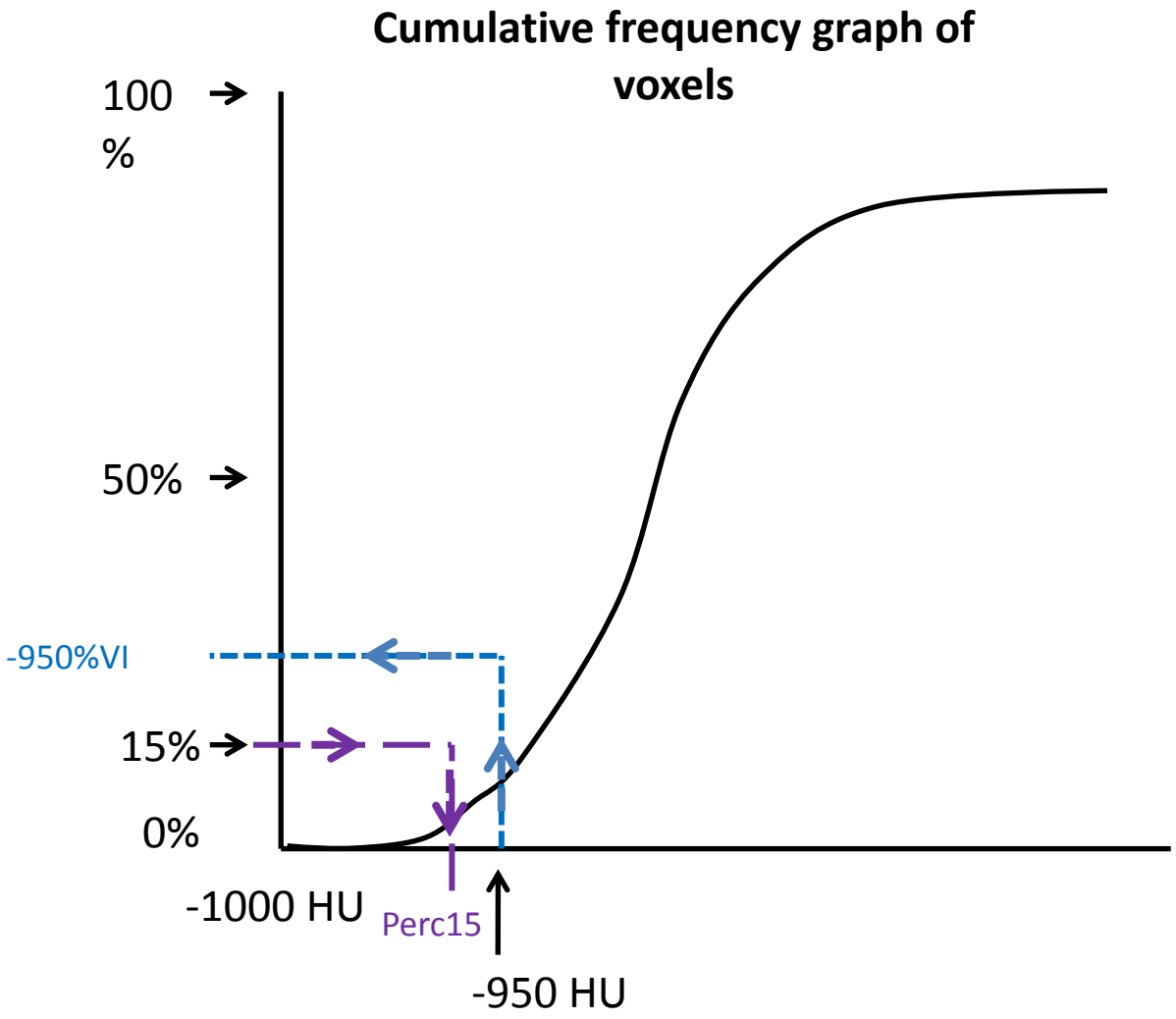


Figure 1.5 Graphical representation of the method of calculating percentile 15 and %VI-950.

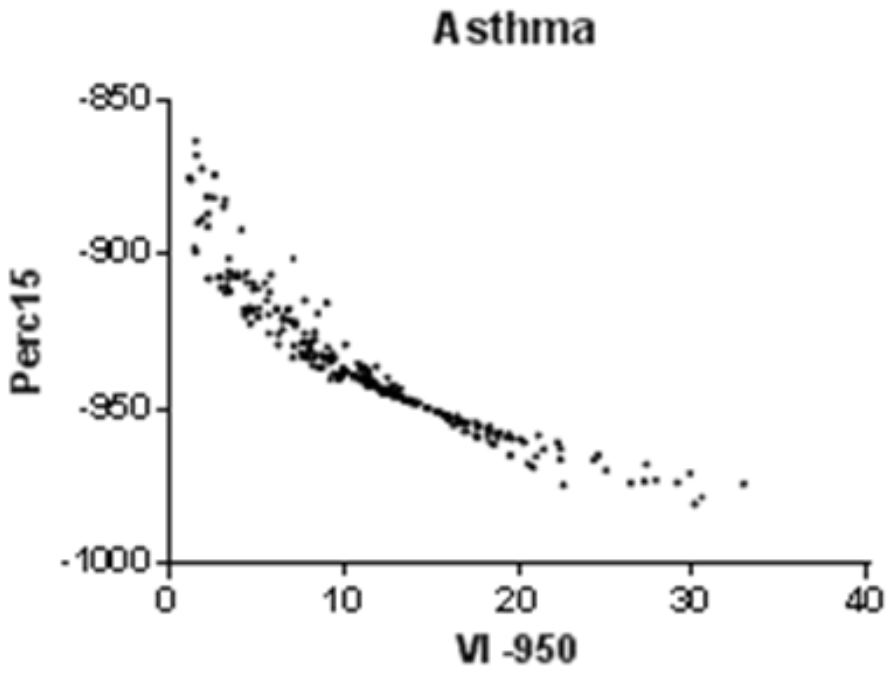


Figure 1.6 Plot of VI -950 against Percentile 15 in asthmatics.

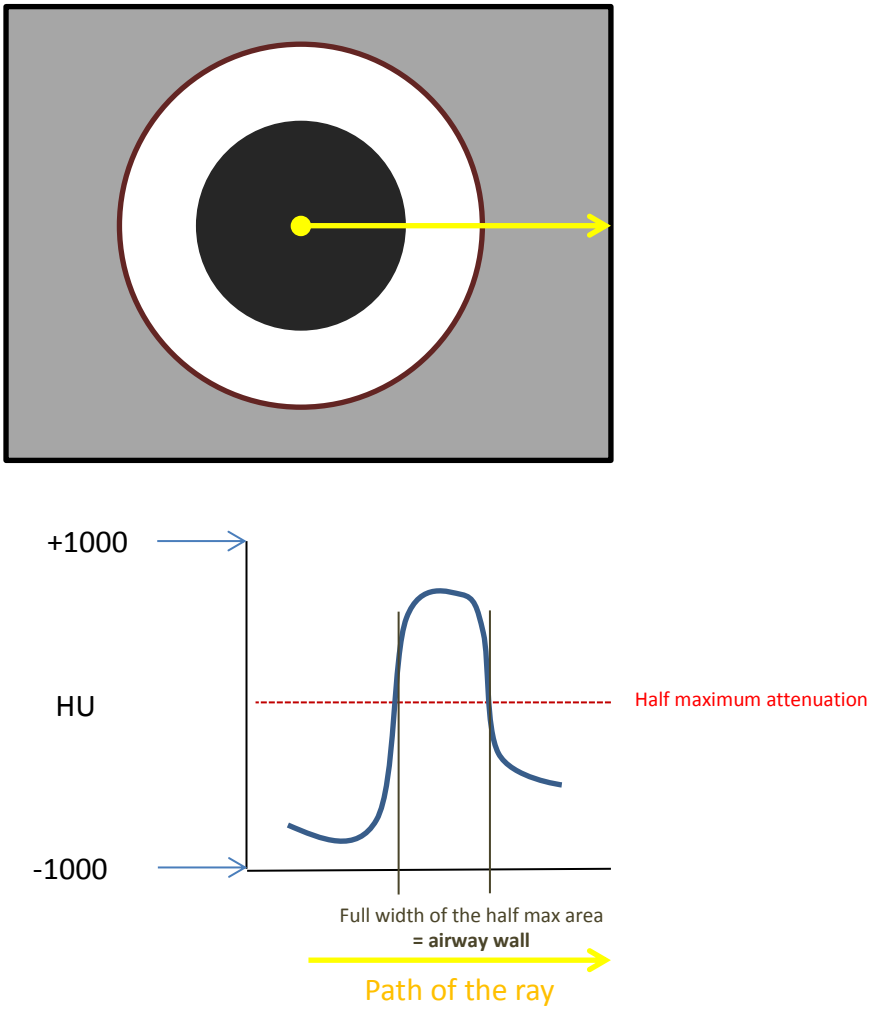


Figure 1.7 Schematic representation of full width half maximum principle. This method plots the HU of the voxels across a "ray" which starts at the lumen centre and goes into the tissues beyond the bronchus. The HU level half way between the maximum and minimum HU levels is noted and this is considered the HU of the wall. The area that is within that level is defined as the airway wall

2 METHODS

2.1 Clinical Methods

2.1.1 Tests in both Asthmatic and COPD cohorts

2.1.1.1 Baseline demographics

The basic demographics that were collected were details such as; clinical history, medication, smoking status and history, age, height, weight, occupational history, exacerbation frequency.

2.1.1.2 Electrocardiogram (ECG)

These were done as routine in the COPD cohort. However it was not done as routine in all the asthmatics. It was done if the subjects were going to have a bronchoscopy, or if they were part of an interventional study.

2.1.1.3 Peripheral blood samples

Routine clinical bloods were taken to include full blood count, in particular eosinophil and neutrophil count.

In Asthmatics total IgE was also collected. If Skin Prick Testing (see section 2.1.2.1), was not done, blood IgEs were obtained where possible in the asthmatic cohort.

If subjects were going on to have a bronchoscopy, then a coagulation screen was also done.

2.1.1.4 Spirometry and lung function tests

Spirometry was done by the clinical team, or as part of the pulmonary function tests, according to ATS/ERS guidelines (162).

The clinical team used a wedge bellow spirometer (Vitalograph, Gold Standard, Buckinghamshire, UK).

Where possible, reversibility was assessed 15-20 minutes after taking 200-400µg of salbutamol via a spacer, or 2.5mg of salbutamol via a nebuliser. FEV_1, FVC were repeated, best effort was recorded.

Full lung function was assessed in respiratory physiology by trained physiologists according to American Thoracic Society (ATS)/European Respiratory Socieity (ERS) guidelines (163,164). Either body plethysmography (Medisoft, constant volume, body box) or helium dilution (Spiro Air, Medisoft, Belguim) was used.

2.1.1.5 Sputum Collection/Induction

All subjects were required to provide a sputum sample. Same participants provided spontaneous sputum samples and others needed induced sputum to provide a sample.

Induced sputum was completed after spirometry and bronchodilator response. Baseline spirometry was measured, as occasionally saline can case bronchospasm. Subjects inhaled 200-400 µg of salbutamol and waited 15-20 mins after which spirometry measurements were repeated. The highest FEV1 value post salbutamol administration was used to calculate any subsequent fall in FEV1 during the test.

Saline was administered in increasing concentrations (3%, 4% and 5% if needed), by an ultrasonic nebuliser (Easyneb II ultrasonic nebuliser. Flow rate up to 1.0 ml/min, FLAEMNUOVA). Subjects were instructed to continue normal tidal breathing via the nebuliser for the duration of the saline administration, (5minutes).

After each administration of saline, subjects were asked to rinse their mouth and blow their noses prior to sputum expectoration. Subjects were asked to attempt to cough sputum into a plastic sputum pot using a deep cough. Several attempts at coughing were made until the sound of the cough became dry and unproductive.

After each administration FEV_1 was repeated, and the test repeated if needed, with a higher concentration of saline as long as the FEV_1 had not fallen by more than or equal to 10% of the highest post-bronchodilator value. If the FEV_1 had fallen by more than or equal to 10% then the same concentration of saline was administered for the remaining induction procedure. Subjects did not breathe saline for > 15 minutes in total.

The test finished when any of these outcomes was reached:

- Sufficient sputum obtained
- Subject unable to tolerate test
- Breathing saline for 15 minutes
- No sputum after administration of 5% saline
- FEV_1 falls by ≥20% baseline value

If subjects FEV_1 had dropped by 20% or more or they experienced severe symptoms such as breathlessness or wheeze a further 200µg of salbutamol was administered and the subjects were monitored until their FEV_1 returned to 95% of baseline pre-bronchodilator FEV_1.

2.1.1.6 Visual Analogue Scores

This questionnaire assessed the subject's perception of the severity of their symptoms. Asthmatic subjects are asked to assess the symptoms of cough, breathlessness and wheeze. Whereas COPD subjects assess the symptoms of cough, dyspnoea, sputum production and sputum purulence.

Each symptom is beside a 100 mm line, this represents a continuous scale, with the left hand side being no symptoms, and the right hand end of the scale representing the worst they have ever felt with that symptom. They are then asked to mark along the 100mm line where they feel they lie along that scale on that day.

This is repeated for each symptom.

The healthcare professional then measures the length of the mark in mm which gives the score for each symptom (165).

2.1.1.7 Bronchoscopy and biopsy sampling

Bronchoscopies were performed by blinded senior clinicians, according to guidelines (166). Endobronchial biopsies were obtained from the carinas of the segmental or subsegmental airways.

Subjects provided informed, written consent for the procedure, which was taken in the setting of a clinical visit weeks or months before the procedure. Consent was also confirmed on the day.

In order to eligible to undergo a research bronchoscopy +/- biopsy +/- BAL subjects had spirometry, ECG and clotting checked. In order to proceed, FEV_1 had to be greater than 1L, ECG had to be stable and clotting normal.

On the day asthmatics and COPD subjects had 5ml nebulised salbutamol pre bronchoscopy and a cannula placed if sedation was requested. All subjects had a local

anaesthetic spray pre procedure. During the procedure all subjects had nasal oxygen and had oxygen sats, pulse and three lead ECG monitoring.

Post bronchoscopy subjects were observed for 2 hours, whilst being nil by mouth, and if stable they would be discharged home.

2.1.2 Tests performed only in Asthma cohorts

2.1.2.1 Skin Prick Testing

Common allergens were tested, such as sensitivity to; dog, cat, house dust mite, grass pollen and moulds. Histamine and saline were used as positive and negative controls respectively. The test was considered positive if, after 15 minutes there was a weal with a diameter 2mm greater than the negative control. Subjects were asked to not take any anti-histamine medication 48-72 hours prior to testing.

2.1.2.2 Fractional exhaled nitric oxide (FE_{NO})

Using an online chemoluminescence analyser (NIOX Mino; Aerocrine, Stockholm, Sweden) FE_{NO} was measured at a flow rate of 50 mL/s (167).

2.1.2.3 Methacholine challenge test (PC20)

$_1$ is measured. Bronchial hyper-responsiveness is diagnosed when there is a 20% drop in FEV_1. The concentration of methacholine needed to induce this change is then documented.

Anti-histamines were withheld for 48 hours prior to testing. Long acting beta agonists and ipratropium bromide were withheld for 24 hours prior to testing. Short acting beta agonists were withheld for 6 hours prior to testing.

This test was contraindicated if subjects had:

- FEV_1 <60% predicted
- FEV_1 < 1L
- Poorly controlled hypertension
- Aortic aneurysm
- Myocardial infarction or stroke in past 3 months

- FEV_1 drop of >10% after inhaling saline

Providing they were fit to continue with the test, they were given methacholine via a Wrights nebuliser, with a nose clip to ensure tidal breathing was entirely via the mouth. The methacholine concentration started at 0.03 mg/ml. Each dose was administered for 2 minutes and doubled with each successive administration to a maximum of 16 mg/ml. FEV_1 was tested at 30 seconds and 90 seconds post administration. If the reading at 90 seconds was less than the reading at 30 then an third reading was taken 3 minutes post administration. The lowest FEV_1 was used to calculate the drop from baseline. If this was 20% or more the test was considered positive at that concentration and the test was stopped.

2.1.2.4 Asthma Control Questionnaire (ACQ)

This questionnaire is set out into seven parts. It requests that the subjects answer the questions in relation to how they have been in the past week.

Six of the seven parts are questions dependant on the subject's perception of their symptoms. A score of ≥ 1.57 represents suboptimal control and a change of 0.5 or more is considered clinically significant (168).

The seventh part to the questionnaire is FEV_1 dependent and is filled out by the healthcare professional. The modified ACQ (averaging the first 6 questions only), has also been validated (169).

Each question has options 0-6 as answers, with 6 always denoting the most severe and 0 most mild.

The questions relate to either how the subject's asthma, or "symptoms of asthma" have been at various time points, such as at night, first thing in the morning, when doing daily activities, or how bad the specific symptoms of shortness of breath and wheeze

have been. The final question for the subject to answer is the average frequency with which they took their short acting beta agonist per day.

2.1.2.5 Asthma Quality of Life Questionnaires with Standardised Activities (AQLQ(S))

This questionnaire is set out into four domains, each with their own set of questions. The four domains are as follows; Symptoms (12 questions), activities (11 questions), emotion (5 questions) and environment (4 questions). Each question is answered with a scale of 1-7, with 7 always representing 7 the best quality of life and 1 the worst. It requests that the subjects answer the questions in relation to how they have been in the previous two weeks. Once filled out, the mean for whole questionnaire, and each domain's mean are calculated (170).

2.1.3 Tests performed only in COPD cohorts

2.1.4.4 Modified Medical Research Council Dyspnoea scale

This is the same as the MRC Dyspnoea scale, except it is graded 0-4 rather than 1-5. It is simply a scale for subjects to state their level of breathlessness, with 0 representing breathlessness only with strenuous exercise and 4 representing breathlessness onset with dressing.

2.1.4.5 St George's Respiratory Questionnaire for COPD patients (SGRQ-C)

This is in two parts. Part 1 is designed to assess how the subject has been over a longer period of time, 1, 3 or 12 months. They are asked to score symptoms of cough, sputum, shortness of breath, wheeze, chest trouble, good days and if the wheeze was worse in the mornings.

Part 2 assesses how they are now and asks various questions about their chest condition and what activities (if any) cause breathlessness. The score is calculated on a SGRQ-C specific excel spreadsheet.

2.2 Laboratory Methods

2.2.1 Sputum processing

Collected sputum was inspected, and samples selected if it was free from salivary contamination. Selected samples were weighed, processed as previously described (171) and the resulting supernatant was assessed for viability, squamous cell contamination and a total cell count was performed using a Neubauer haemocytometer and the tryptan blue exclusion method. A differential cell count was carried out on a Romanowski stained cytospin and was attained by counting more than 400 non squamous cells.

2.2.2 Biopsy processing

Endobronchial biopsies were obtained as described in section 2.1.1.7. Biopsy samples were embedded in glycol methacrylate or paraffin (replication group).

2.2.2.1 Glycol methacrylate samples

Once embedded in glycol methacrylate, sections measuring two micrometeres were cut and stained with Haematoxylin & Eosin (H&E) and/or Periodic acid-Schiff (PAS).

Immunohistochemical staining required several different monoclonal antibodies (mAb) in order to test for cells of a specific phenotype. The following mAbs or appropriate isotope controls were used:

- Anti–mast cell tryptase clone AA1 (Dako UK, Ely, United Kingdom).
- Anti-alpha smooth muscle actin clone 1A4 (Dako UK, Ely, United Kingdom).

- Anti-eosinophil major basic protein clone BMK-13 (Monosan, Uden, The Netherlands).
- Anti-neutrophil elastase clone NP57 (Dako UK, Ely, United Kingdom)A
- Anti-endothelium clone EN4 (Monosan, Uden, The Netherlands).

ZEN 2012 image analysis software for light microscopy, (Carl Zeiss AG, Jena, Germany) was used to evaluate the slides. A single researcher (RB), blinded to the clinical characteristics of the subjects, assessed the slides.

Tissue section areas were measured in H&E and SMA stained sections. Total area, airway smooth muscle area and epithelial area were measured directly, while lamina propria area was calculated by subtracting the all the other areas and the area occupied by vessels and lymphatics from the total section area. All areas were expressed in mm^2 and also as percentages of the total area. All morphometry measurements and cellular counts were performed by one blinded observer on two non-contiguous tissue sections at least 20µm apart from the same biopsy block.

Reticular basement membrane (RBM) thickness was measured at x200 magnification by measuring 50 points 20µm apart according to the method validated by Sullivan et al (172). Epithelial thickness was measures using the method described by Cohen et al (173). Briefly, areas of intact and tangentially orientated epithelium were identified and measured. Subsequently, to calculate the epithelial thickness, this area was divided by the lengths of the corresponding RBM. Both RBM and epithelial thickness were expressed in µm. Vascularity was measured using the Chalkley count, a surrogate of both vessel density and vascular area. As described previously, a Chalkley eyepiece graticule (NG52 Chalkley Point Array, Pyser-SGI Ltd, Edenbridge, UK) was used at

x200 to measure Chalkley counts in four non-overlapping vascular hotspots (1-2/section) (174). The mean Chalkley count (MCC) was calculated as the mean of the four measurements. Epithelial integrity was assessed by measuring the lengths of intact epithelial denuded epithelium. These were expressed as percentage of all the RBM length present in the section. For inflammatory cell counts, submucosal nucleated stained inflammatory cells (eosinophils, mast cells and neutrophils) were counted on the corresponding stained sections and expressed at cells/mm2 of lamina propria.

2.2.2.2 Paraffin samples

Four micrometre sections were cut from the paraffin embedded biopsies and stained with appropriate mAb or corresponding isotype control. This method was used in the replication group of Study 3.2

2.3 Radiological Methods

2.3.1 Scanning protocols

2.3.1.1 Full thoracic Inspiratory and expiratory CT protocol

The scans are done caudo-crainally to try to reduce breathing artefacts.

This scanning protocol was specifically designed to be a high resolution, low dose volumetric scan

The scans were obtained at full inspiration (near TLC) and at the end of expiration (near FRC). All subjects were coached in the breath holding techniques, and practised breath holding, immediately prior to scanning. All asthmatic and COPD subjects were scanned within 60 minutes of receiving 400 micrograms of salbutamol via a spacer

kVp – The voltage going through the x-ray tube. It can be likened to the strength of the beams.

mAs – The current going through the x-ray tube and can be likened to the volume of the beams.

Care dose – This is an in built function to allow the mAs to change over the course of the scan to allow for different mass of the body part(s) being imaged to maintain image quality whilst not over irradiating (e.g. the head would need more mAs to get through the skull than the chest wall and lungs). Care dose is turned off to ensure that the whole thorax is scanned with the same "strength" of radiation beam. If this was not consistent, it would not be possible to know if changes seen in attenuation are due to disease or increased/decreased radiation reaching the detectors.

Det Configuration – (Detector Configuration), The numbers 16 x 0.75 means that 16 detectors with a length of 0.75 mm were used to collect the data (therefore 0.75mm is also the minimum slice thickness).

Rotation time – Is the length of time the tube/detector mechanism takes to cover 360^{o}.

Pitch – The meaning of this term is dependent on scanner type. For the scanners used in these studies, it is a combination of the speed at which the table is moving through the gantry and the rotation time. It can be likened to a telephone wire – a pitch of 1 is a coil with no gaps, all of the body part undergoing investigation has been scanned in each 360^{o} rotation. A pitch of less than 1 means there is overlap in what has been scanned. A pitch of more than 1 means that by the time the 360^{o} rotation has finished the table would have moved, like the telephone wire has been pulled apart.

The radiographer would select the whole thorax from the scout images, using the lateral scout image to ensure lung bases were included.

Reconstructions were done in accordance with the recommended settings by the QCT software producers. Apollo® (by VIDA Diagnostics, Inc), recommended using B35, and the other recons were taken over a range of kernels to allow for the possibility of future software programmes needing different reconstructions.

All scans were analysed by a single observer (RH) using semi-automated software, Apollo® (by VIDA Diagnostics, Inc) and various QCT parameters were obtained. Scans from 76 subjects were analysed by two observers (RH and SG) for assessment of inter-observer repeatability (see section 2.3.1.2).

2.3.1.2 Inter-observer repeatability of co-primary QCT parameters

Inter-observer repeatability was assessed (Cronbach's alpha intra-class correlation [ICC]) between two observers (RH and SG) in 76 subjects for Percentile 15 (ICC=0.996; p<0.001), MLD at inspiration (ICC=0.997; p<0.001) and expiration (ICC=0.997; p<0.001), RB1 LA (ICC=0.873; p<0.001) and TA (ICC=0.873; p<0.001).

2.3.1.3 Limited Thoracic CT scan protocol

Study 3.2 used RB1 measurements from 14 limited scans. The limited scan protocol was devised to specifically image RB1.

The same scanning protocol outlined in table 2.1 (Siemens 16) was used, with the exception of length of the scan. Instead of selecting the whole thorax on the scout image, the radiographer was instructed to scan only 53mm, using the carina as the lower boundry.

Images were reconstructed using the B35 and B70 kernals, (as described in table 2.1).

2.3.1.4 Electron Dense (ED) Rods

In all scans, a lightweight foam box, (LD15, Styrotech Ldt, West Bromich), housing three acrylic rods of known density, was placed on and lightly strapped to the subject's chest.

The three acrylic rods, (LN300, LN450 and "solid water", Gammex – RMI Ldt, Nottingham), were selected as they represented densities seen the lung and blood. LN300, LN450 and "solid water" have and electron density relative to water of 0.28,0.40 and 0.99 respectively.

2.3.2 Analysis of computed tomography scans

For a summary of the main QCT parameters used please see table 2.2

2.3.2.1 Airway Segmentation

In general the main issues surrounding any method of airway segmentation are; airway obstruction, movement artefacts, image reconstruction artefacts, low dose scans (reduces contrast) (175), and severely diseased lungs (176). All these conditions make it hard to segment correctly and runs a high risk of "leakage" of the airway growth into lung parenchyma (177). Numerous different techniques have been developed for airway segmentation (177).

Most segmentation methods have been validated against phantoms rather than histological validation reference (178) lists studies that have used phantoms to validate airways whilst presenting original data looking at histological airway validation. Despite the paucity of studies that have directly compared QCT airway segmentation to histology, the available data does suggest that QCT tends to over-estimate WA and underestimate LA (178,179). Therefore, as long the analysis is all done on the same software then it is comparable, but inter-software comparisons remain difficult.

2.3.2.2 Apollo® (by VIDA Diagnostics, Inc) airway segmentation

Fuzzy connectivity (FC) works on the principle that adjacent voxels belonging to the same object are connected. Although the theory was developed in the late 70s, it wasn't until 1996 that it was used more practically for image segmentation (180).

The strength and similarity of the voxels is measured using an affinity function, depending on the strength of the connection, a value between 0 and 1 is assigned to the affinity function. As long as the value is greater than 0, voxels will be considered to be

fuzzy adjacent, this can be likened to the strength of a link in a chain. Often multiple paths can be made between 2 voxels, the strength of the chain is determined by the weakest link. However the overall level of *connectedness* is determined by the strongest chain (181).

One disadvantage of the FC approach is the processing time. In order to combat this issue, Apollo® (by VIDA Diagnostics, Inc) used small cylindrical regions of interest (ROI). These ROIs reduce the need to process unnecessary voxels (therefore reduces computing time), and will allow quicker detection of leaks (176). They validated their method by comparing it to a well-established, published, region growing algorithm which was developed to analyse low dose scans. They found that their algorithm identifies more airway segments, identifies them more consistently and can reliably segment low dose scans (176).

2.3.2.3 Apollo® (by VIDA Diagnostics, Inc) cross sectional measurements

Detection of the airway walls are guided by the airway tree centrelines and the boundaries of the segmentation. The process is done firstly creating 2D slices in a plane perpendicular to the airway. Each slice is then segmented into wall area and lumen area, and these parameters are measured. This is repeated 10 times at regular intervals along the airway. It is therefore possible to get values from 10% along the airway, 20%, 30% etc. Throughout the studies, the measurements used to obtain the morphometry values was the average of the middle 40% (i.e. at 30%, 40%, 50% and 60% point). This was to reduce the influence of bifurcations of the airways on the values.

2.3.2.4 Apollo® (by VIDA Diagnostics, Inc) lung and lobe segmentation

Most lung segmentation can be divided into direct and indirect methods (182). Direct methods (183-185) use algorithms to "look" for the fissures, as they are usually quite bright thin lines when compared to the rest of the lung tissue. Indirect methods utilise anatomical information to define the area that should belong to each lobe (182). An important part of lung segmentation is to distinguish the lungs from the surrounding cardiovascular and chest wall structures.

Apollo® (by VIDA Diagnostics, Inc) uses a multi-step method and utilises anatomical information such as the airway branches and the vessel segmentation to guide lobar segmentation into a Region of Interest (ROI), and then uses an algorithm to look for contrast within this ROI to pin point fissure lines.

Apollo® (by VIDA Diagnostics, Inc) lung and lobe segmentation process was investigated by Henne et al (186). They obtained human lungs that were not transplantable. There were no restrictions placed on the lungs examined such as underlying disease, age, gender or height of the donor. The ex vivo lungs were scanned after being filled with air to a static pressure of 20-30cm H_2O (~TLC) and these images were analysed using Apollo® (by VIDA Diagnostics, Inc) software.

Once scanned the lungs were deflated the lobes were demarcated. This was done by inflating each lobe and marking the boundaries. The lungs weighed and then the lobes were dissected along the marked lines. They found that the Apollo® (by VIDA Diagnostics, Inc) software accurately estimate the mass of the lungs and lobes.

2.3.2.5 Low attenuation area (terminal airspace) complexity (LAC-D)

Density measurements traditionally quoted in texts focus on the overall area or volume of lung tissue that falls within certain boundaries. For example, using percentile 15 or percentage of voxels under -950 HU as a marker of emphysema.

However it is also possible to assess the number of, and size of, these low attenuation clusters, drawing on the concept of fractal geometry. Fractals are scale free and self-similar, many objects in nature display fractal patterns, including the lungs (187).

A study by Mishima et al (188) demonstrated the existence of a power law distribution of Low Attenuation Clusters (LACs) and the numerical value of *D*, (where *D* represents the gradient of a linear regression log-log plot of cluster size (x) against cumulative frequency (y), Figure 2.1), in COPD and healthy subjects.

The value of *D*, is strongly related to the fractal dimension of the terminal air space, and a low number indicates loss of complexity, as seen in emphysema (188).

2.3.2.6 Pi10, Po20

The use of Pi10 to assess small airways using linear regression from larger airway dimensions was first introduced by Nakano et al (189). They compared geometry of airways on CTs to the geometry of the same airways on histology. They found that the larger airways did accurately predict the geometry of the smaller airways, (airways less that 2mm diameter are below the resolution of most current CT scanners.) It was also noted that CTs overestimated the %WA, especially in those airways less than 7.5mm. Therefore they only used airways larger than 7.5mm diameter in the regression.

Subsequent papers have calculated Pi10 using at least 3 airways with a minimum diameter of 6mm (65,190). Po20 is derived by applying the same concept of using larger airways to predict smaller ones, but rather than predicting the dimensions of the

an airway with a 10mm internal perimeter, an airway with an external perimeter of 20mm is instead predicted.

2.4. Correction methods

2.4.1 Density correction

Recalibrating density measure to take into account blood and air values reduces the effect of tube aging (191,192) and interscanner variability. It is thought that different scanners can have a difference of up to 8 or 9 HU in their measurement of air (137,193). Bakker et al found that this scanner difference can cause changes in lung density of between -3.2HU and 47.8HU (192). These differences are caused by the aging of the x-ray tube and/or replacement and are greater than the variability introduced from users obtaining the measurements needed for recalibration.

For each scan, a regression equation was calculated using 5 densitometry standards, extra thoracic air, aortic blood, and three ED rods (see section 2.3.1.4), as shown in Figure 2.2. Each of the five standards was measured at three separate points and the mean of the three measurements was used in the regression equation.

If the Siemens 128 scanner was used, an extra nine measurements were used to standardise the scans to the Siemens 16, as outlined in section 2.4.3 and Figure 2.3.

2.4.2 Interscanner correction

From 2006-2013 the same Siemens 16 scanner was used until it was replaced with a Siemens 128.

Phantoms were scanned when the new scanner came into use, including the Warwick Density Phantom, (WDP) (Figure 2.4). The WDP has a milled housing with an equivalent density of "solid water", containing nine cores of random and heterogeneous density designed to mimic lung densities. The WDP was scanned in the reference scanner, (Siemens 16), and these readings were taken to be the gold standard to which the Siemens 128 was adjusted.

2.4.3 Morphometry correction

Airway size is affected by the size of the person. In order to compare airways and remove the influence of body habitus, airway measurements are corrected for Body Surface Area (115,194,195). Excluded from this correction are parameters such as %WA and Pi calculations.

$$\text{BSA} = \left(\sqrt{\frac{\text{Height (cm)x Weight (kg)}}{3600}}\right)$$

The reconstruction kernel in which the scans are analysed is also important, and must be standardised (194,196-199). In all the studies B35 was used.

2.5 Radiation safety

According the HPA, [*http://www.hpa.org.uk/Topics/Radiation*], the average UK annual total radiation dose is ~2.7 millisieverts (mSv), whereas in Cornwall it is estimated to be 7.8 mSv. The annual exposure limit for nuclear industry workers is 20mSv, although the average occupational exposure is only 0.18 mSv. Acute radiation effects would be seen at 1000 mSv, and 5000mSV would kill half of those receiving it within a month. In the research studies, we aim to keep radiation exposure of the subjects to 10mSv (research scans only, clinical scan doses are excluded), over 3 years.

Average DLP for the research scans was 213, which equates to an approximate dose of 3.6 mSv (using the thoracic conversion factor of 0.017)

2.6 Tables and Figures

Table 2.1: Scanning protocol

Make	Siemens	Siemens
Model	**16 (sensation)**	**128 Somatom Definition**
Scan Type	Spiral	Spiral
Rotation Time (sec)	0.5	0.5
Det Configuration	16 x 0.75	128 x 0.6
Pitch	1.5	1.5
kVp	120	120
mAs	40	40
Dose modulation	CARE dose off	CARE dose off
Scan Comment	Full thoracic (Insp & Exp)	Full thoracic (Insp & Exp)
Scan Direction	caudio-cranial	caudio-cranial
RECON 1		
Algorithm	B30	B30
Thickness (mm)	0.75	0.75
Interval (mm)	0.5	0.5
DFOV (cm)	Lungs*	Lungs*
RECON 2		
Algorithm	B35	B35
Thickness (mm)	0.75	0.75
Interval (mm)	0.5	0.5
RECON 3		
Algorithm	B60	B60
Thickness (mm)	0.75	0.75
Interval (mm)	0.5	0.5
RECON 4		
Algorithm	B70	B70
Thickness (mm)	0.75	0.75
Interval (mm)	0.5	0.5
RECON 5		
Algorithm	B30	B30
Thickness (mm)	5	5
Interval (mm)	2.5	2.5

Table 2.2 Summary of most frequently used QCT parameters

Parameter	Abbreviation	Units	Equation (where applicable)
Lumen Area*	LA	mm^2	
Total Area*	TA	mm^2	
Wall Area*	WA	mm^2	$(TA - LA)$
Percentage Wall Area	%WA		$\left(100\ x\left(\frac{(TA - LA)}{TA}\right)\right)$
Mean Lung Density Expiratory to Inspiratory ration	$MLD_{E/I}$	HU	
Relative Voxel Change	RVC	HU	$Exp\big((VI - 856) - (VI - 950)\big) - Insp\big((VI - 856) - (VI - 950)\big)$
Percentile 15	Perc15	HU	
Fractal dimensions of low attenuation clusters (inspiration)	LAC-D -950		

*When used clinically, these parameters are corrected for Body Surface Area, BSA. See section 2.4.3.

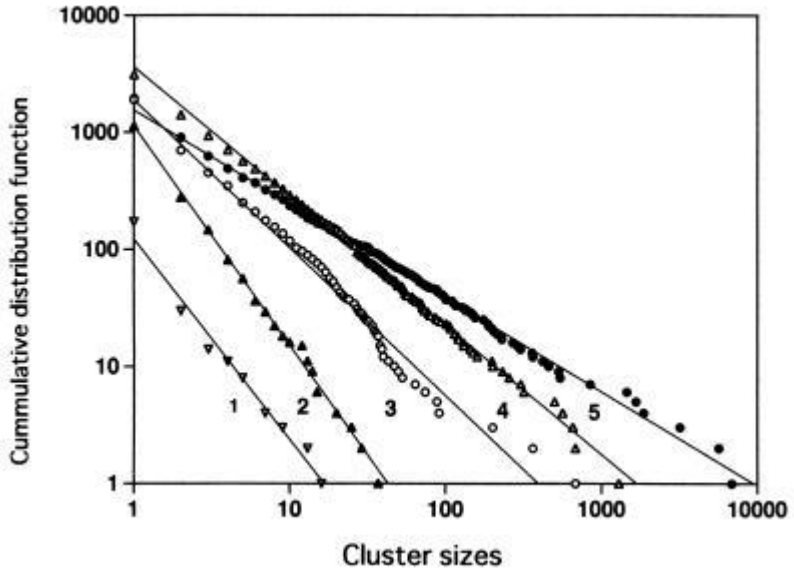


Figure 2.1: Linear regression log-log plot (188)

Linear regression log-log plot of cluster size (x) against cumulative frequency (y) to give *D,* the gradient.

The value of *D*, is strongly related to the fractal dimension of the terminal air space, and a low number indicates loss of complexity, as seen in emphysema

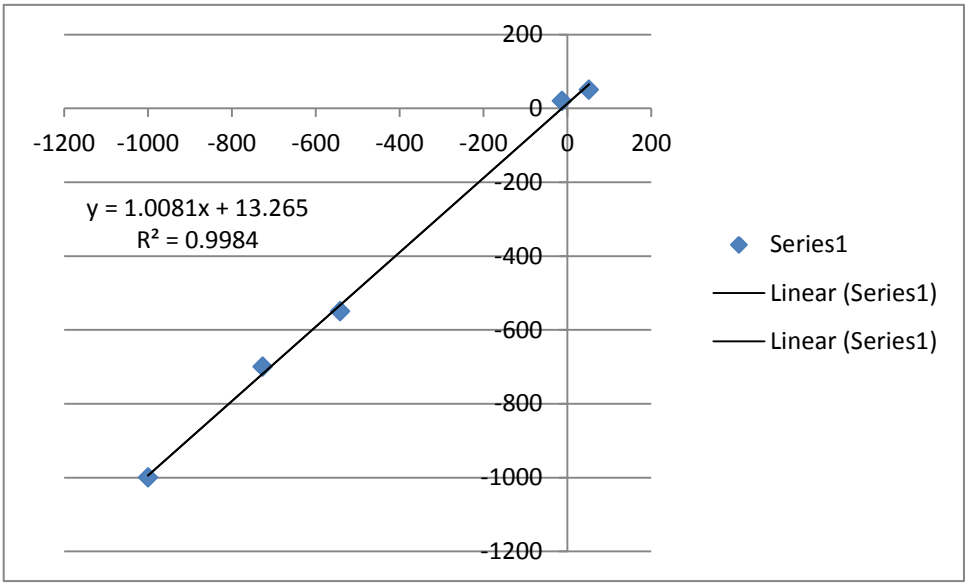


Figure 2.2 Density Correction

The derivation of the regression equation for subject A113's expiratory scan. The linear regression equations were used to standardise all densitometry values for the specific scan for each subject. The equation was performed for every individual scan.

STUDIES

STUDY 3.1

Relationship between lung function and quantitative computed tomography parameters of airway remodelling, air-trapping and emphysema in asthma and COPD: A single centre study

3.1.1 Abstract

3.1.1.1 Background

There is a paucity of studies comparing asthma and COPD based on quantitative thoracic computed tomography (QCT) parameters. The aim of this study is to compare QCT parameters of airway remodeling, air-trapping and emphysema between asthma and COPD and explore their relationship with airflow limitation.

3.1.1.2 Methods

Asthma (n=171), COPD (n=81) and healthy (n=49) subjects, recruited from a single centre, underwent QCT and clinical characterisation.

3.1.1.3 Measurements & main results

Proximal airway percent wall area was significantly increased in asthma (62.5% [2.2]) and COPD (62.7% [2.3]) compared to healthy controls (60.3% [2.2]; $p<0.001$). Air-trapping measured by mean lung density expiratory to inspiratory ratio was significantly increased in COPD (0.922 [0.037]) and asthma (0.852 [0.061]) compared with health (0.816 [0.066]; $p<0.001$). Emphysema assessed by lung density measured by Percentile 15 was a feature of COPD only [COPD -964 (19.62), versus asthma -937 (22.7) and health -937 (17.1); $p<0.001$]. Multiple regression analyses showed that the strongest predictor of lung function impairment in asthma was percent wall area, whereas in COPD and the asthma sub-group with post-bronchodilator FEV_1% predicted <80%, it was air-trapping. Factor analysis of QCT parameters in asthma and COPD subjects combined determined 3 components with percent wall area, air-trapping and Percentile 15 being the highest loading factors. Cluster analysis identified 3 clusters with mild, moderate or severe lung function impairment with corresponding decreased lung density (percentile 15) and increased air-trapping.

3.1.1.4 Conclusions

In asthma and COPD lung function impairment is strongly associated with air trapping with a contribution from proximal airway narrowing in asthma.

3.1.2 Introduction

Asthma and chronic obstructive pulmonary disease (COPD) cause considerable morbidity and consume substantial health-care resources (200,201). Both airway diseases are characterised by airflow obstruction, which is typically variable and reversible in asthma, but fixed in COPD (202). However, there is overlap between the two conditions, particularly between severe asthma and COPD as severe asthma can be characterised by persistent airflow obstruction and some COPD subjects have partially reversible airflow obstruction. Similarly, there is emerging evidence of overlap between asthma and COPD in terms of inflammatory profiles with the former typically associated with eosinophilic and the latter neutrophilic inflammation; there are subgroups of both asthma who have neutrophilic inflammation and COPD sufferers who have and eosinophilic inflammation (113,116,202).

Quantitative computed tomography (QCT) has become an established technique for airway morphometry and lung densitometry in airway disease (24,34,203). This approach allows for quantification of proximal airway remodelling by assessment of airway lumen and wall geometry, air-trapping as an indirect measure of small airway disease and emphysema determined by lung densitometry. QCT has been applied extensively to COPD. Indeed, a systematic review in 2012 found that both markers of emphysema and peripheral airway measurements correlated to airflow obstruction in COPD (204). QCT in COPD is generally accepted as a robust method especially for quantifying emphysema (205). QCT measured emphysema has been shown to predict mortality (206) and has been linked to lung function decline (123). QCT in asthma has demonstrated tremendous heterogeneity in airway remodelling; shown that change in

lumen dimension is an important aspect of proximal airway remodelling (24) and identified that changes in airway geometry are associated with histological features of airway remodelling (25,207,208). Whether the relationships between lung function and QCT parameters are different in asthma and COPD is uncertain.

Our hypotheses were: (1) QCT morphometry and densitometry measures of proximal airway remodelling, air-trapping and emphysema are different between asthma, COPD and healthy subjects, and (2) In asthma and COPD the association between lung function impairment (post-bronchodilator FEV_1 % predicted) and these QCT morphometry and densitometry measures are distinct. The co-primary QCT outcome variables were: for proximal airway remodelling: mean airway lumen area (LA) /body surface area (BSA) and percentage wall area; air-trapping: mean lung density expiratory to inspiratory ratio ($MLD_{E/I}$); and emphysema: Percentile 15. To test our hypotheses we undertook a QCT observational study of asthma and COPD subjects across the spectrum of disease severity and investigated the relationship between lung function and QCT parameters firstly in each disease and secondly in QCT-derived clusters of the disease groups combined.

Some of the results of this study have been previously reported in the form of an abstract (209,210).

3.1.3 Methods

3.1.3.1 Subjects

Adults with COPD (n=81) or asthma (n=171) and healthy control subjects (n=49) were recruited at a single centre, Glenfield Hospital, Leicester. COPD and asthma subjects were recruited from respiratory outpatient clinics and healthy controls were recruited through posters and advertisements placed in public areas including outpatient clinics in the hospital, support group meetings and leisure centres. COPD and asthma subjects fulfilled diagnostic criteria as per GOLD and GINA guidelines respectively (211,212). COPD subjects had >10 pack year smoking history and were >40 years old. Twenty-nine healthy control subjects and 60 subjects with asthma had participated in previous studies (24,34). The study was approved by the Leicestershire Ethics Committee and patients gave their written informed consent.

It was ensured that all subjects with airway disease at the time of their study visit were free from an exacerbation requiring systemic corticosteroids and or antibiotics for at least 6 weeks. All subjects underwent extensive clinical characterization, as described in Methods sections 2.1.1-2.1.3.

3.1.2.2 Computed Tomography

Scans were acquired using the protocol outlined Methods section 2.3. QCT parameters obtained included; morphometry, Lumen Area (LA), Total Area (TA), Wall Area (WA) and percentage Wall Area (%WA). Air-trapping measures were Mean Lung Density Expiratory to Inspiratory ratio ($MLD_{E/I}$) (19) Relative Voxel Change (RVC). Emphysema was quantified using 15^{th} percentile point (Perc15). Fractal dimensions of

the low attenuation clusters on inspiratory scans (LAC-D -950) and on expiratory scans (LAC-D -856) were also measured. Detailed descriptions of QCT parameters are given in Introduction section 1.4.4 and Methods section 2.3.2.

A representative example of an inspiratory and expiratory scan, airway reconstruction from the inspiratory scan and densitometry maps from both the inspiratory and expiratory scans are as shown Figure 3.4.

3.1.2.4 Statistical Analysis

3.1.2.4.1 General analysis

Statistical analyses were performed on IBM SPSS Statistics for Windows, Version 20.0, (Armonk, NY: IBM Corp) and GraphPad Prism version 6 for windows (San Diego California USA). *A priori* subject stratification determined by post-bronchodilator FEV_1% predicted was performed and exploratory outcomes were not tested for multiplicity. Non-parametric and parametric data were presented as median (Interquartile Range [IQR]), or mean (Standard Deviation [SD]) respectively. Comparisons across groups were analysed by parametric and non-parametric ANOVA with *post hoc* testing for pairwise comparisons. Pairwise comparisons were made by t-tests or Mann-Whitney tests as appropriate. Statistical significance was reached if the p value was less than 0.05. Factor analysis and cluster analysis was carried out using IBM SPSS Statistics for Windows, Version 20.0, (Armonk, NY: IBM Corp). The Kaiser criterion was used to select the number of the factors and Wards hierarchical clustering was used to determine the number of clusters, k. Cluster membership was derived using k-means clustering, see online supplement for further details.

3.1.2.4.2 Factor and cluster on COPD and severe Asthma cohort

We undertook *de novo* cluster analysis on the COPD and asthma patients together using the same methodology carried out in Gupta et al (24) but using the QCT variables:

(1) Mean lumen area/body surface area

(2) Mean wall area/body surface area

(3) Mean total area/BSA

(4) Mean % wall area

(5) Expiratory voxel index -856

(6) MLD E/I ratio

(7) Voxel index change of percent voxels between -950 HU and -856 HU on paired inspiratory and expiratory CT scan

(8) Expiratory fractal dimension of low attenuation cluster at threshold of -856 HU

(9) Inspiratory voxel index -950

(10) Percentile 15

(11) Inspiratory fractal dimension of low attenuation cluster at threshold of -950 HU.

The QCT variables listed were first used in a factor analysis with three factors being found allowing for 81% of the variation. The Kaiser criteria to determine the number of factors was used which picking all factors that have an eigen value greater than 1. Varimax rotation was also used to determine the best clinical interpretable factors, see table 3.1.1. The highest loading variables on each factor were taken forward into a cluster analysis. These were mean lumen area adjusted for BSA, Percentile15 and $MLD_{E/I}$.

First hierarchical cluster analysis was applied to determine the number of clusters that best fitted the data. Then the number of clusters determined (3 in this case) (Figure 3.1.2) was inputted into a k-means cluster analysis to determine cluster membership for each patient. Cluster demographics and comparisons are as shown table 3.1.12.

3.1.4 Results

3.1.4.1. Clinical characteristics

The baseline demographics and clinical characteristics of asthma, COPD and healthy subjects are shown in Table 3.1.3. COPD subjects were older, had a greater smoking pack year history, poorer lung functions (airflow limitation, post-bronchodilator FEV_1% predicted < 80%; and airflow obstruction, post-bronchodilator FEV_1/FVC ratio < 70%) and higher neutrophilic airway inflammation compared to asthma. Asthma subjects had higher eosinophilic airway inflammation compared to the other two groups. Body mass index (BMI) of asthma subjects was greater than the COPD subjects. Poorer lung functions were also demonstrated in asthma subjects compared to healthy controls.

3.1.4.2 QCT parameters: Comparison between asthma, COPD and healthy subjects

Examples of CT images for subjects with asthma, COPD or healthy controls are as shown (Figure 3.1.1). The airway morphometry and lung densitometry for subjects with asthma, COPD and healthy subjects are summarised in Table 3.1.4. Segmental airway morphometry is shown in Tables 3.1.5 and 3.1.6. Inter-observer repeatability for QCT parameters was good to excellent. Mean wall area WA/BSA was not significantly different between the three groups. However, the mean percentage wall area was increased in both asthma and COPD subjects compared to healthy controls, with mean LA/BSA being significantly smaller in asthma subjects. The mean LA/BSA was smaller in COPD subjects compared to healthy controls, although it did not reach statistical significance (Table 3.1.4 and Figure 3.1.3a, b). The $MLD_{E/I}$ was increased in both asthma and COPD subjects compared to healthy controls, with highest values seen in

COPD subjects (Table 3.1.4, Figure 3.1.3c). Percentile 15 was decreased only in COPD subjects with comparable values in asthma and healthy subjects (Table 3.1.4, Figure 3.1.3d). Low Attenuation Clusters below -950 HU Fractal Dimension value (LAC-D-950), was significantly decreased in COPD subjects (Figure 3.1.3e). Wall area of theoretical airway with an internal perimeter of 10mm (Pi10 WA) and Percentage wall area of a theoretical airway with an external perimeter of 20mm (Po20 %WA) were increased in both asthma and COPD compared to healthy controls (Table 3.1.4). Age-adjusted comparison of the co-primary QCT parameters between asthma, COPD and healthy subjects was performed as mean age of COPD subjects was higher compared to other groups and all of the comparisons (one-way ANOVA) remained statistically significant ($p<0.001$).

3.1.4.3 Univariate analysis to explore structure and function relationship in asthma and COPD

Correlations between the QCT indices and clinical or physiological parameters are shown in Table 3.1.7 and 3.1.8. Moderate-to-good correlations were observed between QCT parameters and lung physiology indices. Percentile 15 was strongly correlated with Transfer Coefficient (KCO) % predicted in COPD subjects and $MLD_{E/I}$ with Residual Volume (RV) / Total Lung Capacity (TLC)[%] in all three groups. Airflow obstruction was most strongly associated with Percentile 15 and $MLD_{E/I}$ with a weaker association with percentage wall area and LA/BSA in asthma and COPD (Table 3.1.7). Airflow limitation, in asthma subjects was strongly correlated with mean percentage wall area and weakly with $MLD_{E/I}$ and Percentile 15. In contrast, airflow limitation in COPD subjects was most strongly associated with $MLD_{E/I}$ and to a lesser extent with Percentile 15 and percentage wall area (Table 3.1.7, Figure 3.1.4). Sputum neutrophil

count showed positive correlation with mean percentage wall area in asthma subjects and sputum eosinophil count was inversely correlated with mean percentage wall area in COPD subjects. Correlations were also observed between (i) airway narrowing and asthma control, and (ii) between $MLD_{E/I}$ and COPD quality of life (Table 3.1.8).

3.1.4.4 Multiple regression analysis to explore structure and function relationship in asthma and COPD

Multiple linear regression analysis in asthma subjects showed that mean Percentage wall area, $MLD_{E/I}$ and Percentile 15 made a statistically significant contribution to the regression model for prediction of post-bronchodilator FEV1% predicted with mean percentage wall area making the strongest unique contribution. Multiple linear regression analysis in COPD subjects showed that $MLD_{E/I}$ and mean percentage wall area made a statistically significant contribution to the regression model for prediction of post-bronchodilator FEV1% predicted with $MLD_{E/I}$ making the strongest unique contribution (Table 3.1.9).

3.1.4.5 Univariate and multiple regression analysis to explore structure and function relationship in asthma and COPD subjects with airflow limitation

A subset of asthma and COPD subjects with post-bronchodilator FEV_1% predicted <80% were assessed for correlations between the QCT and lung physiology parameters (Table 3.1.10, Figure 3.1.5). The correlations between KCO% predicted or RV/TLC (%) and Percentile 15 or $MLD_{E/I}$ were stronger compared to previous analysis of unselected patients (Table 3.1.10). Post-bronchodilator FEV1% predicted showed

correlations with $MLD_{E/I}$ in asthma subjects and with both $MLD_{E/I}$ and Percentile 15 in COPD subjects. Multiple linear regression analysis demonstrated that in this subset of COPD subjects as well $MLD_{E/I}$ made the strongest unique contribution to the regression model for prediction of post-bronchodilator FEV1% predicted (Table 3.1.11). Multiple regression analysis was not performed in asthma subjects as univariate analysis only showed correlation between post-bronchodilator FEV1% predicted and $MLD_{E/I}$.

3.1.4.6 Asthma and COPD sub-group analysis

We stratified the asthma and COPD subjects into three sub-groups each based on post-bronchodilator FEV_1% predicted, (i) >80% (asthma, n=101; COPD, n=5), (ii) 50-80% (asthma, n=56; COPD, n=43), and (iii) <50% (asthma, n=14; COPD, n=34). As only 5 subjects with COPD had a post-bronchodilator FEV_1% predicted >80% they were excluded from further analyses. The asthma subjects with post-bronchodilator FEV_1% predicted >80% compared to healthy controls, have significantly greater mean percentage wall area with no significant difference in $MLD_{E/I}$ or Percentile 15 (Figures 3.1.6a-d). In asthma sub-group with post-bronchodilator FEV_1% predicted 50-80%, mean percentage wall area was higher and LA/BSA smaller compared to sub-group with post-bronchodilator FEV_1% predicted >80% (Figures 3.1.6a,b). Asthma sub-group with FEV_1% predicted <50% did not show significant difference in airway morphometry compared to other asthma sub-groups. In COPD subjects, the mean percentage wall area and LA/BSA were not significantly different between the sub-groups with post-bronchodilator FEV_1% predicted 50-80% versus <50%. In sub-groups with post-bronchodilator FEV_1% predicted 50-80%, the asthma subjects have greater mean percentage wall area and smaller LA/BSA compared to COPD subjects (Figures 3.1.6a,b).

In both asthma and COPD subjects, sub-groups with lower post-bronchodilator FEV_1% predicted had higher $MLD_{E/I}$ and lower Percentile 15 (Figures 3.1.6c,d). The asthma and COPD sub-groups with similar degree of lung function impairment showed no significant difference in $MLD_{E/I}$ (Figure 3.1.6c). COPD subjects with post-bronchodilator FEV_1% predicted 50-80% showed decreased Percentile 15 compared to asthma subjects with similar degree of lung function impairment (Figure 3.1.6d). In sub-groups with post-bronchodilator FEV_1% predicted <50%, COPD and asthma subjects showed no significant difference in Percentile 15 (Figure 3.1.6d), but the Low Attenuation Clusters below -950 HU Fractal Dimension value was significantly decreased in COPD subjects (Figure 3.1.6e).

3.1.4.7 Unbiased phenotyping of airway disease (asthma and COPD) subjects using factor analysis of QCT parameters

We undertook a *de novo* factor analysis of the QCT parameters in those subjects with asthma or COPD which revealed 3 components with the strongest loading variables being mean LA/BSA, Percentile 15 and $MLD_{E/I}$ (Table 3.1.1). A cluster analysis using these three highest loading variables revealed 3 clusters (Table 3.1.2 and Figure 3.1.2). The 3 clusters had mild (asthma n=40, COPD n=2), moderate (asthma n=94, COPD n=24) and severe (asthma n=25, COPD n=47) lung function impairment respectively with decreased percentile 15 and increased $MLD_{E/I}$ particularly a feature of cluster 3.

3.1.5 Discussion

We describe here the airway morphometry and lung densitometry of asthma and COPD subjects with reference to healthy controls and their relationship with lung function. We found that proximal airway remodelling and air trapping were features of both asthma and COPD. Airway wall area, expressed as a percentage of total area (%WA), was increased in both diseases. Air trapping in subjects with COPD was more severe compared to asthma. Emphysema was only seen in COPD subjects with Percentile 15 being significantly lower compared to other groups. Comparable values of Percentile 15 between asthma and healthy subjects confirm absence of emphysema in asthma. Assessment of structure function relationship revealed a significant contribution of proximal airway remodelling, represented by percentage wall area and air trapping, represented by $MLD_{E/I}$ in prediction of airflow limitation in asthma. In contrast, similar assessment in COPD showed that only QCT-determined air trapping and emphysema contributed to airflow limitation. Both disease groups when further stratified by the degree of lung function impairment showed that in the sub-group with post bronchodilator FEV_1 % predicted < 80%, air trapping remained a significant predictor of lung function impairment. Proximal airway remodelling in this group of subjects did not contribute towards prediction of airflow limitation. With asthma and COPD combined in a factor and cluster analysis the findings were consistent with our *a priori* stratification. Factor analysis revealed 3 components with highest loading factors being measures of proximal airway narrowing, air-trapping and emphysema; and cluster analysis demonstrated 3 clusters that could be distinguished by their degree of airflow obstruction.

Changes in proximal airway geometry in COPD are common and our findings of increased mean segmental percentage wall area compared to control subjects was consistent with previous studies (213). This is consistent with proximal airway remodelling in asthma subjects in the current study as well as previous studies (24,214). Diaz et al. have also demonstrated proximal airway lumen narrowing in mild COPD subjects (215). No significant difference was seen in proximal airway remodelling between asthma and COPD subjects, consistent with previous literature (216). Conversely, other studies report significantly greater proximal airway remodelling in asthma subjects compared to COPD (217,218). In our study asthma sub-group with post-bronchodilator FEV_1% predicted 50-80% have greater mean percentage wall area and smaller LA/BSA compared to COPD subjects with similar degree of airflow limitation. Moreover, airway disease subgroups with post-bronchodilator FEV_1% predicted <50% when compared to healthy controls, proximal airway lumen narrowing was seen in COPD, but not in asthma. These findings highlight the heterogeneity of airway disease and importance of multi-level disease phenotyping and suggest that proximal lumen dimensions in those with severe airflow impairment in asthma might become relatively dilated perhaps to compensate for progressive small airway disease.

Results from COPDgene studies have shown that physiological airway obstruction correlates with both QCT air trapping indices (147,219) and QCT-determined emphysema (219), with the former showing stronger correlations. Similarly, in asthma, QCT-determined air trapping has been associated with increased disease severity (220). Emphysema in asthma has not been extensively studied. However a few studies have suggested that emphysema in asthma subjects is likely secondary to smoking (221). In our study we did not find any evidence of emphysema in asthma subjects, as the

Percentile 15 was comparable to healthy controls. Percentile 15 in asthma sub-group with severe airflow limitation was similar to COPD subgroup with matched airflow limitation, which may suggest that these asthma subjects have emphysema. However, high fractal dimension of low attenuation clusters in the asthma sub-group compared to COPD sub-group indicate that Percentile 15 in this cohort represents air trapping rather than emphysema. Other researchers have found low attenuation on CT in asthma subjects which is comparable to emphysema (222,223) and has been attributed to peribronchial fibrosis or a rupture of dilated bronchial glands, rather than the alveolar disruption as seen in COPD (224). Fractal dimension of the low attenuation cluster is therefore an important QCT parameter in differentiating CT low attenuation secondary to emphysema and air-trapping (188,223).

The findings presented here for COPD are consistent with previous studies and support the view that airflow limitation and obstruction are due to a combination of small airway obliteration and emphysema (109). We found that changes in proximal airway geometry contribute to post-bronchodilator FEV_1% predicted in the multiple regression model for the whole COPD cohort. This is in keeping with previous studies, which have shown that both emphysema and proximal airway remodelling contribute towards the prediction of lung function in COPD (100). Proximal airway geometry, particularly airway lumen narrowing was associated with airflow limitation in asthma. However when the asthma sub-group with airflow limitation was assessed, only air trapping was a significant predictor of lung function suggesting that small airway disease is particularly important in this group. This may be important for our understanding of disease pathogenesis, monitoring response to therapy and identification for therapeutic targets. Importantly, emphysema is absent in asthma subjects with varying degree of

severity and smoking history. Whether the absence of emphysema is a critical distinction between the pathogenesis of asthma and COPD or simply is a consequence of classification of COPD is unclear. Air trapping determined by QCT was closely related to RV/TLC (%) in both asthma and COPD and QCT determined emphysema was related to KCO % predicted in COPD. Even though important differences were observed between asthma and COPD there was marked heterogeneity within both disease groups supporting the view that classification of obstructive airways disease needs to consider multiple dimensions of the disease rather than rely on simple disease labels.

Beyond the associations between QCT and lung function we explored the relationship between QCT and sputum cell counts or health status. The clinical significance of the weak correlations seen between airway inflammation and Percentile 15 or Low Attenuation Clusters below -950 HU Fractal Dimension value, in asthma is uncertain. Proximal airway narrowing in asthma was associated with an increased blood neutrophil count. Previous studies have reported similar relationships in asthma with airway remodelling and lung function decline (214). There were also weak relationships between proximal airway morphometry and health status in asthma with decreased wall and luminal area associated with poorer asthma control and health status. In COPD, increased air trapping, but neither proximal wall remodelling nor emphysema, was weakly associated with poorer health status. How closely changes in airway morphometry or densitometry over time or in response to interventions are related to these clinical outcomes needs to be further investigated.

The major limitation of this report is that it is a cross-sectional study and therefore neither the natural history of disease nor temporal repeatability of the measures was examined. In previous reports QCT is highly repeatable so we are confident that the measures are robust, but longitudinal studies are needed to study the dynamic relationships between airway structure and function. Subjects with COPD were older than those with asthma and healthy controls and therefore age as well as disease effects need to be considered. Importantly, in our study population, age did not influence the differences in QCT parameters between groups for any of the co-primary QCT outcome measures. Although this is the largest study to date comparing QCT parameters in asthma and COPD, to further explore the heterogeneity of QCT in both asthma and COPD further larger studies that include complex phenotyping are required. The investigation of the relationship between QCT and airway inflammation was limited to sputum cell counts and needs to be extended in larger studies of airway inflammation and remodelling determined from bronchial biopsies. In addition the impact of disease exacerbations and exposure to pathogens upon structure-function relationships needs to be further explored.

3.1.6 Conclusion

In conclusion, proximal airway remodelling, and air trapping are QCT features shared by asthma and COPD compared to healthy controls, but emphysema is largely restricted to COPD. In both disease groups air-trapping is an independent major determinant for lung function impairment, with an additional important contribution from proximal airway remodelling particularly in asthma subjects with mild lung function impairment.

3.1.7 Figures and Tables

Table 3.1.1: Factor analysis of QCT variables with combined cohort of both asthma and COPD. The three factors accounted for 81% of the variation of all the variables

	Factors		
	1	**2**	**3**
Mean LA/BSA (mm^2/m^2)	**.99**	.02	-.05
Mean TA/BSA (mm^2/m^2)	.94	.02	.00
Mean WA/BSA (mm^2/m^2)	.98	.02	-.02
Mean %WA	-.78	.03	.16
Insp VI -856	-.06	.73	.60
$MLD_{E/I}$	-.04	.33	**.87**
mean voxel index change	-.18	-.04	.87
Mean fractal 856	.15	.57	.38
Insp VI -950	-.08	.92	.08
Perc15 (HU)	.06	**-.94**	.13
Mean fractal 950	.07	.75	.18

The three factors accounted for 81% of the variation of all the variables

Table 3.1.2 Demographics, both clinical and QCT of clusters in combined cohort of asthma and COPD

		Cluster 1 Asthma n=42 COPD n=2	**Cluster 2** Asthma n=94 COPD n=24	**Cluster 3** Asthma n=25 COPD n=47	**Significance (p value)**
Age (years)		49.7 (13.1)	58.0 (12.4)	62.8 (12.5)	**<0.0001* 0.03∞ 0.001δ**
Gender§	**Female**	73.8%	27.8%	45.8%	**<0.0001**
	Male	26.2%	72.2%	54.2%	
BMI (kg/m^2)		29.0 (6.0)	28.9 (5.7)	28.7 (6.3)	0.97
Smoking status §	**Current Smoker**	4.8%	9.7%	9.3%	**<0.0001**
	Ex-Smoker	31%	68.1%	44.9%	
	Never Smoked	64.3%	22.2%	45.7%	
Pack years (if smoked)		4.8 (10.6)	13.8 (28.0)	34.3 (36.7)	**<0.0001* <0.0001∞** 0.2δ
Severe exacerbations per year		2 (3)	2 (2)	2 (2)	0.51
Blood eosinophil count (x10^9/L)#		0.28 [0.14-0.42]	0.25 [0.15-0.36]	0.23 [0.14-0.29]	0.29
Blood neutrophil count (x10^9/L)#		4.6 [3.4-5.7]	4.4 [3.6-5.7]	4.6 [3.6-5.7]	1
Total sputum cell count 10^6/g#		1.77 [0.92-7.84]	2.33 [1.01-5.10]	3.37[1.23-7.00]	0.27
Sputum % neutrophil#		44.8 [19.3-73.0]	49.3 [22.0-71.3]	61.5 [17.0-88.5]	0.21
Sputum % eosinophil#		0.5 [0-2.3]	1.9[0.3-6.3]	1.0[0.3-2.5]	0.08
Pre BD FEV$_1$ % predicted		82.3 (24.4)	77.6 (23.3)	52.2 (21.7)	**<0.0001* <0.0001∞** 0.77δ
Post BD FEV$_1$ % predicted		89.2 (21.9)	82.7 (23.5)	57.8 (24.0)	**<0.0001* <0.0001∞** 0.38δ
Pre BD FEV$_1$/FVC (%)		72.7 (9.8)	67.0 (12.0)	50.3 (12.5)	**<0.0001* <0.0001∞** 0.28δ
Post BD FEV$_1$/FVC (%)		76.1 (9.6)	68.5 (11.4)	52.2 (12.7)	**<0.0001* <0.0001∞ 0.001δ**
BD response		0.19 (0.24)	0.16 (0.21)	0.18 (0.23)	0.79
KCO % predicted		107 (18.5)	101 (20.3)	79.7 (29.6)	**<0.0001* <0.0001∞** 0.82δ

RV/TLC (%)	37.7 (12)	41.2 (11)	32.7 (12)	**<0.0001*** **<0.0001∞** 0.40δ
Mean LA/BSA (mm^2/m^2)	10.7 (2.94)	11.4 (2.46)	11.0 (3.05)	0.29
Mean TA/BSA (mm^2/m^2)	27.9 (6.19)	29.6 (5.13)	28.5 (6.16)	0.19
Mean WA/BSA (mm^2/m^2)	17.2 (3.32)	18.2 (2.78)	17.4 (3.20)	0.12
Mean %WA	62.7 (2.21)	62.2 (2.05)	62.6 (2.36)	0.3
$MLD_{E/I}$	0.866 (0.056)	0.856 (0.061)	0.910 (0.058)	**<0.0001*** **<0.0001∞** 1.00δ
RVC	-12.93 (11.34)	-23.96 (10.47)	-30.59 (12.22)	**<0.0001*** **<0.0001∞** 0.71δ
Insp VI -950	4.6 (2.0)	13.0 (3.2)	26.6 (6.13)	**<0.0001*** **<0.0001∞** **<0.0001δ**
Exp VI-856	13.3 (8.3)	21.7 (12.6)	45.8 (18.8)	**<0.0001*** **<0.0001∞** **0.004 δ**
$CTLV_{E/I}$	0.65 (0.09)	0.58 (0.11)	0.66 (0.13)	1.00* **<0.0001∞** **0.004δ**
Perc15 (HU)	-905 (14.7)	-943 (8.7)	-972 (11.1)	**<0.0001*** **<0.0001∞** **<0.0001δ**
LAC-D -950	-1.92 (0.20)	-1.84 (0.12)	-1.80 (0.13)	0.22
Pi10 (mm^2)	14.9 (1.28)	15.0 (1.41)	14.8 (1.28)	0.73
Po20 %WA	56.2 (2.37)	56.0 (2.34)	56.0 (2.83)	0.88
%WA (number [%] #)	6 (14.3)	15 (12.7)	11 (15.3)	0.88
$MLD_{E/I}$ (number [%] #)	2 (4.8)	8 (6.8)	20 (27.8)	**<0.0001**
Perc15 (number [%]□)	0 (0)	0 (0)	31 (43.1)	**<0.0001**

Data expressed as mean (SD), # median [IQR], or § proportions. # >2SD of healthy controls, ❖ <2SD of healthy controls. Intergroup comparison: parametric (non-parametric) data, p value for one-way ANOVA (Kruskal-Wallis) has been presented unless the ANOVA (Kruskal-Wallis) was significant ($p<0.05$), in which case the p value has been presented for Tukey (Dunn's) test pairwise comparisons- *cluster 1 versus cluster 3, ∞cluster 2 versus cluster 3, δ cluster1 versus cluster 2. Differences in proportions were tested by Chi Square Test.

Table 3.1.3: Clinical Characteristics of all the subjects with asthma or COPD and healthy controls

		Asthma n= 171	COPD n=81	Healthy n= 49	Significance (p value)
Age (years)		53 (12.8)	69 (8.16)	57 (13.3)	**<0.0001*** 0.07∞ **<0.0001δ**
Gender§	**Female**	51%	33%	39%	**0.03**
	Male	49%	67%	61%	
BMI (kg/m^2)		30 (6)	28 (5)	29 (5)	**0.02*** 0.98∞ 0.07δ
Smoking status §	**Current Smoker**	4%	20%	4%	**<0.0001**
	Ex-Smoker	34%	80%	45%	
	Never Smoked	62%	0%	51%	
Pack years (if smoked)		12.3 (10.6)	50.5 (31.2)	11.7 (9.20)	**<0.0001*** 0.99∞ **<0.0001δ**
Severe exacerbations per year		2.20 (2.58)	2.18 (2.20)	0	1*
AQLQ		4.97 (1.33)	n/a	n/a	
ACQ 6		1.81 (1.15)	n/a	n/a	
SGRQ total		n/a	49.8 (19.1)	n/a	
GOLD/GINA % per group 1,2,3,4 (5)		9, 5, 19, 40, 27	5, 55, 29, 11	n/a	
Total IgE (kU/L)		490 (1785)	ND	83.6 (217)	0.13∞
Blood eosinophil count (x10^9/L)#		0.26 [0.15-0.39]	0.22 [0.14-0.29]	0.13 [0.1-0.2]	0.08* **<0.0001^8∞** **0.03δ**
Blood neutrophil count (x10^9/L)#		4.42 [3.43-5.77]	4.56 [3.7-5.47]	3.74 [3.16-4.46]	1* **0.01∞** **0.005δ**
Total sputum cell count 10^6/g#		2.25 [1.13-5.44]	3.92 [1.32-8.46]	1.64 [0.49-5.7]	0.24* 0.51∞ **0.04δ**
Sputum % neutrophil#		51.8 [35.3-73]	75.5 [39.8-89.8]	75.1 [48.5-90.3]	**0.007*** **0.006∞** 1 δ
Sputum % eosinophil#		2.25 [0.5-8.5]	0.75 [0.25-2]	0.25 [0-0.75]	**<0.0001*** **<0.0001∞** 0.1 δ
Pre BD FEV$_1$ %		78.2 (25.2)	50.5 (17.6)	111 (17.2)	**<0.0001***

predicted				**<0.0001∞ <0.0001δ**
Post BD FEV_1 % predicted	85.3 (24.3)	53.7 (17.2)	113 (18.4)	**<0.0001* <0.0001∞ <0.0001δ**
Pre BD FEV_1/FVC (%)	68.5 (13.3)	50.6 (10.6)	78.5 (5.55)	**<0.0001* <0.0001∞ <0.0001δ**
Post BD FEV_1/FVC (%)	70.7 (12.0)	51.7 (10.2)	78.5 (12.6)	**<0.0001* 0.00015∞ <0.0001δ**
BD response (%)	11.3 (15.1)	8.12 (9.56)	1.78 (4.36)	0.17* **0.000019∞ 0.019 δ**
KCO % predicted	107 (18.4)	74.8 (25.6)	98.9 (13.5)	**<0.0001*** 0.08∞ **<0.0001δ**
RV/TLC (%)	39.7 (12)	55.1 (12)	34.5 (9)	**<0.0001* 0.04∞ <0.0001δ**

Data expressed as mean (SD), # median [IQR], or § proportions

Intergroup comparison: parametric (non-parametric) data, p value for one-way ANOVA (Kruskal-Wallis) has been presented unless the ANOVA (Kruskal-Wallis) was significant ($p<0.05$), in which case the p value has been presented for Tukey (Dunn's) test pairwise comparisons- *asthma versus COPD, ∞asthma versus health, δCOPD versus health. Differences in proportions were tested by Chi Square Test.

n/a – not applicable.

ND – not done

Table 3.1.4 Airway morphometry and lung densitometry of subjects with asthma, COPD and healthy controls

	Asthma n=171	**COPD n=81**	**Health n=49**	**Significance (p value)**
Mean LA/BSA (mm^2/m^2)	11.0 (2.58)	11.3 (3.02)	12.3 (2.75)	0.67* **0.006**∞ 0.08δ
Mean TA/BSA (mm^2/m^2)	28.5 (5.32)	29.3 (6.20)	30.5 (5.40)	0.09
Mean WA/BSA (mm^2/m^2)	17.5 (2.84)	18.1 (3.31)	18.1 (2.76)	0.29
Mean %WA	62.5 (2.19)	62.7 (2.26)	60.3 (2.17)	0.79* **<0.0001**∞ **<0.0001δ**
$MLD_{E/I}$	0.852 (0.061)	0.922 (0.037)	0.816 (0.066)	**<0.0001*** **0.00047**∞ **<0.0001δ**
RVC	-29.3 (12.4)	-12.2 (9.36)	-36.8 (10.2)	**<0.00019*** **0.000268**∞ **<0.0001δ**
Insp VI -950	12.17	23.32	11.40	**<0.0001*** 0.79 **<0.0001δ**
Exp VI-856	20.27	47.57	14.81	**<0.00019*** **<0.05**∞ **<0.0001δ**
$CTLV_{E/I}$	0.58 (0.13)	0.67 (0.18)	0.51 (0.12)	**<0.0001*** **0.009**∞ **<0.0001δ**
Perc15 (HU)	-937 (22.7)	-964 (19.62)	-937 (17.07)	**<0.0001*** 1∞ **<0.0001δ**
LAC-D -950	1.96 (0.104)	1.810 (0.132)	1.989 (0.107)	**<0.0001*** 0.26∞ **<0.0001δ**
Pi10 WA (mm^2)	15.1 (1.42)	15.0 (1.46)	14.4 (1.10)	0.89* **0.011**∞ 0.06δ
Po20 %WA	56.1 (2.57)	56.4 (2.97)	54.6 (1.71)	0.7* **0.001**∞ **0.0002δ**
%WA (number [%] above #)	27 (15.8%)	13 (7.60%)	n/a	1.0

$MLD_{E/I}$ (number [%] above #)	8 (4.68%)	22 (27.16%)	n/a	**<0.0001**
Perc15 (number [%] below □)	7 (4.09%)	26 (32.1%)	n/a	**<0.0001**

Data expressed as mean (SD), # >2SD of healthy controls, ❖ <2SD of healthy controls

Intergroup comparison: p value for one-way ANOVA has been presented unless the ANOVA was significant ($p<0.05$), in which case the p value has been presented for Tukey test pairwise comparisons- *asthma versus COPD, ∞asthma versus health, δCOPD versus health.

n/a – not applicable.

Table 3.1.5 LA/BSA for segmental airways in asthma and COPD subjects and healthy controls

	Asthma	COPD	Healthy	Significance p value
RB1 LA/BSA (mm^2/m^2)	11.3 (4.04)	10.6 (3.68)	11.8 (3.95)	0.25
RB2 LA/BSA (mm^2/m^2)	11.4 (3.87)	11.2 (4.26)	12.1 (4.34)	0.42
RB3 LA/BSA (mm^2/m^2)	15.0 (5.71)	15.3 (5.64)	16.1 (4.58)	0.48
RB4 LA/BSA (mm^2/m^2)	8.42 (3.36)	8.65 (3.12)	9.17 (3.35)	0.37
RB5 LA/BSA (mm^2/m^2)	9.38 (3.01)	9.98 (4.41)	10.9 (2.92)	0.42* **0.02**∞ 0.29δ
RB6 LA/BSA (mm^2/m^2)	13.8 (6.51)	15.9 (13.9)	16.0 (6.95)	0.19
RB7 LA/BSA (mm^2/m^2)	8.86 (4.08)	8.07 (3.07)	9.27 (3.22)	0.19
RB8 LA/BSA (mm^2/m^2)	10.4 (3.32)	10.3 (3.05)	11.9 (3.06)	0.98* **0.02**∞ **0.02δ**
RB9 LA/BSA (mm^2/m^2)	8.83 (3.77)	8.20 (3.35)	9.96 (3.25)	0.41* 0.13∞ **0.02δ**
RB10 LA/BSA (mm^2/m^2)	11.9 (3.84)	11.84 (4.42)	13.38 (3.71)	0.07
LB1 LA/BSA (mm^2/m^2)	8.71 (3.11)	8.43 (3.34)	9.92 (3.18)	0.81* 0.07∞ **0.03δ**
LB2 LA/BSA (mm^2/m^2)	6.04 (2.67)	6.45 (3.23)	7.66 (4.16)	0.63* **0.007**∞ 0.10δ
LB3 LA/BSA (mm^2/m^2)	13.6 (4.65)	14.4 (5.50)	14.2 (4.35)	0.44
LB4 LA/BSA (mm^2/m^2)	8.26 (3.25)	8.08 (3.53)	8.77 (3.83)	0.54
LB5 LA/BSA (mm^2/m^2)	7.58 (2.80)	8.11 (3.09)	8.39 (2.27)	0.16
LB6 LA/BSA (mm^2/m^2)	17.1 (6.86)	18.6 (7.74)	20.4 (5.63)	0.32* **0.01**∞ 0.34δ
LB1+2 LA/BSA (mm^2/m^2)	13.6 (5.32)	15.3 (7.13)	15.7 (5.69)	0.08* 0.08∞ 0.94 δ
LB8 LA/BSA	12.3 (3.96)	13.5 (5.67)	15.0 (5.28)	0.16*

(mm^2/m^2)				**0.002**∞ 0.22δ
LB9 LA/BSA (mm^2/m^2)	10.7 (4.46)	10.4 (4.68)	12.7 (4.83)	0.87* **0.03**∞ **0.02δ**
LB10 LA/BSA (mm^2/m^2)	12.7 (4.40)	12.8 (4.89)	14.1 (4.33)	0.17
Right segmental mean LA/BSA (mm^2/m^2)	10.9 (2.62)	10.8 (3.57)	12.0 (2.62)	0.99* **0.04**∞ 0.06δ
Left Segmental mean LA/BSA (mm^2/m^2)	11.0 (2.79)	11.4 (3.44)	12.4 (3.52)	0.66* **0.02**∞ 0.18δ

Data expressed as mean (SD). Intergroup comparison: p value for one-way ANOVA has been presented unless the ANOVA was significant ($p<0.05$), in which case the p value has been presented for Tukey test pairwise comparisons- *asthma versus COPD, ∞asthma versus health, δCOPD versus health.

Table 3.1.6 %WA for segmental airways for subjects with asthma, COPD and healthy controls

	Asthma	COPD	Healthy	Significance P value
RB1 %WA	62.7 (3.97)	64.0 (3.54)	61.3 (3.39)	0.13* **0.03**∞ **0.001δ**
RB2 %WA	61.8 (3.41)	62.3 (3.61)	59.4 (3.65)	0.54* **<0.0001**∞ **<0.0001 δ**
RB3 %WA	60.1 (3.67)	60.4 (3.91)	57.9 (3.98)	0.79 **0.001**∞ **0.001δ**
RB4 %WA	63.3 (3.63)	63.7 (3.17)	61.7 (3.23)	0.65* **0.02**∞ **0.005δ**
RB5 %WA	62.8 (3.06)	62.6 (3.33)	60.3 (2.38)	0.93* **<0.0001**∞ **<0.0001δ**
RB6 %WA	61.4 (4.52)	61.4 (4.53)	58.1 (3.95)	1* **<0.0001**∞ **0.001δ**
RB7 %WA	64.6 (3.71)	66.0 (2.72)	63.3 (3.19)	**0.02*** 0.07∞ **<0.0001δ**
RB8 %WA	62.8 (3.45)	64.0 (3.16)	60.9 (2.89)	**0.04*** **0.001**∞ **<0.0001δ**
RB9 %WA	63.6 (3.23)	64.4 (2.93)	62.1 (3.56)	0.28* **0.01**∞ **0.001δ**
RB10 %WA	61.2 (3.30)	61.6 (3.44)	59.3 (3.43)	0.71* **0.002**∞ **0.001δ**
LB1 %WA	63.9 (2.88)	64.3 (2.84)	62.1 (2.68)	0.61* **0.001**∞ **<0.0001δ**
LB2 %WA	64.8 (3.04)	64.6 (2.98)	62.6 (4.12)	0.94* **<0.0001**∞ **0.004δ**
LB3 %WA	60.5 (3.92)	60.9 (4.42)	58.9 (3.35)	0.8* **0.04**∞ **0.02δ**
LB4 %WA	62.4 (3.65)	63.1 (3.47)	61.1 (3.73)	0.53* 0.08∞ **0.02δ**

LB5 %WA	63.6 (3.21)	63.8 (2.97)	62.1 (2.45)	0.87* **0.007**∞ **0.006δ**
LB6 %WA	59.4 (4.56)	59.0 (4.78)	56.1 (3.41)	0.85* **<0.0001**∞ **0.001δ**
LB1+2 %WA	62.1 (4.17)	61.3 (4.54)	59.8 (4.34)	0.42* **0.005**∞ 0.14 δ
LB8 %WA	62.5 (3.60)	62.1 (4.30)	59.6 (4.08)	0.73* **<0.0001**∞ **0.003δ**
LB9 %WA	63.4 (3.65)	63.3 (3.42)	61.4 (3.84)	0.96* **0.002**∞ **0.01δ**
LB10 %WA	61.1 (3.88)	61.3 (3.70)	58.7 (3.39)	0.87* **<0.0001**∞ **<0.0001δ**
Right Segmental mean %WA	62.50 (2.29)	62.9 (2.45)	60.4 (2.24)	0.53* **<0.0001**∞ **<0.0001δ**
Left Segmental mean %WA	62.42 (2.34)	62.4 (2.36)	60.3 (2.28)	0.98* **<0.0001**∞ **<0.0001δ**

Data expressed as mean (SD). Intergroup comparison: p value for one-way ANOVA has been presented unless the ANOVA was significant ($p<0.05$), in which case the p value has been presented for Tukey test pairwise comparisons- *asthma versus COPD, ∞asthma versus health, δCOPD versus health

Table 3.1.7 Correlations between clinical outcomes and QCT parameters

	Post-bronchodilator FEV_1 (% predicted) Asthma	**Post-bronchodilator FEV_1 (% predicted) COPD**	**Post-bronchodilator FEV_1/FVC (%) Asthma**	**Post-bronchodilator FEV_1/FVC (%) COPD**
Mean LA/BSA (mm^2/m^2)	.324**	.241*	.218**	.082
Mean TA/BSA (mm^2/m^2)	.287**	.238*	.171*	.084
Mean WA/BSA (mm^2/m^2)	.247**	.226*	.126	.083
Mean %WA	-.417**	-.248*	-.343**	-.121
$MLD_{E/I}$	-.303**	-.697**	-.402**	-.729**
Perc15 (HU)	.178*	.434**	.408**	.554**
LAC-D-950	.190*	.180	.234**	.245*

Pearson's correlation coefficient, * p value <0.05, ** p value<0.005.

Table 3.1.8 Correlations between clinical outcomes and QCT parameters for asthma (upper value) and COPD (lower value)

Asthma COPD	RV/TLC	KCO % predicted	Sputum eosinophils (%)#	Sputum neutrophils (%)#	Blood eosinophils #	Blood neutrophils #	AQLQ#	ACQ6#	SGRQ total#
Mean LA/BSA (mm^2/m^2)	-.147 -.171	-.163 -.089	.031 .121	-.134 .036	-.019 -.033	-.183* -.11	.113 n/a	-.152* n/a	n/a -.034
Mean TA/BSA (mm^2/m^2)	-.115 -.198	-.153 -.084	.044 .096	-.120 .042	-.011 -.033	-.188* -.101	.135 n/a	-.164* n/a	n/a -.040
Mean WA/BSA (mm^2/m^2)	-.078 -.217	-.153 -.075	.064 .086	-.119 .064	-.003 -.027	-.196* -.28	.171* n/a	-.179* n/a	n/a -.059
Mean %WA	.237** .130	.201* .053	-.003 -.236	.094 -.026	.037 -.07	.128 .216	-.049 n/a	.120 n/a	n/a .010
$MLD_{E/I}$	.481** .510**	-.146 -.466**	.026 -.170	.119 .185	.081 .033	.053 .135	-.015 n/a	.030 n/a	n/a .230*
Perc15 (HU)	-.196* -.271*	-.013 .477**	-.239* .047	.084 .005	-.007 .142	.022 -.018	-.028 n/a	.001 n/a	n/a .108
LAC-D-950	-.162 -.170	.083 .484**	.272** .181	-.083 .193	0.156 .025	-.074 -.021	.006 n/a	.024 n/a	n/a -.061

Pearson's correlation coefficient for parametric data, otherwise # Spearman's correlation coefficient for non-parametric data.

*p value <0.05, ** p value<0.005, n/a- not applicable

Table 3.1.9 Multiple regression to determine the strongest independent QCT parameters of post-bronchodilator FEV1% predicted

Dependent variable post-bronchodilator FEV_1% predicted

		Model R^2	**B**	**Std. Error**	**Beta**	**Significance (p value)**
Asthma	%WA	0.254	-3.771	0.778	-0.344	**0.000003**
	$MLD_{E/I}$		-108.021	28.283	-0.271	**0.000194**
	Perc15 (HU)		0.190	0.074	.0181	**0.01**
COPD	%WA	0.542	-1.447	0.644	-0.185	**0.03**
	$MLD_{E/I}$		-283.191	42.260	-0.607	**5 E-9**
	Perc15 (HU)		0.151	0.079	0.173	0.06

Table 3.1.10 Correlations between QCT parameters and clinical outcomes in asthma (upper panel) and COPD subjects (lower value) with FEV1 % predicted <80%

Asthma n=70 COPD n=77	**Post-BD FEV_1 (% pred)**	**Post BD FEV/FVC (%)**	**RV/TLC**	**KCO % pred**	**Sputum eso%#**	**Sputum neut#**	**Blood eso#**	**Blood neut#**	**AQLQ #**	**ACQ6#**	**SGRQ#**
Mean LA/BSA (mm^2/m^2)	-.072 .158	-.085 .043	.049 -.106	.001 -.120	.189 .043	.093 .047	.143 -.033	.134 -.096	.085 n/a	-.153 n/a	n/a -.035
Mean TA/BSA (mm^2/m^2)	-.069 .164	-.110 .044	.082 -.145	-.007 -.129	.203 .021	.092 .042	.152 -.045	-.140 -.087	.112 n/a	-.170 n/a	n/a -.052
Mean WA/BSA (mm^2/m^2)	.070 .164	-.137 .045	.118 -.179	-.007 -.134	.215 .024	.110 .052	.169 -.044	-.160 -.02	.172 n/a	-.208 n/a	n/a -.079
Mean %WA	.020 -.161	-.060 -.094	.057 .046	.064 .043	-.190 -.153	-.053 -.083	-.101 .048	-.103 .186	.068 n/a	.004 n/a	n/a -.026
$MLD_{E/I}$	-.455** -.657**	-.558** -.714**	.611** .482**	-.254 -.422**	.138 -.135	.030 .244	.066 .040	-.158 .187	.168 n/a	-.183 n/a	n/a .254*
Perc15 (HU)	.233 .458**	.493** .559**	-.242 -.264*	-.021 .467**	-.042 .031	.057 -.035	.098 .132	-.118 -.025	-.157 n/a	.089 n/a	N/A .074
Mean fractal -950	-.007 -.239*	-.149 -.248*	.048 .202	-.019 -.481**	.216 -.258	.038 .289*	.183 .077	-.02 -.002	.326** n/a	-.340** n/a	N/A .133

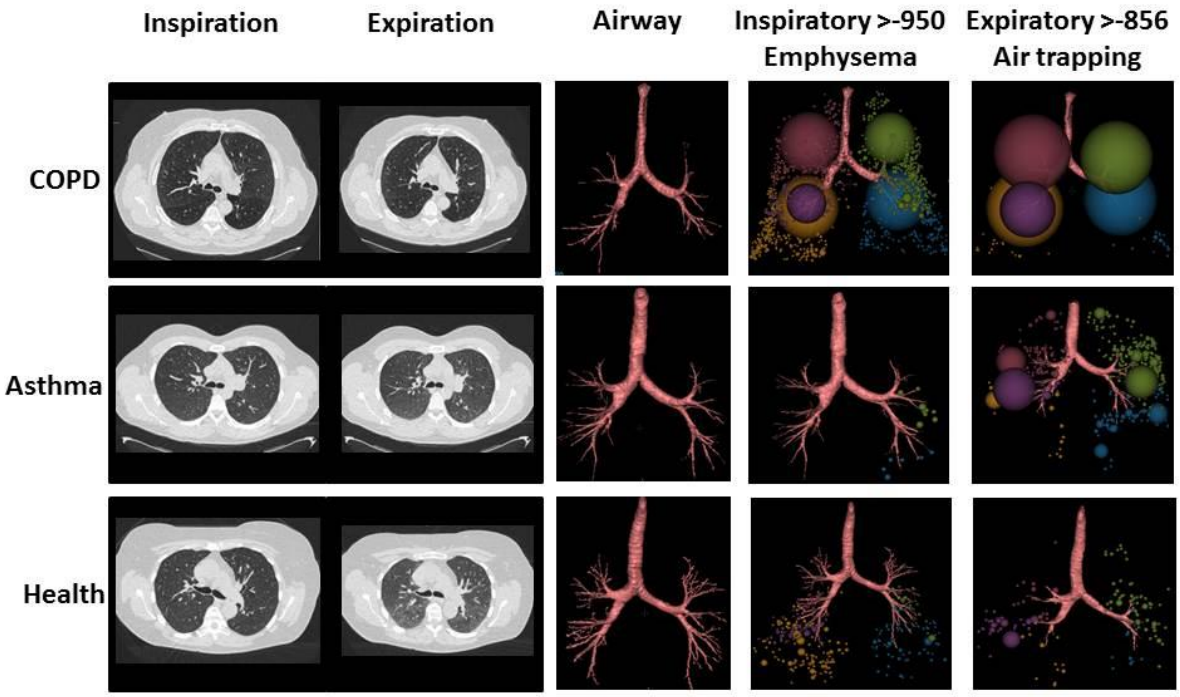
Pearson's correlation coefficient for parametric data, otherwise # Spearman's correlation coefficient for non-parametric data.

*p value <0.05, ** p value<0.005, n/a- not applicable

Table 3.1.11 Multiple regression to determine the strongest independent QCT parameters of FEV1% predicted in those subjects with FEV1 % predicted <80%

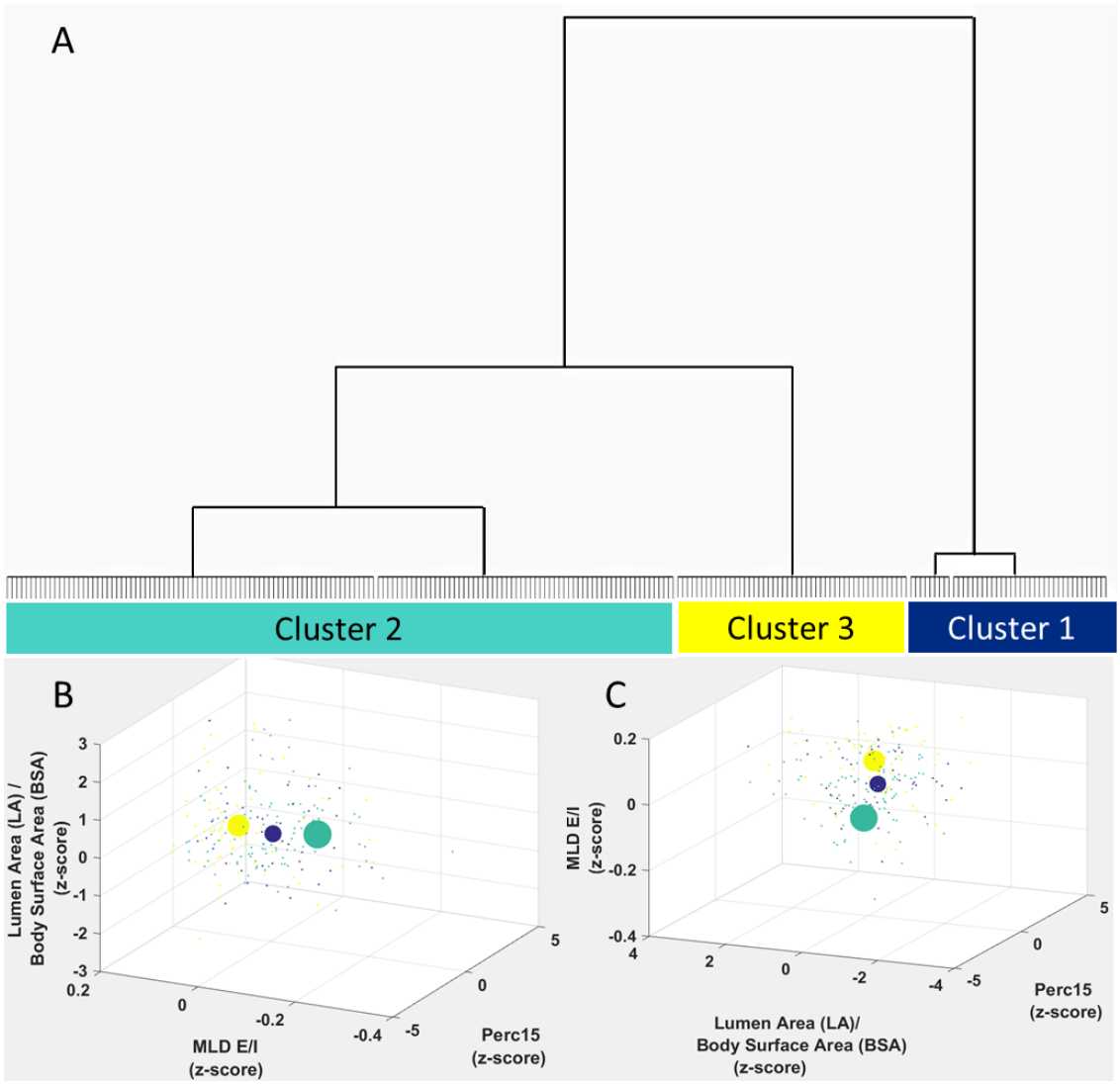
		Model R^2	B	Std. Error	Beta	Significance p value
COPD	$MLD_{E/I}$	.473	-241	41.5	-.567	0.001
	Perc15 (HU)		.165	.073	.221	0.03

Figure 3.1.1 Visual representation of QCT parameters



Visual representation of QCT parameters with CT inspiratory and expiratory axial slices (first 2 columns); illustrate qualitative differences of increased inspiratory volume, emphysema and air trapping in disease versus healthy controls. Column three shows the airways grown by the post processing software, which are reduced in disease. The fourth column shows the Low Attenuating Clusters (LAC) below -950 HU in inspiration, representing areas of emphysema and the final column shows the LAC below -856 in expiration, representing areas of air trapping.

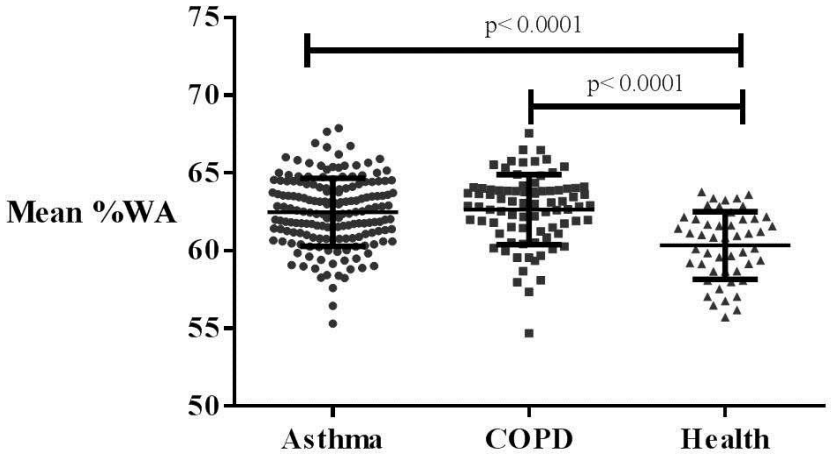
Figure 3.1.2 Cluster dendrogram



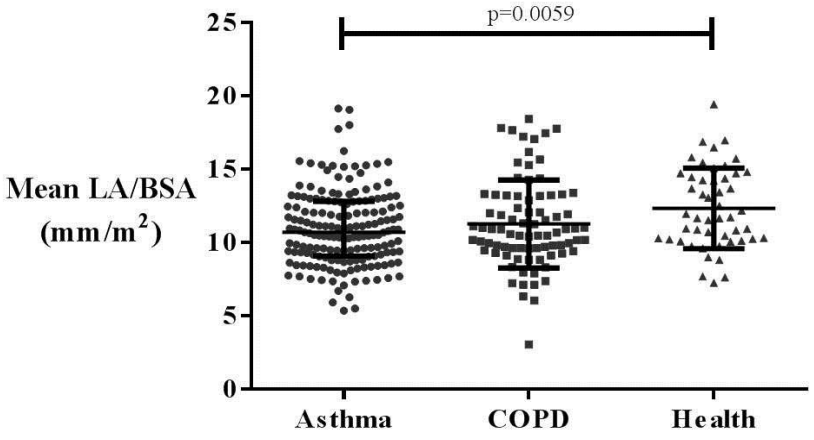
a) The dendrogram to which the number of clusters was determined, k=3, b) and c) two representation s of the clusters on z-scores of three QCT variables, Mean Lung Density Expiratory /Inspiratory (MLD E/I), Percentile 15 and Lumen Area (LA) / Body Surface Area (BSA). Small dots represent individual patients and the large spheres represent the sample sizes of the clusters centred on their multivariate cluster means.

Figure 3.1.3 A&B: Percentage Wall Area and Mean Lumen Area/Body Surface Area

A



B



Dot-plots of airway morphometry QCT parameters for all the subjects with asthma, COPD and healthy controls, these parameters represent changes in proximal airway structure:

a) mean %WA, this shows a significant difference between the healthy cohort and both asthma and COPD. But not between asthma and COPD. b) mean LA/BSA, this shows a significant difference between asthmatics and healthy controls only

Bars and lines represent mean and standard error of the mean.

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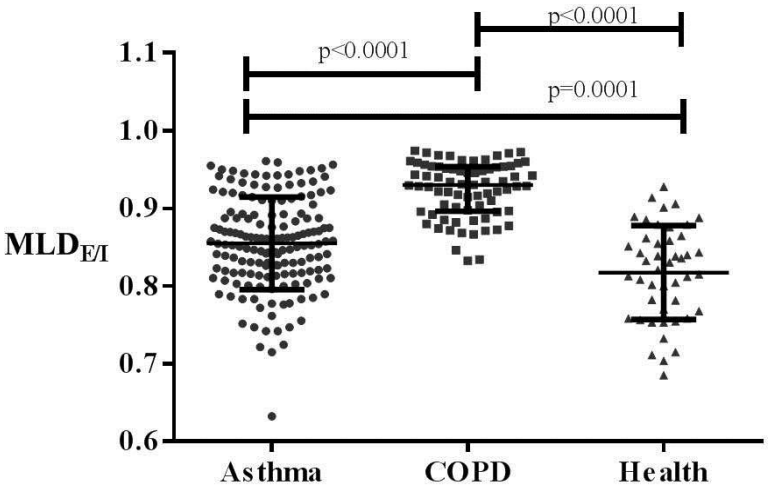
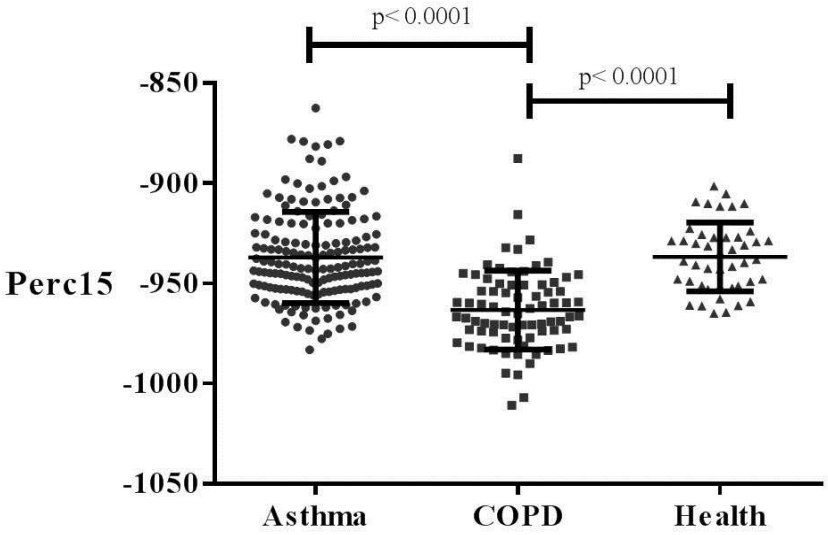
Figure 3.1.3 C&D: Mean Lung Density Expiratory to Inspiratory ratio and Percentile 15

C



D



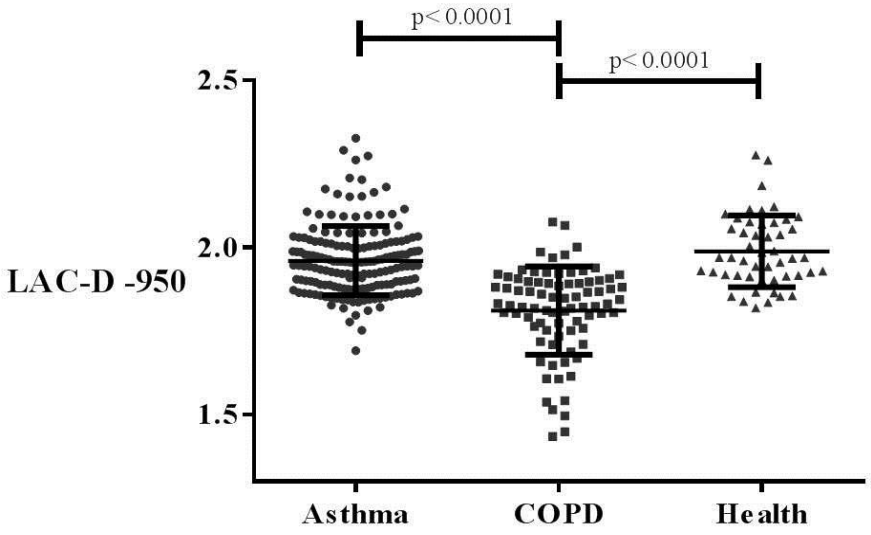
Dot-plots of densitometry QCT parameters for all the subjects with asthma, COPD and healthy controls, $MLD_{E/I}$ represents air trapping/small airway disease and percentile 15 represents emphysema: c) $MLD_{E/I}$ this shows significant differences between all three

groups. d) densitometry- Percentile 15, shows no difference between asthmatics and heatlhy controls, but significant differences between COPD and both asthma and healthy controls.

Bars and lines represent mean and standard error of the mean.

Figure 3.1.3 E: Fractal Dimensions of Low Attenuation Areas below -950

E



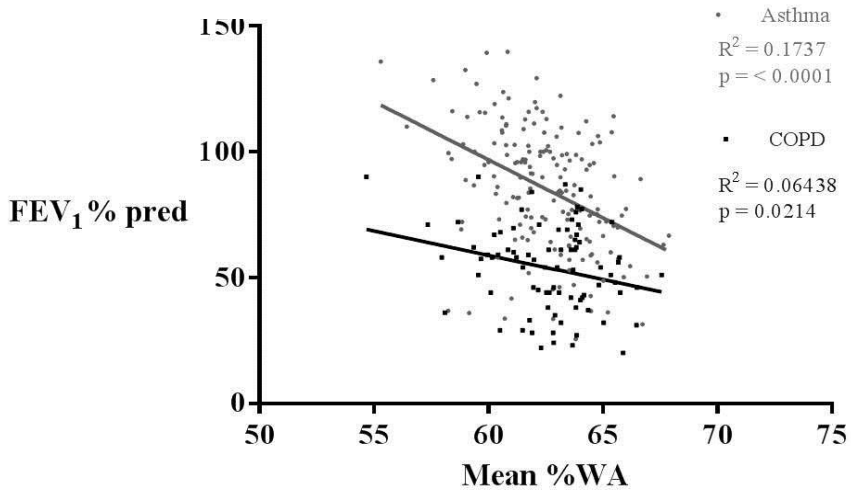
Dot-plots of fractal dimensions of low attenuation areas below -950 HU, this represents the complexity of the low attenuation areas QCT parameters for all the subjects with asthma, COPD and healthy controls:

e) fractal index- LAC-D -950

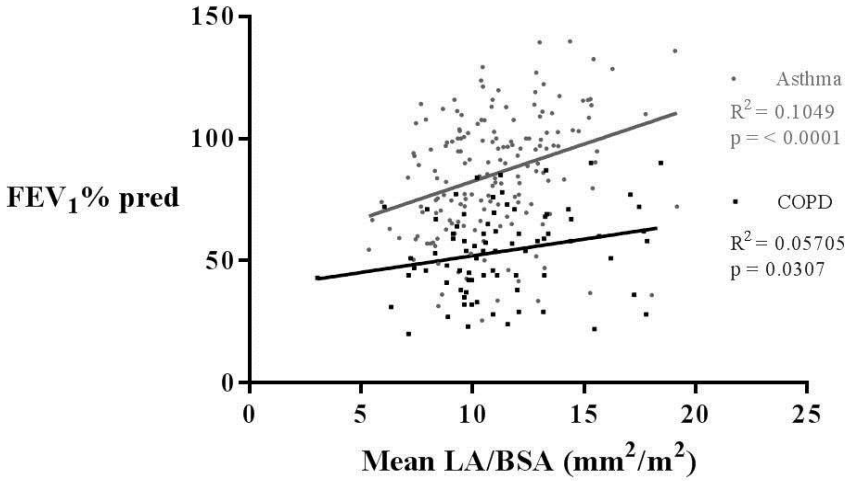
Bars and lines represent mean and standard error of the mean.

Figure 3.1.4 A&B: Percentage WA and LA/BSA

A



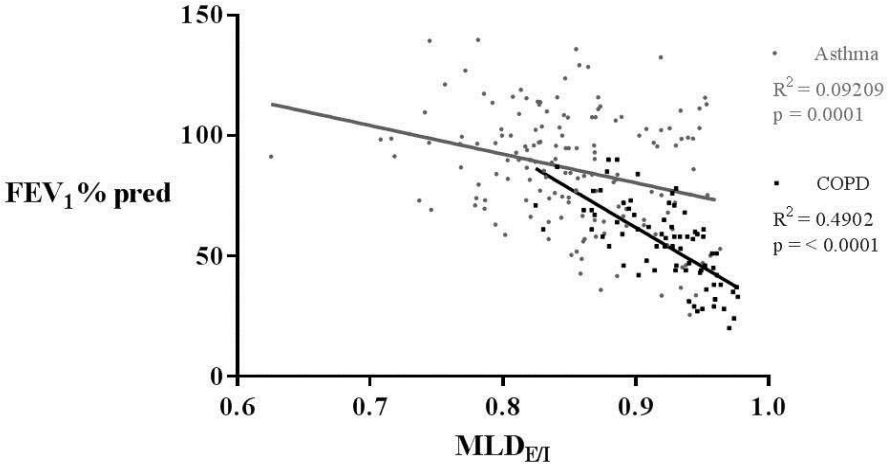
B



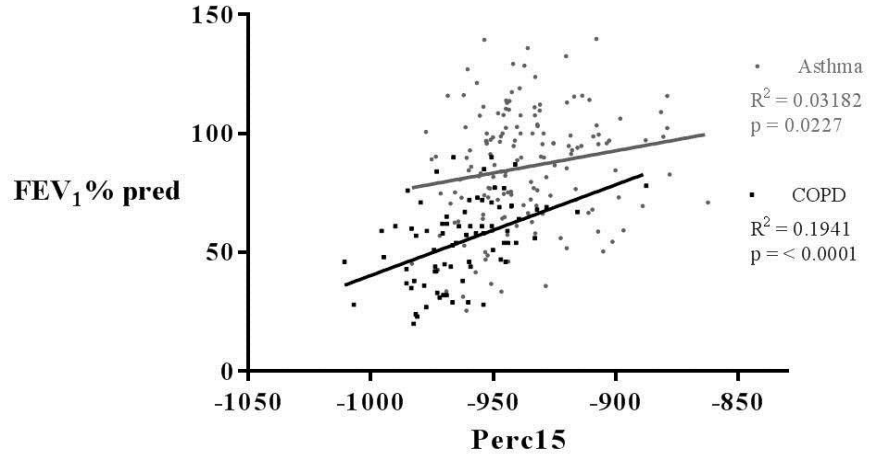
Scatter plot and linear regression of the subjects with asthma (grey circles) and COPD (black squares) showing the relationship between FEV_1 % predicted and the QCT morphometry measures: a) mean %WA, b) mean LA/BSA.

Figure 3.1.4 C&D: $MLD_{E/I}$ and Percentile 15

C



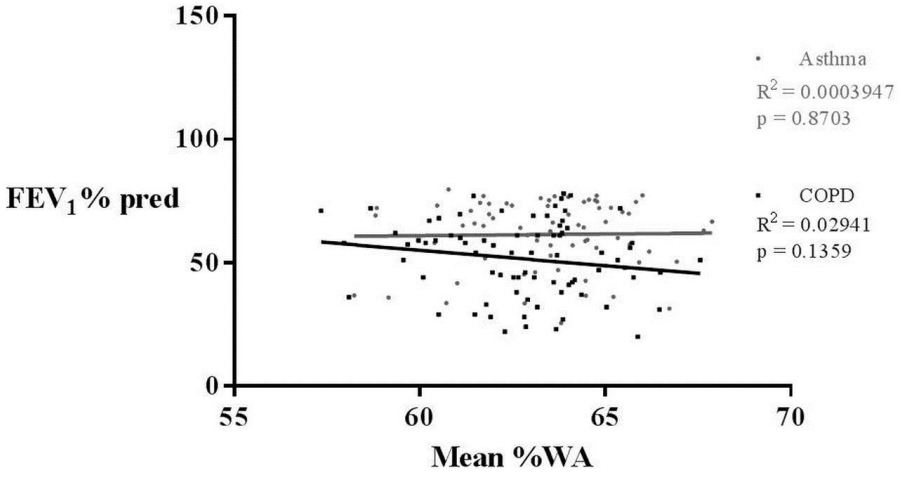
D



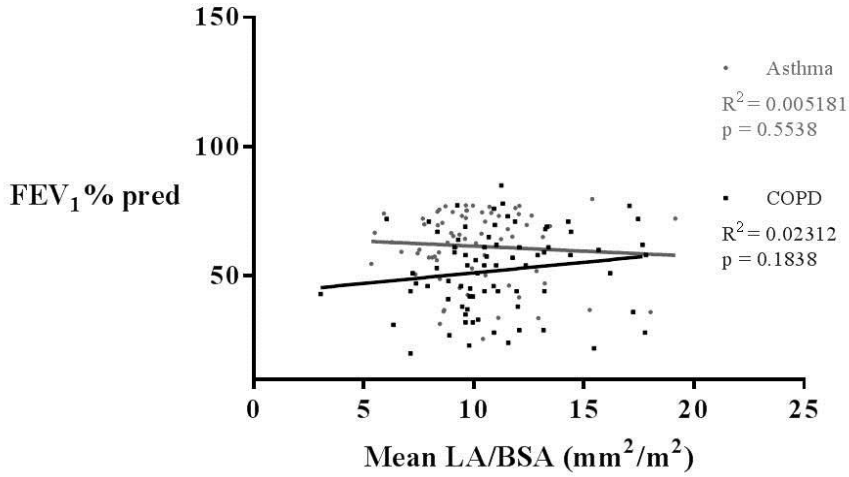
Scatter plot and linear regression of the subjects with asthma (grey circles) and COPD (black squares) showing the relationship between FEV_1 % predicted and the QCT densitometry measures: c) $MLD_{E/I}$ and d) Percentile 15.

Figure 3.1.5 A&B: Percentage WA and LA/BSA

A



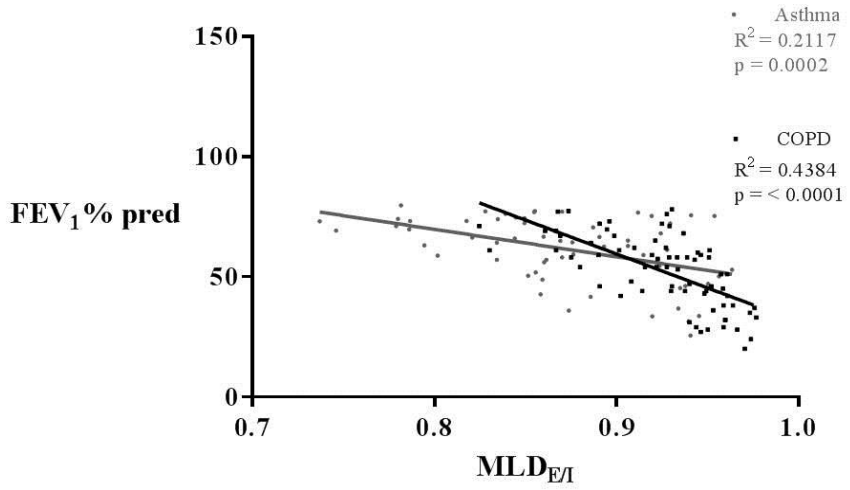
B



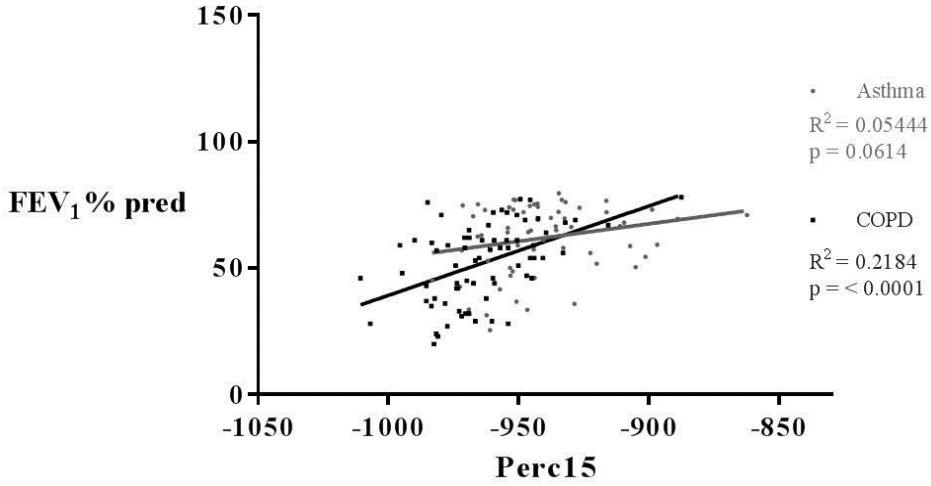
Scatter plot and linear regression of the subjects with an FEV_1 % predicted <80% with asthma (grey circles) and COPD (black squares) showing the relationship between FEV_1 % predicted and the QCT morphometry and densitometry measures: a) mean %WA, b) mean LA/BSA.

Figure 3.1.5 C&D: Percentage WA and LA/BSA

C



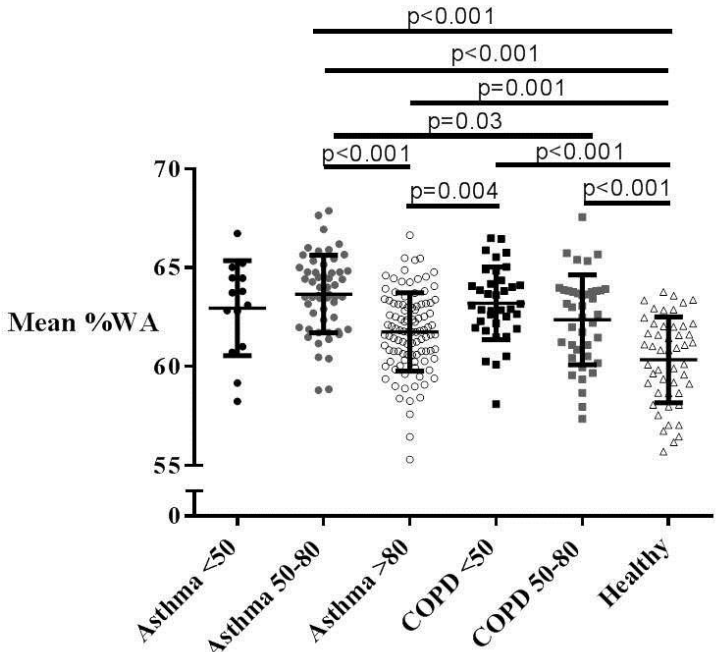
D



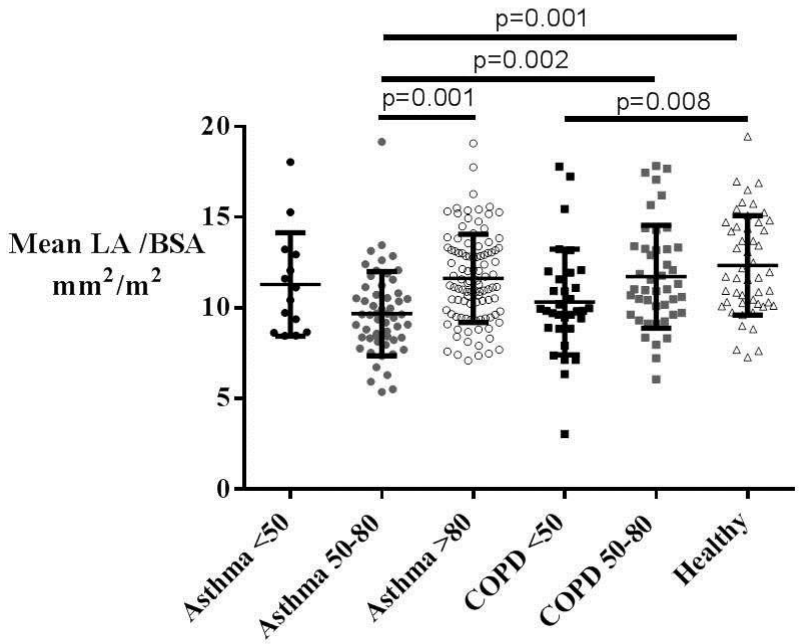
Scatter plot and linear regression of the subjects with an FEV_1 % predicted <80% with asthma (grey circles) and COPD (black squares) showing the relationship between FEV_1 % predicted and the QCT morphometry and densitometry measures: c) $MLD_{E/I}$ d) Percentile 15.

Figure 3.1.6 A&B: Percentage WA and LA/BSA

A



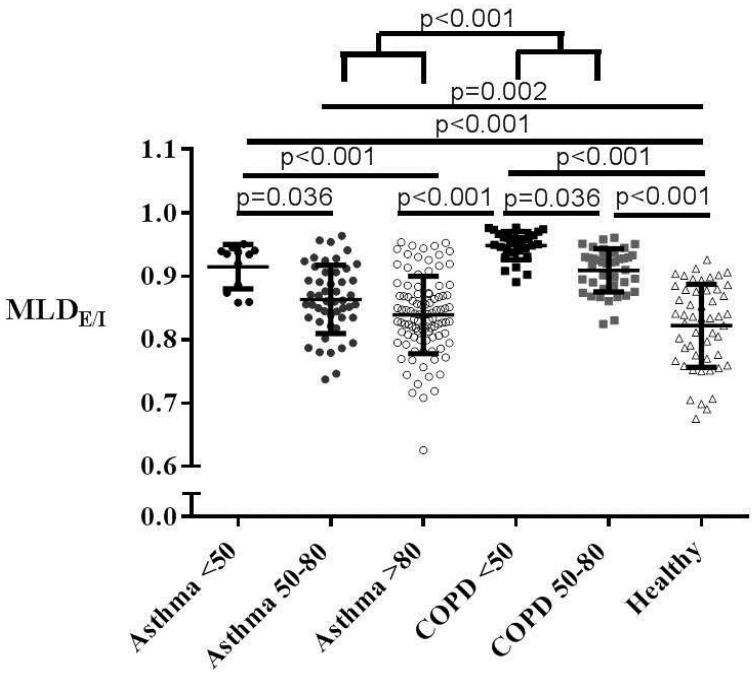
B



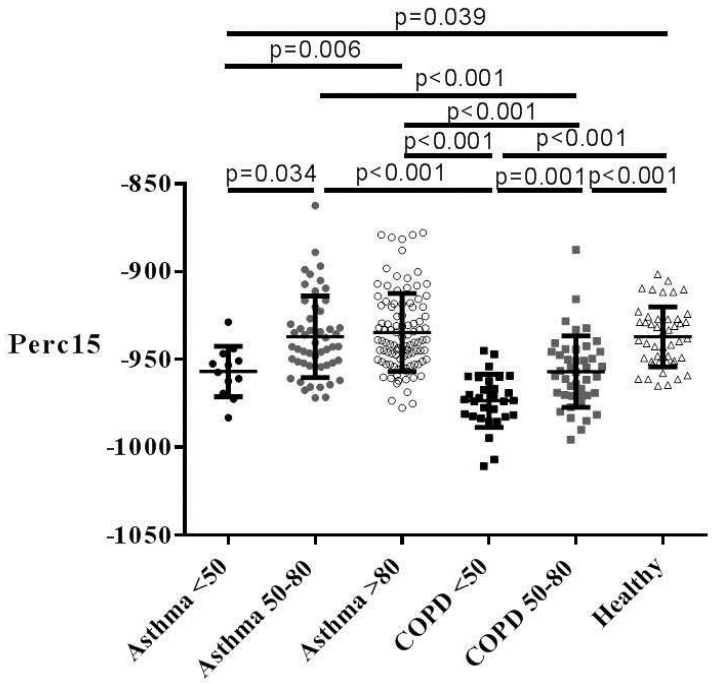
Dot-plots of airway morphometry and densitometry QCT parameters for subjects with asthma (FEV_1 % predicted <50% black circles, 50-80% grey circles, and >80% open circles), COPD (FEV_1 % predicted <50% black squares, 50-80% grey squares) and healthy controls (open triangles): a) mean %WA, b) mean LA/BSA.

Figure 3.1.6: C&D: $MLD_{E/I}$ and Percentile 15

C



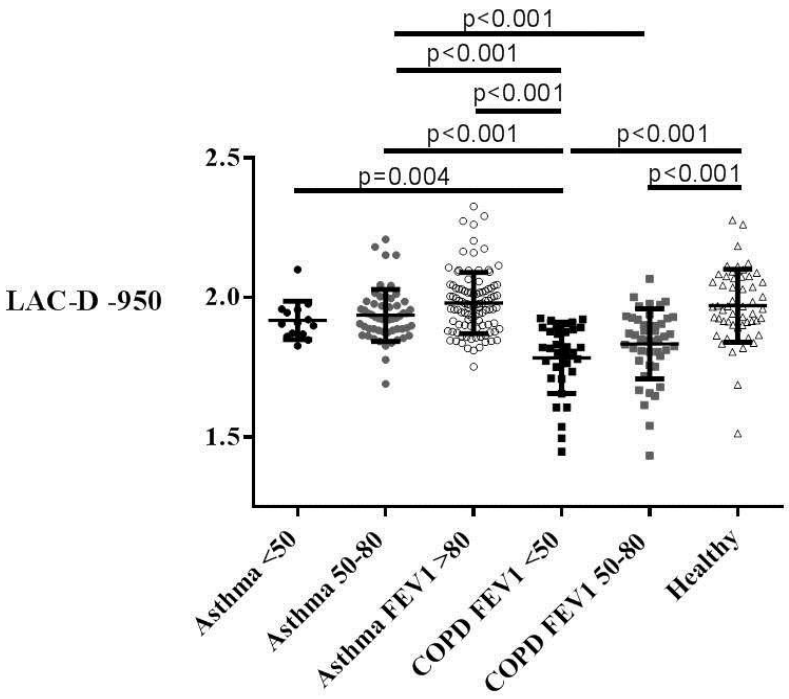
D



Dot-plots of airway morphometry and densitometry QCT parameters for subjects with asthma (FEV_1 % predicted <50% black circles, 50-80% grey circles, and >80% open circles), COPD (FEV_1 % predicted <50% black squares, 50-80% grey squares) and healthy controls (open triangles): c) $MLD_{E/I}$ d) Percentile 15

Figure 3.1.6 E: LAC-D -950

E



Dot-plots of airway morphometry and densitometry QCT parameters for subjects with asthma (FEV_1 % predicted <50% black circles, 50-80% grey circles, and >80% open circles), COPD (FEV_1 % predicted <50% black squares, 50-80% grey squares) and healthy controls (open triangles): e) fractal index- LAC-D -950.

3.2 STUDY 2:
Associations in asthma between quantitative computed tomography and bronchial biopsy-derived airway remodelling

3.2.1 Abstract

3.2.1.1 Background

In asthma the association between quantitative thoracic CT (QCT) and bronchial biopsy-derived airway remodelling is poorly understood. The aim of this study is to determine the relationship between QCT morphometry and densitometry with airway wall structure in bronchial biopsies.

3.2.1.2 Methods

Subjects were recruited from a single centre (n=70) and the bronchial biopsy remodelling features that were the strongest predictors of lung function impairment and QCT-derived proximal airway morphometry (luminal area, wall area and % wall area) and air-trapping (mean lung density expiratory/inspiratory) were determined by step-wise multiple regression. The best predictor of air-trapping was validated in an independent replication group of asthmatics (n=24) from a second centre

3.2.1.3 Measurements and main results

Airway smooth muscle % was the only independent predictor of post-bronchodilator FEV_1 % predicted (R^2=0.22; p=0.001), while both airway smooth muscle % and vascularity (Chalkley count) were predictors of FEV_1/FVC (R^2=0.19; p=0.005 and R^2=0.09; p=0.035 respectively). Epithelial thickness and airway smooth muscle % were predictors of mean segmental bronchial luminal area (R^2=0.12; p=0.02 and R^2=0.12; p=0.015) and wall area (R^2=0.10; p=0.033 and R^2=0.10; p=0.032). Whereas epithelial thickness was the only significant predictor of % wall area (R^2=0.13; p=0.018). Vascularity was the only significant predictor of air-trapping (mean lung density expiratory/inspiratory) (R^2=0.24; p=0.001), which was validated in the replication group (R^2=0.19; p=0.031).

3.2.1.4 Conclusions

In asthma, airway smooth muscle content and vascularity were both associated with airflow obstruction. Proximal airway morphometry by QCT was most strongly associated with epithelial thickness and airway smooth muscle content in a bronchial biopsy, whereas air-trapping was related to vascularity.

3.2.2 Introduction

Asthma remains an important health problem with significant morbidity, mortality and economic burden (7,225). In addition to symptoms, asthma is characterized by variable airflow obstruction, airway inflammation and remodelling (3,7). Airway remodelling is a collective term for the structural changes in the airway wall including epithelial thickness and integrity, airway smooth muscle mass, neoangiogenesis and subepithelial fibrosis (3,7,226) and is related to persistent airflow limitation and airflow obstruction (174,226,227). It is a feature even in childhood disease (228) demonstrating that it can occur early in disease and post-mortem studies of asthma deaths demonstrate airway remodelling in the large and small airways (229,230).

Airway remodelling can be assessed non-invasively by quantitative computed tomography (QCT). This has become an established technique to determine airway morphometry and lung densitometry in asthma (24,25,34,207,208,214,231-234). This approach allows for quantification of proximal airway remodelling by assessment of airway geometry and air-trapping as an indirect measure of small airway disease. QCT in asthma has revealed that the key features of airway remodelling including luminal narrowing, wall thickening and moreover air-trapping are important determinants of airflow obstruction. Some studies have begun to explore the associations between proximal airway geometry and histological features of airway remodelling (25,207,208). However, asthma is a heterogeneous condition with considerable variability in the degree of disordered airway physiology, and the relative changes in airway wall composition and QCT parameters. Thus, these structure-function relationships in asthma remain poorly understood.

Our hypothesis was that airway remodelling determined in bronchial biopsies is associated with i) lung function impairment (post-bronchodilator FEV_1 % predicted) and ii) QCT morphometry and densitometry measures of proximal airway remodelling and air-trapping. The co-primary QCT outcome variables were for proximal airway remodelling: mean airway lumen area / body surface area and wall area % and for air-trapping: mean lung density expiratory to inspiratory ratio. To test our hypothesis we undertook a single-center observational study across the spectrum of disease severity to determine the strongest independent histological features in bronchial biopsies associated with lung function and QCT parameters of airway remodelling. The best immunohistological predictor of air-trapping was validated in an independent replication group of asthmatics from a second center.

3.2.3 Methods

3.2.3.1 Subjects

Subjects were recruited into either test (n=70) or replication (n=24) groups at two independent centres Glenfield Hospital, Leicester, UK and Washington University School of Medicine, St Louis, MO, USA respectively. All subjects were non-smokers with <10 pack-years. All included subjects fulfilled the criteria for the diagnosis of asthma which was defined as: a physician diagnosis of asthma with objective evidence of variable airflow obstruction as indicated by 1 or more of the following: (1) a positive methacholine challenge test defined as a concentration of nebulized methacholine causing a 20% drop in FEV_1 of <8 mg/mL, (2) diurnal maximum peak flow variability of >20% over 2 week time, and (3) improvement of >15% in FEV_1 15 minutes after bronchodilator therapy. Subjects underwent pre- and post-bronchodilator spirometry (albuterol 400mcg), skin prick tests or allergen specific IgE to assess for atopy and those in the test group also underwent sputum induction and processing as described in Methods sections 2.1.1.5 and 2.2.1 respectively.

Persistent airflow limitation was defined as a post-bronchodilator therapy FEV_1<80% predicted. Written informed consent was obtained from all the participants. All the assessments and tests included in this study were approved by the local research ethics committee (The Leicestershire, Northamptonshire, and Rutland Research Ethics Committee and the Washington University School of Medicine Institutional Review Board).

3.2.3.2 Computed tomography

All subjects underwent either limited or full lung CT scans using standardised acquisition protocols as described in Methods section 2.3.2. Limited only scans (Methods section 2.3.1.3) were undertaken in 14 asthmatics (34,231). Scans were analysed using semi-automated software, Apollo® (by VIDA Diagnostics, Inc) in the test group and Pulmonary Workstation, version 2.0 in the replication group (VIDA Diagnostics, Iowa).

In the test group all inspiratory scans were analysed for RB1 morphometry while mean segmental bronchi morphometry was obtained in full lung scans only. 1^{st}-5^{th} generation airways were labelled and measured using the analysis software. Morphological QCT parameters measured included LA/BSA, TA/BSA, WA/BSA and %WA, as described in Introduction section 1.4.4 and Methods section 2.3.2. Estimates of air-trapping were determined in the test and replication groups from the mean lung density on the expiratory/inspiratory scan ($MLD_{E/I}$) and the percentage of lung voxels with a density lower than -856 HU on expiratory scans (VI-856 HU). The co-primary QCT outcome variables were for proximal airway remodelling: mean airway lumen area / body surface area and wall area % and for air-trapping $MLD_{E/I}$.

3.2.3.3 Endobronchial biopsies

Endobronchial biopsies were obtained from segmental and subsegmental carina and either embedded in glycol methacrylate for the test group or paraffin in the replication group as described previously (19,20,25,174).

Two micrometre sections were cut from the glycol methacrylate embedded biopsies and stained with Haematoxylin & Eosin (H&E). Immunohistochemical staining was done with the following mAbs: anti–mast cell tryptase clone AA1 (Dako UK, Ely, United Kingdom), anti-alpha smooth muscle actin clone 1A4 (Dako UK, Ely, United Kingdom), anti-eosinophil major basic protein clone BMK-13 (Monosan, Uden, The Netherlands), anti-neutrophil elastase clone NP57 (Dako UK, Ely, United Kingdom), and anti-endothelium clone EN4 (Monosan, Uden, The Netherlands) or appropriate isotype controls were used.

The endobronchial biopsies were assessed by a single observer blinded to the clinical characteristics (ZEN 2012 image analysis software for light microscopy, Carl Zeiss AG, Jena, Germany) and expressed as the mean of measurements undertaken from a minimum of two sections either from independent biopsies or as non-contiguous tissue sections at least 20μm apart from the same biopsy. Epithelial integrity was assessed by measuring the lengths of intact and denuded epithelium. Lamina reticularis and reticular basement membrane (RBM) and epithelial thickness were measured as described previously (172,173). Vascularity was measured using the Chalkley count, a surrogate of both vessel density and vascular area. As previously described, a Chalkley eyepiece graticule (NG52 Chalkley Point Array, Pyser-SGI Ltd, Edenbridge, UK) was used at x200 to measure Chalkley counts in four non-overlapping vascular hotspots (1-2/section) (174). The mean Chalkley count was calculated from the four measurements. Airway smooth muscle content was determined as the proportion of the total area. Inflammatory cells were expressed as the number of nucleated cells per area of lamina propria.

The strongest independent immunohistological feature of airway remodelling associated with QCT-derived air-trapping identified in the test group was validated in the replication group. Four micrometre sections were cut from the paraffin embedded biopsies and stained with appropriate mAb or corresponding isotype control.

3.2.3.4 Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, California, USA, www.graphpad.com) and IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp, Released 2013, Armonk, NY). Parametric data was expressed as mean (standard deviation, SD) and non-parametric data as median (interquartile range, IQR). Groups were compared using unpaired student t-test and Mann-Whitney U test for parametric and non-parametric data respectively. Proportions were compared using chi-squared test (χ^2). Correlations between variables were expressed using Pearson's correlation. A step-wise multiple regression analysis was undertaken to determine the bronchial biopsy features that were the strongest predictors of post-bronchodilator FEV_1, FEV_1/FVC, and QCT-derived mean segmental bronchial morphometry and air-trapping. Regression data are presented as model-adjusted R^2 Pearson correlations alongside the standardized regression coefficient (β) of the modelled independent variable. A *p*-value of <0.05 was considered statistically significant. An *a priori* decision was made to not test the exploratory outcomes for multiplicity

3.2.4 Results

Baseline demographics and clinical characteristics of subjects with (n=30) and without persistent airflow limitation (post-bronchodilator FEV_1 <80% and ≥80% predicted) (n=40) are shown in Table 3.2.1. There was no difference between the two groups in sex, age, duration of asthma, age of disease onset, smoking status, BMI, sputum eosinophils or sputum neutrophils.

3.2.4.1 Airway inflammation and remodelling univariate correlation with lung function

Subjects with versus those without persistent airflow limitation had significantly higher airway smooth muscle % (33.5 [15.6] versus 20.1 [12.6]%; p<0.001) and increased vascularity (mean Chalkley count) (6.2 [1.6] versus 5.0 [1.9]; p=0.017) as shown in Table 3.2.1 and Figure 3.2.1. However, there was no difference between the two groups in the other measured markers of airway remodelling or inflammation. Airway smooth muscle % was inversely correlated with post-bronchodilator FEV_1 % predicted (r=-0.49; p<0.001) and post-bronchodilator FEV_1/FVC (r=-0.44; p<0.001) as shown in Figure 3.2. Vascularity was also inversely correlated, with post-bronchodilator FEV_1 % predicted (r=-0.3; p=0.026) and post-bronchodilator FEV_1/FVC (r=-0.35; p=0.008). There was no significant correlation between airway inflammation or the other airway remodelling markers in bronchial biopsies and spirometry measurements (Table 3.2.2).

3.2.4.2 CT-derived quantitative morphometry and densitometry univariate correlation with lung function

Subjects with versus those without persistent airflow limitation had significantly narrower mean segmental bronchial luminal areas (9.7 (2.2) versus 11.0 (2.3) mm^2/m^2; p=0.047) and larger mean segmental bronchial wall area % (63.6 (2.0) versus 62.5 (2.1) %; p=0.039) (Table 3.2.3). These differences were more marked in the lower versus upper lobe bronchi (Table 3.2.4). There was significantly more air-trapping in those with versus without persistent airflow limitation as measured by $MLD_{E/I}$ (0.89 [0.05] versus 0.83 [0.05]; p<0.001), and VI-856 HU (%) (32.2 [19.8] versus 15.5 [10.1]; p<0.001) (Table 3.2.3).

3.2.4.3 Univariate correlations between bronchial biopsy airway remodelling and QCT morphometry and air-trapping

Epithelial thickness was significantly correlated with mean segmental bronchial luminal area (r=-0.35; p=0.02), mean segmental bronchial wall area (r=-0.31; p=0.039) and mean segmental bronchial wall area % (r=0.35; p=0.018) (Figure 3.3). Similarly, airway smooth muscle % correlated significantly with mean segmental bronchial luminal area (r=-0.35; p=0.008), mean segmental bronchial wall area (r=-0.32; p=0.015) and mean segmental bronchial wall area % (r=0.27; p=0.045). All the other remodelling and inflammatory markers including vascularity, RBM and submucosal inflammatory cell counts did not have any significant correlation with morphometry indices (Table 3.2.4).

Vascularity was strongly correlated with measures of air-trapping $MLD_{E/I}$ (r=0.49; p<0.001) and VI-856 HU (r=0.53; p<0.001). Airway smooth muscle % was also correlated with $MLD_{E/I}$ (r=0.3; p=0.03) and VI-856 HU (r=0.55; p<0.001) (Figure 3.4).

3.2.4.4 Multivariate analysis of the association between bronchial biopsy immunohistology, lung function and QCT parameters

All airway remodelling and inflammation variables were included in a step-wise multiple regression analysis to examine the predictors of persistent airflow limitation, QCT segmental morphometry and air-trapping. Only airway smooth muscle % was an independent predictor of post-bronchodilator FEV_1 % predicted (R^2=0.24, β=-0.49, p=0.001), while both airway smooth muscle % and vascularity were significant predictors of post-bronchodilator FEV_1/FVC (R^2=0.19, β=-0.40, p=0.003 and R^2=0.09, β=-0.31, p=0.026) respectively). Epithelial thickness and airway smooth muscle % were predictors of mean segmental bronchial luminal area (R^2=0.12, β=-035, p=0.02 and R^2=0.12, β=-0.35, p=0.015), and wall area (R^2=0.10, β=-0.32, p=0.033 and R^2= 0.10, β=0.31, p=0.032). Epithelial thickness was the only independent predictor of mean segmental bronchial wall area % (R^2=0.13, β=0.35, p=0.018). Vascularity was the only predictor of $MLD_{E/I}$ (R^2=0.24, β=0.49, p=0.001), while airway smooth muscle %, vascularity and epithelial thickness all significantly contributed to a model predicting VI-856 HU (R^2=0.31, β=0.49, p<0.001; R^2=0.22, β=0.54, p<0.001 and R^2=0.05; β=0.24, p=0.045 respectively).

3.2.4.5 Validation group: replication of the correlation between vascularity and air-trapping

Vascularity in the bronchial biopsies was the strongest independent predictor of $MLD_{E/I}$. Therefore the relationship between vascularity and $MLD_{E/I}$ was measured in an independent group of asthmatics (n=24). Baseline demographics and clinical characteristics of subjects in the validation group are described in Table 3.2.5. Similar to the primary study group, vascularity was positively correlated with $MLD_{E/I}$ (r=0.44; p=0.031) as well as VI-856 HU (r=0.50; p=0.014) (Figure 3.5).

3.2.5 Discussion

We report here the associations in asthma between bronchial biopsy-derived features of airway inflammation and remodelling with lung function and QCT parameters of proximal airway morphometry and air-trapping. We found that neither airway inflammation nor RBM thickening were related to lung function and QCT parameters. However, airway smooth muscle % and vascularity were both associated with airflow obstruction. Proximal airway morphometry was most strongly associated with epithelial thickness and airway smooth muscle % and air-trapping was related to vascularity. This is the first study to suggest a relationship between airway vascularity and air-trapping. However, we are confident that this observation is robust as we were able to confirm this finding in an independent replication group.

Previous studies have explored the relationship between bronchial biopsy features of remodelling and both FEV_1 % predicted and FEV_1/FVC (reviewed in (226)). As reported here airway smooth muscle mass is typically (226,227), but not always (19), a major determinant of lung function impairment. Increased airway smooth muscle mass is a feature of severe childhood asthma (228) and is described in both the large and small airways in studies of asthma deaths (229,230). Indeed increased airway smooth muscle mass in both the large and small airways is more common than in the large or small airway alone (230). Increased airway vascularity has also been consistently reported in endobronchial biopsies from asthmatics compared to healthy controls and in the small airways from lung resections for lung nodules in subjects with asthma (174,235-239). However, increased vascularity was not a feature observed in fatal asthma (240). We and others have reported that increased vascularity is associated with lung function impairment (174) and confirmed this finding in the current study. The

relationship between airway inflammation and lung function impairment is more contentious with some reports suggesting an association whereas others have not been able to reveal associations (reviewed in 4). We found that other features of remodelling namely epithelial thickening, RBM thickening and submucosal airway inflammation was not associated with lung function.

Proximal airway morphometry assessed by QCT is abnormal in asthma with luminal narrowing and airway wall thickening (24). These changes are weakly associated with lung function impairment. We found that epithelial thickening and airway smooth muscle % were related to QCT airway morphometry features of remodelling as described previously (25,207,208), but not other bronchial biopsy measures of remodelling or inflammation. Interestingly, although airway vascularity was associated with lung function impairment it was not associated with proximal airway morphometry.

We have extended previous studies of the relationship between endobronchial features of remodelling and QCT parameters to include measures of air-trapping. We found that both airway smooth muscle % and vascularity were associated with air-trapping in univariate analysis, but that vascularity alone was an independent and significant predictor of $MLD_{E/I}$ in our step wise linear regression. In comparative studies of asthma and chronic obstructive pulmonary disease we found that QCT measures of air-trapping are stronger predictors of lung function impairment than changes in proximal airway morphometry. It is therefore intriguing that increased vascularity measured in the proximal airway is related to air-trapping a measure of small airway dysfunction. Previous studies suggest that the degree of vascularity in the proximal airway tracks

with findings in the small airway (235,240), but of note we did not directly measure remodelling from small airway samples. Due to the novelty of our finding we sought to validate our finding in an independent replication group. In spite of differences in the processing of the endobronchial biopsies we found a remarkably similar relationship between airway vascularity and QCT-derived air-trapping in the replication group compared with our initial analyses.

Taken together these data support an important role for airway smooth muscle mass in proximal airway remodelling and possibly to a lesser extent in the smaller airway with both likely to be contributing to lung function impairment. Epithelial thickness plays a role in proximal airway remodelling, but is not related to airway dysfunction. Airway vascularity is not associated with proximal airway remodelling, but is associated with air-trapping and lung function impairment. Whether increased vascularity promotes small airway closure secondary to oedema or due to direct effects upon airway wall thickness is unknown. Interestingly, there are no reports of effects of corticosteroids upon airway smooth muscle mass, whereas in most although not all studies of the effects of corticosteroids upon airway vasculature demonstrate a decrease in vascularity with a concomitant improvement in lung function (236-239). In our study subjects were all receiving inhaled corticosteroid therapy suggesting that the remaining vascularity is resistant to corticosteroid therapy. Whether improvements in airway vascularity in response to corticosteroid or other therapies are related to improvements in air-trapping requires further study.

This study has a number of potential limitations. Although this is the largest study to date comparing immunohistology with QCT parameters of airway remodelling it

remains a relatively small study. It is also cross-sectional and future longitudinal studies of the natural history of asthma and response to therapies should consider inclusion of endobronchial biopsy and imaging parameters to further determine the structure-function relationships. Importantly, we did not standardise the location of the sampling of the endobronchial biopsies with a corresponding airway identified by QCT and whether this is important to determine the heterogeneity within an individual will be important in future studies. However, we did reduce the variability of QCT parameters within an individual by using the mean airway morphometry derived from multiple airways. Critically, our comparisons between QCT air-trapping were with proximal rather than distal airway samples. As discussed above it is likely that these proximal airway samples reflected similar changes in the smaller airways, but notwithstanding this likelihood further studies are required to compare QCT parameters of the small airway with distal sampling such as transbronchial biopsies.

3.2.6 Conclusion

In conclusion, we have found important associations between endobronchial biopsy and QCT measures of airway remodelling with lung function. We found that airway smooth muscle mass and airway vascularity are related to airflow obstruction with airway smooth muscle mass likely contributing more to large than small airway remodelling, whereas increased vascularity appears to be related to air-trapping possibly due to small airway remodelling.

3.2.6 Tables and Figures

Table 3.2.1 Demographics, clinical and laboratory characteristics

Characteristic		All patients (n = 70)	Post-bronchodilator FEV_1<80% (n =30)	Post-bronchodilator FEV_1≥80% (n = 40)	*p*-value
Age (y)		49 (12)	52 (12)	47 (13)	0.095
Male (%)		57	67	50	0.163
Caucasian (%)		93	93	93	0.893
Asthma duration (y)		24 (18)	29 (20)	20 (15)	0.059
BMI (kg/m^2)		29.9 (5.6)	30.3 (5.9)	29.6 (5.4)	0.644
Ex-smokers (%)		19	27	13	0.131
Atopy (%)		81	77	81	0.659
GINA class	**GINA 5, n (%)**	22 (31)	13 (43.3)	9 (23)	0.084
	GINA 4, n (%)	34 (49)	15 (50.0)	19 (48)	
	GINA 3, n (%)	6 (9)	0 (0.0)	6 (15)	
	GINA 1&2, n (%)	8 (11)	2 (6.7)	6 (16)	
Inhaled BDP equivalent (µg/24h)		1289 (689)	1444 (658)	1173 (698)	0.104
Pre-Bronchodilator FEV_1 (L)		2.46 (0.92)	1.76 (0.65)	2.98 (0.73)	<**0.001**
Pre-Bronchodilator FEV_1 (% predicted)		78.8 (24.6)	55.1 (14.3)	96.5 (12.8)	<**0.001**
Pre-Bronchodilator FEV_1/FVC (%)		66.7 (13.3)	55.7 (11.2)	74.9 (7.7)	<**0.001**
Post-Bronchodilator FEV_1 (L)		2.63 (0.91)	1.98 (0.63)	3.14 (0.77)	<**0.001**
Post-Bronchodilator FEV_1 (% predicted)		84.8 (23.3)	62.1 (12.8)	101.8 (12.0)	<**0.001**
Post-Bronchodilator FEV_1/FVC (%)		69.5 (12.5)	59.3 (11.0)	77.1 (6.8)	<**0.001**
Induced sputum					
Sputum eosinophils (%)[#]		4.5 [1.4-18.8]	5.3 [2.0-23.1]	4.2 [0.03-10.0]	0.185
Sputum neutrophils (%)[#]		46.5 [25.6-63.5]	49.7 [36.8 - 68.4]	44.1 [17.6-63.2]	0.066
Immunohistochemistry					
Submucosal eosinophils ($cells/mm^2$)[#]		19.6 [8.0-32.7]	19.2 [8.3-35.4]	19.9 [8.0-28.3]	0.947
Submucosal neutrophils ($cells/mm^2$)[#]		5.8 [2.2-20.8]	4.3 [2.1-15.5]	9.6 [2.3-24.6]	0.294
Submucosal mast cells ($cells/mm^2$)[#]		15.7 [5.4-33.6]	13.8 [6.2-37.1]	15.7 [5.3-22.9]	0.149

RBM thickness (µm)	12.3 (3.9)	12.3 (4.4)	12.3 (3.6)	0.974
Airway smooth muscle %	25.8 (15.4)	33.5 (15.6)	20.1 (12.6)	**<0.001**
Vascularity (mean Chalkley count)	5.5 (1.8)	6.2 (1.6)	5.0 (1.9)	**0.017**
Epithelial Thickness (µm)	62.0 (16.8)	65.1 (17.5)	59.7 (16.2)	0.257
Intact epithelium % [#]	27.8 [12.5-49.7]	36.2 [15.2-54.2]	22.9 [9.8-45.0]	0.466

Mean (SD) unless stated; [#] median [IQR]. BDP- Beclomethasone dipropionate

Table 3.2.2 Univariate correlations between primary QCT parameters and lung function, airway inflammation and remodelling

	Post-BD FEV1 (% pred)	Post BD FEV/FVC (%)	Submucosal eosinophils #	Submucosal neutrophils #	Submucosal mast cells #	Airway smooth muscle %	RBM thickness	SubmucosalVascularity	Epithelial Thickness	Intact epithelium
Mean LA/BSA (mm2/m2)	0.23	0.17	-0.26	0.05	-0.13	-0.35**	-0.13	0.06	-0.35*	0.04
Mean WA/BSA (mm2/m2)	0.16	0.06	-0.27	-0.02	-0.14	-0.32*	-0.18	0.04	-0.31*	0.06
Mean %WA	-0.28*	-0.29*	0.22	-0.28	0.10	0.27*	-0.01	-0.09	0.35*	-0.08
MLDE/I	-0.45**	-0.56***	0.03	-0.31	-0.15	0.3*	0.05	0.49***	0.07	-0.09
VI-856HU	-0.51***	-0.70***	-0.06	-0.17	0.00	0.55***	0.07	0.53***	0.07	-0.09

Pearson's correlation coefficient for parametric data, otherwise # Spearman's correlation coefficient for non-parametric data.

p value $<0.05^{*}$, $<0.01^{**}$ p value$<0.001^{***}$

Table 3.2.3 QCT morphometry and air-trapping parameters

Characteristic	All patients (n = 56)	Post-bronchodilator FEV_1<80% (n =29)	Post-bronchodilator FEV_1≥80% (n = 25)	*p*-value
CT-derived quantitative morphometry				
Mean segmental bronchial lumen area/BSA (mm^2/m^2)	10.4 (2.3)	9.7 (2.2)	11.0 (2.3)	**0.047**
Mean segmental bronchial wall area/BSA (mm^2/m^2)	17.0 (2.6)	16.5 (2.9)	17.5 (2.4)	0.133
Mean segmental bronchi wall area %	63.0 (2.2)	63.6 (2.0)	62.5 (2.1)	**0.039**
CT-derived measures of air-trapping				
$MLD_{E/I}$	0.85 (0.06)	0.89 (0.05)	0.83 (0.05)	**<0.001**
VI-856HU (%)	22.6 (17.0)	32.2 (19.8)	15.5 (10.1)	**<0.001**

Mean (SD), VI-856 HU- % of lung voxels with a density lower than -856 HU on expiratory scans

Table 3.2.4 Quantitative morphometry

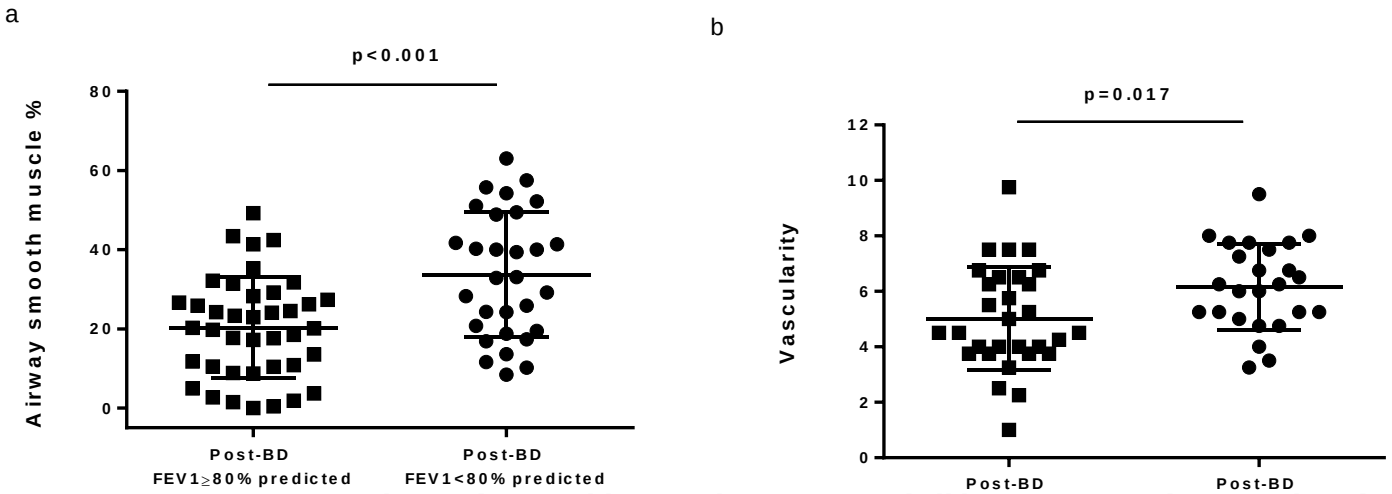
Characteristic	All patient	Post-bronchodilator FEV_1<80%	Post-bronchodilator FEV_1≥80%	*p*-value
RB1 LA/BSA (mm^2/m^2)	10.2 (3.7)	10.3 (3.6)	10.3 (3.5)	0.858
RB1 wall area percentage	64.0 (4.3)	63.9 (4.2)	64.0 (4.2)	0.718
RB2 LA/BSA (mm^2/m^2)	10.4 (3.0)	10.2 (3.0)	10.6 (3.1)	0.667
RB2 wall area percentage	62.5 (3.3)	62.9 (3.3)	62.1 (3.3)	0.406
RB3 LA/BSA (mm^2/m^2)	14.7 (6.6)	14.0 (5.4)	15.3 (7.5)	0.496
RB3 wall area percentage	60.3 (3.7)	60.5 (4.5)	60.2 (3.0)	0.817
RB4 LA/BSA (mm^2/m^2)	8.4 (3.7)	7.7 (3.3)	9.0 (3.9)	0.211
RB4 wall area percentage	63.1 (4.2)	63.6 (4.3)	62.8 (4.1)	0.474
RB5 LA/BSA (mm^2/m^2)	9.3 (3.3)	8.6 (2.8)	9.8 (3.6)	0.184
RB5 wall area percentage	62.9 (3.1)	63.6 (2.7)	62.3 (3.3)	0.12
RB6 LA/BSA (mm^2/m^2)	12.8 (5.0)	11.1 (4.1)	14.2 (5.3)	**0.024**
RB6 wall area percentage	62.3 (4.3)	63.0 (4.0)	61.3 (4.5)	0.261
RB7 LA/BSA (mm^2/m^2)	8.2 (3.1)	7.6 (2.8)	8.7 (3.4)	0.203
RB7 wall area percentage	65.1 (3.4)	65.8 (3.5)	64.4 (3.2)	0.165
RB8 LA/BSA (mm^2/m^2)	9.7 (2.9)	8.6 (2.4)	10.5 (3.0)	**0.013**
RB8 wall area percentage	63.3 (3.2)	64.5 (2.7)	62.4 (3.4)	0.023
RB9 LA/BSA (mm^2/m^2)	9.0 (4.1)	8.4 (3.7)	9.5 (4.4)	0.303
RB9 wall area percentage	63.9 (3.8)	64.3 (3.1)	63.5 (4.1)	0.422
RB10 LA/BSA (mm^2/m^2)	11.4 (3.8)	10.1 (3.2)	12.5 (3.9)	**0.015**

RB10 wall area percentage	61.9 (3.2)	63.2 (3.1)	60.8 (3.1)	**0.006**
LB1 LA/BSA (mm^2/m^2)	8.6 (2.7)	9.4 (2.9)	8.1 (2.4)	0.097
LB1 wall area percentage	64.1 (2.9)	63.5 (3.2)	64.6 (2.8)	0.199
LB2 LA/BSA (mm^2/m^2)	6.3 (3.2)	6.5 (3.2)	6.2 (3.2)	0.796
LB2 wall area percentage	64.7 (3.8)	64.6 (3.6)	64.7 (4.0)	0.96
LB3 LA/BSA (mm^2/m^2)	12.7 (4.9)	13.1 (5.4)	12.4 (4.6)	0.627
LB3 wall area percentage	61.3 (4.4)	61.9 (5.0)	60.9 (3.9)	0.414
LB4 LA/BSA (mm^2/m^2)	7.9 (3.2)	8.2 (3.6)	7.6 (2.9)	0.498
LB4 wall area percentage	62.7 (4.2)	62.6 (4.5)	62.8 (4.1)	0.874
LB5 LA/BSA (mm^2/m^2)	7.1 (2.0)	6.6 (1.8)	7.4 (2.2)	0.161
LB5 wall area percentage	64.2 (2.7)	65.2 (2.5)	63.4 (2.5)	**0.015**
LB6 LA/BSA (mm^2/m^2)	15.2 (6.4)	12.8 (5.1)	17.2 (6.8)	**0.009**
LB6 wall area percentage	61.0 (4.7)	62.2 (4.4)	59.9 (4.7)	0.068
LB1+2 LA/BSA (mm^2/m^2)	13.4 (5.0)	12.4 (4.9)	14.1 (5.0)	0.216
LB1+2 wall area percentage	62.3 (4.2)	63.1 (4.8)	61.6 (3.7)	0.219
LB8 LA/BSA (mm^2/m^2)	11.5 (4.1)	11.1 (5.0)	11.9 n(3.4)	0.511
LB8 wall area percentage	63.5 (3.5)	64.7 (3.5)	62.7 (3.4)	**0.042**
LB9 LA/BSA (mm^2/m^2)	9.4 (3.7)	8.4 (3.5)	10.1 (3.7)	0.107
LB9 wall area percentage	64.5 (3.1)	65.3 (3.1)	63.9 (3.1)	0.105
LB10 LA/BSA (mm^2/m^2)	11.2 (3.6)	9.7 (2.6)	12.3 (3.9)	**0.009**
LB10 wall area percentage	62.0 (3.1)	62.8 (3.0)	61.4 (3.2)	0.103

Table 3.2.5 Demographics, clinical and laboratory characteristics

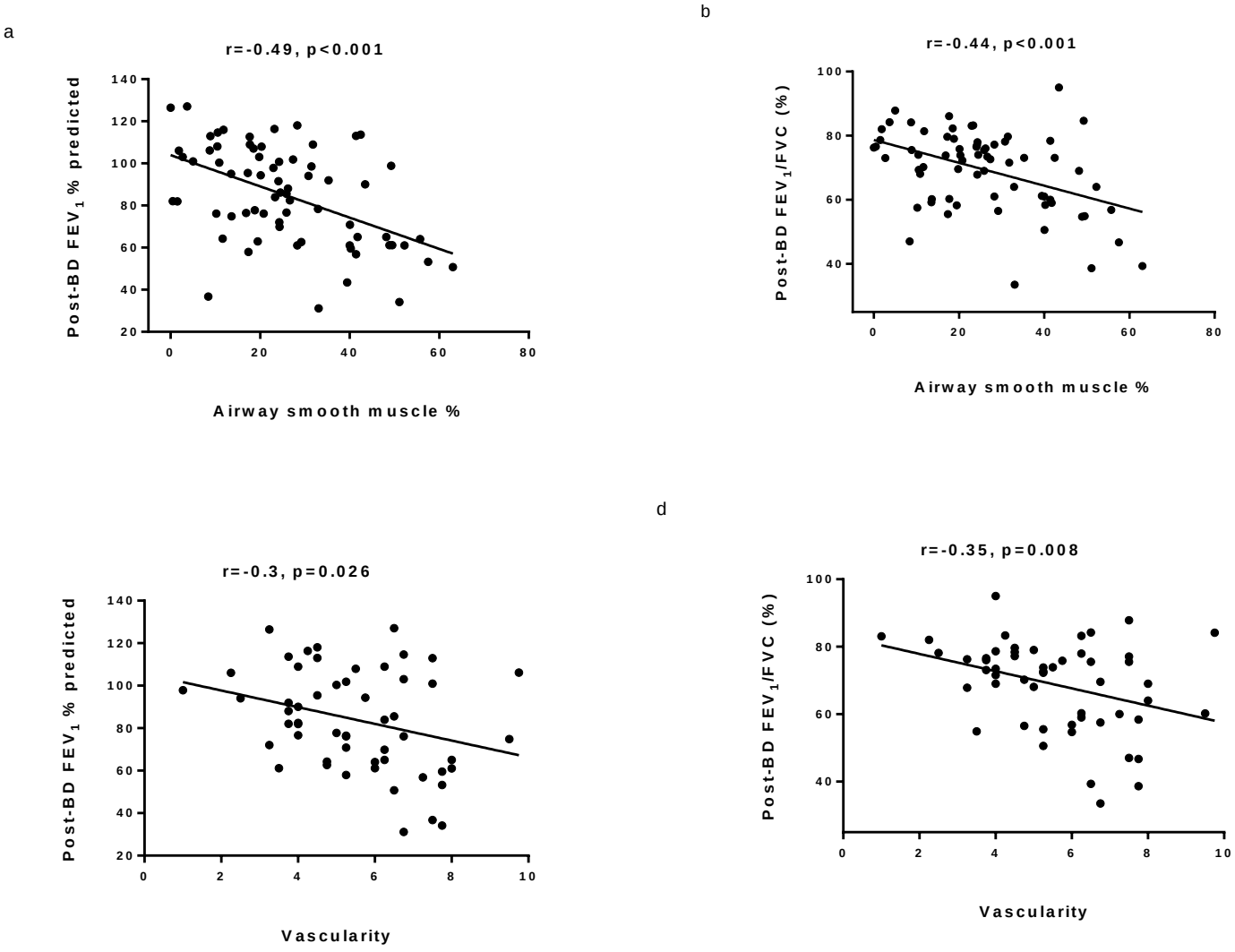
Characteristic		Subjects (n = 24)
Age (y)		33.8 (11.6)
Male (%)		37.5
Caucasian (%)		42.1
Asthma duration (y)		20.1 (11.1)
BMI (kg/m^2)		28.9 (5.7)
Age of asthma onset (y)		15.5 (14.3)
Ex-smokers (%)		4.2
Atopy (%)		79.2
GINA class	**GINA 5, %**	16.7
	GINA 4, %	50
	GINA 3, %	0
	GINA 1&2, %	33.3
Inhaled BDP equivalent (µg/24h)		1506 (938)
Pre-Bronchodilator FEV_1 (L)		2.7 (0.9)
Pre-Bronchodilator FEV_1 (% predicted)		83.1 (17.5)
Pre-Bronchodilator FEV_1/FVC (%)		83.8 (10.4)
Post-Bronchodilator FEV_1 (L)		3.0 (0.9)
Post-Bronchodilator FEV_1 (% predicted)		92.0 (13.7)
Post-Bronchodilator FEV_1/FVC (%)		88.1 (10.3)
Vascularity (mean Chalkley count)		4.2 (1.0)
MLD E/I		0.81 (0.05)
€VI-856HU		9.1 (12.1)

Figure 3.2.1 Comparing airway smooth muscle percentage and vascularity in those with and without persistent airflow limitation



a) Airway smooth muscle % and b) vascularity (mean Chalkley count) in subjects with and without persistent airflow limitation (post-bronchodilator [BD] FEV_1 <80% and ≥80% predicted).

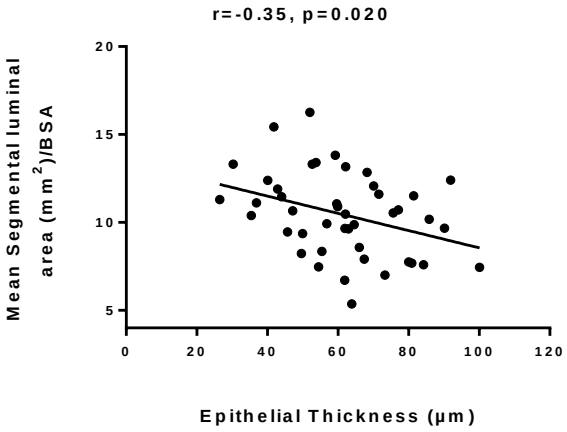
Figure 3.2.2 Scatterplots of correlations, airway smooth muscle



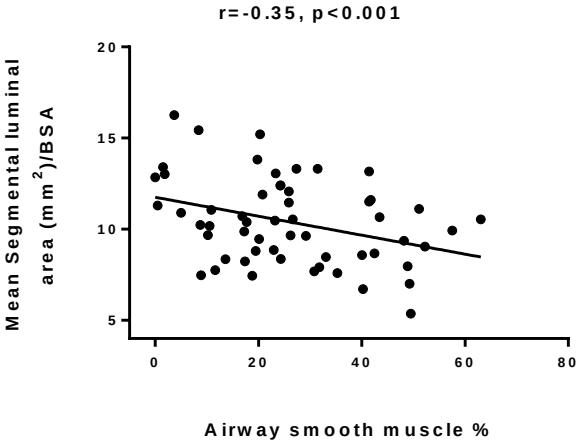
Scatterplots showing correlations of post-bronchodilator [BD] FEV_1 and FEV_1/FVC with a) and b) airway smooth muscle % and c) and d) vascularity.

Figure 3.2.3 Scatterplots of correlations, epithelial thickness

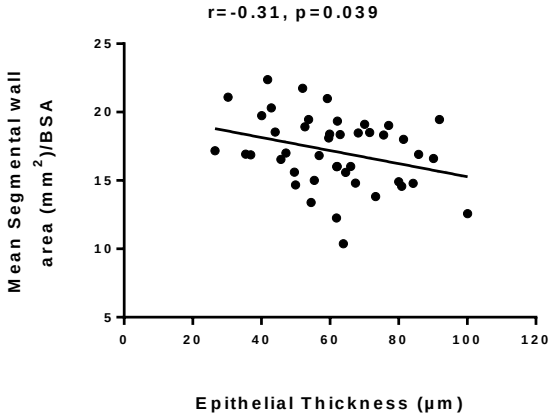
a



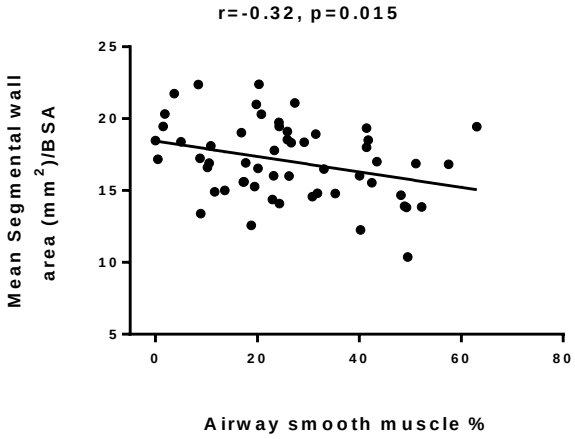
b



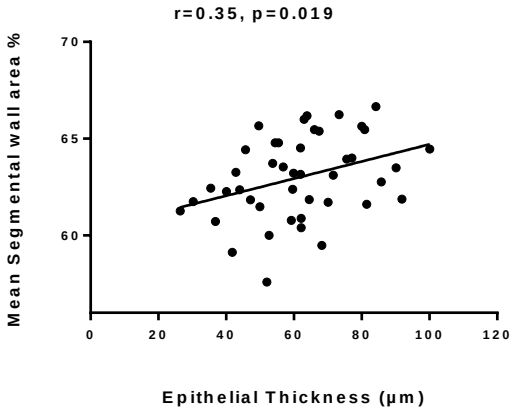
c



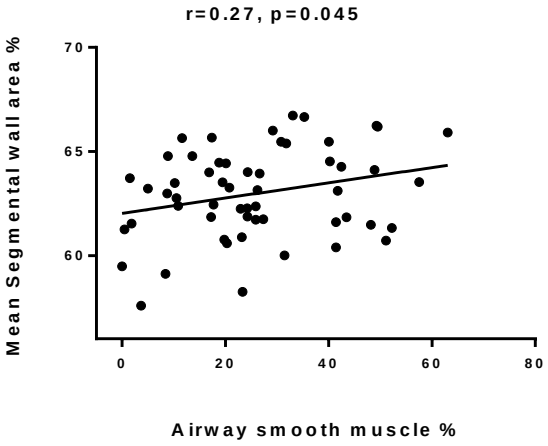
d



e

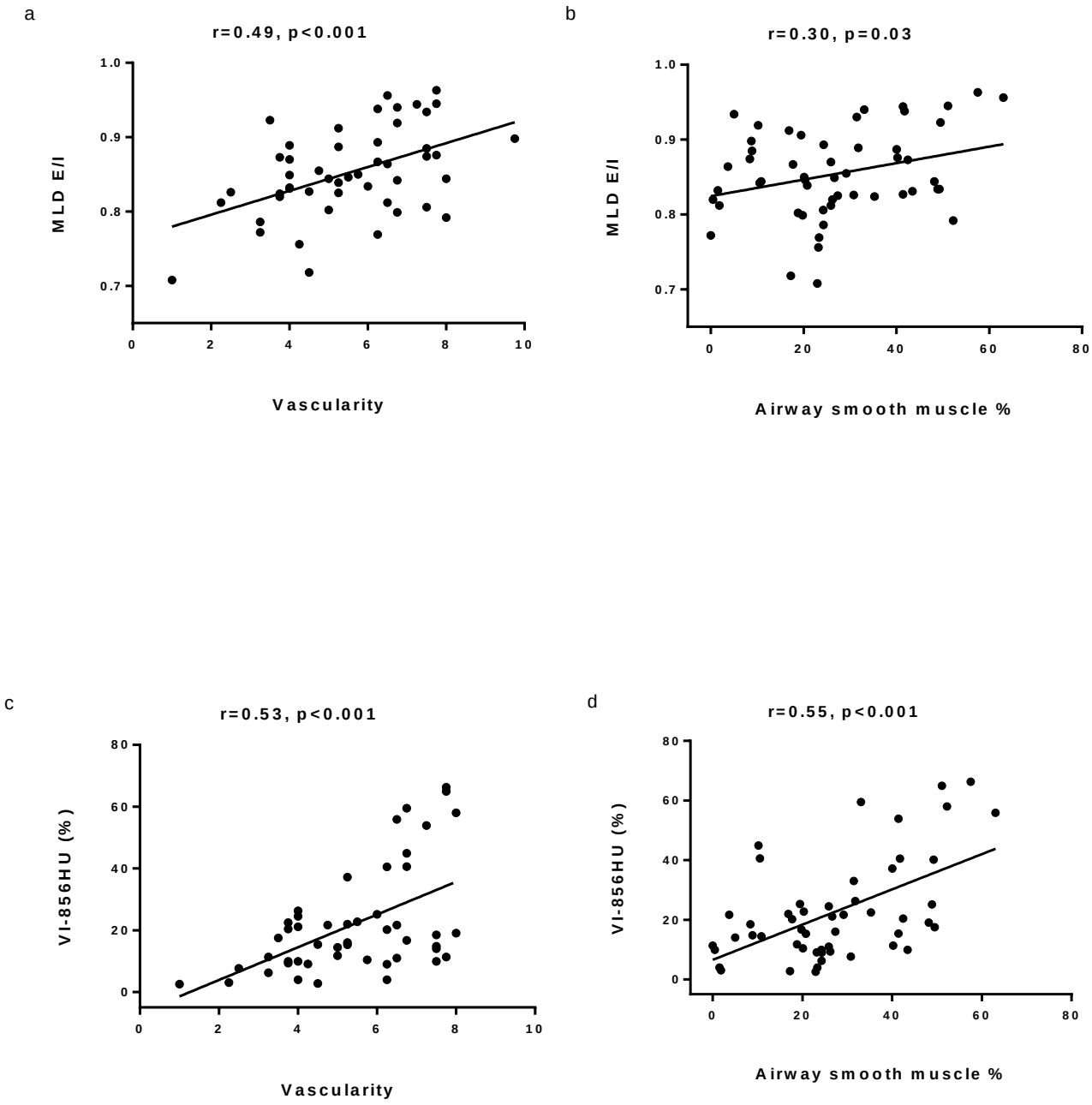


f



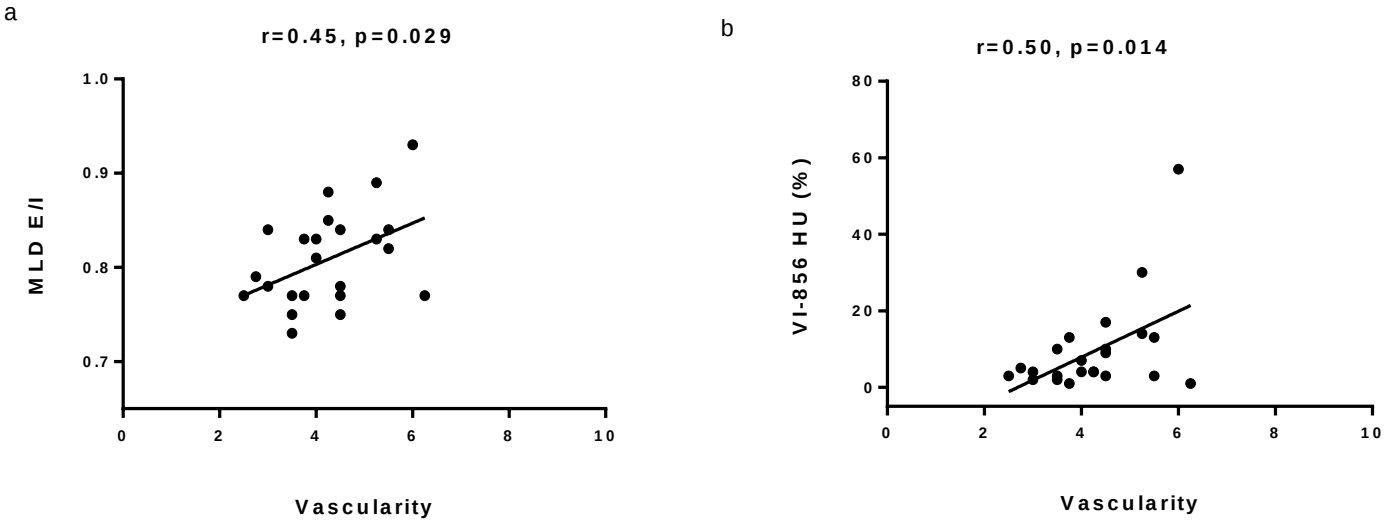
Scatterplots showing correlations of epithelial thickness and airway smooth muscle % with a) and b) mean segmental bronchial luminal and c) and d) wall area and e) and f) wall area %.

Figure 3.2.4 Scatterplots of correlations, vascularity



Scatterplots showing correlations of vascularity and airway smooth muscle % with a) and b) $MLD_{E/I}$ and c) and d) VI-856 HU.

Figure 3.2.5 Scatterplots of correlation, vascularity and air trapping in the replication group



Validation of the association between vascularity and air-trapping in the replication group showing scatterplots of vascularity with a) $MLD_{E/I}$ and b) VI-856 HU.

3.3 STUDY 3:

Randomised controlled trial of the prostaglandin D2 receptor 2 antagonist fevipiprant in persistent eosinophilic asthma

3.3.1 Abstract

3.3.1.1 Background

Eosinophilic airway inflammation is often present in asthma and interventions that reduce it result in improved clinical outcomes. Antagonism of the prostaglandin D2 receptor 2 (DP2) may reduce eosinophilic airway inflammation.

3.3.1.2 Methods

We performed a single-centre, 12-week, randomized, double-blind, placebo-controlled, parallel-group clinical trial of the DP2 receptor antagonist fevipiprant (QAW039) 225mg twice per day orally in 61 subjects with persistent moderate-to-severe asthma and an elevated sputum eosinophil count. The primary outcome was the change in sputum eosinophil percentage from baseline to post-treatment. Secondary and exploratory outcomes included changes in Asthma Control Questionnaire score (ACQ-7), standardised Asthma Quality of Life Score (AQLQ(S)), forced expiratory volume in one second (FEV1), and bronchial submucosal inflammation. This trial is registered with ClinicalTrials.gov (NCT01545726).

3.3.1.3 Measurements & main results

Sputum eosinophil percentage fell from a geometric mean of 5·4% at baseline to 1·1% post-treatment in the fevipiprant group and from 4·7% at baseline to 3·9% post-treatment in the placebo group (between group difference 3·5-fold; 95% confidence interval 1·7 to 7·0; p = 0·0014). Bronchial submucosal eosinophils were reduced 2.5-fold in the fevipiprant group compared to placebo (p = 0.040). ACQ-7 score fell by 0·32 points in the fevipiprant group compared to placebo (p = 0·17) and by 0.56 points in the subgroup with poor control (≥1.5 points) at baseline (p = 0.046). In the fevipiprant group compared to placebo AQLQ(S) improved by 0.59 points (p = 0.0080) and post-bronchodilator FEV1 improved by 0.16 L (p = 0.021). Fevipiprant displayed a favourable safety profile, with no serious adverse events reported.

3.3.1.4 Conclusions

Fevipiprant reduces eosinophilic airway inflammation in patients with persistent asthma and raised sputum eosinophil counts despite inhaled corticosteroid treatment. This is associated with improved lung function and asthma-related quality of life, and a favourable safety profile.

3.3.2 Introduction

Asthma is a chronic inflammatory airway disease that is characterised by heterogeneity with respect to clinical phenotype and response to therapy (241). Eosinophilic airway inflammation, mediated by type 2 immunity, is a common feature of asthma (241). Treatment strategies that specifically target eosinophilic airway inflammation substantially reduce exacerbations of asthma in those patients with uncontrolled eosinophilic airway inflammation, and to a lesser extent improve lung function and asthma control (34,38,43,44,242,243).

There is increasing evidence that prostaglandin D2 (PGD2), acting upon the DP2 receptor, also known as receptor homologous molecule expressed on T-helper 2 cells (CRTH2), may play an important role in mediating eosinophilic airway inflammation in asthma. The DP2 receptor mediates the migration of T-helper 2 (TH2) cells, delays their apoptosis and stimulates them to produce the cytokines IL-4, IL-5 and IL-13 (244-246). DP2 also influences the migration of and cytokine release from type 2 innate lymphoid cells (247), and importantly the receptor is expressed by eosinophils, and directly mediates their chemotaxis and degranulation (248,249). The number of DP2+ cells in the bronchial submucosa increases with increasing severity of asthma (32). DP2 is also expressed on airway epithelial cells and directly promotes their migration and differentiation (32). DP2 is therefore a highly promising novel drug target in the treatment of asthma. Fevipiprant (QAW039) is an orally administered highly selective and potent antagonist of the DP2 receptor, but not to the more general homeostatic PGD2 receptor DP1.

We tested the hypothesis that, in patients with sputum eosinophilia (≥ 2%) and persistent, moderate-to-severe asthma, 12-weeks' treatment with fevipiprant at a dose of 225mg twice per day, on top of conventional treatment, reduces the levels of eosinophils in induced sputum compared to placebo. Secondary objectives were to determine the effects of fevipiprant on asthma symptoms, as measured by the seven-point Asthma Control Questionnaire (ACQ-7) (168), and to assess safety and tolerability of fevipiprant. Exploratory objectives included assessment of the effect of fevipiprant on the forced expiratory volume in one second (FEV1), lung volumes using body plethysmography, health-related quality of life as measured by the standardised Asthma Quality of Life Questionnaire (AQLQ(S)) (170), airway inflammation and remodelling in bronchial biopsies and airway morphometry and lung density assessed by quantitative computed tomography (CT).

3.3.3 Methods

3.3.3.1 Subjects

Participants were older than 18 years of age and had a clinical diagnosis of asthma that was supported by one or more objective criteria.

1. An increase in forced expiratory volume (FEV_1) of ≥ 12% and ≥ 200ml from its pre-bronchodilator value following the inhalation of 400μg salbutamol.
2. A provoked fall in FEV_1 of 20% by methacholine at ≤ 16mg/ml while on inhaled corticosteroids (ICS)
3. A change in FEV_1 of > 12% over two non-exacerbation-related measurements during the previous year.

Participants were recruited from a regional refractory asthma clinic providing tertiary care for a population of 4 million people. Suitable participants were also identified from secondary care asthma and general respiratory clinics in the region, and through screening of local primary care databases.

Inclusion criteria were:

1. current treatment with ICS
2. A sputum eosinophil count of ≥ 2% at screening
3. Either an Asthma Control Questionnaire (ACQ-7) score ≥ 1·5 at randomization or ≥ 1 exacerbations (requiring higher than the patient's normal dose of systemic corticosteroids for ≥ 3 days) in the past 12 months.

Exclusion criteria were:

1. Serious coexisting illness
2. Pregnancy or lactation
3. The possibility of conception
4. History of malignancy within the previous five years
5. Recent (within 6 weeks of screening) lower respiratory tract infection or exacerbation of asthma requiring oral prednisolone
6. The use of omalizumab within 6 months before randomization into the study
7. The use of immunosuppressive medication (except low-dose [≤ 10mg prednisolone per day] oral corticosteroids) within 30 days before randomization

All subjects provided written informed consent. The study protocol was approved by the National Research Ethics Committee (Leicestershire, Northamptonshire and Rutland, approval no. 11/EM/0402) and the United Kingdom Medicines and Healthcare Products Regulatory Agency. The trial was registered with ClinicalTrials.gov (NCT01545726) and EudraCT (2011-004966-13).

3.3.3.2 Design of the study

The study was a single-centre, randomised, double-blind, placebo-controlled, parallel-group clinical trial conducted from February 2012 through June 2013. The funding organisation (Novartis Pharmaceuticals) supplied the study drug and placebo.

The study design is illustrated in Figure 3.3.1a. Participants were given the option of undergoing bronchoscopy at the baseline and post-treatment visits as part of the study. Patients attended a screening visit (Visit 1, Day -21), at which inclusion/exclusion criteria were reviewed, an induced sputum sample was collected and cell count was performed, in order to assess eligibility based upon a sputum eosinophil count, and demographic and clinical details were collected. Regular treatment was kept constant from this time point until the end of the study.

One week later, a two-week single-blind placebo run-in period was commenced (Visit 2, Day -14). Following this, patients attended a baseline visit (Visit 3, Day 0), at which the inclusion and exclusion criteria were again assessed, taking into account the ACQ-7 score. If patients fulfilled the criteria, they proceeded to undertake the remainder of the study visit tests, (outlined in Table 3.3.1) and were then randomized in a 1:1 ratio to receive either fevipiprant at a dose of 225 mg twice per day, or an identical placebo.

Patients attended a mid-treatment visit (Visit 4, Day 42), and a post-treatment visit (Visit 5, Day 84). At the post-treatment visit, patients began a six-week single-blind placebo washout period, and then attended an end-of-study visit (Visit 6, Day 126).

Criteria for withdrawal from the study were defined a priori, and included withdrawal of informed consent, asthma exacerbation, pregnancy, and adverse events for which continued exposure to the study drug would be detrimental.

All tests performed at the baseline and post-treatment visits were carried out on the same day, with the exception of bronchoscopy, which was performed on a separate day not more than seven days following the other tests, but not on the day immediately following them, due to the possibility of interaction between the sputum induction procedure and bronchial biopsies.

The time interval between the two testing days was kept constant for each patient between the baseline and post-treatment visits

Safety was assessed at each study visit on the basis of patient-reported adverse events, physical examination, vital signs, haematology, blood chemistry, urinalysis and an electrocardiogram.

3.3.3.3 Randomisation and masking

Randomisation was performed by the trial pharmacist using previously generated treatment allocation cards, and was stratified by whether or not participants were receiving treatment with regular oral corticosteroids, and whether they were undergoing bronchoscopy. All other site staff, patients and sponsor personnel remained blinded to treatment allocation until the study had been completed and the trial database locked. Results of sputum and blood eosinophil counts subsequent to the baseline visit were not disclosed to the investigators during the study because of the expected anti-eosinophilic effects of fevipiprant.

3.3.3.4 Statistical analysis

The primary outcome of the study was the change in sputum eosinophil percentage between the baseline visit and the post-treatment visit. As sputum eosinophil percentage is known to follow a log-normal distribution, the analysis was based on a log10-transformed scale with results back-transformed to obtain the within-group ratios of geometric means at the end of treatment compared to baseline, as well as their ratio. We report the reciprocal of these ratios as fold-reductions from baseline, and as a measure of how many times greater the reduction in the fevipiprant group was compared to the reduction in the placebo group, respectively.

The secondary outcome was the change from baseline to post-treatment with respect to ACQ-7 score. Exploratory outcomes included the change from baseline to post-treatment with respect to ACQ-7 score in the subgroup with baseline score ≥ 1·5, AQLQ(S) score, FEV1 and submucosal eosinophil count on bronchial biopsy. Statistical analyses were performed using SAS/STAT software, versions 9·3 and 9·4 of the SAS System for AIX (SAS Institute Inc., Cary, NC, USA) and Prism 6 (GraphPad, La Jolla, CA, USA). Changes in efficacy outcomes from the baseline to post-treatment visits were analysed using an analysis of covariance (ANCOVA) model, with treatment as the fixed effect. Randomisation strata and baseline values of efficacy variables were entered as factors in the ANCOVA model for analysis of the primary outcome, secondary outcome and exploratory outcomes. Efficacy outcomes were analysed by intention to treat and safety outcomes were analysed by treatment received. One patient was assigned to fevipiprant but incorrectly dispensed placebo at the mid-treatment visit. One patient was assigned to fevipiprant but incorrectly dispensed placebo throughout the course of the study. They were included in the fevipiprant group for efficacy analyses, but the latter patient was included in the placebo group for safety analyses. The planned sample size of 60 randomised patients was calculated so that at least 24 patients per arm would complete the post-treatment assessment in order to ensure 80% power at the two-sided 5% significance, assuming a 50% reduction in sputum eosinophil percentage with fevipiprant (250).

3.3.4 Results

Participants were recruited between Feb 10, 2012 and Jan 30, 2013. A total of 117 patients attended a screening visit, of which 61 fulfilled the inclusion and exclusion criteria and were randomised (Figure 3.3.1b). Thirty-one patients were assigned to receive placebo and 30 to receive fevipiprant. Four patients withdrew in the placebo group and three patients in the fevipiprant group, in each case due to an exacerbation of asthma. The randomised groups were well-matched for baseline characteristics, as shown in Table 3.3.2. Efficacy outcomes are shown in Figures 3.3.2-3.3.4, and in Tables 3.3.3-3.3.5.

The geometric mean sputum eosinophil percentage fell from 5·4% at baseline to 1·1% post-treatment in the fevipiprant group, and from 4·7% at baseline to 3·9% post-treatment in the placebo group. The ratio of geometric means post-treatment to baseline for the sputum eosinophil percentage was 0·78 (1·3-fold reduction) in the placebo group and 0·22 (4·5-fold reduction) in the fevipiprant group, with a 3·5-fold (95% confidence interval [CI] 1·7 to 7·0-fold) greater reduction in the fevipiprant group compared to placebo (p = 0·0014).

The mean ACQ-7 score fell by 0·32 points from baseline to post-treatment in the fevipiprant group compared to the change seen with placebo, but this improvement did not reach statistical significance (95% CI -0·78, 0·14; p = 0·17). However, among the subset of patients (n = 40) uncontrolled at baseline (ACQ-7 score ≥ 1·5), the mean ACQ-7 score fell by 0·56 points compared to placebo, which was both clinically and statistically significant (95% CI -1·12, -0·01; p = 0·046). The mean AQLQ(S) score improved by 0·59 points in the fevipiprant group compared to placebo, which was statistically significant (95% CI 0·16, 1·03; p =

0·0080). The mean post-bronchodilator FEV1 increased by 0·16L from baseline to post-treatment in the fevipiprant group compared to placebo, with a statistically significant difference between the groups (95% CI 0·03, 0·30; p = 0·021). There were no significant differences between the groups with respect to changes in pre-bronchodilator FEV1. There were no significant changes in peripheral blood eosinophil count or exhaled nitric oxide in either group.

Paired bronchial biopsies (baseline and post-treatment) were obtained in 14 patients in the fevipiprant group and 12 patients in the placebo group. We observed a 2·5-fold greater reduction in bronchial submucosal eosinophil numbers from baseline to post-treatment in the fevipiprant group compared to the placebo group (p = 0·040). There was a 1·7-fold reduction in bronchial epithelial eosinophil numbers from baseline in favour of fevipiprant, but the treatment difference did not reach statistical significance. Subjects treated with fevipiprant demonstrated a 27·8 percentage point increase in the proportion of intact epithelium (95% CI 2·9, 52·7; p = 0·030), and a 26·6 percentage point reduction in the proportion of denuded epithelium (95% CI -44·9, -8·3; p = 0·0062), compared to the change seen with placebo. Changes in epithelial integrity were not significantly correlated with changes in sputum or bronchial mucosal eosinophilic inflammation, as shown in Figure 3.3.5.

Functional residual capacity (FRC) fell by 0·31 L in the fevipiprant group compared to the change seen with placebo (95% CI -0·62, -0·001; p = 0·049) and expiratory CT lung volume fell by 216 cm^3 in the fevipiprant group compared to the placebo group (95% CI -391, -40; p = 0·017). We observed a significant negative correlation between the change in expiratory CT lung volume and the change in post-bronchodilator FEV_1, taking the fevipiprant and

placebo groups together (R = -0·317, p = 0·041), and this correlation was more pronounced with CT-derived lower lobe lung volume (R = -0·523, p = 0.0004) (Figure 3.3.6). Positive correlations were also observed between changes in expiratory CT lung volume and changes in both residual volume (RV) and the ratio of RV to total lung capacity (TLC) measured using body plethysmography, but these correlations only reached statistical significance with CT-derived lower lobe lung volumes (R = 0·374, p = 0·014 for RV; R = 0·361, p = 0·017 for RV/TLC).

Significant positive correlations were observed between changes in plethysmographic and CT lung volumes, as shown in Figure 3.3.6.

Outcomes measured following the 6 week washout period returned to baseline without any significant differences between baseline and post-washout for any outcome. Fevipiprant had an acceptable side-effect profile throughout the study period. Total adverse events and adverse events within each organ class were balanced between the two treatment groups. There were no deaths or serious adverse events reported, and no patient withdrawals suspected by the investigator to be related to the study drug, as shown in Table 3.3.6.

3.3.5 Discussion

We found that fevipiprant significantly reduced eosinophilic inflammation in the sputum and bronchial submucosa compared to placebo in patients with persistent, moderate-to-severe asthma and sputum eosinophilia. Fevipiprant significantly improved AQLQ(S) scores, post-bronchodilator FEV_1 and functional residual capacity compared to placebo in all patients, and ACQ-7 scores in the sub-group of patients who had poor asthma control at baseline (ACQ-7 $\geq 1{\cdot}5$ points). Exploratory analyses of bronchial biopsies suggested that fevipiprant led to improvements in epithelial integrity, but did not affect epithelial goblet cell number or MUC5A expression.

The magnitude of reduction in eosinophilic inflammation reported here was comparable to that observed with mepolizumab (34,243). Unlike mepolizumab (34,243), and other anti-IL5(R) targeted biologics reslizumab and benralizumab, fevipiprant did not have any significant effect on the blood eosinophil count. This suggests that DP2 receptor blockade attenuates the migration of eosinophils into the airway tissues, but is unlikely to have a substantial effect upon release from the bone marrow although it might exert a small indirect effect through a reduction in circulating IL-5 (246,247). Previous interventional studies have shown that anti-eosinophilic treatments or strategies exert their major therapeutic effect through the reduction in asthma exacerbations (34,38,44,242,243), although effects on FEV1 have also been observed, particularly in patients with blood eosinophilia (43,44). The treatment period in this study was not long enough to observe a significant effect on exacerbations. Whether fevipiprant reduces the frequency of exacerbations in patients with eosinophilic asthma is an important question for future studies.

We noted a prompt return to baseline values following a six-week placebo wash-out period in the fevipiprant group with respect to sputum eosinophil percentage, ACQ-7 and AQLQ(S) scores, and post-bronchodilator FEV1. There were no statistically significant differences between baseline values and those recorded following the placebo wash-out. This suggests that the short-term improvements in asthma quality of life and post-bronchodilator FEV1 seen with fevipiprant were driven by reversible processes rather than underlying disease modification. However, we observed significant improvements in epithelial integrity following 12 weeks of treatment with fevipiprant compared to placebo. Whether this effect was a consequence of reduced eosinophilic inflammation which is known to cause epithelial damage or a direct effect upon epithelial repair and differentiation as observed in vitro (32) remains uncertain, although the lack of an association between changes in sputum eosinophil counts and epithelial integrity in response to fevipiprant favours a direct mechanistic effect upon the epithelium.

Previous clinical trials of DP2 receptor antagonists in asthma have yielded mixed results. The compound OC000459 was found to improve pre-bronchodilator FEV1 and asthma quality of life in steroid-free patients (251), with a subsequent study finding that the beneficial effect was confined to patients with a baseline peripheral blood eosinophil count >250/μl (252). However, this compound has not yet been tested in patients with moderate-to-severe asthma. AMG853, a dual DP1 and DP2 antagonist, was not effective in improving asthma symptoms or either pre- or post-bronchodilator FEV1 in patients with moderate-to-severe asthma (253), but there is evidence that DP1 and DP2 stimulation may have opposing effects on a number of inflammatory mechanisms (254). The efficacy of BI671800 was evaluated in two separate randomised controlled trials, one in steroid-naïve adults with asthma, and one in patients receiving inhaled fluticasone (255). In both cases, six weeks of treatment resulted in modest

but statistically significant improvements in pre-bronchodilator FEV1 compared to placebo. In these previous studies patient selection was not based upon evidence of eosinophilic airway inflammation. Previous experience has shown that targeting anti-eosinophilic therapies to patients with evidence of uncontrolled type 2 inflammation is associated with more clear evidence of efficacy (34,38,43,44,243), and the positive results obtained in our study should therefore not be extrapolated to an unselected group of patients with moderate-to-severe asthma.

One limitation of our study is the relatively small sample size undertaken in a single centre. However, the effect size in our primary outcome the sputum eosinophil count was large and other positive clinical outcomes showed both statistically and clinically important differences between the fevipiprant and placebo groups. Furthermore, our study design allowed a significant loss of efficacy to be demonstrated when fevipiprant was stopped. In contrast to many clinical trials the clinical outcomes in the group that received placebo were typically worse following intervention compared to their baseline, suggesting deterioration in this group. The lack of a positive placebo effect in this study may be explained by the fact that many of the participants were drawn from a tertiary refractory asthma clinic, and their treatment had previously been fully optimised. We also included a two-week single-blind placebo run-in period prior to the baseline visit specifically in order to minimise the placebo effect. Finally, our inclusion and exclusion criteria mandated a six-week period of clinical stability before patients could participate in the study, thus minimising the potential for changes to occur as a result of regression to the mean. A further limitation of the study was that two dispensing errors occurred, with one patient randomised to fevipiprant and receiving placebo throughout, and a second randomised to fevipiprant and receiving placebo in the second half of the treatment period. Since efficacy outcomes were analysed by intention to

treat, this could have increased the chance of a type II error. However, when efficacy outcomes were analysed by treatment received there were no significant changes in the results obtained (data not shown).

3.3.6 Conclusion

The DP2 receptor antagonist fevipiprant is effective at attenuating eosinophilic airway inflammation in patients with persistent eosinophilic asthma, and appears to have a favourable safety profile over a 12-week treatment period. There is evidence that fevipiprant improves lung function and asthma-related quality of life, as well as expiratory air trapping and epithelial integrity. Longer-term multi-centre studies are required to confirm these findings and to investigate the effect of fevipiprant on asthma exacerbations.

3.3.7 Tables and Figures

Table 3.3.1: Summary of visit days and tests

Visit (Day)	Tests carried out
Visit 1 (-21)	Inclusion/exclusion criteria assessed Induced sputum & sputum analysis Demographic and clinical details
Visit 2 (-14)	Inclusion/exclusion criteria assessed ACQ & AQLQ PC20 or reversibility Skin prick test
Visit 3 (1)	Inclusion/exclusion criteria assessed ACQ & AQLQ Blood haematology & biochemistry Spirometry Induced sputum & sputum analysis HRCT Bronchoscopy* FENO
Visit 4 (42)	ACQ & AQLQ Blood haematology & biochemistry Spirometry Induced sputum & sputum analysis
Visit 5 (84)	ACQ & AQLQ Blood haematology & biochemistry Spirometry Induced sputum & sputum analysis HRCT Bronchoscopy* FENO
Visit 6 (126)	ACQ & AQLQ Blood haematology & biochemistry

	Spirometry Induced sputum & sputum analysis FENO

*Bronchoscopy timings as outlined in 3.3.3.2 Design of the study

Table 3.3.2 Baseline characteristics of randomised population

Characteristic	**Fevipiprant (n = 30)**	**Placebo (n = 31)**
Sex (no. of subjects) Male Female	 18 12	 13 18
Age (yr) Mean Range	 50 20 – 80	 50 19 – 68
Duration of asthma (yr)	32 ± 16	29 ± 15
Body-mass index (kg/m^2)	31·0 ± 5·9	29·6 ± 6·0
Positive atopic status (% of subjects)	87	84
Number of exacerbations in previous year	1.8 ± 1.7	2.2 ± 2.8
Number of patients (%) with rhinosinusitis	12 (40.0)	11 (35.5)
Number of patients (%) with nasal polyps	5 (16.7)	3 (9.7)
Total IgE (U/ml) Median Interquartile range	 414 216 – 863	 388 181 – 1121
FEV_1 before bronchodilator use (% of predicted value)	72·5 ± 23·8	75·1 ± 27·3
FEV_1/FVC before bronchodilator use (%) Median Interquartile range	 68·0 46·7 – 73·6	 69·2 52·1 – 73·5
Improvement in FEV_1 after bronchodilator use (%) Median Interquartile range	 9·3 5·5 – 12·6	 12·0 6·1 – 29·9
Eosinophil count in sputum (%) ¶	5·31 (2·77)	4·24 (4·03)
Eosinophil count in blood ($\times 10^9$/L) ¶	0·28 (1·31)	0·28 (0·79)

$FENO_{50}$ (ppb)	30 ± 24	48 ± 43
Score on Asthma Control Questionnaire	1·9 ± 0·8	2·2 ± 0·9
Score on Asthma Quality of Life Questionnaire	5·4 ± 1·1	5·0 ± 1·0
Inhaled corticosteroid dose (beclomethasone dipropionate equivalent [µg]) Median Interquartile range	 1600 800 – 1600	 1000 800 – 1600
Use of long-acting beta-agonists (% of subjects)	90	84
Regular use of oral prednisolone (% of subjects)	23	23
Global Initiative for Asthma treatment step (number of patients) Step 2 Step 3 Step 4 Step 5	 1 1 21 7	 1 4 19 7

Plus-minus values are means ± standard deviation (SD) unless otherwise stated.

¶ Expressed as geometric mean (coefficient of variation)

Table 3.3.3 Outcome Measures at Baseline and Post-Treatment in the Full Analysis Set Population. (Parameters in italics are ratio change. Non italics are absolute change)

Outcome	Baseline values		Post-treatment values		Change from baseline to post-treatment			
	Fevipiprant	Placebo	Fevipiprant	Placebo	Fevipiprant (N = 30)	Placebo (N = 31)	Treatment difference (Fevipiprant vs placebo)	P value
Eosinophil count in sputum (%) ‡	*5·42 (287·65)*	*4·65 (391·44)*	*1·12 (0·65, 1·93)*	*3·88 (2·26, 6·67)*	*0·22 (0·13, 0·39)*	*0·78 (0·45, 1·33)*	*0·29 (0·14, 0·58)*	*0·0014*
Eosinophil count in blood ($\times 10^9$/L) ‡	*0·29 (95·03)*	*0·28 (80·63)*	*0·29 (0·23, 0·36)*	*0·32 (0·25, 0·41)*	*1·01 (0·79, 1·28)*	*1·13 (0·89, 1·43)*	*0·89 (0·66, 1·20)*	*0·44*
$FE_{NO_{50}}$ (ppb)	37·72 (4·75)	43·67 (6·97)	34·88 (3·97)	38·48 (4·32)	-5·82 (-13·79, 2·16)	-2·21 (-10·90, 6·48)	-3·60 (-13·93, 6·72)	0·49
ACQ-7 score	1·91 (0·15)	2·22 (0·16)	1·89 (0·18)	2·21 (0·18)	-0·18 (0·18)	0·14 (0·18)	-0·32 (-0·78, 0·14)	0·17
ACQ-7 score in subjects with baseline ≥1·5†	2·37 (0·11)	2·57 (0·15)	1·69 (0·22)	2·25 (0·23)	-0·37 (0·22)	0·20 (0·23)	-0·56 (-1·12, -0·01)	0·046
ACQ-6 score	1·71 (0·18)	2·11 (0·17)	1·66 (0·19)	2·11 (0·19)	-0·26 (-0·65, 0·13)	0·19 (-0·20, 0·59)	-0·45 (-0·96, 0·05)	0·077
AQLQ score Total	5·43 (0·20)	5·02 (0·18)	5·48 (0·17)	4·89 (0·17)	0·27 (-0·07, 0·61)	-0·33 (-0·66, 0·01)	0·59 (0·16, 1·03)	0·0080

AQLQ score Symptoms	5·22 (0·21)	4·73 (0·20)	5·25 (0·22)	4·62 (0·21)	0·28 (-0·15, 0·72)	-0·34 (-0·77, 0·09)	0·63 (0·07, 1·18)	0·028
AQLQ score Activities	5·58 (0·20)	5·28 (0·20)	5·70 (0·15)	5·16 (0·15)	0·28 (-0·03, 0·59)	-0·26 (-0·57, 0·05)	0·54 (0·14, 0·93)	0·0087
AQLQ score Emotions	5·48 (0·26)	4·90 (0·21)	5·50 (0·22)	4·65 (0·21)	0·33 (-0·10, 0·76)	-0·53 (-0·95, -0·11)	0·86 (0·31, 1·40)	0·0027
AQLQ score Environmental	5·62 (0·27)	5·28 (0·23)	5·74 (0·19)	5·19 (0·18)	0·30 (-0·08, 0·67)	-0·25 (-0·62, 0·12)	0·55 (0·07, 1·02)	0·025
Pre-bronchodilator FEV_1 (L)	2·27 (0·17)	2·27 (0·18)	2·35 (0·07)	2·28 (0·07)	0·08 (-0·06, 0·22)	0·004 (-0·14, 0·15)	0·07 (-0·10, 0·25)	0·41
Pre-bronchodilator FEV_1/FVC (%)	63·67 (2·85)	64·23 (2·17)	65·49 (0·88)	63·61 (0·88)	1·54 (-0·22, 3·29)	-0·34 (-2·12, 1·43)	1·88 (-0·38, 4·14)	0·10
Post-bronchodilator FEV_1 (L)	2·49 (0·17)	2·71 (0·19)	2·66 (0·05)	2·50 (0·05)	0·06 (-0·05, 0·17)	-0·10 (-0·21, 0·01)	0·16 (0·03, 0·30)	0·021
Post-bronchodilator FEV_1/FVC (%)	66·90 (2·82)	69·72 (2·14)	70·42 (1·02)	67·31 (1·03)	2·10 (0·05, 4·15)	-1·00 (-3·07, 1·06)	3·11 (0·46, 5·75)	0·023
RV (L)	2·78 (0·22)	2·87 (0·23)	2·66 (0·13)	2·79 (0·12)	-0·17 (-0·42, 0·09)	-0·03 (-0·29, 0·22)	-0·14 (-0·46, 0·19)	0·40
TLC (L)	6·49 (0·29)	6·41 (0·29)	6·40 (0·13)	6·37 (0·13)	-0·04 (-0·30, 0·21)	-0·08 (-0·33, 0·18)	0·04 (-0·29, 0·36)	0·83

RV/TLC (%)	42·28 (2·30)	44·29 (2·49)	40·70 (1·30)	43·08 (1·32)	-2·64 (-5·24, -0·04)	-0·260 (-2·92, 2·40)	-2·38 (-5·69, 0·93)	0·15
FRC (L)	3·90 (0·26)	3·73 (0·24)	3·58 (0·12)	3·88 (0·12)	-0·23 (-0·48, 0·01)	0·08 (-0·17, 0·32)	-0·31 (-0·62,-0·001)	0·049
KCO (% predicted)	108·93 (4·39)	104·87 (3·28)	106·47 (1·72)	109·01 (1·70)	-0·32 (-3·78, 3·14)	2·22 (-1·20, 5·63)	-2·54 (-6·90, 1·83)	0·25

Baseline and post-treatment values are mean (standard error), change from baseline to post-treatment is mean change (lower limit, upper limit of 95% confidence interval), and treatment difference is mean change in fevipiprant group minus mean change in placebo group (lower limit, upper limit of 95% confidence interval), unless otherwise stated**. Post-treatment and changes from baseline to post-treatment are covariate-adjusted (least square mean) values.**

‡ Baseline values are geometric mean (% coefficient of variation), post-treatment values are geometric mean (lower limit, upper limit of 95% confidence interval), change from baseline to post-treatment is geometric mean fold-change (lower limit, upper limit of 95% confidence interval), and treatment difference is ratio of geometric mean fold-change in fevipiprant group to geometric mean fold-change in placebo group (lower limit, upper limit of 95% confidence interval).

†N = 18 in fevipiprant group and N = 22 in placebo group

Table 3.3.4 Bronchial biopsy outcome measures

Outcome	Baseline values		Post-treatment values		Change from baseline to post-treatment			
	Fevipiprant	Placebo	Fevipiprant	Placebo	Fevipiprant (N = 14)	Placebo (N = 12)	Treatment difference (Fevipiprant vs placebo)	P value
Inflammatory cells*								
Eosinophils/mm^2 lamina propria	13·9 (23·5)	9·1 (39·4)	6·7 (28·8)	15·8 (33·3)	0·6 (0·3, 1·0)	1·4 (0·7, 2·7)	0·4 (0·2, 1·0)	0·040
CD3+ cells/mm^2 lamina propria	9·0 (37·7)	11·1 (31·2)	14·4 (29·8)	14·6 (31·0)	1·6 (0·7, 3·9)	1·3 (0·5, 3·3)	1·2 (0·3, 4·3)	0·75
Mast cells/mm^2 lamina propria	5·5 (36·7)	8·9 (26·5)	4·7 (27·4)	11·9 (28·5)	0·9 (0·5, 1·5)	1·3 (0·8, 2·3)	0·6 (0·3, 1·4)	0·25
Neutrophils/mm^2 lamina propria	1·3 (38·5)	3·1 (36·5)	1·3 (31·8)	3·5 (33·1)	1·0 (0·4, 2·4)	1·1 (0·4, 2·8)	0·9 (0·2, 3·2)	0·84
Eosinophils/mm^2 epithelium	2·5 (45·9)	2·2 (42·8)	2·4 (50·5)	3·5 (67·3)	1·0 (0·4, 2·8)	1·5 (0·4, 5·4)	0·7 (0·2, 2·9)	0·59
CD3+ cells/mm^2 epithelium	2·0 (38·8)	3·9 (53·8)	4·1 (47·0)	3·2 (56·8)	1·7 (0·5, 5·4)	0·8 (0·2, 3·2)	2·0 (0·3, 12·0)	0·42
Mast cells/mm^2 epithelium	0·8 (21·2)	1·0 (22·7)	1·7 (32·8)	1·8 (39·2)	1·9 (1·0, 3·4)	1·8 (0·9, 3·6)	1·0 (0·4, 2·6)	0·93
Neutrophils/mm^2	0·7	2·1	0·8	0·8	1·1	0·4	3·1	0·044

epithelium	(8·6)	(53·2)	(18·7)	(22·2)	(0·6, 2·3)	(0·2, 0·8)	(1·0, 9·1)	
Mast cells/mm^2 airway smooth muscle	3·4 (43·4)	4·3 (51·1)	2·9 (53·8)	4·8 (56·6)	0·7 (0·2, 1·9)	1·1 (0·4, 3·0)	0·6 (0·1, 2·6)	0·48
Tissue remodeling†								
MUC5AC cells/mm intact epithelial length	38·3 (6·7)	24·6 (7·9)	55·6 (7·6)	37·1 (7·9)	12·8 (-7·3, 32·9)	10·2 (-7·6, 27·9)	2·7 (-24·1, 29·5)	0·84
MUC5AC cells/mm^2 intact epithelial area	738·2 (134·8)	461·6 (128·2)	836·6 (140·7)	666·6 (146·9)	37·0 (-349·0, 423·4)	192·9 (-148, 533·7)	-156 (-671, 359·3)	0·53
Percentage of intact epithelial area positive for MUC5AC	5·4 (2·6)	4·7 (1·7)	10·6 (2·6)	4·5 (2·7)	4·7 (-1·1, 10·5)	-0·2 (-5·3, 4·9)	4·9 (-2·8, 12·6)	0·20
Goblet cells/mm intact epithelial length	13·7 (4·2)	11·6 (3·3)	22·3 (4·2)	23·2 (4·8)	7·9 (-0·6, 16·5)	11·6 (3·6, 19·7)	-3·7 (-15·5, 8·0)	0·51
Goblet cells/mm^2 intact epithelial area	287·8 (83·6)	209·7 (48·5)	366·9 (90·7)	457·0 (103·4)	58·9 (-147, 265·2)	247·4 (51·7, 443·0)	-188 (-473, 95·9)	0·18
Vessel score (mean Chalkley count)	5·8 (0·3)	6·6 (0·5)	5·9 (0·4)	5·8 (0·4)	0·1 (-0·8, 1·0)	-0·8 (-1·8, 0·1)	0·9 (-0·4, 2·2)	0·17
Intact epithelium (% of total length)	28·0 (6·5)	47·0 (7·9)	51·7 (6·9)	42·9 (7·1)	23·7 (6·4, 41·0)	-4·1 (-22·0, 13·8)	27·8 (2·9, 52·7)	0·030
Partially intact epithelium (% of total length)	39·0 (4·3)	39·2 (6·4)	34·0 (4·8)	35·3 (5·0)	-5·0 (-19·6, 9·5)	-3·9 (-18·9, 11·2)	-1·2 (-22·1, 19·7)	0·91
Denuded epithelium (% of total length)	33·0 (6·7)	13·8 (4·1)	14·3 (5·5)	21·7 (5·7)	-18·6 (-31·4, -5·9)	8·0 (-5·2, 21·2)	-26·6 (-44·9, -8·3)	0·0062

Epithelial thickness (μm)	54·3 (4·5)	64·0 (5·8)	67·3 (4·3)	58·4 (4·9)	10·3 (-5·6, 26·2)	-5·1 (-21·9, 11·7)	15·4 (-7·7, 38·5)	0·18
RBM thickness (μm)	14·9 (1·2)	10·4 (1·0)	11·3 (1·1)	13·4 (1·2)	-1·5 (-3·8, 0·7)	0·6 (-1·9, 3·0)	-2·1 (-5·4, 1·2)	0·20

*Baseline and post-treatment values are geometric mean (% coefficient of variation), change from baseline to post-treatment is geometric mean fold-change (lower limit, upper limit of 95% confidence interval), and treatment difference is ratio of geometric mean fold-change in fevipiprant group to geometric mean fold-change in placebo group (lower limit, upper limit of 95% confidence interval).

†Baseline and post-treatment values are mean (standard error), change from baseline to post-treatment is mean change (lower limit, upper limit of 95% confidence interval), and treatment difference is mean change in fevipiprant group minus mean change in placebo group (lower limit, upper limit of 95% confidence interval).

Table 3.3.5 Quantitative computed tomography and densitometry

Outcome	Baseline values		Post-treatment values		Change from baseline to post-treatment			
	Fevipiprant	Placebo	Fevipiprant	Placebo	Fevipiprant (N = 23)	Placebo (N = 26)	Treatment difference (Fevipiprant vs placebo)	P value
RB1 wall area / BSA (mm^2/m^2)	19·74 (1·09)	19·59 (0·94)	20·89 (1·50)	18·37 (1·41)	1·15 (-0·89, 3·18)	-1·23 (-3·14, 0·68)	2·38 (-0·41, 5·17)	0·093
RB1 luminal area / BSA (mm^2/m^2)	12·54 (1·45)	11·11 (0·79)	14·22 (2·05)	11·02 (1·92)	1·68 (-1·55, 4·90)	-0·09 (-3·12, 2·93)	1·77 (-2·65, 6·19)	0·42
RB1 percentage wall area (%)	62·7 (1·3)	64·5 (0·8)	63·2 (1·1)	63·1 (1·1)	0·4 (-1·3, 2·2)	-1·4 (-3·0, 0·3)	1·8 (-0·6, 4·2)	0·14
Average wall area / BSA (mm^2/m^2)	17·3 (0·5)	17·7 (0·6)	17·6 (0·6)	17·8 (0·6)	0·3 (-0·5, 1·1)	0·1 (-0·7, 0·9)	0·2 (-0·9, 1·3)	0·75
Average lumen area / BSA (mm^2/m^2)	10·6 (0·5)	10·9 (0·6)	10·7 (0·6)	11·5 (0·6)	0·2 (-0·6, 1·0)	0·6 (-0·2, 1·3)	-0·4 (-1·5, 0·7)	0·45
Average percentage wall area (%)	63·2 (0·5)	62·8 (0·4)	62·9 (0·3)	62·3 (0·3)	-0·3 (-1·0, 0·4)	-0·5 (-1·1, 0·1)	0·2 (-0·7, 1·1)	0·67
Inspiratory MLD (HU)	-829·1 (7·7)	-837·2 (6·9)	-839·7 (5·4)	-846·5 (5·1)	-10·6 (-20·7, -0·6)	-9·3 (-18·7, 0·2)	-1·4 (-15·2, 12·4)	0·84
Expiratory MLD (HU)	-704·8 (15·0)	-719·1 (10·6)	-706·6 (12·7)	-732·7 (11·7)	-1·0 (-11·5, 9·6)	-13·0 (-22·8, -3·2)	12·0 (-2·4, 26·4)	0·099
MLD E/I	0·851 (0·016)	0·861 (0·013)	0·841 (0·014)	0·865 (0·013)	-0·010 (-0·026, 0·005)	0·003 (-0·012, 0·017)	-0·013 (-0·034, 0·008)	0·22
Inspiratory VI <-950	13·7	14·3	15·2	14·9	1·5	0·7	0·8	0·42

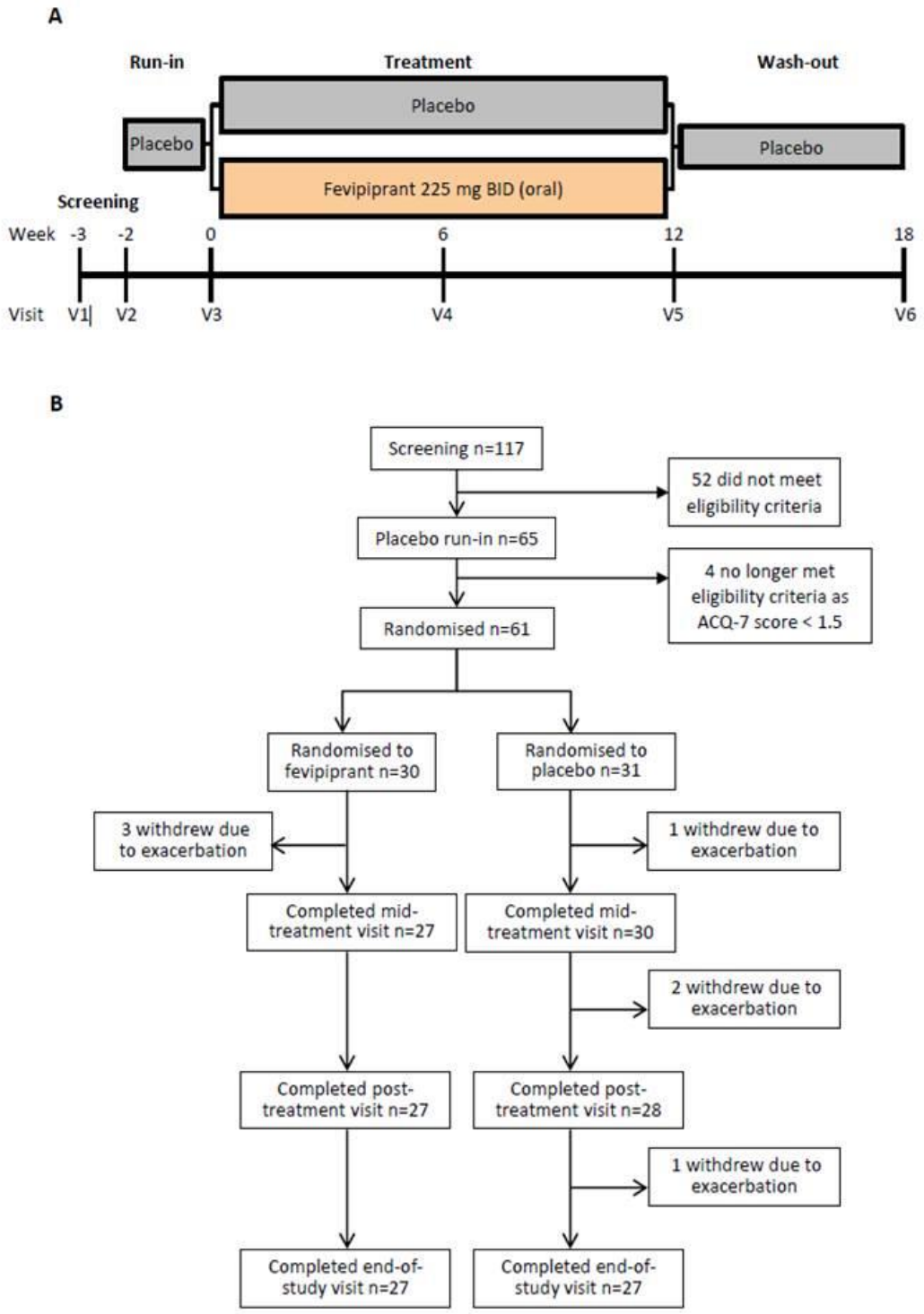
HU (cm^3)	(1·4)	(1·2)	(1·2)	(1·2)	(0·0, 3·1)	(-0·8, 2·1)	(-1·2, 2·9)	
Expiratory VI <-856 HU (cm^3)	21·4 (3·7)	22·0 (2·8)	21·8 (3·4)	24·1 (3·1)	0·4 (-2·0, 2·8)	2·2 (-0·1, 4·4)	-1·8 (-5·1, 1·5)	0·27
CTLV expiratory (cm^3)	3040 (199)	3209 (188)	3004 (225)	3420 (206)	-10 (-138, 118)	205 (86, 325)	-216 (-391, -40)	0·017
CTLV inspiratory (cm^3)	5221 (252)	5588 (297)	5419 (266)	5809 (251)	198 (-58, 454)	222 (-19, 462)	-24 (-375, 328)	0·89
CT lung volume E/I	0·588 (0·026)	0·583 (0·030)	0·557 (0·026)	0·582 (0·024)	-0·030 (-0·066, 0·006)	-0·006 (-0·040, 0·027)	-0·024 (-0·073, 0·026)	0·34
P_{15} (HU)	-939·7 (5·3)	-942·4 (4·0)	-946·5 (3·6)	-947·2 (3·4)	-6·9 (-13·2, -0·5)	-4·8 (-10·7, 1·2)	-2·1 (-10·8, 6·6)	0·63
Pi10 (mm^2)	15·6 (0·4)	14·8 (0·2)	16·0 (0·4)	15·0 (0·4)	0·3 (-0·2, 0·9)	0·2 (-0·3, 0·8)	0·1 (-0·7, 0·9)	0·76
Po20 (%)	56·5 (0·3)	56·5 (0·4)	57·8 (0·7)	56·8 (0·7)	1·3 (0·0, 2·6)	0·4 (-0·9, 1·6)	0·9 (-0·9, 2·7)	0·32

Baseline and post-treatment values are mean (standard error), change from baseline to post-treatment is mean change (lower limit, upper limit of 95% confidence interval), and treatment difference is mean change in fevipiprant group minus mean change in placebo group (lower limit, upper limit of 95% confidence interval).

Table 3.3.6 Summary of Adverse Events

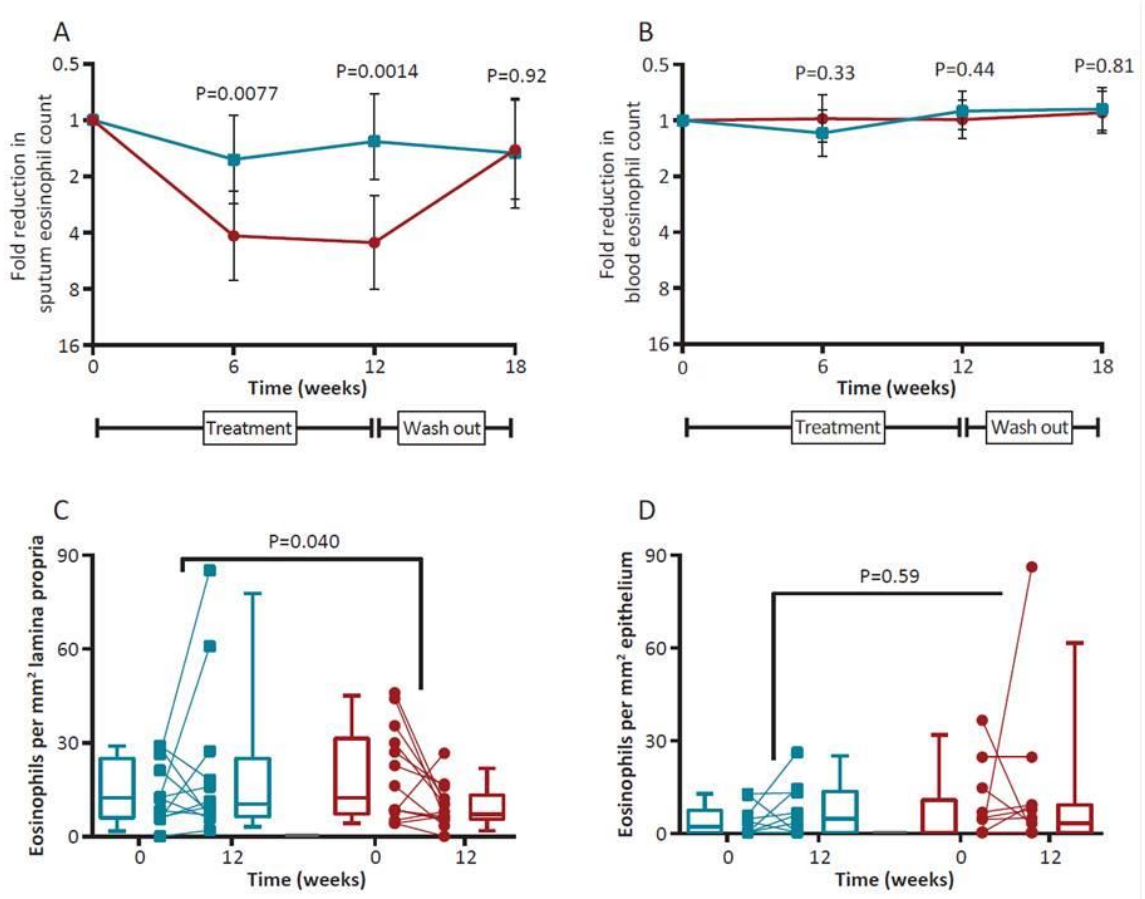
Group	**Period between baseline and post-treatment visits**		**Period between baseline and end-of-study visits**	
	Fevipiprant (N=29) n (%)	**Placebo (N=32)** n (%)	**Fevipiprant (N=29)** n (%)	**Placebo (N=32)** n (%)
Patients with at least 1 AE	**21 (72·4)**	**25 (78·1)**	**24 (82·8)**	**26 (81·3)**
Primary system organ class				
Infections and infestations	8 (27·6)	8 (25·0)	11 (37·9)	10 (31·3)
Respiratory, thoracic and mediastinal disorders	6 (20·7)	9 (28·1)	12 (41·4)	15 (46·9)
Gastrointestinal disorders	3 (10·3)	6 (18·8)	5 (17·2)	8 (25·0)
Nervous system disorders	3 (10·3)	6 (18·8)	3 (10·3)	8 (25·0)
Injury, poisoning and procedural complications	5 (17·2)	1 (3·1)	5 (17·2)	2 (6·3)
Musculoskeletal and connective tissue disorders	1 (3·4)	3 (9·4)	3 (10·3)	3 (9·4)
General disorders and administration site conditions	3 (10·3)	1 (3·1)	3 (10·3)	2 (6·3)
Blood and lymphatic system disorders	2 (6·9)	0	2 (6·9)	0
Eye disorders	0	2 (6·3)	0	2 (6·3)
Investigations	1 (3·4)	1 (3·1)	2 (6·9)	2 (6·3)
Metabolism and nutrition disorders	2 (6·9)	0	2 (6·9)	0
Cardiac disorders	0	1 (3·1)	0	1 (3·1)
Renal and urinary disorders	1 (3·4)	0	2 (6·9)	0
Reproductive system and breast disorders	0	1 (3·1)	0	1 (3·1)
Skin and subcutaneous tissue disorders	0	1 (3·1)	1 (3·4)	1 (3·1)
Immune system disorders	0	0	0	1 (3·1)
Surgical and medical procedures	0	0	0	1 (3·1)
Vascular disorders	0	0	0	1 (3·1)

Figure 3.3.1 Summary of study protocol and participant flow



Panel A shows the timings of study visits and treatment allocations. Panel B shows the number of patients who attended screening, were randomised, and completed each of the study visits.

Figure 3.3.2 Comparison of eosinophilic inflammation outcomes between the study groups



Panels A and B show fold-reductions in sputum and blood eosinophil counts respectively at each study visit compared to the baseline visit, in the placebo (blue square) and fevipiprant (orange circle) groups. P values refer to differences between the study groups with respect to change from the baseline visit. Panels C and D show lamina propria and epithelial eosinophil numbers respectively at the baseline and post-treatment visits, in the placebo (blue square) and fevipiprant (orange circle) groups. Box and whisker plots show the median, 25th and 75th percentiles as a box, and the 10th and 90th percentiles as whiskers. P values refer to differences between the study groups with respect to change from the baseline visit to the post-treatment visit.

Figure 3.3.3 Comparison of patient-reported and lung function outcome measures between the study groups

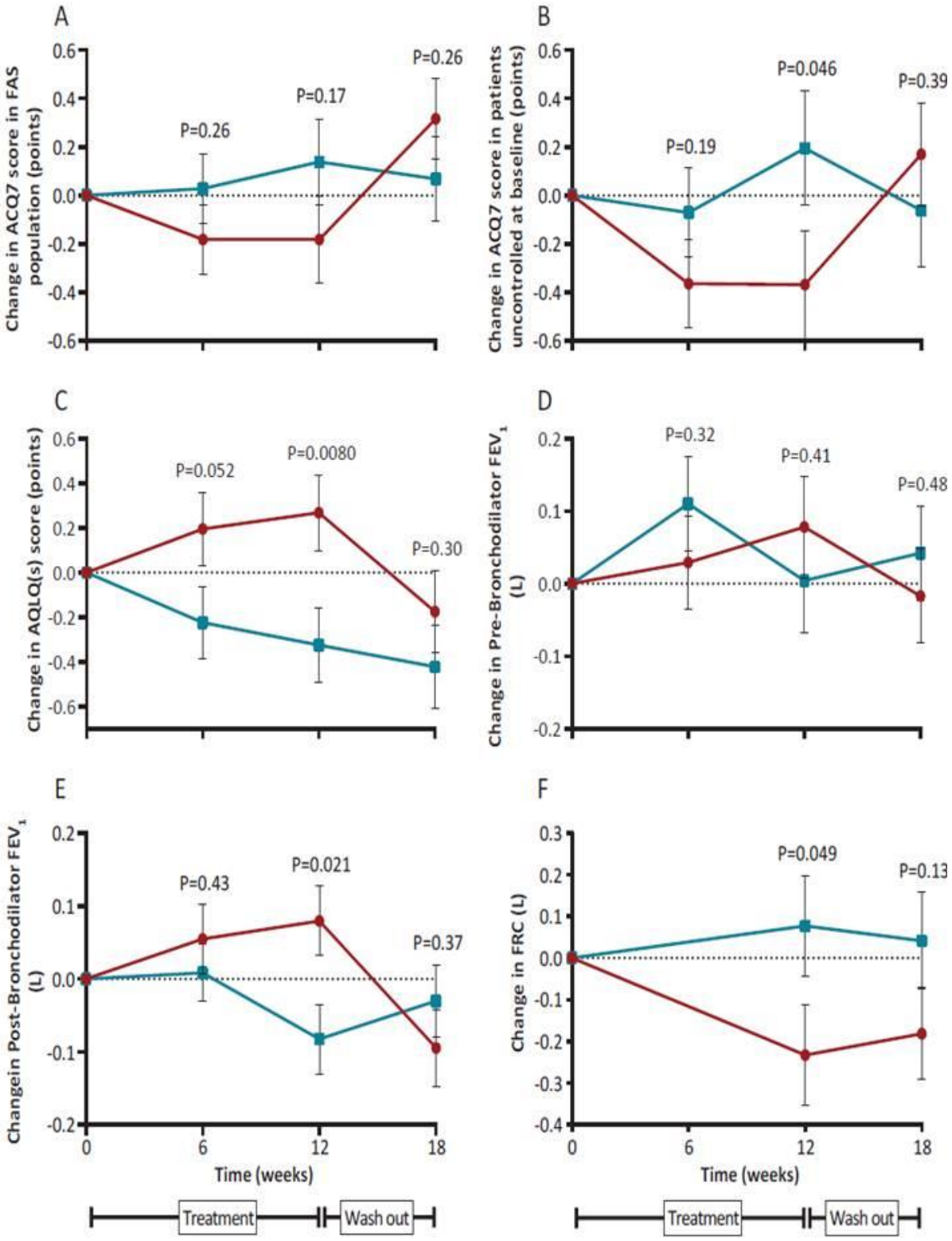
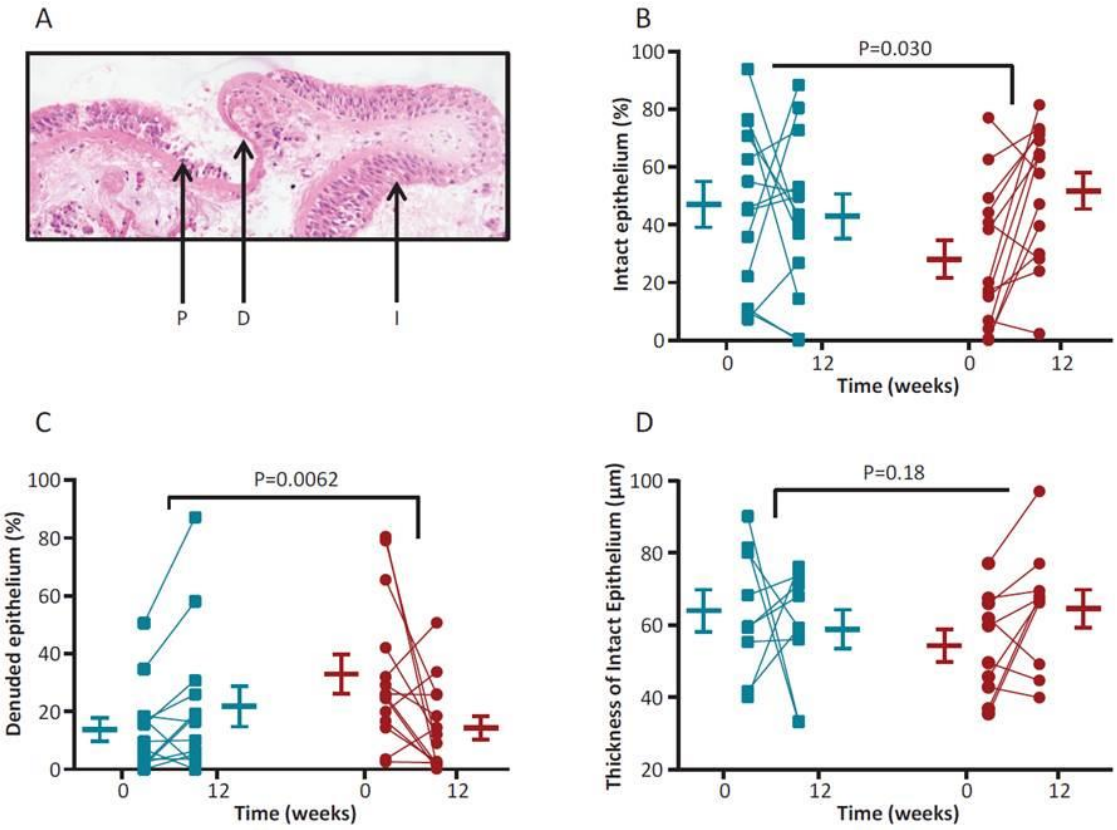


Figure 3.3.3 cont…

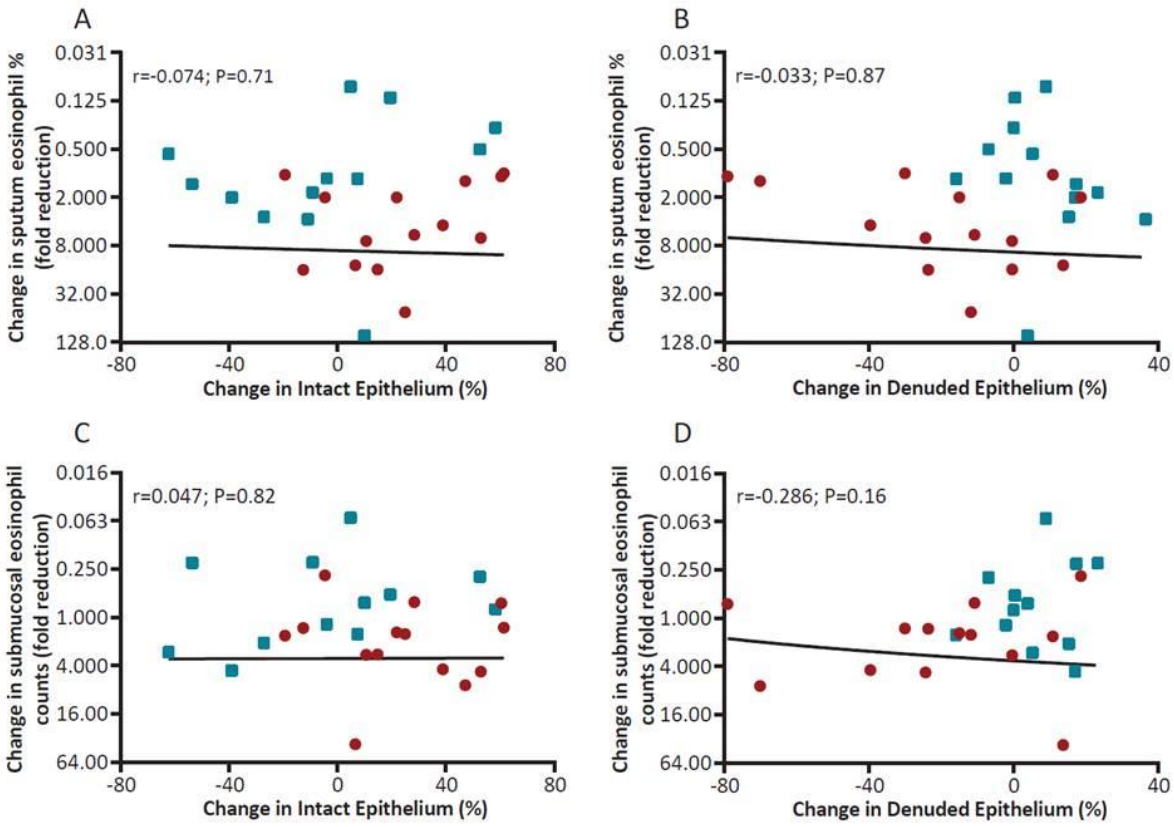
Changes compared to the baseline visit are shown in the placebo (blue square) and fevipiprant (orange circle) groups with respect to Asthma Control Questionnaire score (ACQ7) in the Full Analysis Set (FAS, Panel A), ACQ7 in the subgroup with a baseline value $\geq 1{\cdot}5$ (Panel B), standardised Asthma Quality of Life Questionnaire score (AQLQ(S), Panel C), forced expiratory volume in one second (FEV_1) performed before the administration of a bronchodilator (Panel D), FEV_1 performed after the administration of a bronchodilator (Panel E), and functional residual capacity (FRC, Panel F). P values refer to differences between the study groups with respect to change from the baseline visit.

Figure 3.3.4 Comparison of epithelial damage outcome measures between the study groups



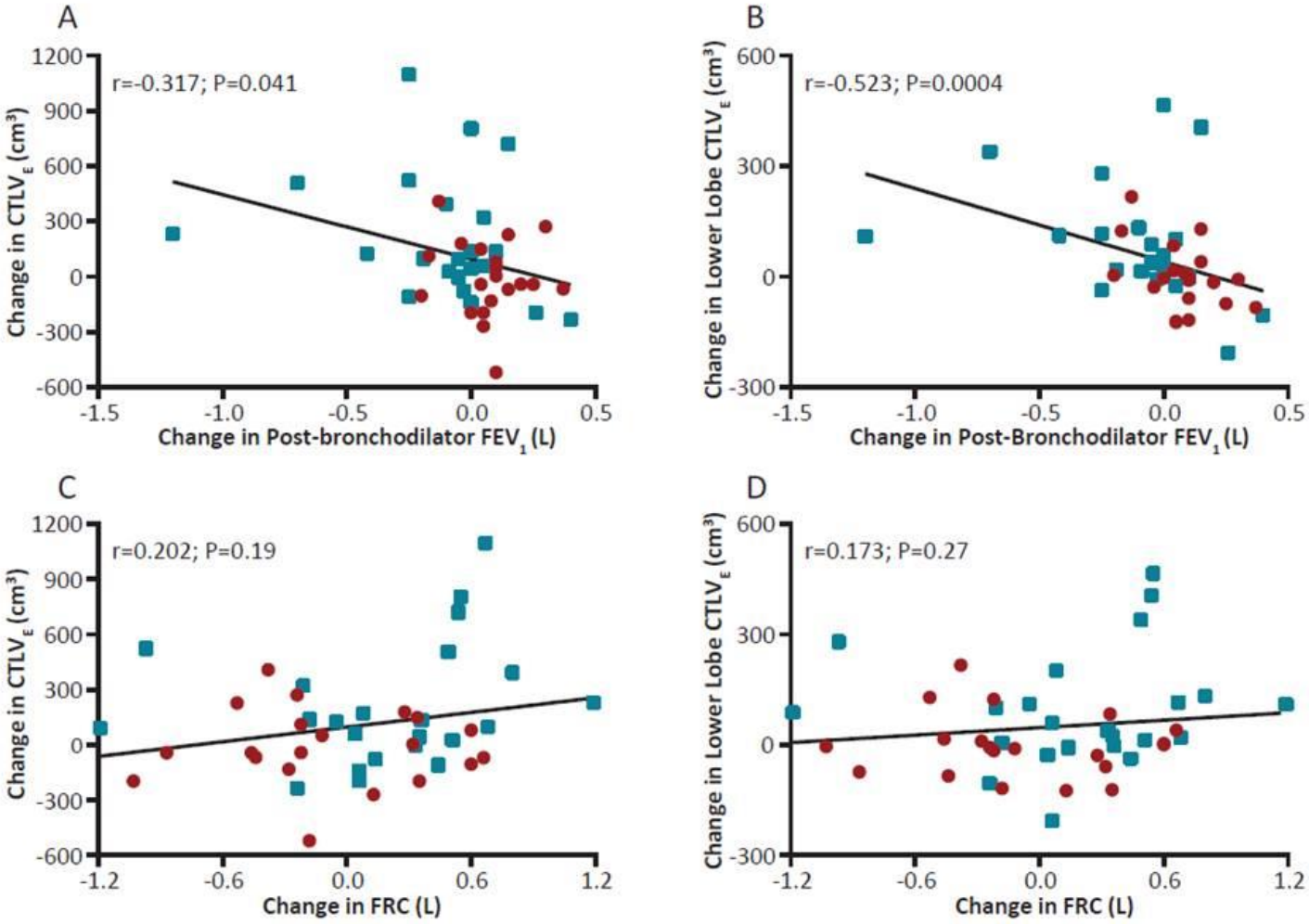
Panel A shows a photomicrograph of a bronchial biopsy specimen demonstrating the appearance of intact epithelium (I), partially denuded epithelium (P) and denuded epithelium (D). Panels B-D show percentage of epithelium that is intact, percentage of epithelium that is denuded and thickness of intact epithelium respectively at the baseline and post-treatment visits, in the placebo (blue square) and fevipiprant (orange circle) groups. Error bars indicate the mean plus or minus the standard error of the mean. P values refer to differences between the study groups with respect to change from the baseline visit to the post-treatment visit.

Figure 3.3.5 Correlations between changes in eosinophilic airway inflammation and changes in epithelial damage between the baseline and post-treatment visits



Panels A and B show correlations between fold-change in sputum eosinophil count and change in intact or denuded epithelial percentage respectively. Panels C and D show correlations between fold-change in submucosal eosinophil count and change in intact or denuded epithelial percentage respectively. Participants in the placebo and fevipiprant groups are represented by blue squares and orange circles respectively, and best-fit linear regression lines are shown for the combined group. Spearman correlation coefficients (r) and associated P values are shown

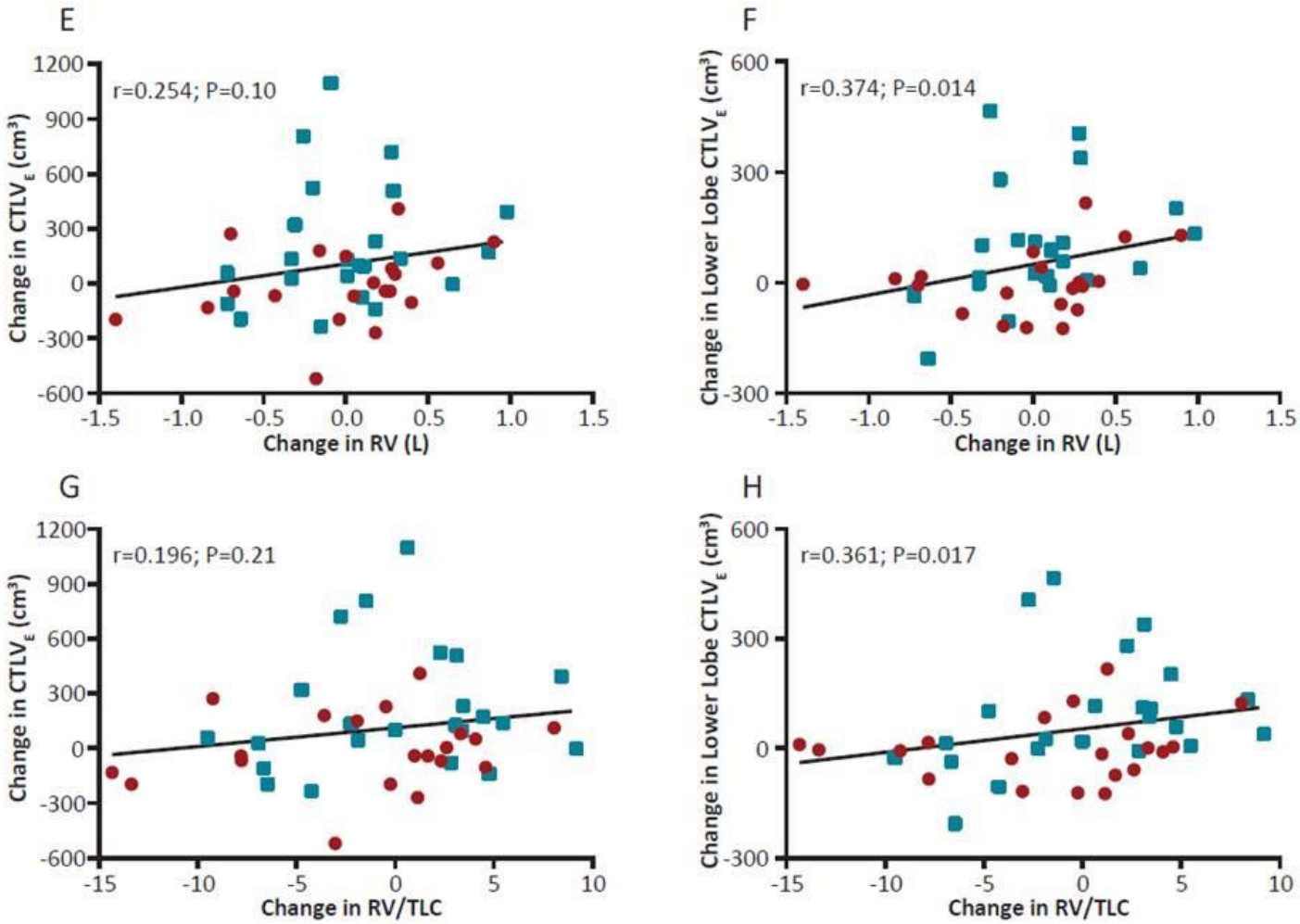
Figure 3.3.6 A-D Correlations between changes in computed tomography-derived lung volumes and changes in lung function outcomes between the baseline and post-treatment visits



Legend on page 196

Participants in the placebo and fevipiprant groups are represented by blue squares and orange circles respectively

Figure 3.3.6 E-H Correlations between changes in computed tomography-derived lung volumes and changes in lung function outcomes between the baseline and post-treatment visits



Legend on page 196

Participants in the placebo and fevipiprant groups are represented by blue squares and orange circles respectively

Figure 3.3.6 Correlations between changes in computed tomography-derived lung volumes and changes in lung function outcomes between the baseline and post-treatment visits

Correlations are shown between changes in expiratory computed tomography-derived lung volumes ($CTLV_E$) in the whole lung or specifically the lower lobes, and changes in post-bronchodilator forced expiratory volume in one second (FEV_1, Panels A and B), functional residual capacity (FRC, Panels C and D), residual volume (RV, Panels E and F), and the ratio of residual volume to total lung capacity (RV/TLC, Panels G and H). Participants in the placebo and fevipiprant groups are represented by blue squares and orange circles respectively, and best-fit linear regression lines are shown for the combined group. Spearman correlation coefficients (r) and associated P values are shown.

4 CONCLUSIONS

4.1 Final Discussion

Airway wall remodelling, alongside airway inflammation is thought to be an integral part of disease pathogenesis in both asthma and COPD. It is a coverall term for histological changes in the airways that is not inflammation. Research into remodelling and inflammation in asthma and COPD have been ongoing for decades, however the natural history of remodelling, the potential for reversal and its relationship to inflammation remain largely unknown.

Gas trapping and emphysema are also features seen in obstructive airway diseases, the former thought to be related to disease in the small distal airways and the latter a consequence of long standing small airway disease. Again, thought to relate back to remodelling and inflammation.

Understanding remodelling is of high importance, and traditionally it has been studied using ex vivo tissue samples and requires invasive methods to collect samples. There are numerous difficulties with traditional methods. Firstly tissue collection in living subjects is invasive. A bronchoscopy is an unpleasant experience. For the most part it is avoided in research, in those with very severe disease as it is not without risks. Secondly only relatively small samples can be easily collected. Certainly there is no scope for large 'organ level' studies particularly in asthma. Thirdly histological and pathological analysis requires the tissue/organ to be ex vivo.

QCT is quick, painless, non-invasive, can be done in large numbers and allows in vivo analysis at organ level. However an obvious consideration for QCT is radiation exposure. In this thesis all CTs were acquired using a low dose protocol, and all studies

had a limit of 10mSv exposure over three years. This is a very conservative approach when national regulations set a maximum limit of 20mSv per year for individuals who work with radiation, such as nuclear power station workers.

This thesis has demonstrated that QCT gives us the ability to quantify and assess the structure of the lungs without using invasive procedures. It enables in vivo assessment, and when done responsibility and with careful monitoring, repeated in vivo assessment.

Imaging is therefore a unique opportunity to probe the lungs and QCT in particular is able to provide a lot of data regarding structure, and how structre relates to function.

I will now summarise the findings of each of the studies included in this thesis, evaluate how the study has demonstrated the role QCT can play in providing insights into obstructive airway diseases. I will also discuss key questions, future directions and the limitations of this thesis.

4.2 Study 3.1: Relationship Between Lung Function and Quantitative Computed Tomography Parameters of Airway Remodelling, Air-trapping and Emphysema in Asthma and COPD: A Single Center Study

This study demonstrated a few key points. It confirmed the heterogeneity within each disease. In all QCT measures there was overlap between all three groups, with some individuals from both COPD and asthma cohorts falling into the same ranges as the healthy subjects. In diseases such as asthma and COPD where there is a great deal of heterogeneity, a tool like QCT, which is able to assess large numbers of subjects, gives more scope for seeing trends and patterns which might otherwise be missed.

Indeed, despite the heterogeneity seen, it was possible to assess the differences of QCT derived measures of proximal airways. This study showed that when looking at all COPD subjects, and all asthmatic subjects, they differed significantly to healthy controls by having larger percentage wall areas in their segmental bronchi, and smaller lumen areas, (although the latter only reached statistical significance with asthmatics). Differences were also made more apparent when stratifying subjects according to airflow limitation, suggesting that structural changes seen on QCT are linked to established physiological measures of disease severity.

Another key point is the result of the univariate and multivariate analysis. Interestingly, when looking at the asthmatic cohort as a whole, (including those with no airflow limitation), mean percentage wall area is a significant contributor to FEV_1 % predicted, whereas $MLD_{E/I}$ is the only contributing factor in asthmatics with airflow limitation. This requires further investigation, however knowledge of interplay between airway structure and lung function, such as this may have a significant impact on directing

future therapies such as bronchial thermoplasty, which currently focuses on severe asthmatics, who will often have established fixed airflow obstruction.

However it was the assessment of QCT derived measures of emphysema and air trapping where curious variation between asthma and COPD occurred. As expected, COPD subjects had significantly worse measures of emphysema than healthy controls, and asthmatics were no different to healthy controls, with the exception of the asthmatic group who had severe airflow limitation. This latter group was in fact, statistically, no different to COPD subjects and different to the other asthmatics as well the healthy controls. Interestingly, the assessment of the structure of these low density areas suggested that despite similar overall levels of low density, they were structurally different between the asthmatics with severe airflow limitation and COPD subjects, hinting at a different underlying cause. This is a novel QCT insight into the phenotypic differences between asthma and COPD, and the ability of QCT to identify new aspects of the remodelling process in both diseases. However it does need to be tested in large numbers, and ideally would need pathological investigation and corroboration. Nevertheless it does demonstrate how QCT can probe the lungs in vivo and provide new insights and new routes for investigation.

In both COPD and asthma, the QCT measure of air trapping, $MLD_{E/I}$, was shown to be consistently different between the diseases, and when compared to healthy controls, and very important contributor to FEV_1 % predicted in both diseases. Therefore additional investigation of this parameter and what it represents is of great interest. This study suggests it is a major player in asthmatics with airflow limitation and raises the

question, would targeted local therapies and/or systemic therapies achieve better control?

Other than investigating QCT parameters in relation to measures of obstructive airflow, this study identified weak relationships in asthma between proximal airway morphometry and blood neutrophilia, worse health status and poorer asthma control. In COPD, $MLD_{E/I}$ was associated with poorer health status. However in both groups, these relationships were weak and few, if any, conclusions should be drawn from them from this study alone. However this does provide a prompt to use QCT to probe the structure of the lungs of inflammatory subgroups e.g. neutrophilic asthmatics.

Key questions have been raised by this study, although a large cross section of subjects with varying degrees of airflow obstruction has been studied, and the observations that have been made comparing the groups across the spectrum of airflow limitation are fascinating, this study cannot comment on the natural history of either disease as there is no longitudinal element. Understanding the natural history of the disease and how it appears on QCT is an area of great interest. Does QCT have the potential to improve the selection of subjects for particular management routes? In particular treatments focused on remodelling and structure, (as opposed to interventions that influence short term inflammation), such as bronchial thermoplasty. This thesis has supplied the evidence that suggests this is an area that shows great potential and deserves further investigation.

Although this study has shown structural changes in the airways and lungs of asthma and COPD sufferers, it has been unable to shed light on what these changes could be as there has been no pathological/histological element to it. In COPD there is greater scope for acquiring lung tissue for histological analysis, so headway is being made at looking at QCT and pathological findings. However the same cannot be said for asthma and this remains a key area to investigate.

QCT links with inflammation has been touched upon in this study, but was limited to sputum and blood counts. Further detailed analysis of this area is needed in future work with subjects undergoing much more detailed clinical work up of their inflammatory profile.

4.3 Study 3.2: Associations in asthma between quantitative computed tomography and bronchial biopsy-derived airway remodelling

This study begins to address some key questions that remained from study 3.1. It has investigated the relationship of QCT to changes seen in bronchial biopsies of asthmatic subjects. It has also begun to probe the significance of $MLD_{E/I}$ in asthma.

Here we see that QCT parameters of proximal airway morphometry are associated with epithelial thickness and percentage airway smooth muscle,(with the latter influencing airflow limitation), but not airway inflammation. Suggesting that what is seen on QCT is due to disease led remodelling rather than acute inflammatory responses in the airway, as was indicated by study 3.1.

$MLD_{E/I}$ was associated with vascularity and lung function. Increased vascularity in asthmatics is well established, but this is the first time a link has been demonstrated between air trapping and vascularity. Study 3.1 has shown that the measure of air trapping, as seen with the parameter $MLD_{E/I}$, plays an important role in airflow limitation in asthmatics. When that observation is considered alongside the association with increased vascularity in bronchial biopsies and $MLD_{E/I}$ seen in this study, but no other histological features, (including inflammatory markers); it suggests that this aspect of asthmatic airway pathology deserves thorough further investigation, focusing on comparing vascularity and its effects in asthmatics with and without fixed airflow obstruction. This again demonstrates how QCT is able to expand on, and provide novel information to traditional methods of investigating remodelling in asthma.

The tissue samples in this study were taken from bronchial biopsies. So although there is this intriguing association of vascularity with a QCT marker of air-trapping, and thus we assume small airway disease, the small airways themselves were not sampled. Further work to look at the histological make up of lung parenchyma in asthmatics and compare it to QCT is key.

Another area for further study is looking at treatments that target vascularity and assess their impact on air trapping and lung function. Corticosteroids could be a good initial starting point as it is thought to decrease vascularity and are commonly used. However any new drugs in development that are thought to impact vascularity would be wise to consider assessing air trapping, as measured by $MLD_{E/I}$.

4.4 Study 3.3: Randomised controlled trial of the prostaglandin D2 receptor 2 antagonist fevipiprant in persistent eosinophilic asthma

This study was based on a 12 week, randomized, double-blind, placebo-controlled, parallel-group clinical trial of a new drug, fevipiprant. DP2 recruits and activates eosinophils. It was therefore anticipated that fevipiprant would reduce sputum eosinophilia and improve spirometry.

This trial found that fevipiprant did indeed significantly reduce sputum eosinophilia. It also significantly improved FEV_1, AQLQ(S) scores and FRC when compared to subjects who had been taking the placebo. QCT measures showed that the expiratory lung volume decreased significantly in those taking fevipiprant when compared to the placebo group. Confirming the clinical finding of reduced FRC.

No other QCT parameters showed any significant change. Despite these apparently negative results, this has still added valuable understanding to the interpretation of QCT parameters in asthma.

Firstly, the main change seen in the biopsy results was epithelial integrity, and markers of airway inflammation, which demonstrated no link with QCT parameters in Study 3.2. Of note,, study 3.1 showed that although QCT parameters were significant contributors in the linear regression model for FEV_1, they only accounted for about a quarter of it in asthmatics. This, taken alongside the reduced sputum eosinophil count, suggests the effect fevipiprant had on FEV_1 worked via factors likely linked directly to the inflammatory process in the airways. Studies 3.1 and 3.2 have already suggested

that short term change in inflammation is not what QCT is best able to measure, again demonstrating the robustness of QCT as it has maintained the same story.

Secondly, this study was powered to assess sputum eosinophil changes. QCT was an exploratory outcome, and it is likely the study was under powered for QCT. This too may have contributed to lack of QCT changes. Nonetheless it is reassuring that despite probably under powering QCT did not produce random anomalous results.

This drug is still in development and still requires larger and longer studies. This current trial was not long enough to assess the impact of fevipiprant on exacerbation rate. Further study of this drug would be an excellent opportunity to assess the effects of intervention on lung structure both histologically and with QCT. This drug alters the inflammatory state of the subject. Longer studies of this drug would also provide good insight not long into longitudinal QCT, but also the effect of long term changes to an asthmatic's inflammatory profile, a question this thesis does not address.

4.5 Key questions and future directions

From the concluding discussion, the following questions have been posed:

1. Can QCT measure and quantify the natural history of obstructive airway diseases?

Study 3.1 showed how those with obstructive airway disease have distinct features depending on degree of airflow obstruction. However we cannot comment on disease progression. Despite presence of clear subdivisions, there is still a great deal of heterogeneity within both the asthmatic and COPD cohorts, once again highlighting the complexity of obstructive airway diseases. We do not know if those who have mild-moderate airflow obstruction go on to develop severe airflow limitation, or if the QCT changes seen are permanent or variable. Although study 3.1 nicely demonstrated different QCT features according to different levels of airflow obstruction, we cannot assume a QCT finding leads to certain clinical picture; indeed it is not known if any feature observed on QCT is a precursor to developing airflow obstruction. Longitudinal studies are needed to begin to answer this question, as this thesis has not touched upon this aspect.

This study also showed that once airflow limitation set in, percentage wall area no longer had a significant role in FEV_1 % predicted. Currently a new therapy, bronchial thermoplasty is being trialled to assess its impact on asthmatics, however it is only being used in those with severe uncontrolled asthma. If QCT could help assess and predict the natural history of asthma, it could have a dramatic impact in the choice of subjects for novel therapies, such as bronchial thermoplasty and systemic therapies.

2. Does QCT have any role in assessing airway inflammation in obstructive airway diseases?

Study 3.1 and 3.2 touched on the associations between inflammation and QCT. A weak correlation was found in asthmatics between QCT morphometry and blood neutrophilia. However in study 3.1, the inflammatory profile of the subjects was not the focus of the study, therefore the correlations cannot be interpreted to any great level, but their presence can be noted and their meaning considered. Interestingly, no correlations were seen with the inflammatory markers and QCT parameters in COPD. Inflammation in both asthmatics and COPD sufferers is notoriously volatile, therefore it should be stressed that a single cross sectional sample may not adequately reflect the normal state of that person's airways.

Considering the results this thesis, it would seem unlikely that the QCT parameters examined in this thesis would have a major role in all subtypes of inflammation, or have a role in identifying short term inflammatory changes. However it is very possible that QCT could have a role in helping phenotype sub groups, e.g. neutrophilic asthmatics, or have a role in identifying changes seen after long term intervention that has been targeted at a particular inflammatory profile. These scenarios have not been addressed in this thesis.

Another area to consider are QCT parameters that are currently under development and are beginning to filter into use, such as bronchial wall attenuation. By assessing the bronchial wall attenuation, this parameter would be more likely to reflect changes at a cellular level, than simply assessing wall area. However this feature was unavailable on

the Apollo® (by VIDA Diagnostics, Inc), platform at the time the scans were analysed for this thesis.

3. How do the images and measurements seen on QCT represent changes in histology?

There is definitely a lot more work to be done with comparing QCT and histology in asthma. Study 3.2 was one of the biggest and most comprehensive study to date looking at this in asthma. It certainly cannot be considered a comprehensive investigation of the relationship of QCT and histology in asthma, but it very clearly demonstrates that QCT does reflect histological changes. This study suggests further and more thorough investigation of QCT and histology would yield exciting and more novel insights into the remodelling process in asthma.

However there were limitations and areas where it could be improved. For example there was no standardisation of site of biopsy sample. An obvious, easy, improvement to this study would be to collect biopsies from standard locations, and compare these directly to the relevant airway on QCT, (although this would still not be a direct comparison as biopsies are taken from the carina whereas QCT assesses the middle 40% of the airway). However better localisation would also allow comparison with the relevant lobe densitometry. Another factor to consider is that this study only collected proximal airway tissue samples. Transbronchial biopsies would allow the distal airways to be studied directly. However they do carry a much higher risk of complications such as bleeding and pneumothorax.

Another area of interest, which has not been addressed in this thesis, but is very relevant to the assessment of the relationship between QCT and histology, is micro CT.

Micro CT however, needs much larger tissue samples than biopsies and is only ex vivo, any study looking at this is likely to be much smaller and more complex. On the other hand radiation exposure of the tissue samples would not be of any concern.

Currently the interpretation of low density areas on QCT are based on work done with COPD histology. Using these parameters in asthma enables us to compare both asthma and COPD, which is very valuable, but leaves us unsure of exactly how interpret unexpected findings such as the presence of "emphysema" in asthmatics. The QCT parameter of LAC-D-950, suggested that this low density area in asthmatics is structurally more like low density areas seen in healthy subjects rather than COPD subjects. But without histological assessment these remain educated assumptions and best guesses.

Studies looking at ex vivo lungs/lung tissue in asthma and comparing histological findings to QCT and micro CT findings would be of great interest, and would provide huge insight into this area.

However it is a testament to the work already done in managing and treating asthma that such samples are very hard to come by.

4.6 Limitations

Some limitations of this thesis have been addressed whilst looking at the key questions and future directions, by considering how the studies could be improved, what questions have arisen that have not been answered. However there are further, more general limitations that should be acknowledged.

In this thesis, I have only used one QCT analysis platform. On the one hand this fact provides continuity and comparability throughout, and is an important and necessary feature to ensure coherency of this thesis.

However, this also means any systematic errors the platform may have remain unidentified and uncorrected. In study 3.1, where cross sectional comparisons are made across clinically defined groups, this is less of an issue as computational errors are generally standard. If for example, Apollo® (by VIDA Diagnostics, Inc), over estimates wall area, all groups would have had wall area overestimated.

This limitation needs to be considered mainly in regards to study 3.2 where QCT was directly compared to histological features. If there were any systematic errors, would this have changed findings? As the association between vascularity and air trapping was a novel finding, this was assessed by a test group, who were able to repeat the findings. The test group used PulmoWorkstation (by VIDA Diagnostics, Inc), the predecessor to Apollo® (by VIDA Diagnostics, Inc). Although technically a different platform, similar algorithms would have been used by both. Therefore the possibility of undetected technical errors still remains possible, and this limitation should be taken into account

Another limitation to consider is user error in analysing and labelling the airways. Airway and lung segmentation are automatic with Apollo® (by VIDA Diagnostics, Inc). However in very diseased airways, where lumens are narrowed, and mucus plugs are present, segmentation becomes difficult, judgement is required when deciding when to pursue the analysis of an airway and when to stop. Another area where user judgement was required was where anatomy was not standard. This was rarely a problem with upper lobes, but lower lobes would often have slight variations. Throughout this thesis, I analysed all the scans, with the exception of a small selection which was only used for inter user comparisons, not for any of the study results. I ensured that I followed the same steps and tried to apply the same rules to branch labelling for all scans. However there will be a degree of error introduced due to all these factors. To a certain degree, these errors are unavoidable and are present in all research based on biological systems as there will always be natural variation. Nonetheless it should be acknowledged and its impact considered.

This thesis has looked at different aspects of using QCT to provide insight into the heterogeneity of obstructive airway diseases. However, in order to do this, different studies, with different study protocols have been used. For example, the very process of comparing COPD and asthma produces some important distinctions between the groups simply due to the way the diseases are defined and diagnosed. For example, in this thesis, all COPD subjects were smokers, whereas most asthmatics were not. There was also a significant difference in age, however this issue was addressed in study 3.1 and was not found to have a statistically significant influence. Using subjects with clear clinical diagnoses is on the one hand necessary; however it may also introduce subtle

bias and confounding factors despite careful attempts to assess such factors and correct for them where necessary.

4.7 Concluding remarks

QCT in its current form cannot replace traditional methods of investigating remodelling in asthma and COPD. However they complement each other. Histological investigation addresses what is happening on a cellular level, and is often limited to smaller study numbers. QCT on the other hand addresses what is happening on the organ level and can used in very large numbers to observe patterns in large and varied populations, bringing with it new and insightful observations that would otherwise have been missed. Questions that have arisen from this thesis have the ability to direct further research. Again these would have been missed if traditional methods alone were employed.

This thesis has looked at the use of QCT in comparing and interrogating the lungs of those with obstructive airway diseases as well as healthy controls and has demonstrated the robustness of QCT measures when examined alongside histology and when used in intervention. This thesis has demonstrated that QCT is able to provide valuable insight into the phenotypic heterogeneity of asthma and COPD.

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