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

Rachid Berair[1,6], Ruth Hartley[1,6], Vijay Mistry[1], Ajay Sheshadri[2], Sumit Gupta[1], Amisha Singapuri[1], Sherif Gonem[1], Richard P. Marshall[3], Ana R. Sousa[3], Aarti Shikotra[1], Richard Kay[4,5], Andrew Wardlaw[1], Peter Bradding[1], Salman Siddiqui[1], Mario Castro[2] and Christopher E. Brightling[1]

Affiliations: [1]Institute for Lung Health, NIHR Respiratory Biomedical Research Unit, Dept of Infection, Immunity and Inflammation, University of Leicester, Leicester, UK. [2]Dept of Internal Medicine, Washington University School of Medicine, St Louis, MO, USA. [3]GlaxoSmithKline, Uxbridge, UK. [4]Novartis Pharmaceuticals, Basel, Switzerland. [5]Medpace (UK) Ltd, Stirling, UK. [6]These authors contributed equally to this work.

Correspondence: Chris E. Brightling, Institute for Lung Health, University of Leicester, Groby Road, Leicester, LE3 9QP, UK. E-mail: ceb17@le.ac.uk

@ERSpublications
Airway remodelling in asthma biopsies is associated with proximal airway QCT-derived morphometry and air trapping http://ow.ly/ucoA308Jv9F

Cite this article as: Berair R, Hartley R, Mistry V, *et al*. Associations in asthma between quantitative computed tomography and bronchial biopsy-derived airway remodelling. *Eur Respir J* 2017; 49: 1601507 [https://doi.org/10.1183/13993003.01507-2016].

ABSTRACT Airway remodelling in asthma remains poorly understood. This study aimed to determine the association of airway remodelling measured on bronchial biopsies with 1) lung function impairment and 2) thoracic quantitative computed tomography (QCT)-derived morphometry and densitometry measures of proximal airway remodelling and air trapping.

Subjects were recruited from a single centre. Bronchial biopsy remodelling features that were the strongest predictors of lung function impairment and QCT-derived proximal airway morphometry and air trapping markers were determined by stepwise multiple regression. The best predictor of air trapping was validated in an independent replication group.

Airway smooth muscle % was the only predictor of post-bronchodilator forced expiratory volume in 1 s (FEV_1) % pred, while both airway smooth muscle % and vascularity were predictors of FEV_1/forced vital capacity. 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), whereas epithelial thickness was the only predictor of wall area % (R^2=0.13; p=0.018). Vascularity was the only significant predictor of air trapping (R^2=0.24; p=0.001), which was validated in the replication group (R^2=0.19; p=0.031).

In asthma, airway smooth muscle content and vascularity were both associated with airflow obstruction. QCT-derived proximal airway morphometry was most strongly associated with epithelial thickness and airway smooth muscle content, whereas air trapping was related to vascularity.

This article has supplementary material available from erj.ersjournals.com

Received: July 27 2016 | Accepted after revision: Jan 20 2017

Introduction

Asthma remains an important health problem with significant morbidity, mortality and economic burden [1, 2]. In addition to symptoms, asthma is characterised by variable airflow obstruction, airway inflammation and remodelling [2, 3]. Airway remodelling is a collective term for the structural changes in the airway wall, including epithelial thickness and integrity, increased airway smooth muscle mass, neoangiogenesis, and subepithelial fibrosis [2–4], and is related to persistent airflow limitation and airflow obstruction [4–6]. It is a feature of asthma even in children [7], demonstrating that it can occur early in the disease course; and it affects both large and small airways as evident from *post mortem* studies of asthma deaths [8, 9].

Macroscopic airway remodelling can be assessed noninvasively by quantitative computed tomography (QCT). This has become an established technique to determine airway morphometry and lung densitometry in asthma [10–19]. 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 [10–12]. 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 1) lung function impairment (post-bronchodilator forced expiratory volume in 1 s (FEV_1) % pred) and 2) QCT morphometry and densitometry measures of proximal airway remodelling and air trapping. The co-primary QCT outcome variables were: 1) for proximal airway remodelling: mean airway lumen area/body surface area and wall area %, and 2) for air trapping: mean lung density expiratory/inspiratory ratio (MLD E/I). To test our hypothesis we undertook a single-centre 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 asthmatic subjects from a second centre.

Methods

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 nonsmokers 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 one or more of the following: 1) a positive methacholine challenge test defined as a concentration of nebulised methacholine causing a 20% drop in FEV_1 of <8 $mg \cdot mL^{-1}$, 2) diurnal maximum peak flow variability of >20% over 2 weeks and 3) improvement of >15% in FEV_1 15 min after bronchodilator therapy. Subjects underwent pre- and post-bronchodilator spirometry (albuterol 400 µg), skin prick tests or allergen-specific IgE to assess for atopy, and those in the test group also underwent sputum induction and processing. Persistent airflow limitation was defined as a post-bronchodilator therapy FEV_1 % pred <80%. Written informed consent was obtained from all the participants. All subjects were on optimal asthma treatment and free of exacerbation for at least 6 weeks prior to recruitment into the study. 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).

Computed tomography

All subjects underwent either limited or full lung CT scans performed following administration of albuterol using standardised acquisition protocols as described previously [11, 16]. Limited-only scans from the

Support statement: This work was part funded by Wellcome Trust Senior Fellowship (C.E. Brightling), Airway Disease Predicting Outcomes through Patient Specific Computational Modelling (AirPROM) project (funded through FP7 EU grant), Leicester National Institute for Health Research (NIHR) Respiratory Biomedical Research Unit, Novartis and GlaxoSmithKline (GSK). Some of the biopsy and computed tomography data mentioned here were baseline data for a proportion of patients who were enrolled into a phase II randomised controlled trial (QAW039A2208) that was sponsored by Novartis Pharmaceuticals. S. Gupta is a NIHR Clinical Lecturer and is funded by a research and career development training scheme. This paper presents independent research funded by the NIHR. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Dept of Health. Funding information for this article has been deposited with the Open Funder Registry.

Conflict of interest: Disclosures can be found alongside this article at erj.ersjournals.com

aortic arch to the carina done at full inspiration (near total lung capacity) were undertaken in 14 asthmatic subjects [13, 14]. All other subjects had full lung inspiratory and expiratory (near-functional residual capacity) CT scans. Scans were analysed using semi-automated software: Apollo in the test group and Pulmonary Workstation version 2.0 in the replication group (VIDA Diagnostics, Coralville, IA, USA).

In the test group all inspiratory scans were analysed for the right upper lobe apical segmental bronchus (RB1) morphometry, while mean segmental bronchi morphometry was obtained in full lung scans only. First- to fifth-generation airways were labelled and measured using the analysis software. Morphological measurements of segmental airways (third-generation airways) were obtained along each centre-line voxel of the lumen perpendicular to the long axis on each airway and averaged over the middle third of the airway segment. Lumen area and total area were measured directly, while wall area was derived using the following calculation: wall area=total area−lumen area. Total, lumen and wall area were measured in mm^2, and all corrected for body surface area, which was calculated using the Mosteller formula, and expressed as $mm^2 \cdot m^{-2}$ body surface area [20]. Percentage wall area was calculated as follows: wall area %=100×wall area/total area. Estimates of air trapping were determined in the test and replication groups from MLD E/I on the expiratory/inspiratory scan and the percentage of lung voxels with a density lower than –856 HU (VI-856 HU) on expiratory scans (supplementary figure S1). The co-primary QCT outcome variables were: for proximal airway remodelling mean segmental airway lumen area/body surface area and wall area %, and for air trapping MLD E/I.

Endobronchial biopsies

Fibreoptic bronchoscopy was performed according to the British Thoracic Society guidelines [21]. All patients received albuterol prior to the procedure. 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 [6, 11, 22, 23].

Sections of 2 µm were cut from the glycol methacrylate-embedded biopsies and stained with haematoxylin/eosin. Immunohistochemical staining was done with the following mAbs: anti-mast cell tryptase clone AA1 (Dako, High Wycombe, UK), anti-α-smooth muscle actin clone 1A4 (Dako), anti-eosinophil major basic protein clone BMK-13 (Monosan, Uden, The Netherlands), anti-neutrophil elastase clone NP57 (Dako) and anti-endothelium clone EN4 (Monosan) or appropriate isotype controls.

The endobronchial biopsies were assessed by a single observer blinded to the clinical characteristics (ZEN 2012 image analysis software for light microscopy; Carl Zeiss, Jena, Germany) and expressed as the mean of measurements undertaken from a minimum of two sections either from independent biopsies or on noncontiguous tissue sections at least 20 µm apart from the same biopsy. Epithelial integrity was assessed by measuring the lengths of intact and denuded epithelium. These were expressed as percentage of all the reticular basement membrane (RBM) length present in the section. RBM and epithelial thickness were measured as described previously [24, 25]. 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, Edenbridge, UK) was used at ×200 to measure Chalkley counts in four nonoverlapping vascular hotspots (one or two per section) (supplementary figure S2) [6]. The mean Chalkley count was calculated from the four measurements. In 24 biopsies we compared the Chalkley count with computerised pixel counting in the lamina propria and found these measures were strongly correlated ($r=0.83$; $p<0.0001$). Airway smooth muscle content was determined as the proportion of the total area. Inflammatory cells were expressed as the number of nucleated cells/area of lamina propria.

The intraclass correlation coefficients for the within-donor measurements made by a single blinded observer were: airway smooth muscle % area 0.87, epithelial thickness 0.85 and vascularity 0.65.

The strongest independent immunohistological feature of airway remodelling associated with QCT-derived markers of air trapping identified in the test group was validated in the replication group. Sections of 4 µm were cut from the paraffin-embedded biopsies and stained with appropriate mAb or corresponding isotype control.

Statistical analysis

Statistical analysis was performed using Prism version 6.00 for Windows (GraphPad, La Jolla, CA, USA) and SPSS Statistics for Windows version 22.0 (IBM, Armonk, NY, USA). Parametric data were expressed as mean±SD and nonparametric data as median (interquartile range). Groups were compared using the unpaired t-test and Mann–Whitney U-test for parametric and nonparametric data, respectively. Proportions were compared using the Chi-squared test. Correlations between variables were expressed using Pearson's correlation. A stepwise multiple regression analysis was undertaken to determine the bronchial biopsy features that were the strongest predictors of post-bronchodilator FEV_1, FEV_1/forced vital capacity (FVC)

and QCT-derived mean segmental bronchial morphometry and air trapping. Regression data are presented as model-adjusted R^2 Pearson correlations alongside the standardised regression coefficient (β) of the modelled independent variable. A p-value of <0.05 was considered statistically significant.

Results

Baseline demographics and clinical characteristics of subjects with (n=30) and without (n=40) persistent airflow limitation (post-bronchodilator FEV_1 % pred <80% and ⩾80%, respectively) are shown in table 1. There was no difference between the two groups in terms of sex, age, duration of asthma, age of disease onset, smoking status, smoking pack-years, body mass index, sputum eosinophils or sputum neutrophils.

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) (table 1 and figure 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 % pred (r=−0.49; p<0.001) and post-bronchodilator FEV_1/FVC (r=−0.44; p<0.001) (figure 2). Vascularity was also inversely correlated with post-bronchodilator FEV_1 % pred (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

TABLE 1 Demographics and clinical/laboratory characteristics

	All	Post-bronchodilator FEV_1 % pred <80%	Post-bronchodilator FEV_1 % pred ⩾80%	p-value
Patients n	70	30	40	
Age years	49±12	52±12	47±13	0.095
Male %	57	67	50	0.163
Caucasian %	93	93	93	0.893
Asthma duration years	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
Smoking pack-years	0.0 (0.0–0.0)	0.0 (0.0–3.9)	0.0 (0.0–0.0)	0.109
Atopy %	81	77	81	0.659
GINA class n (%)				
GINA 5	22 (31)	13 (43.3)	9 (23)	0.084
GINA 4	34 (49)	15 (50.0)	19 (48)	
GINA 3	6 (9)	0 (0.0)	6 (15)	
GINA 1 and 2	8 (11)	2 (6.7)	6 (16)	
Inhaled BDP equivalent µg·24 h^{-1}	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*
FEV_1 % pred	78.8±24.6	55.1±14.3	96.5±12.8	<0.001*
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*
FEV_1 % pred	84.8±23.3	62.1±12.8	101.8±12.0	<0.001*
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				
Tissue eosinophils cells·mm^{-2} of lamina propria	19.6 (8.0–32.7)	19.2 (8.3–35.4)	19.9 (8.0–28.3)	0.947
Tissue neutrophils cells·mm^{-2} of lamina propria	5.8 (2.2–20.8)	4.3 (2.1–15.5)	9.6 (2.3–24.6)	0.924
Tissue mast cells cells·mm^{-2} of lamina propria	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 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

Data are presented as mean±SD or median (interquartile range), unless otherwise stated. FEV_1: forced expiratory volume in 1 s; BMI: body mass index; GINA: Global Initiative for Asthma; BDP: beclomethasone dipropionate; FVC: forced vital capacity; RBM: reticular basement membrane. *: p<0.05.

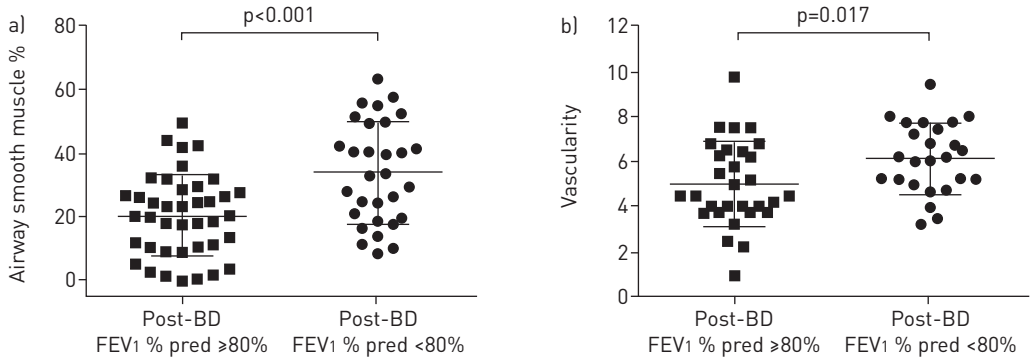

FIGURE 1 a) Airway smooth muscle % and b) vascularity (mean Chalkley count) in subjects with and without persistent airflow limitation (post-bronchodilator (BD) forced expiratory volume in 1 s (FEV_1) % pred <80% and ⩾80%). Data are presented as individual data points with mean±SD.

biopsies and spirometry measurements (supplementary table S1). Smoking history including pack-years was not associated with any of the remodelling or inflammatory features in bronchial biopsies.

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 $mm^2 \cdot m^{-2}$; p=0.047) and larger mean segmental bronchial wall area % (63.6±2.0% *versus* 62.5±2.1%; p=0.039) (table 2). These differences were more marked in the lower *versus* upper lobe bronchi (supplementary table S2). 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-856HU (32.2±19.8% *versus* 15.5±10.1%; p<0.001) (table 2).

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

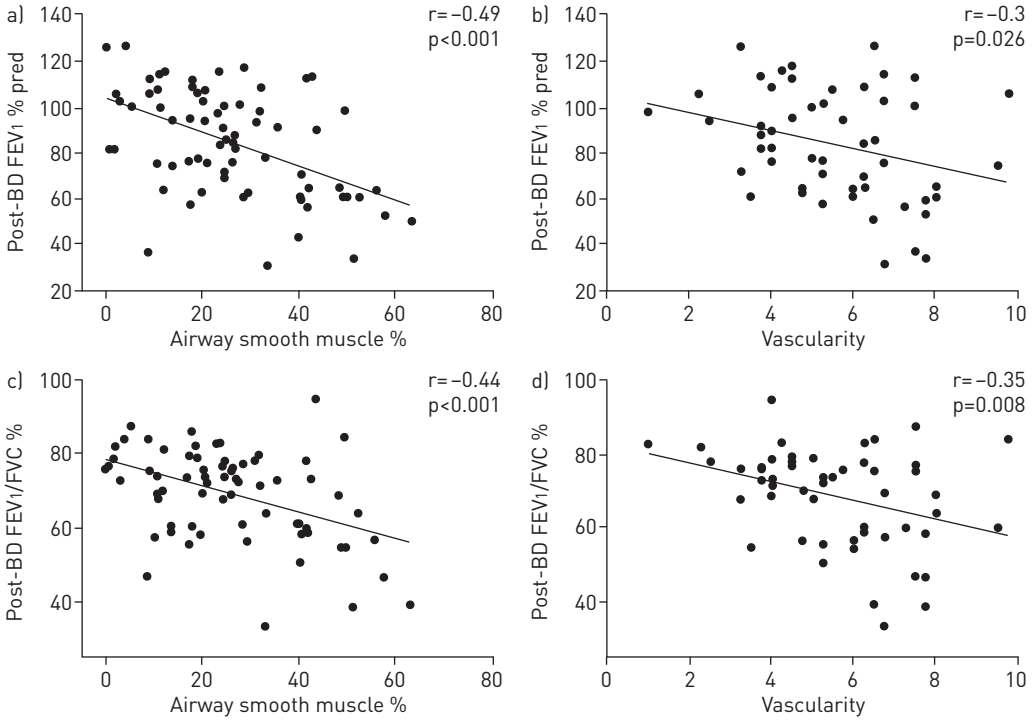

FIGURE 2 Scatterplots showing correlations of post-bronchodilator (BD) a, b) forced expiratory volume in 1 s (FEV_1) % pred and c, d) FEV_1/forced vital capacity (FVC) with a, c) airway smooth muscle % and b, d) vascularity.

TABLE 2 Quantitative computed tomography (QCT) morphometry and air trapping parameters

	All	Post-bronchodilator FEV_1 % pred <80%	Post-bronchodilator FEV_1 % pred ⩾80%	p-value
Patients n	56	29	25	
CT-derived quantitative morphometry				
Mean segmental bronchial lumen area/BSA $mm^2 \cdot m^{-2}$	10.4±2.3	9.7±2.2	11.0±2.3	0.047*
Mean segmental bronchial wall area/BSA $mm^2 \cdot m^{-2}$	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*

Data are presented as mean±SD, unless otherwise stated. FEV_1: forced expiratory volume in 1 s; BSA: body surface area; MLD E/I: mean lung density expiratory/inspiratory ratio on the expiratory/inspiratory scan; VI-856HU: percentage of lung voxels with a density lower than –856 HU on expiratory scans. *: p<0.05.

% (r=0.35; p=0.018) (figure 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 inflammatory cell counts in the lamina propria did not have any significant correlation with morphometry indices (table S1).

Vascularity was strongly correlated with measures of air trapping MLD E/I (r=0.49; p<0.001) and VI-856HU (r=0.53; p<0.001). Airway smooth muscle % was also correlated with MLD E/I (r=0.3; p=0.03) and VI-856HU (r=0.55; p<0.001) (figure 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 stepwise 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 % pred

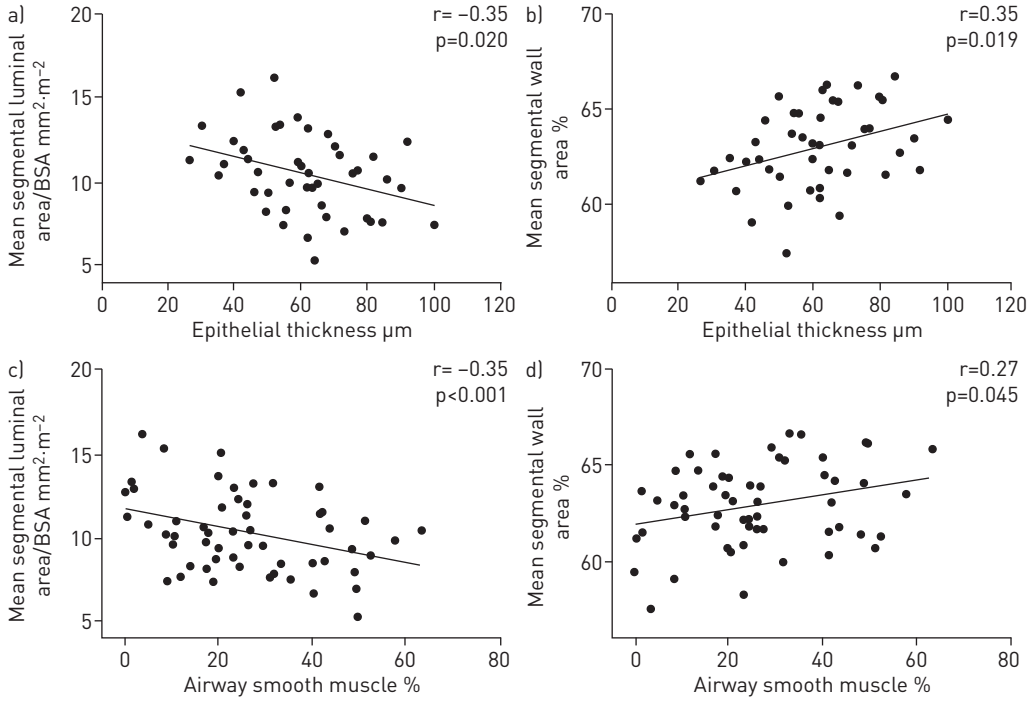

FIGURE 3 Scatterplots showing correlations of a, b) epithelial thickness and c, d) airway smooth muscle % with a, c) mean segmental bronchial luminal/body surface area (BSA) and b, d) mean segmental wall area %.

(R^2=0.24, β=−0.49; p=0.001), while both airway smooth muscle % and vascularity were significant predictors of post-bronchodilator FEV1/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=−0.35; p=0.02 and R^2=0.12, β=−0.35; p=0.015, respectively) and wall area (R^2=0.10, β=−0.32; p=0.033 and R^2=0.10, β=0.31; p=0.032, respectively). 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-856HU (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).

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

Vascularity in the bronchial biopsies was the only independent predictor of MLD E/I. Therefore the relationship between vascularity and MLD E/I was measured in an independent group of asthmatic subjects (n=24). Baseline demographics and clinical characteristics of subjects in the validation group are described in supplementary table S3. Similar to the primary study group, vascularity was positively correlated with MLD E/I (r=0.44; p=0.031) as well as VI-856HU (r=0.50; p=0.014) (figure 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 FEV1 % pred and FEV1/FVC (reviewed in [4]). As reported here airway smooth muscle mass is typically [4, 5], but not always [22], a major determinant of lung function impairment. Increased airway smooth muscle mass is a feature of severe childhood asthma [7], and is described in both the large and small

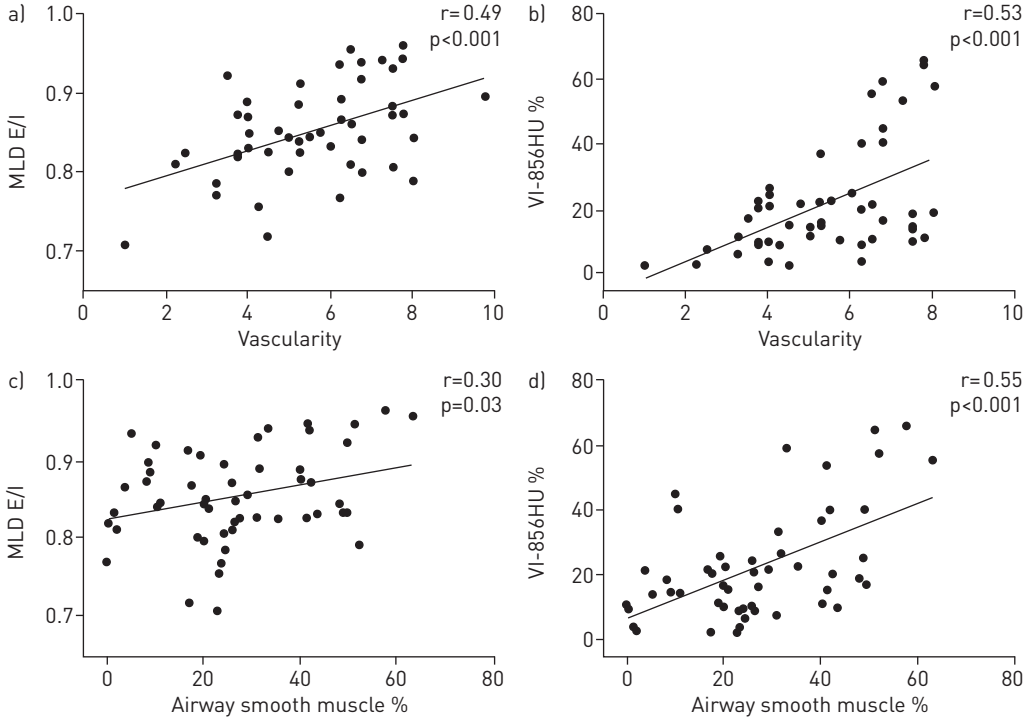

FIGURE 4 Scatterplots showing correlations of a, b) vascularity and c, d) airway smooth muscle % with a, c) mean lung density on the expiratory/inspiratory scan (MLD E/I) and b, d) percentage of lung voxels with a density lower than −856 HU on expiratory scans (VI-856HU).

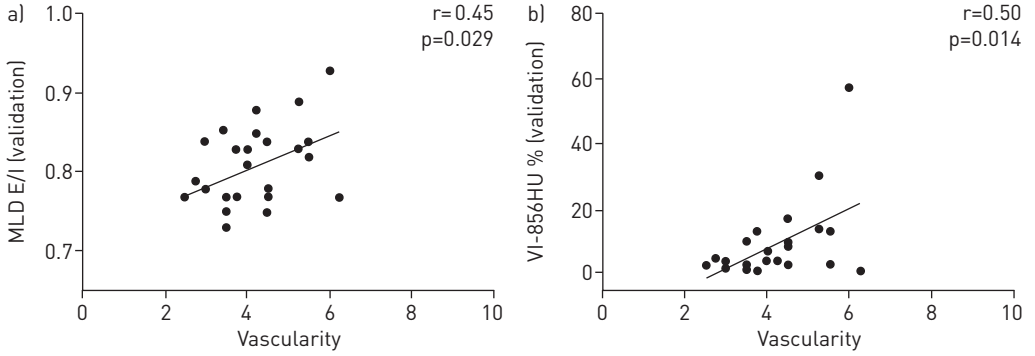

FIGURE 5 Validation of the association between vascularity and air trapping in the replication group showing scatterplots of vascularity with a) mean lung density on the expiratory/inspiratory scan (MLD E/I) and b) percentage of lung voxels with a density lower than –856 HU on expiratory scans (VI-856HU).

airways in studies of asthma deaths [8, 9]. Indeed, increased airway smooth muscle mass in both the large and small airways is more common than in the large or small airway alone [9]. The Chalkley count method used to assess vascularity in this study is a stereological method used commonly in cancer studies and is well validated for inter- and intra-observer variability, disease progression and mortality [26, 27]. Furthermore, the method has been previously used to measure vascular remodelling in asthma with studies showing greater mean Chalkley counts in asthmatic subjects compared with control and correlation with asthma severity [6]. Increased airway vascularity, neoangiogenesis, has been consistently reported in endobronchial biopsies from asthmatic subjects compared with healthy controls and in the small airways from lung resections for lung nodules in subjects with asthma [6, 28–32]. However, increased vascularity was not a feature observed in fatal asthma [33]. We and others have reported that increased vascularity is associated with lung function impairment [6], 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 Berair and Brightling [4]). Interestingly, in our study, other features of remodelling, *i.e.* epithelial thickening, RBM thickening and airway inflammation, were not associated with lung function indices.

Proximal airway morphometry assessed by QCT is abnormal in asthma with luminal narrowing and airway wall thickening [16]. 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 [10–12], 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 stepwise 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 [19]. 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 [28, 33], 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 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 [29–32]. 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 therefore 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 to explore 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 and demonstrated good within-donor repeatability of the key structural wall components in bronchial biopsies. Critically, our comparisons between QCT air trapping were with proximal rather than distal airway samples. As discussed earlier, 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. In contrast to Kasahara *et al.* [10], our study has shown no correlation between RBM and QCT-derived morphometry parameters. However, similar to our observation, Saglani *et al.* [34] also failed to demonstrate such correlation. Another limitation of this study relates to using endobronchial biopsies to measure airway remodelling. These biopsies are small and only sample the superficial layer of the airway, and thus cannot determine the changes in airway structure in relationship to the whole depth of the airway wall. However, despite this shortcoming, endobronchial biopsies remain the best *in vivo* tool to assess the structural changes in the airway wall contributing to airway remodelling [35].

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.

Acknowledgements

Author contributions: R. Berair, R. Hartley and C.E. Brightling contributed to study concept and design, acquisition, analysis and interpretation of data. V. Mistry, A. Sheshadri, A. Shikotra, A. Singapuri and S. Gonem contributed to data acquisition and analysis. S. Gupta, R.P. Marshall, A.R. Sousa, R. Kay, A. Wardlaw, P. Bradding, S. Siddiqui and M. Castro contributed to acquisition and interpretation of the data. All authors critically appraised and approved the final manuscript and are accountable for the work.

References

1 World Health Organization. Global Surveillance, Prevention and Control of Chronic Respiratory Diseases: A Comprehensive Approach. 2007. www.who.int/respiratory/publications/global_surveillance/en/index.html Date last accessed: January 10, 2017.
2 Chung KF, Wenzel SE, Brozek JL, *et al.* International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343–373.
3 Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. 2015. www.ginasthma.org Date last accessed: December 11, 2015.
4 Berair R, Brightling CE. Asthma therapy and its effect on airway remodelling. *Drugs* 2014; 74: 1345–1369.
5 Benayoun L, Druilhe A, Dombret MC, *et al.* Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003; 167: 1360–1368.
6 Siddiqui S, Sutcliffe A, Shikotra A, *et al.* Vascular remodeling is a feature of asthma and nonasthmatic eosinophilic bronchitis. *J Allergy Clin Immunol* 2007; 120: 813–819.
7 O'Reilly R, Ullmann N, Irving S, *et al.* Increased airway smooth muscle in preschool wheezers who have asthma at school age. *J Allergy Clin Immunol* 2013; 131: 1024–1032.
8 James A, Elliot JG, Jones RL, *et al.* Airway smooth muscle hypertrophy and hyperplasia in asthma. *Am J Respir Crit Care Med* 2012; 185: 1058–1064.
9 Elliot JG, Jones RL, Abramson MJ, *et al.* Distribution of airway smooth muscle remodelling in asthma: relation to airway inflammation. *Respirology* 2015; 20: 66–72.
10 Kasahara K, Shiba K, Ozawa T, *et al.* Correlation between the bronchial subepithelial layer and whole airway wall thickness in patients with asthma. *Thorax* 2002; 57: 242–246.
11 Aysola RS, Hoffman EA, Gierada D, *et al.* Airway remodeling measured by multidetector CT is increased in severe asthma and correlates with pathology. *Chest* 2008; 134: 1183–1191.
12 Montaudon M, Lederlin M, Reich S, *et al.* Bronchial measurements in patients with asthma: comparison of quantitative thin-section CT findings with those in healthy subjects and correlation with pathologic findings. *Radiology* 2009; 253: 844–853.
13 Siddiqui S, Gupta S, Cruse G, *et al.* Airway wall geometry in asthma and nonasthmatic eosinophilic bronchitis. *Allergy* 2009; 64: 951–958.
14 Haldar P, Brightling CE, Hargadon B, *et al.* Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009; 360: 973–984.

15 Gupta S, Siddiqui S, Haldar P, *et al.* Quantitative analysis of high-resolution computed tomography scans in severe asthma subphenotypes. *Thorax* 2010; 65: 775–781.
16 Gupta S, Hartley R, Khan UT, *et al.* Quantitative computed tomography-derived clusters: redefining airway remodeling in asthmatic patients. *J Allergy Clin Immunol* 2014; 133: 729–738.
17 Gupta S, Hartley R, Singapuri A, *et al.* Temporal assessment of airway remodeling in severe asthma using quantitative computed tomography. *Am J Respir Crit Care Med* 2015; 191: 107–110.
18 Haldar P, Brightling CE, Singapuri A, *et al.* Outcomes after cessation of mepolizumab therapy in severe eosinophilic asthma: a 12-month follow-up analysis. *J Allergy Clin Immunol* 2014; 133: 921–923.
19 Hartley RA, Barker BL, Newby C, *et al.* Relationship between lung function and quantitative computed tomographic parameters of airway remodeling, air trapping, and emphysema in patients with asthma and chronic obstructive pulmonary disease: a single-center study. *J Allergy Clin Immunol* 2016; 137: 1413–1422.
20 Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med* 1987; 317: 1098.
21 Du Rand IA, Blaikley J, Booton R, *et al.* British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: accredited by NICE. *Thorax* 2013; 68; Suppl. 1, i1–i44.
22 Brightling CE, Bradding P, Symon FA, *et al.* Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; 346: 1699–1705.
23 Siddiqui S, Mistry V, Doe C, *et al.* Airway hyperresponsiveness is dissociated from airway wall structural remodeling. *J Allergy Clin Immunol* 2008; 122: 335–341.
24 Sullivan P, Stephens D, Ansari T, *et al.* Variation in the measurements of basement membrane thickness and inflammatory cell number in bronchial biopsies. *Eur Respir J* 1998; 12: 811–815.
25 Cohen L, Xueping E, Tarsi J, *et al.* Epithelial cell proliferation contributes to airway remodeling in severe asthma. *Am J Respir Crit Care Med* 2007; 176: 138–145.
26 Fox SB, Leek RD, Weekes MP, *et al.* Quantitation and prognostic value of breast cancer angiogenesis: comparison of microvessel density, Chalkley count, and computer image analysis. *J Pathol* 1995; 177: 275–283.
27 Hansen S, Grabau DA, Rose C, *et al.* Angiogenesis in breast cancer: a comparative study of the observer variability of methods for determining microvessel density. *Lab Invest* 1998; 78: 1563–1573.
28 Hashimoto M, Tanaka H, Abe S. Quantitative analysis of bronchial wall vascularity in the medium and small airways of patients with asthma and COPD. *Chest* 2005; 127: 965–972.
29 Chetta A, Zanini A, Foresi A, *et al.* Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. *Am J Respir Crit Care Med* 2003; 167: 751–757.
30 Hoshino M, Takahashi M, Takai Y, *et al.* Inhaled corticosteroids decrease vascularity of the bronchial mucosa in patients with asthma. *Clin Exp Allergy* 2001; 31: 722–730.
31 Orsida BE, Li X, Hickey B, *et al.* Vascularity in asthmatic airways: relation to inhaled steroid dose. *Thorax* 1999; 54: 289–295.
32 Orsida BE, Ward C, Li X, *et al.* Effect of a long-acting beta2-agonist over three months on airway wall vascular remodeling in asthma. *Am J Respir Crit Care Med* 2001; 164: 117–121.
33 Carroll NG, Cooke C, James AL. Bronchial blood vessel dimensions in asthma. *Am J Respir Crit Care Med* 1997; 155: 689–695.
34 Saglani S, Papaioannou G, Khoo L, *et al.* Can HRCT be used as a marker of airway remodelling in children with difficult asthma? *Respir Res* 2006; 7: 46.
35 Jeffery P, Holgate S, Wenzel S, *et al.* Methods for the assessment of endobronchial biopsies in clinical research: application to studies of pathogenesis and the effects of treatment. *Am J Respir Crit Care Med* 2003; 168: S1–S17.