Airway Elastin is increased in severe asthma and relates to proximal wall area: histological and computed tomography findings from the U-BIOPRED severe asthma study

Short running title: Elastin is increased in severe asthma

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Take-home message
Elastin deposition in biopsy tissue relates to airway wall thickening

Manuscript word count: XXXX /5000

Number of tables: 3

Number of figures: 2

Author contributions:

SJW lead the immunopathological analysis for the U-BIOPRED study including the analysis presented here. JAW and HMP undertook the staining and analysis described. SB and CB were responsible for the CT part of this study. KFC, DS, BD, BB, NK, TS and PHH were leads at the clinical centres undertaking the bronchoscopy aspects of this work and SRS and PHH were the bronchoscopy study leads. DS and ARS were responsible for the clinical data analysis for the main study. KFC, RD and PJS designed and were the leads for the U-BIOPRED study.

Abstract

Background: Airway remodelling, which may include goblet cell hyperplasia / hypertrophy, changes in epithelial integrity, accumulation of extracellular matrix components, smooth muscle hypertrophy and thickening of the *lamina reticularis,* is a feature of severe asthma and contributes to the clinical phenotype.

Objective: Within the U-BIOPRED severe asthma study, we have assessed histological elements of airway remodelling and their relationship to computed tomography (CT) measures of proximal airway dimensions.

Methods: Bronchial biopsies were collected from two severe asthma groups, one non-smoker (SAns, n=28) and one current/ex-smoker (SAs/ex, n=13), and a mild-moderate asthma group (MMA, n=28) classified and treated according to GINA guidelines, plus a healthy control group (HC, n=33). A Movat's pentachrome technique was used to identify mucin, elastin and total collagen in these biopsies. The number of goblet cells (mucin+) were counted as a percentage of the total number of epithelial cells and the percentage mucin epithelial area measured. The percentage area of elastic fibres and total collagen within the submucosa were also measured, and the morphology of the elastic fibres classified. Participants in the asthma groups also had a CT scan to assess large airway morphometry.

Results: The submucosal tissue elastin percentage was higher in both severe asthma groups (16.1% SAns, 18.9% SAs/ex) compared to the HC (9.7%) but did not differ between asthma groups. There was a positive relationship between elastin and airway wall area measured by CT (n= 18-20, rho=0.544, p=0.024), which also related to an increase in elastic fibres with a thickened lamellar morphological appearance. Mucin epithelial area and total collagen were not different between the four groups. Due to small numbers of suitable CT scans it was not feasible to compare airway morphometry between the asthma groups.

Conclusion: These findings identify a link between extent of elastin deposition and airway wall thickening in severe asthma.

Word count 298/300

INTRODUCTION

Severe asthma has a heterogeneous clinical phenotype and pathology. This pathology includes airway remodelling, features of which may be goblet cell hyperplasia / hypertrophy, changes in epithelial integrity, accumulation of extracellular matrix components, smooth muscle hypertrophy and thickening of the *lamina reticularis* [1-4]. In this current study we focus on the evaluation of goblet cell hyperplasia / hypertrophy and the presence of the extracellular matrix components, collagen and elastin, and how these relate to central airway wall and lumen measurements by computed tomography (CT). This study was undertaken as data regarding changes in these parameters, in relation to disease are conflicting.

Goblet cell hyperplasia has been observed in the lungs of patients who have died from asthma [5,6] and is also seen in bronchial biopsy tissue in milder asthma [7], although not consistently so [8]. To date there are no reported studies in living severe asthmatics.

Collagens 1, 3 and 5 form part of the extracellular matrix in the bronchial wall, contributing to the structural support of the airway [1, 2]. Whilst there is a report of increased submucosal collagen [9] in bronchial biopsies from mild and moderate asthma compared to healthy controls, other studies have not consistently reproduced this finding [10-13]. In severe asthmatics, Benayoun *et al* [12] and Chakir *et al* [13] have reported an increase in submucosal collagen compared to both milder asthmatics and healthy controls but again this finding in biopsy tissue is inconsistent [11,14]. Furthermore, post-mortem studies have not reported any differences in total collagen content in the lungs of cases of fatal asthma as compared to that in non-asthmatic deaths [15, 16].

Elastin, which contributes to airway patency and elastic recoil, has been reported to be increased in the central airways [17] and in longitudinal bundles [18] , but decreased in the distal airways [19] in fatal asthma compared to non-asthma controls. Whereas, no disease related differences were observed by Godfrey [20]. An increase in the proportion of elastin within the airway smooth muscle has been observed in cases of fatal asthma compared to non-fatal asthma [21]. In bronchial biopsies from mild and moderate

asthma no difference in the proportion of elastic fibres, as compared to healthy controls, is reported and this is not affected by corticosteroid treatment [20]. Both Mauad *et al* [17] in fatal asthma and Bousquet *et al* [22] in living asthmatics report a change in the appearance of the elastic fibres. The elastic fibres in the superficial layer appearing more fragmented and wispy whilst those in the deeper layer are condensed and thickened (lamellar).

Imaging of the lungs of asthmatics by computed tomography (CT) [23, 24] has shown increased air-trapping, airway wall thickening and decreases in lumen area. Several groups have investigated the relationship between these CT changes and remodelling changes identified by histological methods. Some groups have demonstrated relationships others have not. In moderate asthmatics a positive relationship is observed between percent wall area (as a fraction of total airway area) and wall thickness, as measured by CT, with the thickness of the reticular basement membrane (RBM) in proximal airway biopsies [25], whereas in the SARP (severe asthma research programme) study [26] and the study of Berair *et al* [27], this relationship is not observed. Percent wall area, segmental wall area and wall thickness are reported to be positively correlated with the thickness of the bronchial epithelium [26, 27]. Berair *et al* [27] also observe a positive relationship between segmental wall area percent and airway smooth muscle percent in biopsies. However, other studies have not observed any relationship between CT and histological measures of remodelling [28, 29] although Lederlin *et al* [29] did observe that airway wall attenuation correlated with mast cell infiltration into the airway smooth muscle.

Due to these conflicting findings and the need to better understand the remodelling pathology in severe asthma and its relationship to the clinical assessment by CT, the U-BIOPRED study of severe asthma has explored the relationship between CT central wall parameters and proximal airway wall biopsy changes of remodelling.

METHODS

Study design

As previously described in detail [30], the U-BIOPRED (Unbiased Biomarkers for the Predictions of Respiratory Disease Outcomes) multi-centre pan-European severe asthma study included three adult asthma groups; severe non-smokers (SAn), severe current/ex-smokers (SAs/ex) and those with mild-moderate disease (MMA), classified and treated according to the Global Initiative for Asthma (GINA) guidelines, as well as a healthy control (HC) group. Participants underwent detailed clinical phenotyping, had induced sputum and blood collected for inflammatory cell profiling and 'omics analysis and exhaled nitric oxide fraction (FeNO) measured. This study was approved by an ethics review committee at each centre (France: Independent Ethics Committee sud Mediterranee 2100-A01681-40, Germany: Hanover Medical School Ethics Committee, 5938, Netherlands: Medical Ethics Committee, Academic Medical Centre, University of Amsterdam, METC 10/207 #11.17.0430, Sweden: Regional Independent Ethics committee, Stockholm, 2011/1254-31/3 , UK: NRES committee South West 10/H0721/66) and all participants gave written informed consent.

Bronchoscopy procedure, biopsy collection and analysis

The bronchoscopy cross-sectional sub-study, for the collection of airway samples has been described in detail by Wilson *et al* [31]. In brief, participants underwent bronchoscopy, with the collection of airway samples, including endobronchial biopsies, in accordance with standardised protocols across each of the eight participating clinical centres. Up to two of these biopsies from each bronchoscopy, were fixed 10% neutral buffered formalin and embedded in paraffin wax and were used for this current study. Of the 139 bronchoscopsies that biopsies for paraffin embedding were collected from, 102 participants had biopsies that were suitable for staining , having a submucosal area greater than 0.25mm^2 and / or 0.1mm of intact epithelium. Two 4µm sections were cut from each biopsy and stained with a Movat's pentachrome technique [32] to identify features of remodelling, including mucin, elastic fibres and collagen. The number of goblet cells (mucin positive) were counted as a percentage of the total number of epithelial cells & the percentage mucin epithelial area as a fraction of total epithelial area, measured using computerised image

analysis (Zeiss KS400 software, Image Associates, UK) to thresholding on features of interest based on RGB colour composition, in lengths of intact epithelium. The percentage area of elastic fibres and collagen within the submucosa, excluding glands, smooth muscle, cartilage and the *lamina reticularis*, was also measured using the same approach (see Figure 1, in supplementary material for an illustration of this method). We also made a qualitative assessment of the morphological appearance of the elastic fibres classifying them as wispy, lamellar or mixed as described by Bousquet *et al* [22]. The observers (HMP and JAW) undertaking the image analysis were blinded to the participant grouping and ID.

Computed Tomography

The clinical characterisation of the participants also included CT. Volumetric whole lung scans were obtained at full inspiration (total lung capacity) and full expiration (residual volume) using a standardised protocol for each scanner manufacturer and model. All participants were coached in the breath-holding techniques, and practiced breath-holding immediately prior to scanning. Participants were scanned 10–60 minutes after receiving 400 µg salbutamol. Post-processing was performed using the VIDA Apollo software (VIDA Diagnostics, Iowa, USA) as described previously [33, 34]. Quantitative CT parameters included large airway morphometry (measured in mm^2): lumen area (LA), wall area (WA) and percentage wall area $\left(WA\% = 100\ x\left(\frac{WA}{WA+LA}\right)\right)$.

Statistical analysis

Data was initially analysed by ANOVA to test for differences between groups and then where relevant, either non-parametric or parametric analysis applied to evaluate the significance of group differences using SPSS (version 19). The Spearman's rank test was applied to test for pair-wise correlations. We also included our previously published data [31] for *lamina reticularis* thickness and airway smooth muscle area fraction when testing for these relationships.

RESULTS

The demographics for the participants included in this study are summarised in table 1. The participants in both severe asthma groups were older, had a higher body mass index (BMI) and lower forced expiratory volume in one second (FEV1), forced expiratory flow (FEF25-75) than both the MMA and healthy controls. Peak expiratory flow (PEF) and specific airways conductance (sGaw) were also lower in both severe groups compared to healthy controls, and in the SAs/ex, but not the San, these measures were both lower than the MMA. The MMA had a lower FEV_1, FEF25-75, PEF and SGAW than the HC.

Biopsy remodelling features

Representative images of the Movat's pentachrome staining are shown in figure 1. This dye-based technique enables the identification of mucin, elastic and collagen in one section. Summary data is shown in table 2. A full data set was not available for all parameters, as some cases did not have any intact epithelium for mucin measurements and in a few cases the submucosa showed signs of crush artefact so collagen and elastin could not be assessed. The numbers included for each parameter are shown in table 2. There was no difference in the mucin or collagen quantification between the four groups. The median (IQR) percentage of elastic fibres in the bronchial submucosa was significantly higher in both severe groups (SAns 16.1% [10.6-24.9], SAs/ex 18.9% [15.4-26.1]) than in the HC (9.7 [7.1-14.5], p=0.025 & p=0.003 respectively, figure 2A). In most cases these elastic fibres had a mixed appearance (wispy and lamella). However, a lamellar elastic appearance was more frequent in the severe asthmatics than the HC, where a wispy phenotype was more predominant (figure 2B).

Computed Tomography

For the bronchoscopy cohort included in this study, suitable CT images for analysis were only available for 18 expiratory scans and 20 inspiratory scans the majority of these being for the SAn group (n=12)(Table 3). Therefore, it was not reasonable to make between groups comparisons of these data. CT scans had to be excluded for the following reasons: i) deviations in the CT acquisition protocol, ii) technical errors in data capture or transfer, iii) error in CT procedure identified by lung density being greater in the inspiratory

compared to expiratory scans, or iv) CT and body plethysmography lung volumes, when compared, being discrepant by more than 3 SD from the mean difference.

Relationship between biopsy remodelling, CT measures and lung function data

Due to the low number of participants with CT data we tested for relationships between biopsy remodelling data and all participants with CT data (n=17-18 asthma, n=1-2 HC). We observed a positive relationship between the percentage elastin in the submucosa and the percent wall area (expiratory) (figure 2C), which also related to the appearance of the elastin fibres (figure 2D). There was no relationship between mucin, collagen, ASM fraction or *lamina reticularis* thickness and CT measures of remodelling.

We also did not observe any relationship between remodelling features and measures of lung function.

DISCUSSION

In this study, which is the largest to date to assess submucosal airway remodelling in severe asthma, we have observed more elastin in the airways of severe asthmatics, irrespective of smoking status, compared to healthy controls. The elastic fibres in the asthmatics were thickened and more lamellar in appearance than in the HC. The amount of elastin in the airways and its appearance had a relationship to airway wall area, assessed by CT. There was no relationship between the amount of elastin and the demographic or clinical measures reported here.

The proportion of elastin we observed in our severe asthma groups is similar to that reported by Godfrey *et al* [20] in the airway wall in asthma deaths and Araujo [21] in airway smooth muscle. Neither of these studies report differences between fatal asthma and non-asthma controls. Increases in elastin have been reported in the central airways in fatal asthma compared to non-asthma controls [17,18]. These elastic fibres were observed to be similar in appearance to those observed in our study, being thickened and more lamellar in appearance, as has also been reported by Bousquet *et al* [22].

The differences in findings with respect to elastin content in relation to disease between some of the previous studies and our work could reflect the airway compartments studied, sample type and number of samples included. Our study in bronchial biopsies taken from the large airways, showing increased elastin in severe asthma compared to healthy controls, concurs with the findings of Mauad *et al* [17] and Carroll *et al* [18], who examined central airways in post-mortem tissue from patients who had died of asthma, observing increased elastin compared to non-asthma controls. The study of Godfrey *et al* [20], also in central airways, did not observe a difference between fatal asthma and non-asthma, however, this study was small, with only five fatal asthma cases. Whilst we report similar proportions of elastin to that of Araujo *et al* [21], they did not see a difference compared to non-asthma. Our studies differ in the compartment within the airway that was assessed, with Araujo *et al* looking at airway smooth muscle and our study the airway submucosa. Which could account for the differences in group comparisons. Our

results also differ to the findings in distal airways [19] which may reflect the differing role of elastin in these compartments.

This increase in elastin could be part of the remodelling / repair response that is well documented in asthma [1-4]. Smooth muscle cells, fibroblasts and myofibroblasts are sources of elastin in normal lung [35,36]. The cytokines TNFα, IL1-β and TGFβ, all of which are elevated in asthma [3,37], can induce myofibroblasts to synthesise increased amounts of elastin [36]. The study of Shifren *et al* [38] reports upregulation of elastin expression by myofibroblasts in the lung fibrotic disease bronchiolitis obliterans. This leads us to speculate that the myofibroblast, known to be important in the asthma remodelling response, could be contributing to the increase in elastin we have observed in this study. This is further supported by the study of Carroll *et al* [18], who observed elastin staining to be in close proximity to myofibroblasts, both of which were increased in fatal asthma.

Increased bronchoconstriction has, in mild asthmatics, been shown to drive a remodelling response [39], this could also be a possible mechanism initiating the remodelling changes we have observed. The presence of increased bronchoconstriction could also account for the increased lamellar appearance of the elastin, as reported by Mauad *et al* [17].

The positive relationship we observed between elastin and percent wall area is novel. We also noted that this was associated with an altered appearance of the elastic fibres, with the higher percentage of elastin and greater wall area having elastic fibres with thickened lamellar appearance as shown in figure 1. This suggests that as the amount of elastin in the airway increases and thickens so does the airway wall area. We did not observe any relationship between airway remodelling measured by CT with mucin, collagen, ASM fraction or *lamina reticularis* assessed histologically. This result for *lamina reticularis* concurs with that of Aysola *et al* [26] and Berair *et al* [27] but not that of Kasahara *et al* [25], who reported a positive relationship. These differences in findings could reflect the asthmatic population included in the studies. We, Aysola [26] and Berair [27] included severe asthmatics in our studies, whereas that of Kasahara did not.

A positive relationship between airway smooth muscle fraction assessed in biopsies with both airway luminal area and airway wall measured by CT has previously been reported [27], but we did not observe this. We also did not see the inverse relationship between FEV_1 percent predicted and airwall thickness measured by CT as in the studies of Kasahara *et al* [25] and Aysola *et al* [26]. This could be due to low numbers of participants with CT measures in our study.

In this study we did not observe a relationship between these structural changes and measures of lung function. In mild asthma, an inverse correlation between PC_{20} methacholine and airway elastin content in paraffin embedded bronchial biopsies, is observed [40] implying that increasing severity of airway hyperreactivity is linked with increased elastin. Howarth *et al* [41] report that airway hyperreactivity remains abnormal in severe asthma despite therapy. However, in the UBIOPRED study PC_{20} methacholine was not assessed, so we are unable to explore relationships between this aspect of abnormal physiology and airway tissue morphology.

Our data showing that total submucosal collagen did not vary with disease concurs with the observations of others in mild, moderate and severe asthmatics [10-12,14] and in asthma deaths [15, 16], but does differ from the reports of Wilson *et al* [9], Benayoun *et al* [12] and Chakir *et al* [13]. In these latter studies the methodological approaches employed differ from the present study, which may explain the differences in results. These three studies employed immunohistochemistry to stain for collagen 1 or 3. Wilson *et al* [9] quantified staining using computerised image analysis to a depth of 30-50 microns beneath the SBM and both Benaynoun *et al* [12] and Chakir *et al* [13] used a scoring system. Whereas in the present study we have stained for total collagen and focused specifically on collagen within the sub-mucosa, excluding collagen within the *lamina reticularis* layer. The study of Kaminska et al [28] is the only previously reported study that has examined the relationship between CT measures of remodelling and submucosal collagen. Like our current study they did not observe any relationships.

Our study, which is larger than previously reported studies assessing goblet cell hyperplasia in asthma[5-8], demonstrates that there is no change in goblet cell number or mucin fraction within the bronchial epithelium in either severe or moderate asthma, when assessed in bronchial biopsies. This concurs with the findings of Lozewicz *et al* [8] in mild asthma but does not support the findings of Ordonez *et al* [*7* who reported an increase in the volume density of mucin in mild asthma or from findings in autopsy based studies [5-6]. This difference in findings could be a reflection of the increased numbers in our study, which included 27 severe asthmatics (SAn & SAs/ex), 23 MMA and 33 HC, which better reflects the true sample population. The largest study previously included bronchial biopsies from 13 asthmatics and 12 healthy controls.

As previously reported [31] we did not observe any disease related differences in the thickness of the *lamina reticularis* or the proportion of airway smooth muscle as a fraction of the submucosa in these steroid-treated asthmatics as compared to healthy controls. We also did not observe, in this current study, any relationship with CT measures of remodelling and these morphological measures. This lack of relationship concurs previous studies [26-29].

Although this is one of the largest studies assessing submucosal remodelling in bronchial biopsies from living severe asthmatics that yielded some novel findings this study does have some limitations. The low number of suitable CT images that could be analysed meant we were unable to compare remodelling mesured by CT across the different asthma groups or with HC. This could have also lead to a type II error.

In conclusion this study has revealed increased elastin in severe asthma that relates to CT scan measures of airway wall thickening. It also supports previous work showing that submucosal collagen deposition and goblet cell number, do not differ with asthma disease severity and that this, and airway smooth muscle and *laminar reticularis* thickness do not relate to CT measures of remodelling.

ACKNOWLEDGEMENTS

The bronchoscopy centres acknowledge the help received from nursing and other health care workers in the conduct of this study and the Histochemistry Research Unit in Southampton acknowledges the help from Helen Pickett and Jon Ward in assisting with the analysis of the tissue biopsies. The U-BIOPRED study would have not been possible without the Innovative Medicines Initiative (IMI) funding provided by the European Union (EU) and the European Federation of Pharmaceutical Industries and Associations (EFPIA).

This research presented in this paper was co-funded by the National Institute for Health Research (NIHR) Leicester Biomedical Research Centre, Airway Disease Predicting Outcomes through Patient Specific Computational Modelling (AirPROM) project (funded through 7th EU framework grant, 270194). This paper presents independent research funded by the National Institute for Health Research (NIHR). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

The authors also recognise that without the voluntary help from the asthmatic participants and the healthy control volunteers this study would not have been possible and are indebted to their generosity.

Members of the U-BIOPRED study group are detailed in the supplementary acknowledgements.

Conflict of interests statement

SJW, JAW, HMP, SB, ARS, PJS, BD, BB, NK, TS, PHH have no conflicts of interest to declare.

KFC has no conflict of interests related to the study; outside of this study he has received honoraria for participating in Advisory Board meetings of GSK, AZ, Roche, Novartis, Merck, BI, TEVA and Shionogi regarding treatments for asthma, chronic obstructive pulmonary disease and chronic cough and has also been renumerated for speaking engagements.

DS receives speaker fees from AZ, GSK and Novartis, and travel fees from AZ and Novartis.

CEB has no conflict of interests related to the study; during the conduct of the study CEB received paid to his Institution grants and personal fees from GSK, AZ, Novartis, Sanofi, Regeneron, BI, Chiesi, Roche/Genentech, Mologic, 4DPharma and Gossamer, outside the submitted work

RD reports receiving fees for lectures at symposia organised by Novartis, AstraZeneca and TEVA, consultation for TEVA and Novartis as member of advisory boards, and participation in a scientific discussion about asthma organised by GlaxoSmithKline. He is a co-founder and current consultant, and has shares in Synairgen, a University of Southampton spin out company.

The data that support the findings of this study are available from the corresponding author upon reasonable request

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FIGURE LEGENDS

Figure 1: Representative images showing Movats Pentachrome staining in a severe asthmatic (a), mild-moderate asthmatic (b) and healthy control (c) Goblet cells (G) (cyan) can be seen in the epithelium, collagen (C)(yellow) is distributed throughout the mucosa with some muscle (M)(red). The elastic fibres (E)(black) had a mixed appearance (lamellar and wispy), the lamellar appearance being more prominent in the severe asthmatics and wispy in the healthy controls. Scale bar is 50µm.

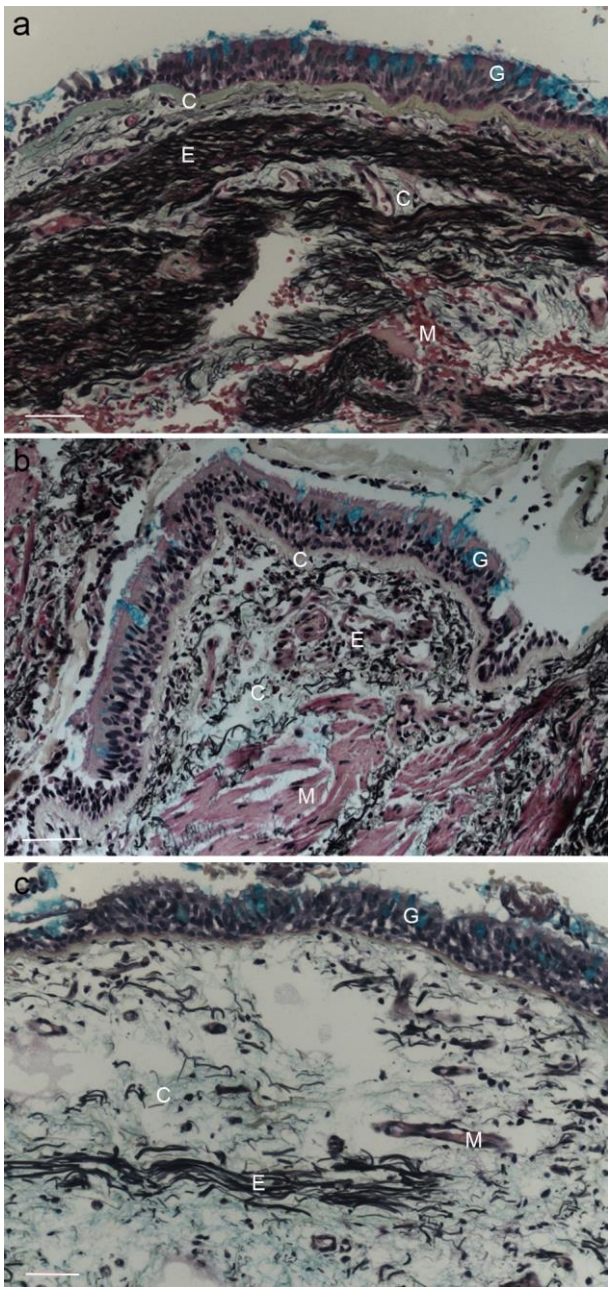


Figure 2: Elastin in the bronchial submucosa and relationship to CT wall area: The fraction (%) of elastin is shown (a) in severe non-smokers (●), severe current/ ex-smokers (■), mild-moderate (▲) asthmatics and in healthy controls (▼). Median values (--) and significant differences between the groups are indicated. The appearance of these elastic fibres (b) was classified as wispy, lamellar or mixed. There was a positive relationship between submucosal elastin and percent wall area (expiratory) measured by CT (c), this also related to the appearance of the elastin (d). Data for severe asthmatics taking oral corticosteroids is shown as open symbols.

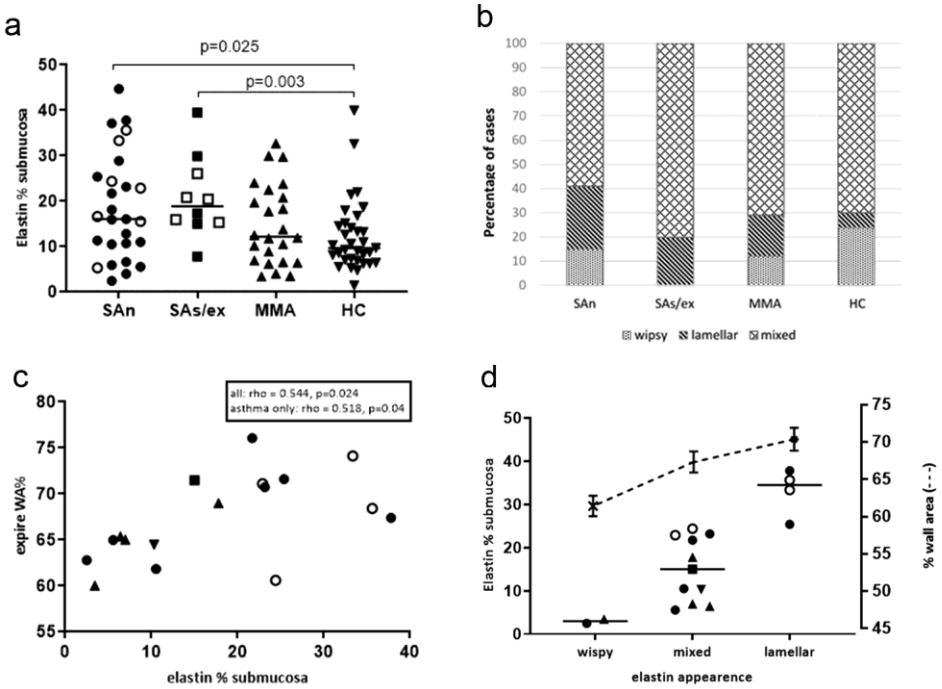


Table 1: Participant demographics – remodelling study

	severe asthma non-smoker (SAn)	severe asthma current or ex-smoker (SAs/ex)	mild-moderate asthma (MMA)	healthy control (HC)
Number of participants	28	13	28	33
Age #~•▲ [mean years (SE)]	49.1 (2.82)	55.1 (1.75)	42.1 (2.57)	40.2 (2.39)
Gender [n]	female: 16 male: 12	female: 5 male: 8	female: 15 male:13	female:9 male:24
Body Mass Index #~•▲ [mean kg/m^2 (SE)]	30.1 (1.3)	30.1 (1.9)	26.3 (1.0)	25.5 (0.6)
Forced Expiratory volume in one second#~•▲▪ [mean % predicted (SE)]	73.1 (4.02)	66.7 (4.78)	88.91 (4.25)	102.30 (2.08)
Reversibility [change FEV_1 % predicted, pre-post salbutamol (SE)]	9.22 (1.68)	7.16 (4.65)	8.29 (1.90)	not measured
Forced Expiratory Flow 25-75% #~•▲▪ [mean (SE)]	39.24 (4.79)	33.97 (7.06)	61.34 (4.44)	86.95 (4.18)
Peak Expiratory Flow ~•▲▪ [mean % predicted (SE)]	82.55 (4.61)	68.98 (5.92)	88.00 (6.13)	105.37 (2.47)
Specific Airways Conductance ~•▲▪ [mean (SE)]	1.12 (0.20)	0.90 (0.11)	1.47 (0.14)	1.95 (0.20)
Smoking history [% subjects]	never: 86 ex (<5 pack yrs): 14	current: 23 ex (>5 pack yrs):77	never: 89 ex (<5 pack yrs): 11	never: 79 ex (<5 pack yrs): 21

Differences ($p<0.05$) are indicated: # SAn vs MMA, ~ SAn vs HC, • SAs/ex vs MMA, ▲ SAs/ex vs HC, ▪ MMA vs HC

Table 2: Summary data – biopsy remodelling

	severe asthma non-smoker (SAn) (n=28)	**severe asthma current or ex-smoker (SAs/ex)(n=13)**	**mild-moderate asthma (MMA)(n=28**	**healthy control (HC) (n=33)**
Number of samples analysed for each biopsy parameter	mucin n=17 elastin n=27 collagen n=27	mucin n=10 elastin n=10 collagen n=11	mucin n=23 elastin n=24 collagen n=26	mucin n=26 elastin n=33 collagen n=33
Mucin % epithelial cells positive (IQR)	9.1 (5.8-12.8)	17.8 (6.2-24.7)	13.1 (6.7-16.2)	10.5 (5.3-14.7)
Mucin % epithelial area positive (IQR)	4.8 (3.0-8.8)	10.6 (1.8-20.7)	7.8 (3.7-13.1)	5.3 (2.5-8.5)
Elastin % submucosal area positive (IQR) ~▲	16.1 (10.6-24.9)	18.9 (15.4-26.1)	12.2 (6.8-31.7)	9.7 (7.1-14.5)
Collagen % submucosal area positive (IQR)	23.5 (15.1-27.9)	21.7 (17.5-30.2)	20.4 (14.9-29.5)	22.1 (18.0-24.7)
***Lamina reticularis* thickness** (um) (IQR) previously published in [30]	8.9 (7.3-10.2)	8.7 (7.9-9.9)	9.0 (7.7-9.7)	8.8 (8.0-9.2)
ASM volume fraction (IQR) previously published in [30]	0.3 (0.2-0.4)	0.3(0.2-0.4)	0.3 (0.3-0.5)	0.3 (0.2-0.4)

All data are medians and IQR. Differences ($p<0.05$) are indicated: ~ SAn vs HC, ▲ SAs/ex vs HC

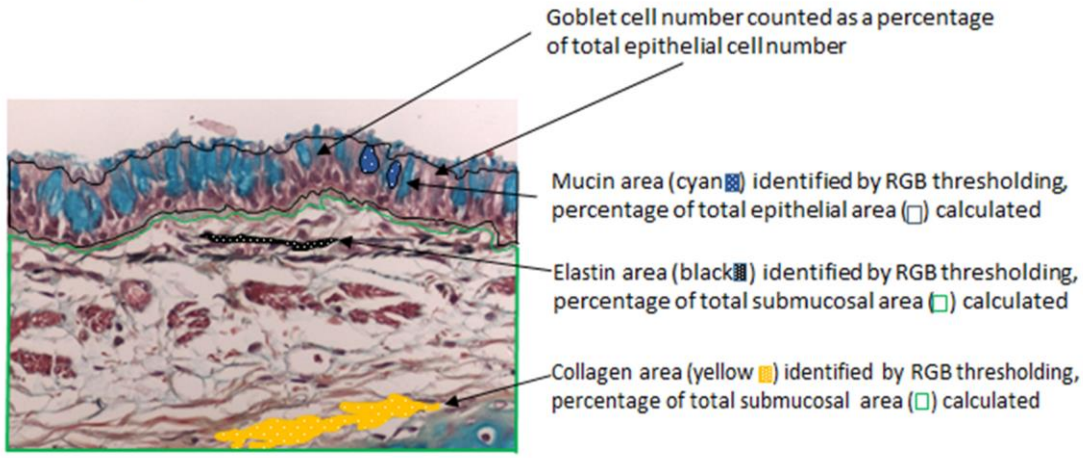
Table 3: Summary data – Computed tomography

		severe asthma non-smoker (SAn)	severe asthma current or ex-smoker (SAs/ex)	mild-moderate asthma (MMA)	healthy control (HC)
Number of scans	expire	12	1	4	1
	inspire	12	1	5	2
Wall area %	expire	68.0 (1.42)	71.5	64.9 (1.41)	64.5
	inspire	63.9 (0.43)	68.8	63.0 (1.66)	65.3 (0.16)
Wall area mean	expire	35.3 (2.12)	33.4	28.8 (2.53)	29.1
	inspire	33.0 (2.11)	41.1	32.2 (1.87)	31.7 (3.28)
Wall area median	expire	33.5 (2.17)	30.0	29.0 (3.59)	28.8
	inspire	33.9 (1.81)	40.9	33.5 (2.23)	35.5 (5.02)
Lumen area mean	expire	19.9 (2.10)	12.7	17.0 (2.56)	16.8
	inspire	22.0 (1.31)	21.2	25.7 (4.14)	22.9 (4.48)
Lumen area median	expire	15.5 (0.97)	12.0	16.1 (3.3)	15.9
	inspire	19.1 (0.96)	18.5	20.1 (2.40)	18.8 (2.80)

Data sets> 1 are mean (SE)

Supplement:

Figure 1: Image analysis method for sections stained with Movats pentachrome
The four analysis measurements made are illustrated below



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