



ORIGINAL ARTICLE

Quantitative assessment of airway remodelling and response to allergen in asthma

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ABSTRACT

Background and objective: In vivo evaluation of the microstructural differences between asthmatic and non-asthmatic airways and their functional consequences is relevant to understanding and, potentially, treating asthma. In this study, we use endobronchial optical coherence tomography to investigate how allergic airways with asthma differ from allergic non-asthmatic airways in baseline microstructure and in response to allergen challenge.

Methods: A total of 45 subjects completed the study, including 20 allergic, mildly asthmatic individuals, 22 non-asthmatic allergic controls and 3 healthy controls. A 3-cm airway segment in the right middle and right upper lobe were imaged in each subject immediately before and 24 h following segmental allergen challenge to the right middle lobe. Relationships between optical airway measurements (epithelial and mucosal thicknesses, mucosal buckling and mucus) and airway obstruction (FEV₁/FVC (forced expiratory volume in 1 s/forced vital capacity) and FEV₁% (FEV₁ as a percentage of predictive value)) were investigated.

Results: Significant increases at baseline and in response to allergen were observed for all four of our imaging metrics in the asthmatic airways compared to the non-asthmatic airways. Epithelial thickness and mucosal buckling exhibited a significant relationship to FEV₁/FVC in the asthmatic group.

Conclusion: Simultaneous assessments of airway microstructure, buckling and mucus revealed both structural and functional differences between the mildly asthmatic and control groups, with airway buckling seeming to be the most relevant factor. The results of this study

SUMMARY AT A GLANCE

Endobronchial optical coherence tomography is used to investigate the microstructural features of airway in allergic individuals with and without asthma, before and after allergen challenge. Quantification of these features reveals significant differences between the asthmatic and non-asthmatic airways both at baseline and in response to allergen.

demonstrate that a comprehensive, microstructural approach to assessing the airways may be important in future asthma studies as well as in the monitoring and treatment of asthma.

Key words: asthma, bronchoscopy and interventional techniques, radiology and other imaging, respiratory function test, respiratory structure and function.

INTRODUCTION

Asthma is a disorder of the airways characterized by chronic inflammation, airway remodelling and airway hyperresponsiveness. It is estimated to affect over 300 million people worldwide, and there is a large healthcare burden associated with the portion of the population classified as severe asthmatics who have poorly controlled symptoms.¹ Among the different types of asthma, allergic asthma is the most common, accounting for approximately 60% of the asthmatic population in the United States.² Although historically asthma has been regarded as an inflammatory disease, the failure of anti-inflammatory treatments to sufficiently control asthma in a crucial subset of patients emphasizes the need to better understand the pathogenesis of airway remodelling in asthma. This highlights the need to investigate more sophisticated approaches to researching and

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monitoring the microstructural features of airway remodelling.

Some of the key features of asthma include airway wall thickening, bronchoconstriction, excessive mucus production and epithelial changes.^{2–10} Although there are techniques, such as bronchial biopsy, that allow some of these features to be assessed, the need to remove tissue and sample distortion mitigate their overall usefulness. One possible solution for studying asthma at the microstructural level that has received only minimal attention is endobronchial optical coherence tomography (OCT). OCT is a high-resolution, high-speed, volumetric imaging modality that has been described as the optical analogue to ultrasound.^{11,12} A significant advantage to using endobronchial OCT to study airway remodelling is its compatibility with bronchoscopy. Research techniques such as segmental allergen challenge (SAC)¹³ as well as therapies such as bronchial thermoplasty¹⁴ are bronchoscopic procedures, and incorporating endobronchial OCT imaging into the procedure introduces minimal additional time and risk to the subject.

In this study, we use endobronchial OCT to assess airway microstructure, mucosal buckling and mucus content in mild allergic asthmatic (AA), non-asthma allergic control (AC) and healthy control (HC) human volunteers. The advantage of using endobronchial OCT is that these measurements can be performed from a single volumetric scan. We conduct imaging (i) at baseline to assess airway remodelling and (ii) following an SAC to investigate the transient microstructural and functional response to the allergen. We correlate in vivo measurements with FEV₁/FVC (forced expiratory volume in 1 s/forced vital capacity) and FEV₁% (FEV₁ as a percentage of predictive value) measurements obtained at baseline to determine the relationship between these baseline imaging measurements and airway obstruction. This comprehensive study demonstrates both underlying structural and functional differences between mildly asthmatic and non-asthmatic individuals and establishes a methodology for future asthma research with endobronchial OCT.

METHODS

Subject enrolment

The Partners Healthcare Institutional Review Board granted approval for this study (protocol #2007P001050) and participants gave their informed written consent. Prior to enrolment, subjects were screened for eligibility with a complete medical history, baseline spirometry, methacholine challenge and allergen skin testing, as previously described.¹³ All subjects underwent allergen skin prick testing, and all subjects enrolled into the AA or AC groups had a positive skin prick test to either cat hair or dust mite extract, with the threshold level determined by titration. For subjects allergic to both, cat hair extract was selected for use in the study. All HC subjects had a negative reaction to skin test. Asthmatics were classified as previously described.¹⁵ All asthmatic volunteers had a PC20 (concentration of methacholine needed to produce a 20% fall of FEV₁ from baseline) of less than 20 mg/mL at baseline. Baseline airway obstruction was assessed with

spirometry (FEV₁/FVC and FEV₁%). The subjects were asked to refrain from using inhaled steroids for 2 weeks prior to bronchoscopy and bronchodilator for 12 h prior. A detailed description of the inclusion/exclusion criteria for each of the three study groups can be found in Appendix S1 (Supplementary Information).

Segmental allergen challenge

Comprehensive details regarding the SAC procedure have previously been published.^{16–18} Briefly, a baseline bronchoalveolar lavage (BAL) was conducted in the lingula (4 × 30 mL of normal saline) and 2 mL of diluent and allergen (at the lowest concentration of cat hair or dust mite extract that elicited a positive skin prick test) was subsequently administered to the anterior segment of the right upper lobe (RUL) and the lateral segment of the right middle lobe (RML), respectively. The HC group was administered a dose of 500 bioequivalent allergen units per mL (BAU/mL) of cat hair extract in the RML. Twenty-four hours following allergen challenge, a second bronchoscopy procedure was performed and BAL was performed in the diluent and allergen-challenged lung segments. Cell differential counts were obtained from the collected fluid using a cytocentrifuge.¹⁶

OCT system

The details of our OCT system have been previously described.^{12,19} The axial resolution of the system was approximately 10 µm, and the penetration depth in soft tissue 2–3 mm. The fibre optic imaging catheter used a monolithic side-viewing ball lens design with a beam spot size of ~30 µm. The 360° cross-sectional images of the airway were acquired at a rate of 33 frames per second, and longitudinal scanning was performed at a rate of 1 mm per second. The resultant longitudinal pitch between frames was ~33 µm.

OCT procedure

Endobronchial OCT was performed in the RUL and the RML immediately prior to the SAC on day 1 and immediately prior to the BAL on day 2 (Fig. S1, Supplementary Information). Imaging was performed by inserting the OCT catheter (1.65 mm outer diameter, ~2 m length) through the working channel of the bronchoscope to extend approximately 3 cm into the selected airway segment. Care was taken to ensure consistency in the generation of the airway imaged, and the placement of the bronchoscope with respect to the airway was used as a reference. Volumetric OCT of each 3 cm segment was subsequently conducted in 30 s. Images were displayed in real time and the raw data were saved for post-procedure analysis.

OCT image analysis

OCT images were processed according to standard processing techniques.²⁰ Four distinct metrics were quantified per data set: epithelial thickness, mucosal thickness, mucosal buckling (as a measure of bronchoconstriction) and mucus contrast (an optical assessment of mucus content²¹). Analysis of all excluding mucus contrast was performed using ImageJ (National

Institute of Health, Bethesda, MD, USA). Measurements for these features were only performed in frames that did not contain any bronchial branching. Epithelial thickness and mucosal thickness were measured at approximately 2 mm intervals along each imaged segment, excluding the rare non-cartilaginous region that may have been observed distally in some data sets.

Mucosal thickness was measured from lumen to cartilage perichondrium (Fig. 1A). Epithelial thickness was measured from lumen to basement membrane²² (Fig. 1B). Mucosal buckling was identified by the visible presence of at least three distinct folds in the airway wall (Fig. 1C). For quantification purposes, we defined a fold as a bend in the airway wall where the width perpendicular to the wall was (approximately) at least as great as its width tangential to the wall. Mucosal buckling was quantified as the percentage of frames (from the entire data set) where at least three distinct folds were observed. Mucus (Fig. 1D) was segmented and assessed automatically.²¹ The final values are reported as the average of each of the measurements obtained per segment. Volumetric images were generated with Osirix (Pixmeo, Bernex, Switzerland).

Statistical analysis

Data are means $\pm$ SD. Statistical analysis was performed in Prism (GraphPad Software, La Jolla, CA,

USA). Baseline differences between study groups were assessed using Kruskal–Wallis with multiple comparisons performed using Dunn's test. Differences in response to allergen were assessed using a two-way analysis of variance (ANOVA) with multiple comparisons performed using Sidak's test. The factors assessed in the allergen response analyses were group (AA, AC and HC) and time (pre- and post-allergen), the latter of which was assessed using repeated measures. Correlations between spirometry and imaging metrics were assessed based on the strength of Pearson's correlation coefficients. Comparisons were considered statistically significant for $P < 0.05$.

RESULTS

From 2011 to 2016, 22 AA, 22 AC and 3 HC study volunteers were enrolled in this study. Two AA subjects were unable to undergo the second day of imaging due to excessive coughing and $FEV_1 < 50\%$ predicted, respectively. The results presented in this manuscript represent data obtained from the remaining study subjects; 20 AA, 22 AC and 3 HC. In two cases, an individual from the AA group received a bronchodilator on the second day of the study as recommended by a physician but were able to complete the study. Patient

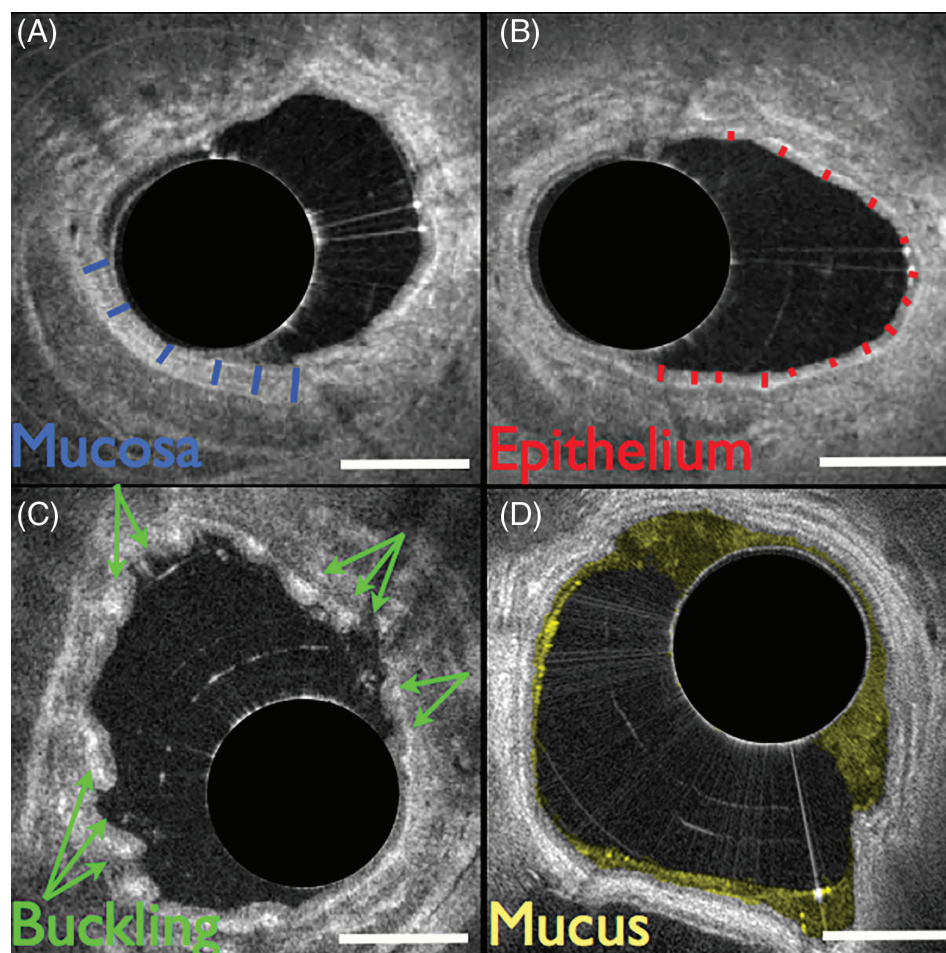


Figure 1 Structural airway features measured with OCT. (A) Mucosal thickness was measured from lumen to cartilage perichondrium, indicated with blue lines. (B) Epithelial thickness was measured from lumen to basement membrane, indicated with red lines. (C) Mucosal buckling was calculated as the percentage of frames in the data set that exhibited buckling, indicated with arrows. (D) Mucus was automatically segmented and colour-coded in yellow. OCT, optical coherence tomography. Scale bars: 1 mm.

Table 1 Subject characteristics

	AA	AC	HC	P-value
Subjects, <i>n</i>	20	22	3	
Age, years, mean (SD)	28.7 (9.3)	27.2 (7.7)	24 (2.3)	0.935
Female, <i>n</i> (%)	11 (61.1)	10 (45.5)	2 (66.6)	0.546
FEV ₁ , mean (SD)				
Litres	3.31 (0.79)	4.02 (0.68)	3.76 (0.34)	0.071
% Predicted	88.9 (10.6) [†]	103.1 (12.9)	102.7 (7.77)	0.004
FVC, mean (SD)				
Litres	4.46 (1.31)	4.83 (0.82)	4.30 (0.34)	0.306
% Predicted	100.3 (12.2)	104.9 (14.0)	101.7 (7.51)	0.294
FEV ₁ /FVC, mean (SD)	0.76 (0.08) ^{†‡}	0.84 (0.05)	0.87 (0.02)	0.0005
PC20, mg/mL, mean (SD)	3.50 (5.38) ^{†‡}	>25	>25	<0.0001
Allergen, <i>n</i> (%)				
<i>Dermatophagoides pteronyssinus</i>	12 (60)	10 (45.5)	—	
Cat hair extract	8 (40)	12 (54.5)	—	
Allergen dose, BAU/mL, mean (SD)				
<i>D. Pteronyssinus</i>	226.2 (339.0)	110.1 (108.2)	—	0.847 [§]
Cat hair extract	158.7 (172.0)	751.7 (418.6)	500 (0)	0.0042 [§]

[†]*P* < 0.05 for post hoc comparison with AC.

[‡]*P* < 0.05 for post hoc comparison with HC.

[§]HC group excluded and Mann–Whitney test was used for statistical analysis.

AA, allergic asthmatic; AC, allergic control; BAU, bioequivalent allergen unit; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; HC, healthy control; PC20, concentration of methacholine needed to produce a 20% fall of FEV₁ from baseline.

characteristics and pulmonary function tests are provided in Table 1.

shows the relationship between FEV₁/FVC and the AA and AC measurements.

Baseline airway morphology and mucus

Figure 2A–C shows representative OCT cross-sectional images obtained from each of the study groups. Baseline measurements are reported as an average of both the RML and the RUL measurements per subject, as there was no statistically significant difference observed between locations within each study subject. The mean diameter of all airways imaged was 2.8 mm (± 0.7 mm). An increase in epithelial and mucosal thickness and mucosal buckling was observed in AA study subjects compared to AC subjects (mucosal thickness: *P* = 0.0073; epithelial thickness: *P* = 0.0082; mucosal buckling: *P* = 0.0003) and compared to HC subjects (mucosal thickness: *P* = 0.0029; epithelial thickness: *P* = 0.0376; mucosal buckling: *P* = 0.0031) (Fig. 2G–I). Representative airway cross-sectional images highlighting the mucus are shown for the three groups in Figure 2D–F. Mucus contrast was found to be higher for the AA group compared to the AC group (*P* = 0.0154) (Fig. 2J).

OCT and spirometry

To determine if OCT measures of airway morphology at baseline are reflected in global airway obstruction, we investigated the relationship between these metrics and both FEV₁/FVC and FEV₁% for the AA and AC groups. No correlation was observed for any of the metrics and FEV₁%; however, both epithelial thickness and mucosal buckling were found to be correlated to FEV₁/FVC in the AA group (*P* = 0.03 for epithelial thickness and *P* = 0.003 for mucosal buckling). Figure 3

SAC response

BAL results for some potentially relevant cellular data are shown in Figure S2 (Supplementary Information). An increase in epithelial thickness, mucosal thickness and mucosal buckling measured in the RML compared to baseline values was observed in response to allergen in both AA and AC groups (Fig. 4A–C). While epithelial and mucosal thicknesses increased in both the AA and AC groups (*P* = 0.0003 AA, *P* = 0.0004 AC for mucosal thickness; *P* = 0.0002 AA, *P* = 0.0075 AC for epithelial thickness), mucosal buckling was only increased in the AA group (*P* = 0.0006 AA). An increase in mucosal buckling was also observed in response to diluent in the AA group (*P* = 0.0054). No change was observed in the HC subjects.

The results of the mucus measurements are depicted in Figure 4D. Post-allergen, mucus contrast was increased in the AA group relative to both the baseline of the AA group (*P* = 0.0011) and the post-allergen AC group (*P* < 0.0001). A volumetric representation of an allergen-challenged AA airway segment highlighting the change in both mucus volume and heterogeneity compared to the pre-allergen airway is depicted in Figure 5.

DISCUSSION

In this study, we assessed airway remodelling in asthma on a microscopic scale, in vivo, using endobronchial OCT. Three groups were included in this study: AA, AC and HC subjects. Fewer HC subjects were enrolled in this study as we were primarily

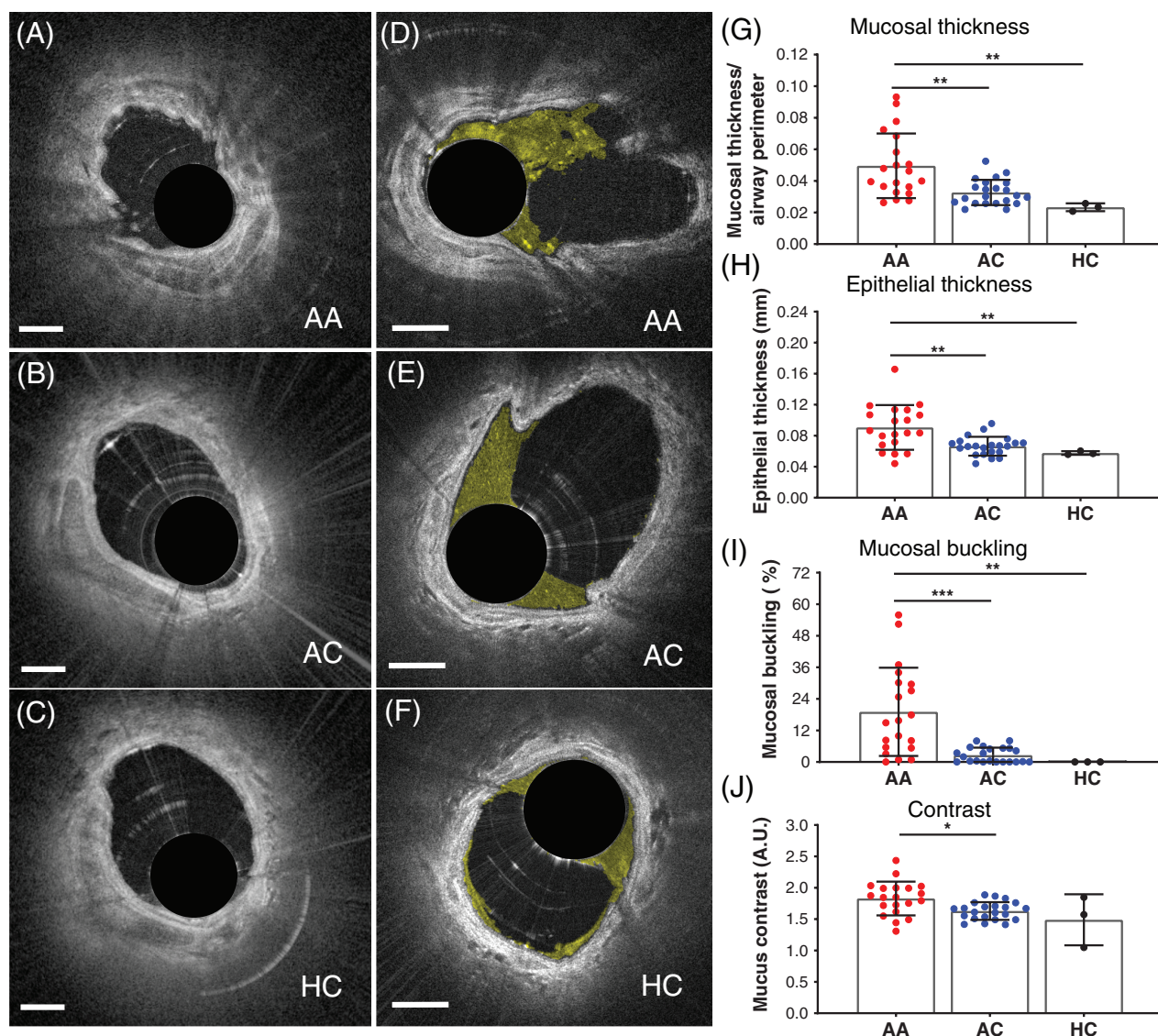


Figure 2 Representative cross-sectional images comparing pre-challenge airway wall morphology (A–C) and mucus (D–F) for the three groups included in the study. Mucus was colour-coded in yellow. (A,C) AA, (B,E) AC and (C,F) HC. (G–J) Airway morphology and mucus metrics measured pre-challenge for each of the three groups. (G) Mucosal thickness, (H) epithelial thickness, (I) percentage of frames exhibiting mucosal buckling and (J) mucus contrast. AA, allergic asthmatic; AC, allergic control; A.U., arbitrary unit; HC, healthy control. Scale bars: 1 mm. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

interested in comparing AA and AC individuals. The measurements for the HC group demonstrated less variability than those of the AA and AC groups, and are included to provide some context for our data and so that possible baseline values may be inferred. The features that we measured were epithelial thickness, mucosal thickness, airway buckling and mucus. Baseline differences between groups were observed for each of the features. Correlations with FEV_1/FVC in the AA group alone were observed for both epithelial thickness and mucosal buckling. The fact that our microstructural assessments have a stronger relationship with FEV_1/FVC than $FEV_1\%$ is consistent with previous observations involving wall thickness as a measure of remodelling with computed tomography.²³

Post-allergen, there were significant differences between the AA and AC groups for every metric except

mucosal thickness. While significance was observed in both the AA and AC groups for mucosal and epithelial thickness, mucosal buckling and mucus contrast were only significantly different in the AA group. Mucus contrast demonstrated the most significant post-allergen difference between the AA and AC groups ($P < 0.0001$). Although mucus contrast is likely influenced by factors such as cellular debris, we have already demonstrated that mucus contrast correlates with mucin content obtained from BAL.²¹ Although further tests are necessary to establish correlations, this metric seems to accurately capture the appearance of mucus in our images (refer to the more solid appearing mucus in the AA airways of Figs 2,5).

Perhaps, the most interesting and unique aspect of this study was the differences in mucosal buckling. Previously, it has been shown that airway wall thickness

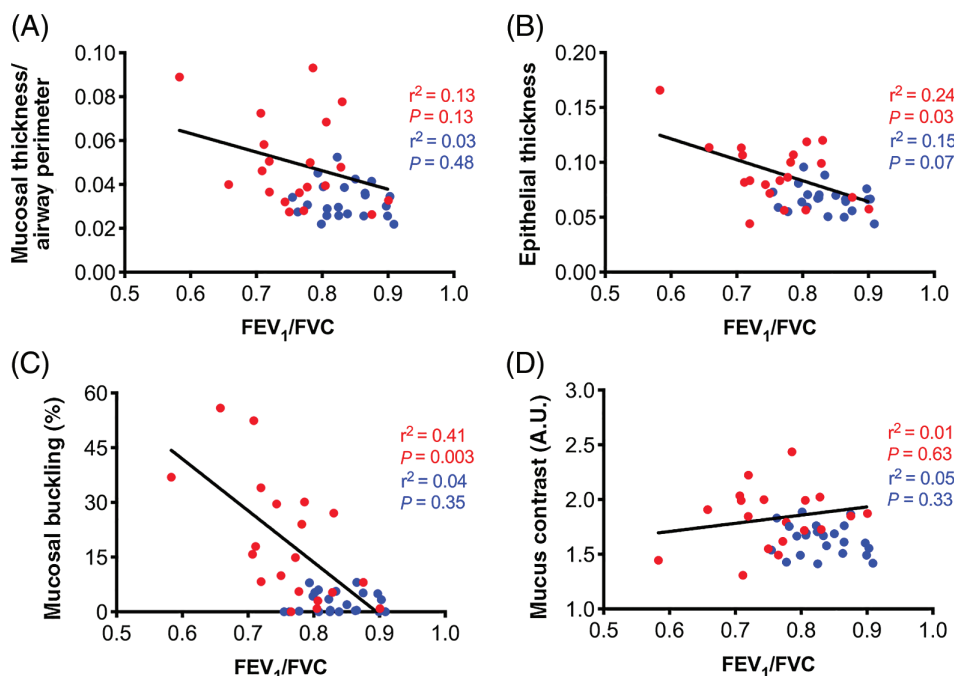


Figure 3 Correlations between FEV_1/FVC and (A) mucosal thickness, (B) epithelial thickness, (C) mucosal buckling and (D) mucus contrast, using baseline measurements for each. Values from both the AA (red dots) and AC (blue dots) group are included. Correlations are presented both AA and AC, separately. Trend line is included for AA only. AA, allergic asthmatic; AC, allergic control; A.U., arbitrary unit; FEV_1 , forced expiratory volume in 1 s; FVC, forced vital capacity.

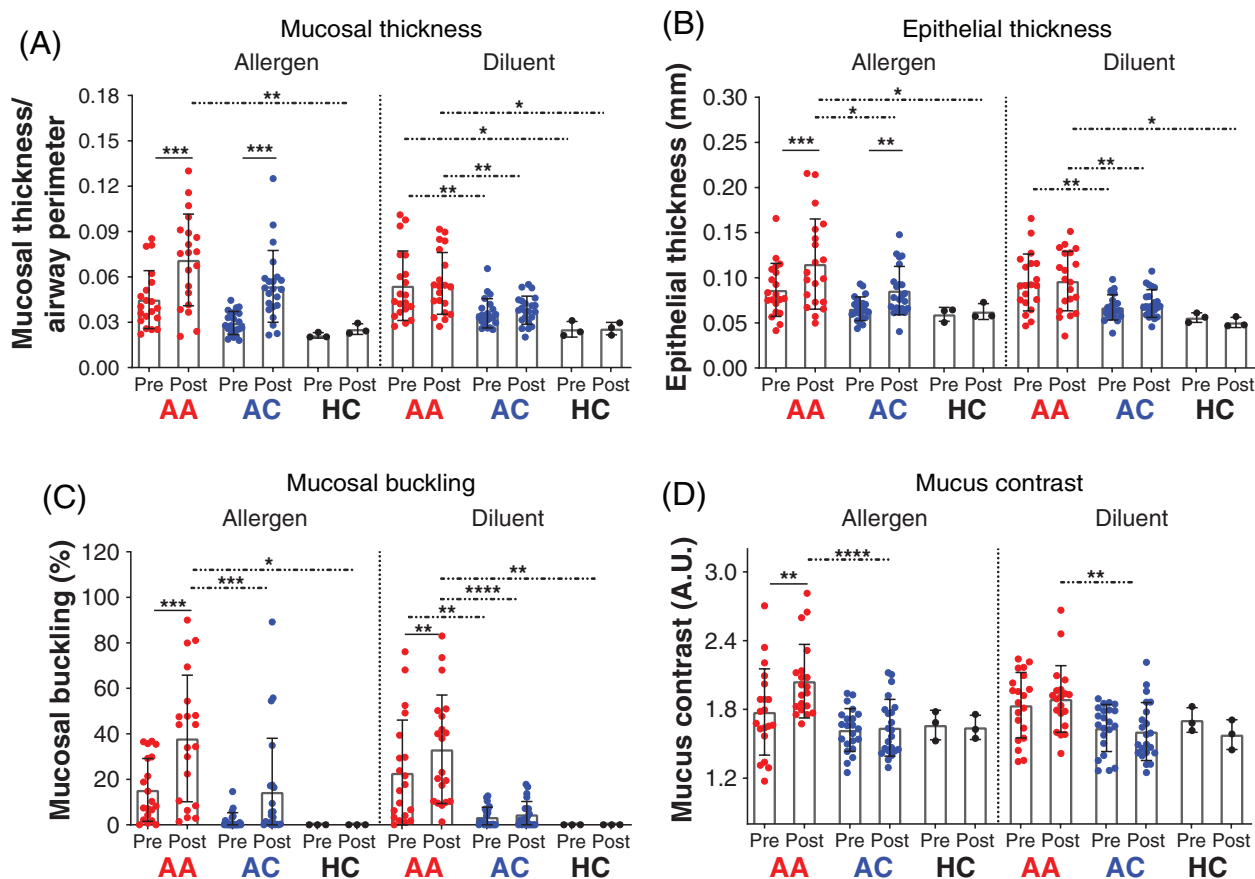


Figure 4 The four OCT metrics included in the study measured pre- and post-allergen (right middle lobe) and diluent (right upper lobe) for each of the three groups. (A) Mucosal thickness. (B) Epithelial thickness. (C) Mucosal buckling. (D) Mucus contrast. AA, allergic asthmatic; AC, allergic control; A.U., arbitrary unit; HC, healthy control; OCT, optical coherence tomography. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

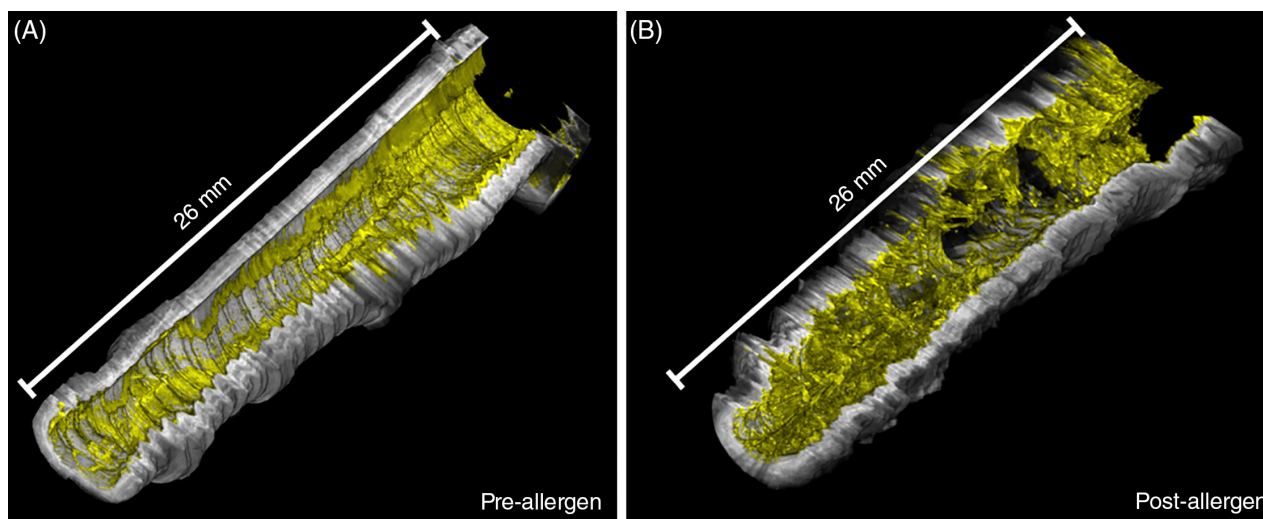


Figure 5 Volumetric renderings of mucus in a right middle lobe airway segment of an allergic asthmatic study subject pre-challenge and 24 h post-challenge. The airway wall was rendered in greyscale and the mucus false-coloured in yellow. (A) Pre-allergen. (B) Post-allergen.

assessed with OCT was strongly correlated with lung function,²⁴ but in our study the granularity of the distinct microstructural metrics shows mucosal buckling to be the strongest correlate. Additionally, buckling exhibited the most significant difference post-allergen between the two groups. Here, we make the reasonable assumption that the buckling is primarily a consequence of bronchoconstriction,²⁵ and therefore consider it a surrogate measure for airway smooth muscle tone. In this study, we observed several indications that suggest the possibility of airway smooth muscle abnormality²⁶ in the mild asthmatic group: increase in the baseline tone, hyperresponsiveness as indicated by the increased buckling in the diluent airway and resistance to relaxation as indicated by the increased buckling 24 h following the allergen administration. Our results, coupled with the results of a pivotal study which found that bronchoconstriction promotes airway remodelling in asthma²⁷ as well as our own pilot study that demonstrated increased muscle thickness in three mild asthmatics,¹⁹ add to the increasingly compelling evidence that abnormalities of airway smooth muscle can be a crucial component in the pathogenesis of asthma. Further investigation into the relationship between bronchoconstriction and airway smooth muscle in asthma, along with the continued development of technology which can directly assess airway smooth muscle,¹⁹ may therefore be important for therapies targeting airway smooth muscle such as bronchial thermoplasty that aim to mitigate asthma symptoms.^{14,28–30}

In conclusion, endobronchial OCT was used to measure mucus and airway microstructure in allergic individuals with and without asthma to examine alterations in mild asthma and determine how each correlated to airflow obstruction. We observed evidence of airway remodelling in individuals with mild asthma, and a difference in reaction to allergen challenge between the mildly asthmatic and non-asthmatic airways. Even with only two 3-cm airway segments imaged per individual, a relationship was observed between baseline

remodelling and baseline airway obstruction, highlighting the physiological relevance of even mild airway remodelling. The results of this study demonstrate the potential of endobronchial OCT for both asthma research and monitoring airway remodelling in asthma, offering a minimally invasive approach for measuring multiple important features of remodelling simultaneously.

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Disclosure statement

M.J.S. reports personal fees from LX Medical, VIDA Diagnostics and NinePoint Medical, and grants from Boston Scientific and Biogen Idec outside the submitted work. L.P.H. reports personal fees from LX Medical outside the submitted work.

Abbreviations: AA, allergic asthmatic; AC, allergic control; BAL, bronchoalveolar lavage; FEV₁, forced expiratory volume in 1 s; FEV₁%, FEV₁ as a percentage of predictive value; FVC, forced vital capacity; HC, healthy control; OCT, optical coherence tomography; RML, right middle lobe; RUL, right upper lobe; SAC, segmental allergen challenge.

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Supplementary Information

Additional supplementary information can be accessed via the *html* version of this article at the publisher's website.

Appendix S1 Detailed inclusion and exclusion criteria.

Figure S1 Study overview.

Figure S2 Cell counts per mL of BAL fluid.

SUPPLEMENTARY INFORMATION

Quantitative assessment of airway remodeling and response to allergen in asthma

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Appendix S1- Detailed Inclusion and Exclusion Criteria

Healthy Controls (HC)

Inclusion criteria:

1. Willing and able to give informed consent.
2. Age between 18 and 50 years.
3. Non-allergic as defined by a negative reaction to a panel of prick skin tests.
4. No history of allergic rhinitis or asthma.
5. No history of allergic symptoms caused by cats or dust mite allergen exposure.
6. Life-long nonsmokers of cigarettes (defined as a lifetime total of less than 5 pack-years and none in 5 years).
7. Normal spirometry (i.e. FEV1 and FVC of at least 90% of predicted).
8. Methacholine PC20 of > 16 mg/ml.

Exclusion Criteria:

- 1) Women of childbearing potential who are documented to be pregnant (based on urine β -HCG testing), are sexually active and not using contraception, are seeking to become pregnant, or who are nursing.
- 2) BMI > 35 kg/m².
- 3) Participation in a research study involving a drug or biologic during the 30 days prior to the study.
- 4) Intolerance to albuterol, atropine, lidocaine, fentanyl, or midazolam.
- 5) Antihistamines within 7 days of the screening visit.
- 6) Presence of diabetes mellitus, congestive heart failure, ventricular arrhythmias, history of a cerebrovascular accident, renal failure, history of anaphylaxis, or cirrhosis.
- 7) Use of systemic steroids, inhaled steroids, beta blockers and monoamine oxidase inhibitors within 1 month of the screening visit.
- 8) Antibiotic use for respiratory disease within 1 month of the characterization visit or a respiratory tract infection within 6 weeks of the bronchoscopy visits.

- 9) Subjects with a high possibility of poor compliance with the study.
- 10) Second-hand cigarette smoke exposure or indoor furry pets except in the case of dog, if the subject is not allergic to the dog and the subject has a negative skin test to dog.
- 11) Exposure to asbestos or silica.
- 12) Other lung diseases, such as sarcoidosis, bronchiectasis or active lung infection.
- 13) Non-English speakers.
- 14) History of coagulopathy, thrombocytopenia, pulmonary hypertension, and/or use of anticoagulants/anti-platelet drugs.

Allergic Asthmatics (AA)

Inclusion Criteria:

- 1) Willing and able to give informed consent.
- 2) Age between 18 and 50 years.
- 3) Asthma as defined by the National Asthma Education and Prevention Program's definition.
- 4) Mild to moderate asthma severity with baseline FEV1 determined at the characterization visit that is no less than 75 % of the predicted value after bronchodilator administration.
- 5) Bronchodilator-responsive airway obstruction and/or a methacholine PC20 of < 16 mg/ml.
- 6) Clinical history of allergic symptoms to cat or dust mite allergen and demonstrated skin reactivity. A positive allergen prick test was defined as at least a 3 mm wheal diameter to an extract concentration of > 0.056 BAU/AU.
- 7) Life-long absence of cigarette smoking (defined as a lifetime total of less than 5 pack-years and none in 5 years).

Exclusion Criteria:

1. Women of childbearing potential who are documented to be pregnant (based on urine β -HCG testing), are sexually active and not using contraception, are seeking to become pregnant, or who are nursing.
2. BMI > 35 kg/m².
3. The presence of spontaneous asthmatic episode within the previous 6 weeks.
4. Participation in a research study involving a drug or biologic during the 30 days prior to the study.
5. Intolerance to albuterol, atropine, lidocaine, fentanyl, or midazolam.
6. Antihistamines within 7 days of the screening visit.
7. Presence of diabetes mellitus, congestive heart failure, ventricular arrhythmias, history of a cerebrovascular accident, renal failure, history of anaphylaxis, or cirrhosis.
8. Use of systemic steroids, increased use of inhaled steroids, beta blockers and monoamine oxidase inhibitors.
9. Antibiotic use for respiratory disease within 1 month of the characterization visit or a respiratory tract infection within 6 weeks of the bronchoscopy visits.
10. A history of asthma-related respiratory failure requiring intubation.
11. Quantitative skin-prick test positive reaction down to an allergen concentration of 0.056 BAU or AU/ml.
12. Subjects with a high possibility of poor compliance with the study.
13. Having second-hand cigarette smoke exposure or indoor furry pets except in the case of dog, if the subject is not allergic to the dog and the subject has a negative skin test to dog.
14. Exposure to asbestos or silica.

15. Other lung diseases, such as sarcoidosis, bronchiectasis or active lung infection.
16. Use of omalizumab (anti-IgE monoclonal antibody) for 6 months.
17. Immunotherapy with cat or dust mite extract now or in the past.
18. Non-English speakers.
19. History of coagulopathy, thrombocytopenia, pulmonary hypertension, and/or use of anticoagulants/anti-platelet drugs.

Allergic Non-Asthmatic Controls (AC)

Inclusion Criteria:

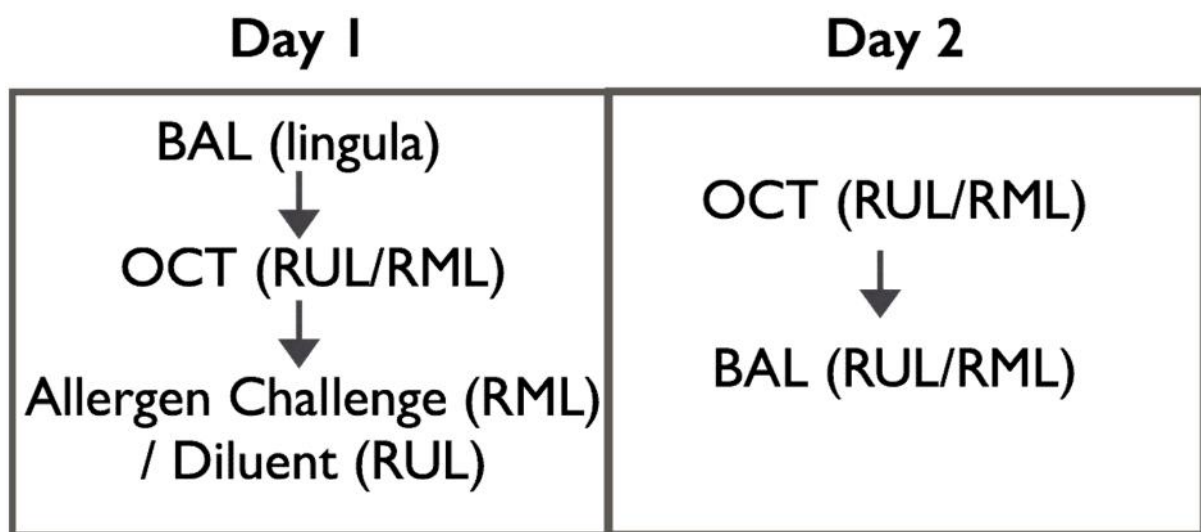
1. Willing and able to give informed consent.
2. Age between 18 and 50 years.
3. History of either (a) allergic rhinitis (with one or more of the following symptoms: nasal congestion, sneezing, runny nose, postnasal drainage), (b) allergic conjunctivitis (ocular itching, tearing and/or swelling) or (c) contact allergy associated with cat dander or dust mite and a positive allergy test to the same allergen.
4. Baseline FEV1 and FVC determined at the characterization visit that is no less than 90 % of the predicted value.
5. Clinical history of allergic symptoms to cat or dust mite allergen and demonstrated skin reactivity. A positive allergen prick test was defined as at least a 3 mm wheal diameter to an extract concentration of > 0.056 BAU/AU.
6. Life-long absence of cigarette smoking (defined as a lifetime total of less than 5 pack-years and none in 5 years).

Exclusion Criteria:

1. A history of asthma.
2. A methacholine PC20 < 16 mg/ml.
3. Women of childbearing potential who are documented to be pregnant (based on urine β -HCG testing), are sexually active and not using contraception, are seeking to become pregnant, or who are nursing.
4. BMI > 35 kg/m².
5. The presence of spontaneous asthmatic episode within the previous 6 weeks.
6. Participation in a research study involving a drug or biologic during the 30 days prior to the study.
7. Intolerance to albuterol, atropine, lidocaine, fentanyl, or midazolam.
8. Antihistamines within 7 days of the screening visit.
9. Presence of diabetes mellitus, congestive heart failure, ventricular arrhythmias, history of a cerebrovascular accident, renal failure, history of anaphylaxis, or cirrhosis.
10. Use of systemic steroids, inhaled steroids, beta blockers and monoamine oxidase inhibitors.
11. Antibiotic use for respiratory disease within 1 month of the characterization visit or a respiratory tract infection within 6 weeks of the bronchoscopy visits.
12. Quantitative skin-prick test positive reaction down to an allergen concentration of 0.056 BAU or AU/ml.
13. Subjects with a high possibility of poor compliance with the study.
14. Having second-hand cigarette smoke exposure or indoor furry pets except in the case of dog, if the subject is not allergic to the dog and the subject has a negative skin test to dog.
15. Exposure to asbestos or silica.

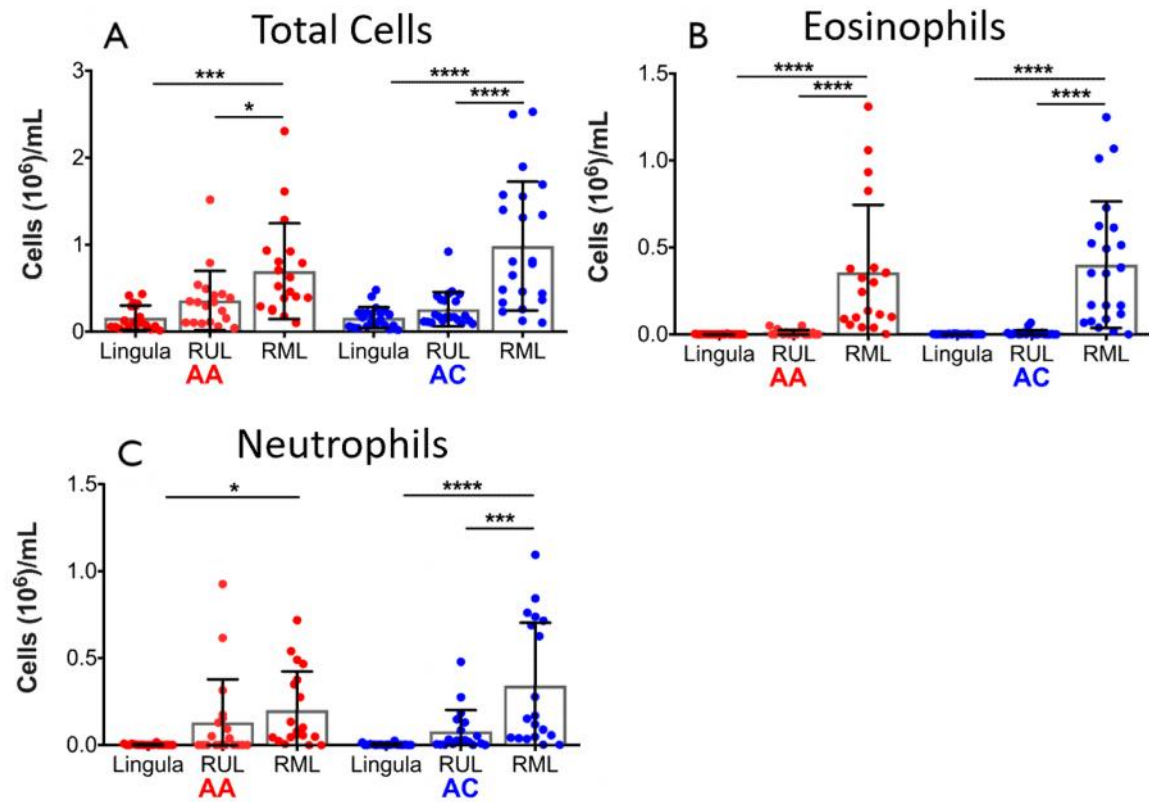
16. Other lung diseases, such as sarcoidosis, bronchiectasis or active lung infection.
17. Use of omalizumab (anti-IgE monoclonal antibody) for 6 months.
18. Immunotherapy with cat or dust mite extract now or in the past.
19. Non-English speakers.
20. History of coagulopathy, thrombocytopenia, pulmonary hypertension, and/or use of anticoagulants/anti-platelet drugs.

Figure S1- Study Overview.



Overview of the procedures conducted on day 1 (pre-allergen) and day 2 (post-allergen) of the study.

Figure S2- Cell counts per mL of BAL fluid.



(a) Total cell counts from the AA and AC subjects pre-challenge (lingula) and post-challenge (RML and RUL), analysed with two-way ANOVA (airway segment and subject group, using repeated measures for segment factor). (b) Eosinophil counts obtained from the same. (c) Neutrophil counts obtained from same. AA, allergic asthmatic; AC, allergic control. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.