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ORIGINAL ARTICLE

Asthma and Rhinitis

The relationship of airway structural changes to blood and bronchoalveolar lavage biomarkers, and lung function abnormalities in asthma

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Abstract

Background: Airway structural changes are important in asthma pathology and require further investigations.

Objective: We sought to evaluate which computed tomography (CT) indices, bronchial histological traits, or blood and bronchoalveolar lavage (BAL) biomarkers correlate best with lung function abnormalities in asthma.

Methods: In 105 white adult asthmatics (53 with a component of fixed airflow obstruction), we determined airway cross-sectional geometry of two proximal (the right upper lobe apical segmental and the left apicoposterior) and two distal (the right and the left basal posterior) bronchi, quantified the low-attenuation lung area (LAA%), and analysed clusters based on airway CT-metrics. We also performed bronchofiberoscopy with BAL and endobronchial biopsy, assessed blood and BAL biomarkers, including interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, IL-23, interferon (INF)γ and periostin, together with circulating a disintegrin and metalloproteinase domain-containing protein (ADAM)33, and investigated interplays between analysed variables.

Results: Patients with fixed airflow limitation were characterized by lower lumen area and increased wall area and wall thickness ratios in distal airways, accompanied by raised LAA%. They had also higher blood neutrophilia, blood and BAL eosinophilia, increased circulating fibrinogen, periostin, and ADAM33. Blood neutrophilia, serum high density lipoproteins, thyroid-stimulating hormone, and shortened activated partial thromboplastin time were determinants of thicker reticular basement membrane (RBM). BAL eosinophilia was the only positive predictor of collagen I accumulation. Surprisingly, we observed a negative correlation between RBM thickening and collagen I deposit. Cluster analysis based on CT-metrics of the right lower lobe basal posterior bronchus revealed three well-separated clusters similar in age, asthma duration, and BMI, but different in RBM thickness, collagen I accumulation, and inflammatory markers.

Conclusions and clinical relevance: Airway remodelling traits are mainly related to the Th_2 profile, higher circulating ADAM33, and blood neutrophilia. Lung function

abnormalities and RBM thickening correlate better with CT-metrics of distal than proximal airways.

KEYWORDS
airway remodelling, asthma, biomarkers, computed tomography, histology

1 | INTRODUCTION

In relation to airway pathology, asthma is characterized by airway lumen narrowing and wall thickening caused by chronic inflammation and hyperresponsiveness of the bronchi.[1] Numerous studies have shown that airway walls are thicker in both mild and severe asthma.[2] These airway structural changes involve all airway components, including epithelium, reticular basement membrane (RBM), submucosa, mucous glands, and smooth muscles. Other related factors enclose mucosal oedema, bronchial vessel congestion, and proliferation.[3] Despite observed histological abnormalities, thickening of the airway wall is not usually related to lumen narrowing. The bronchial lumen may be obstructed rather due to shortening of the smooth muscle layer surrounding the bronchus, accumulation of fluid, cells or mucus, and airway wall collapse.[2] Moreover, the extent to which airway wall structural changes reflect uniquely an airway remodelled phenotype or clinically severe asthma remains unknown. Emerging non-invasive methods for quantifying airway remodelling pathology include chest computed tomography (CT).[4,5] Imaging variables, such as airway diameter, wall area, wall thickness, and air trapping, have been found to be important metrics when differentiating patients with severe asthma from those with non-severe type of the disease and healthy subjects.[5] Thickening of the right upper lobe apical segmental bronchus (RB1) has been demonstrated to associate with airflow obstruction and epithelial thickening,[6] as well as airway wall hyperresponsiveness.[7] Moreover, proximal airway wall thickening measured at RB1 has been shown to be related to the appropriate indices obtained for bronchi generations third through sixth.[7] However, identification of specific asthma traits, such as blood biomarkers, CT indices, or histological changes that correlate best with asthma severity and progression, requires further investigation. In the present study, we sought to evaluate relationships between lung function abnormalities, CT indices of airway remodelling, RBM thickening, collagen type I and IV deposits in bronchial biopsies, as well as blood and bronchoalveolar lavage (BAL) biomarkers in 105 adult clinically stable asthmatics. We compared CT-derived dimensions of proximal and distal airways, and measured low-attenuation lung area (LAA%) by the conventional threshold level of −950 Hounsfield units,[8] to show which of those indices correlate best with lung function, histology, and asthma biomarkers. The LAA% determines the percentile quantity of voxels showing a value lower than preset attenuation from all voxels within lung tissue and is usually employed for evaluation of emphysema.[9,10] In asthma, however, it might be also related to the excessive narrowing of large and small airways with subsequent air trapping and hyperinflation.[2,10] Standard air trapping measurement requires inspiratory and expiratory CT scans with exposure to a double radiation dose. The LAA% is automatically assessed in standard inspiratory CT examination by simple quantitative CT software.

2 | METHODS

2.1 | Patients

We enrolled 114 white, non-smoking, clinically stable adult asthmatics: 60 with fixed airflow limitation and 54 without spirometric evidence of airway obstruction before or after bronchodilator. Fixed airflow limitation was defined as a forced expiratory volume in 1 second (FEV_1)/vital capacity (VC) index lower than 0.7 or FEV_1 below 0.8 of predicted value postbronchodilator. Diagnosis of asthma was established by a physician, based on a history of recurrent respiratory symptoms (wheeze, cough, shortness of breath, and chest tightness) together with currently and/or historically documented postbronchodilator increase in FEV_1 of at least 200 mL and 12% from the baseline.[11] Atopic status was confirmed by a positive skin prick testing for at least one inhaled allergen (Allergopharma, Reinbeck, Germany). All asthma medications, except of biological treatment, were permitted, including oral corticosteroids at a daily dose equivalent to ≤10 mg of prednisolone, unless the doses were unchanged during the preceding 3 months. Asthma patients could not be exacerbated in the last 6 months. Severity of asthma was categorized according to the Global Initiative for Asthma (GINA) 2018 guidelines.[11] Definitions of "mild," "moderate," and "severe" asthma are provided in Appendices. Asthma symptom control was assessed based on result of asthma control test. Scores 20-25 were classified as "well-controlled asthma," 16-19 as "not well-controlled," while 5-15 as "very poorly controlled asthma." Spirometry, bronchial reversibility test with 400 μg of albuterol, and postbronchodilator body plethysmography were performed in all subjects according to the American Thoracic Society standards,[12] using a Jaeger MasterLab spirometer (Jaeger-Toennies GmbH).

Representatives of both groups were 18-70 years old. Ex-smokers were eligible for the study if they stopped smoking at least 5 years ago, with a history of less than 7 pack-years. The exclusion criteria included pregnancy or breast feeding, any acute illness, congestive heart failure, atrial fibrillation, myocardial infarction or stroke in a history, cancer, hyper- or hypothyroidism, liver injury, and chronic kidney disease (stage 3 or more). Subjects were eligible if they had arterial hypertension, diabetes mellitus, hypercholesterolaemia or stable coronary heart disease. Definitions of comorbidities were provided in Appendices.

The study was approved by the Ethics Committee of the Jagiellonian University (approval number: KBET/151/B/2013). All subjects gave written informed consent to participate in the study.

2.2 | Lung CT with evaluation of the airways cross-sectional geometry and LAA%

Lung CT was performed 1 hour after administration of 400 μg albuterol using 64-raw multidetector computed tomography (Aquilion TSX-101A, Toshiba Medical Systems Corporation), without administration of intravenous contrast medium. The airway cross-sectional geometry was quantified automatically by adapted by us AW Server Software (General Electric Healthcare) in four bronchi: RB1, the left apicoposterior bronchus (LB1 + 2), the right and the left lower lobe basal posterior bronchi (RB10 and LB10, respectively). After identifying the studied bronchus, the operator placed a seed point in the airway lumen, while the software automatically quantified airway geometry parameters: average diameter of the lumen and airway, average wall thickness, lumen and wall area, wall area ratio (WAR), and wall thickness ratio (WTR). Wall thickness was calculated based on average outer and inner bronchial diameters. The WAR was defined as an average difference between outer and inner wall area divided by the outer wall area. The WTR was calculated as an average value of the ratio of the wall thickness and the airway diameter. CT-based indices were measured two times by an independent radiologist (JZ) on different days. The mean value was included in a subsequent analysis. The LAA% was quantified automatically using the Volume Viewer 11.3 software (General Electric Healthcare) and 1 mm soft tissue reconstruction algorithm. More detail on CT investigations is provided in Appendices.

Among initially enrolled patients three subjects were excluded due to lung tumour, lobar atelectasis, and bronchiectases, respectively. Two subjects were excluded because of emphysema. In the next four patients, appropriate measurements were not possible. Finally, 53 subjects with fixed airflow limitation and 52 with normal postbronchodilator spirometry were included into the study.

2.3 | Bronchofiberoscopy, endobronchial biopsy and BAL

Bronchofiberoscopy was performed according to the guidelines of the American Thoracic Society [13] using the bronchofiberoscope BF 1T180 (Olympus) with local anaesthesia (2% lidocaine) and in mild sedation (0.05-0.1 mg fentanyl and 2.5-5 mg midazolam, both intravenously). During this procedure, BAL was performed with 200 mL of 0.9% saline given to the right middle lobe bronchus and 2-3 bronchial biopsy specimens were taken from the right lower lobe (the carina between B9 and B10). First 10 mL of obtained BAL fluid (BALF) was discarded, while the next sample was used for the study. Collected tissue samples were immediately fixed in 10% neutral buffered formalin solution (Sigma-Aldrich) and sent to the Pathology Department for further investigations.

2.4 | BALF analysis

The cytospin preparations (Thermo Scientific) were made from BALF and stained with May-Grünwald Giemsa dye. In each preparation, one thousand cells were counted. The results were shown as a percentage of all inflammatory cells (with exception of epithelial cells). Remaining BALF sample was centrifuged at 2000× *g* for 20 minutes; supernatant was frozen in aliquots and stored at −70°C until analysis.

2.5 | Histological examination

Tissue specimens were routinely processed and embedded in paraffin. The 2 μm sections were prepared and stained by haematoxylin and eosin. The slides were photographed using Nikon D5300 camera attached to the Zeiss Axioscope microscope with 100× oil immersion lens. The images were measured by the AnalySIS 3.2 software (Soft Imaging System GmbH) with a custom-built application written in Imaging C language. The images were acquired along the epithelial surface of the biopsy, including the entire basement membrane. Only sections covered by well preserved and non-tangentially cut epithelial cells were analysed. The RBM thickness was measured perpendicularly to the epithelium, according to the orthogonal intercept method proposed by Ferrando et al[14] (see also Figure S1 in Appendices) with an arbitrary-distance software tool of the analysis system. For each patient, at least 30 individual RBM measurements were performed at intervals of 9.5 μm. Notice that no stereological randomization was attempted because of type of the specimens (small biopsies). To reduce the obliquity effect of sectioning, the results were expressed as harmonic mean. The "harmonic mean" term was defined in Appendices.

Immunohistochemistry was performed in the routine manner from paraffin blocks in 2 μm sections using rabbit polyclonal anti-collagen I and mouse monoclonal anti-collagen IV antibodies (Abcam, Cambridge, United Kingdom, both). For visualization, we applied UltraVision Quanto Detection system with HRP DAB (Thermo Fisher Scientific). The results were scored semi-quantitatively. In case of collagen I, the staining was scored as 0 (lack of staining), 1 (very faint staining), 2 (evident positive stain), and 3 (strong reaction), and the percentage of stroma showing reactivity was assessed. In case of collagen IV, the staining was only scored as 0 (lack of staining), 1 (very faint staining), 2 (evident positive stain), and 3 (strong reaction).

Histological data were obtained from 45 patients with fixed airflow limitation and from 43 with normal spirometry. In other cases, bronchial specimens were not representative for reliable results.

2.6 | Laboratory investigations

Fasting blood samples were drawn from the antecubital vein using minimal stasis between 8:00 and 11:00 am. Lipid profile, glucose, urine, creatinine, alanine aminotransferase, thyroid-stimulating hormone (TSH), fibrinogen, activated partial thromboplastin time (aPTT), prothrombin time (PT), as well as complete blood cell and platelet count were assayed by routine laboratory techniques.

High-sensitivity C-reactive protein (hsCRP) and immunoglobulin E (IgE) were measured by latex nephelometry (Siemens). Blood samples were drawn into serum tubes and centrifuged at 2000× *g* for 20 minutes. The supernatant was frozen in aliquots and stored at –70°C.

Commercially available high-sensitivity immunoenzymatic assays (ELISAs) were used to measure levels of interleukin (IL)-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A, and interferon (INF)γ (eBiosciencea, Vienna, Austria) in blood and BALF. Blood and BALF levels of IL-23 (eBiosciencea) and periostin (Phoenix Pharmaceuticals), as well as circulating a disintegrin and metalloproteinase domain-containing protein (ADAM)33 (Cloud-Clone Corp.) were assessed by standard ELISA.

2.7 | Statistical analysis

For statistical analyses, we applied STATISTICA 12.5 software. Data distributions were verified by the Shapiro-Wilk test. Continuous variables were reported as median with interquartile range, or mean with standard deviation, and were compared by the Mann-Whitney *U* test, or unpaired *t* test, as appropriate. Categorical variables were provided as percentages and were compared by the chi-square test. Lung CT indices and results of histological examinations were log-transformed (or entered in original scale if normally distributed), and a one-way covariance analysis (ANCOVA) was performed to adjust for age, sex, body mass index (BMI), and smoking in the past. To test associations between continuous variables, the Spearman rank correlation test or the univariate linear regression model adjusted for the same confounders was applied. Independent determinants of RBM thickening, collagen I accumulation, mean WTR, and LAA% were established in multiple linear regression models. To calculate odds ratio (OR) with 95% confidence interval (CI), variables were divided by the median value as a cut-off point. A cluster analysis reported here was performed using the k-means clustering method, based on CT-metrics of RB10. We obtained four clusters, but one of them consisted of only one representative with extremely small lumen area and extremely large WAR and thus was excluded from the further analysis. Three remaining clusters were compared by the covariance analysis (ANCOVA), Kruskal-Wallis test or chi-square test, as appropriate.

Results were considered significant when the *P*-value was less than 0.05. In each case of multiple comparisons, Bonferroni correction was applied.

3 | RESULTS

3.1 | Patient groups

As shown in Table 1, both asthma subsets were well matched for demographic factors, including sex, height, BMI, place of residence (urban or rural area) and smoking habit in the past, albeit subjects with fixed airflow limitation were older. Both subgroups were also similar in a prevalence of atopy, comorbidities, asthma duration, symptom control, and severity (Table 1). In basic laboratory tests, patients with fixed airflow limitation were characterized by higher white blood cell count, as well as increased number of neutrophils, monocytes, and eosinophils in peripheral blood (Table 2). They had also slightly higher total cholesterol level and serum urea (all values within normal range) (Table 2). Moreover, in subjects with fixed airflow limitation we documented higher percentage of eosinophils in BALF and increased circulating fibrinogen (Table 2).

3.2 | BALF and blood biomarkers

Level of IL-5 was below the detection point in each serum sample and in almost all BALFs. Likewise, IL-4 in serum and INFγ in BALF were measurable only in a few subjects.

Concentrations of IL-10, IL-17A, and IL-23 in BALFs were above the detection point in about 15% of individuals, IL-4 in 30% of them, while IL-6 and IL-12p70 were detected in the majority of analysed BALF samples. No difference was observed between both patient subsets in BALF cytokines (Table 2).

Blood levels of IL-12p70, IL-17A, and IL-23 were above the detection point in about 30% of subjects, IL-10 in more than 60% of them, while IL-6 was detected in almost all individuals. As shown in Table 2, both asthma subsets were similar in circulating cytokine levels, except for INFγ, which was lower in subjects with fixed airflow limitation. Analyses limited to patients with cytokines above the detection point demonstrated that IL-10 was lower, while IL-23 higher in those with fixed airway obstruction (0.58 [0.42-1.07], n = 35 vs 0.77 [0.47-1.27] pg/mL, n = 32, $P = .03$ and 41.3 [21.2-74.6], n = 17 vs 19 [6.6-40.7] pg/mL, n = 18, $P = .04$, respectively). These patients were also characterized by raised serum periostin and ADAM33, correlated well to each other ($R = 0.38$, $P = .03$).

Other associations regarding blood and BALF biomarkers are provided in Appendices.

3.3 | Lung CT and airway remodelling indices

Figure 1 depicts how the airway geometry parameters were automatically measured in computer scans.

Table 3 shows results of airway CT-metrics, total lung volume, and LAA% evaluated automatically by the appropriate CT software. Subjects with fixed airflow limitation were characterized by smaller lumen diameter and area, as well as increased WAR and WTR in both distal airways, that is RB10 and LB10 (Table 3). They had also lower lumen diameter and higher WAR of RB1, albeit no difference regarding LB1 + 2 (Table 3). Fixed airflow limitation was also related to the higher LAA%.

Relationships between corresponding CT-derived airway remodelling indices as well as their associations with pulmonary function test results are presented in Appendices. Referring CT variables correlated well to each other, particularly regarding WARs and WTRs (Appendices, Table S1). On the other hand, the most potent associations between bronchial dimensions and pulmonary function parameters were documented for RB1 and RB10 (Appendices, Table S2).

TABLE 1 Demographic and clinical characteristics of the subjects studied

	Reversible bronchial obstruction n = 52	Fixed airflow limitation n = 53	*P*-value
Age, years	52 (42-59)	58 (52-65)	.002
Male gender, n (%)	11 (21)	18 (34)	.21
Height, m	1.64 ± 0.09	1.65 ± 0.1	.78
Body mass index, kg/m^2	27.8 (24.7-30.8)	26.4 (25.5-32)	.4
Past smoking, n (%)	15 (29)	17 (32)	.88
Pack-years of smoking	0 (0-4)	0 (0-7)	.46
Atopy, n (%)	24 (46)	26 (49)	.92
Living primarily in inner-city environments, n (%)	34 (65)	31 (58)	.6
Gastroesophageal reflux disease, n (%)	16 (31)	20 (38)	.58
Arterial hypertension, n (%)	18 (35)	26 (49)	.19
Diabetes mellitus, n (%)	6 (12)	11 (21)	.31
Hypercholesterolaemia, n (%)	10 (19)	15 (28)	.39
Coronary heart disease, n (%)	2 (4)	5 (9)	.45
Medications used			
Antihypertensives, n (%)	17 (33)	23 (43)	.35
Statins, n (%)	8 (15)	12 (23)	.48
Aspirin, n (%)	6 (12)	10 (19)	.21
Asthma duration, years	8 (3-18)	10 (6.5-20)	.78
Asthma severity (GINA)			
Persistent mild, n (%)	8 (15)	5 (9)	.13
Persistent moderate, n (%)	21 (40)	17 (32)	
Persistent severe, n (%)	23 (44)	31 (58)	
Asthma treatment			
Inhaled corticosteroids, n (%)	52 (100)	53 (100)	.5
Long-acting β_2-agonists, n (%)	31 (60)	38 (72)	.22
Montelukast, n (%)	9 (17)	4 (8)	.22
Theophylline, n (%)	4 (8)	9 (17)	.25
Oral corticosteroids, n (%)	8 (15)	14 (26)	.25
Asthma symptom control[a]			
Well-controlled asthma, n (%)	18 (34)	15 (28)	
Not well-controlled asthma, n (%)	17 (33)	17 (32)	
Very poorly controlled asthma, n (%)	17 (33)	21 (40)	.71
FEV_1 before bronchodilator, L	2.79 ± 0.75	1.79 ± 0.8	<.001
FEV_1 before bronchodilator, % of the predicted value	101 (93-111.8)	66.7 (54.1-80.6)	<.001
FEV_1 after bronchodilator, L	2.92 ± 0.71	2.07 ± 0.95	<.001
FEV_1 after bronchodilator, % of the predicted value	104.3 (96.5-117.2)	79.2 (62.8-87.2)	<.001
VC before bronchodilator, L	3.75 (3.17-4.13)	2.76 (2.12-3.83)	<.001
VC after bronchodilator, L	3.74 (3.13-4.17)	2.89 (2.45-4.01)	.01
FEV_1/VC (before bronchodilator)	73.47 (68-78.65)	59.05 (51.68-63.82)	<.001
FEV_1/VC (after bronchodilator)	77.14 (73.05-81.88)	65.4 (54.5-68.6)	<.001
Total lung capacity, L	5.71 (5.17-6.34)	6.04 (5.2-7.2)	.19

(Continues)

TABLE 1 (Continued)

	Reversible bronchial obstruction n = 52	Fixed airflow limitation n = 53	*P*-value
Total lung capacity, % of the predicted value	109.3 (102.4-119.8)	113.9 (101-120.6)	.42
Residual volume, L	2.02 (1.66-2.32)	2.86 (2.35-3.69)	<.001
Residual volume, % of the predicted value	114.05 (100.1-127)	151 (119.9-172.5)	<.001

Note: Categorical variables are presented as numbers (percentages), continuous variables as median and interquartile range, or mean and standard deviation, as appropriate.

Abbreviations: FEV_1, forced expiratory volume in 1 s; FEV_1/VC, forced expiratory volume in 1 s/vital capacity; GINA, Global Initiative for Asthma; L, litre; n, number; VC, vital capacity.

[a]Asthma symptom control was assessed according to the asthma control test result.

3.3.1 | Mean wall thickness

Mean wall thickness was related to pulmonary function variables, such as FEV_1 (% of predicted value) (β = −0.2 [95%CI: −0.28 to −0.12], *P* = .01), VC (β = −0.11 [95%CI: −0.16 to −0.06], *P* = .02), and residual volume (RV) (β = 0.18 [95%CI: 0.11-0.26], *P* = .01). It remained also in positive relationships with inflammatory markers, such as hsCRP (β = 0.23 [95%CI: 0.13-0.32], *P* = .02) and fibrinogen (β = 0.25 [95%CI: 0.16-0.34], *P* = .01), white blood cell count (β = 0.26 [95%CI: 0.19-0.33], *P* = .008), number of neutrophils, monocytes, and basophiles in peripheral blood (β = 0.37 [95%CI: 0.3-0.44], *P* < .001; β = 0.26 [95%CI: 0.19-0.33], *P* = .001; β = 0.17 [95%CI: 0.09-0.25], *P* = .04, respectively), as well as IgE level (β = 0.31 [95%CI: 0.22-0.4], *P* = .001) and circulating ADAM33 (β = 0.16 [95%CI: 0.09-0.24], *P* = .03). Likewise, BALF biomarkers determined mean wall thickness, including BAL eosinophilia (β = 0.24 [95%CI: 0.17-0.32], *P* = .01) and INFγ (β = 0.15 [95%CI: 0.08-0.23], *P* = .047).

A multiple regression model showed that the only positive predictor of mean WTR and WAR was blood neutrophilia (β = 0.28 [95%CI: 0.18-0.38] and (β = 0.43 [95%CI: 0.31-0.55], respectively, both *P* < .001).

3.3.2 | LAA%

As expected, LAA% was strongly associated with height (β = 0.26 [95%CI: 0.2-0.32], *P* < .001). However, it was also correlated with pulmonary function parameters, including inverse relationship with FEV_1 (% of predicted value) (β = −0.19 [95%CI: −0.28 to −0.04], *P* = .04) and FEV_1/VC (β = −0.22 [95%CI: −0.32 to −0.13], *P* = .02), as well as positive with VC (β = 0.27 [95%CI: 0.21-0.33], *P* < .001), RV (β = 0.39 [95%CI: 0.31-0.47], *P* < .001), total lung capacity (TLC) (β = 0.42 [95%CI: 0.36-0.48], *P* < .001), and total lung volume measured automatically in lung CT (β = 0.41 [95%CI: 0.35-0.47], *P* < .001). Many laboratory variables determined LAA%, including red blood cell count (RBC) (β = 0.24 [95%CI: 0.17-0.31], *P* = .01) and high density lipoproteins cholesterol (HDL) (β = 0.27 [95%CI: 0.13-0.4], *P* = .047), as well as circulating IL-12p70 (β = 0.42 [95%CI: 0.34-0.5], *P* < .001), and IL-17A (β = 0.18 [95%CI: 0.1-0.27], *P* = .048). A multiple regression model showed that LAA% was independently determined by number of neutrophils in peripheral blood (β = 0.22 [95%CI: 0.14-0.3]), haemoglobin level (β = 0.23 [95%CI: 0.15-0.31]), and wall area of RB10 (β = 0.2 [95%CI: 0.12-0.28]) (*F* = 33.8, *P* < .001, for this model).

3.4 | Histological investigation

Histology results are presented in Table 4. Both asthma subsets were similar in RBM thickness, as well as collagen I and IV deposits in bronchial biopsies (Table 4). Figure 2 shows sample images of immunohistochemistry.

Reticular basement membrane thickness was related to height (β = 0.32 [95%CI: 0.2-0.43], *P* = .03), and was higher in men compared to women (7.15 [6.7-7.9] vs. 5.98 [5.2-7.56], *P* = .02). Smoking in the past and comorbidities had no impact on this parameter, as well as atopy and place of living. Likewise, pulmonary function variables, asthma symptom score, and asthma severity did not determine RBM thickness. Surprisingly, RBM thickening remained in a positive relationship with TSH (β = 0.35 [95%CI: 0.23-0.46], *P* = .02) and HDL (β = 0.31 [95%CI: 0.2-0.43], *P* = .03), as well as in a negative with coagulation times, such as PT (β = −0.29 [95%CI: −0.44 to −0.18], *P* = .02) and aPTT (β = −0.48 [95%CI: −0.58 to −0.39], *P* < .001). Among BALF biomarkers, RBM thickness was positively related to the periostin and INFγ (β = 0.34 [95%CI: 0.24-0.45], *P* = .02 and β = 0.51 [95%CI: 0.36-0.66], *P* = .002, respectively).

A multiple regression model showed that the number of neutrophils in peripheral blood (β = 0.28 [95%CI: 0.21-0.35]) and LAA% (β = 0.22 [95%CI: 0.15-0.29] were the most important independent determinants of thicker RBM, with an unexpected very strong negative contribution of aPTT (β = −0.49 [95%CI: −0.59 to −0.42]) (*F* = 28.7, *P* < .001 for this model).

Collagen I accumulation, reflected by the percentage of stroma showing reactivity for collagen I in bronchial biopsies, was also not related to the lung function, asthma symptom score and severity, as well as atopy, and place of residence. Interestingly, its increased deposits were demonstrated in those with gastroesophageal reflux disease (40 [25-75]%, n = 35 vs 25 [17-60]%, n = 53, *P* = .002). Other comorbidities had no impact on this parameter. Collagen I deposits were related to BALF eosinophilia and periostin (β = 0.21

TABLE 2 Laboratory variables

	Reversible bronchial obstruction n = 52	Fixed airflow limitation n = 53	*P*-value
Basic laboratory tests			
Haemoglobin, g/dL	13.45 (13-14.5)	13.85 (13-14.5)	.66
Red blood cells, 10^6/μL	4.65 ± 0.41	4.69 ± 0.5	.71
White blood cells, 10^3/μL	6.22 (5.27-7.33)	7.44 (6.39-9.25)	.0005
Neutrophils, 10^3/μL	3.07 (2.7-4.1)	3.7 (2.9-4.7)	.04
Lymphocytes, 10^3/μL	1.93 (1.58-2.43)	2.2 (1.58-2.61)	.46
Monocytes, 10^3/μL	0.56 (0.48-0.74)	0.71 (0.53-0.9)	.02
Blood platelets, 10^3/μL	222.5 (193-247)	225.5 (191-265)	.73
Total cholesterol, mmol/L	4.66 ± 0.9	5.2 ± 0.95	.02
High density lipoproteins, mmol/L	1.33 (1.12-1.56)	1.34 (1.03-1.74)	.91
Triglycerides, mmol/L	1.36 (0.96-1.9)	1.5 (1.09-2.4)	.39
Glucose, mmol/L	4.9 (4.6-5.3)	5.15 (4.8-5.6)	.14
Urea, mmol/L	4.5 (3.9-5.05)	5.35 (4.4-6.3)	.009
Creatinine, μmol/L	68 (63-77)	71 (61-81)	.39
Alanine transaminase, IU/L	29 (22-39)	27 (20-42)	.5
Asthma and inflammatory biomarkers (blood)			
Eosinophilia/μL	220 (130-310)	400 (180-680)	.008
Immunoglobulin E, IU/mL	71.5 (24.2-388)	87.8 (43-511)	.51
C-reactive protein, mg/L	1.64 (0.53-8)	4.53 (0.58-9.38)	.4
Fibrinogen, g/L	3.1 (2.8-3.5)	3.45 (3.2-4.2)	.03
Periostin, ng/mL	0.28 (0.24-0.33)	0.38 (0.31-0.51)	.01
Interleukin 4, pg/mL	0.005 (0.005-0.005)	0.005 (0.005-0.005)	1
Interleukin 5, pg/mL	0.005(0.005-0.005)	0.005 (0.005-0.005)	1
Interleukin 6, pg/mL	0.75 (0.43-1.29)	1.106 (0.479-2.39)	.15
Interleukin 10, pg/mL	0.6 (0.23-1.13)	0.54 (0.35-0.9)	.87
Interleukin 12 (p70), pg/mL	0.005 (0.005-1.2)	0.005 (0.005-1.25)	.76
Interleukin 17A, pg/mL	0.005 (0.005-0.159)	0.005 (0.005-0.12)	.97
Interleukin 23, pg/mL	0.005 (0.005-15.93)	0.005 (0.005-21.2)	.69
Interferon γ, pg/mL	0.07 (0.005-0.54)	0.005 (0.005-0.05)	.02
A disintegrin and metalloproteinase domain-containing protein 33, ng/mL	0.73 (0.2-1.29)	1.32 (0.4-2.37)	.008
Asthma and inflammatory biomarkers (bronchoalveolar lavage fluid)			
Periostin, ng/mL	0.86 (0.79-0.98)	0.81 (0.72-0.95)	.35
Interleukin 4, pg/mL	0.005 (0.005-0.163)	0.005(0.005-0.279)	.92
Interleukin 5, pg/mL	0.005 (0.005-0.005)	0.005 (0.005-0.005)	.86
Interleukin 6, pg/mL	0.78 (0.35-1.12)	0.51 (0.005-1.28)	.12
Interleukin 10, pg/mL	0.005 (0.005-0.005)	0.005(0.005-0.005)	.73
Interleukin 12 (p70), pg/mL	0.09 (0.06-0.12)	0.07 (0.05-0.1)	.17
Interleukin 17A, pg/mL	0.005 (0.005-0.005)	0.005 (0.005-0.005)	.91
Interleukin 23, pg/mL	0.005 (0.005-0.005)	0.005 (0.005-0.005)	.68
Interferon γ, pg/mL	0.005 (0.005-0.005)	0.005 (0.005-0.005)	.86
Bronchoalveolar lavage cellularity			
Macrophages, %	88 (75.8-92.7)	83 (56.5-90)	.04
Lymphocytes, %	7.5 (4.7-19)	7.75 (3.5-14))	.72
Neutrophils, %	3 (2-4)	3.8 (2-11)	.07
Eosinophils, %	0.5 (0-1)	1 (0.1-3)	.02

Note: Variables are presented as median and interquartile range or mean and standard deviation, as appropriate.

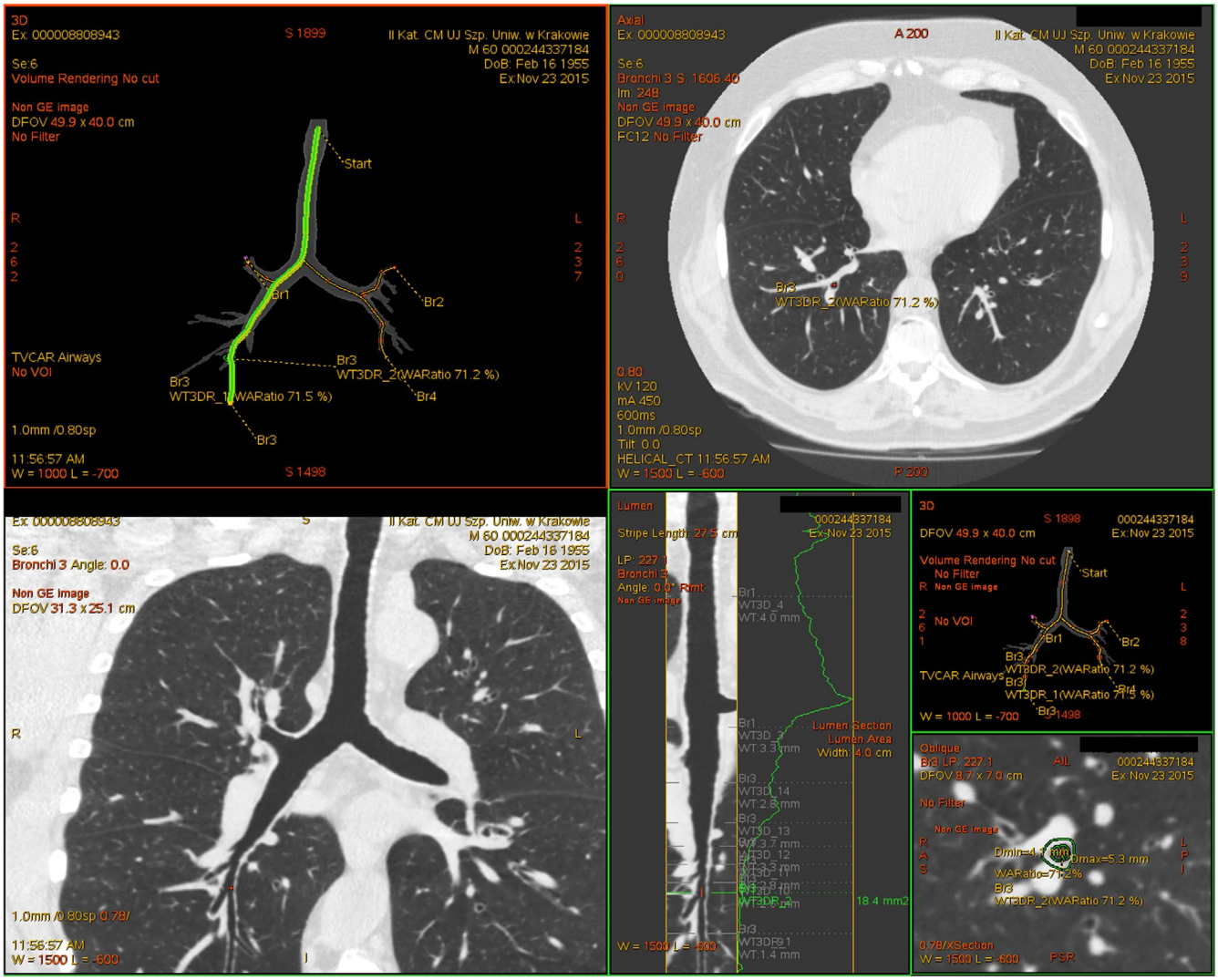

FIGURE 1 Automatic measurement of airway remodelling indices in lung computer tomography scans. On the bottom, right side is shown cross-sectional geometry of the right posterior basal bronchus (RB10)

[95%CI: 0.13-0.28], P = .04 and (β = 0.4 [95%CI: 0.35-0.66], P = .01, respectively).

A multiple linear regression model showed that BALF eosinophilia was the only positive predictor of collagen I accumulation (β = 0.26 [95%CI: 0.15-038]). Other independent determinants, including FEV_1 (β = −0.21 [95%CI: −0.34 to −0.09] and WAR RB10 (β = −0.51 [95%CI: −0.63 to −0.37], had a negative impact on that parameter (F = 5.81, P = .004 for the model).

Surprisingly, we demonstrated a weak, but significant negative association between RBM thickening and percentage of stroma showing collagen I reactivity in immunohistochemistry (β = −0.21 [95%CI: −0.31 to −0.11], P = .04) (see also Figure 2).

3.4.1 | Relation of airway CT-metrics to histological investigations

Reticular basement membrane thickness was not related to RB1 or LB1 + 2 CT-derived airway geometry indices. On the contrary, it remained in positive relationships with CT-metrics of distal airways, including airway diameter, area and wall thickness of RB10 (β = 0.25 [95%CI: 0.16-0.36], P = .01; β = 0.29 [95%CI: 0.21-0.39], P = .008; and β = 0.27 [95%CI: 0.19-0.36], P = .02, respectively) and LB10 (β = 0.23 [95%CI: 0.13-0.33], P = .049; β = 0.28 [95%CI: 0.18-0.38], P = .04; and β = 0.19 [0.09-0.29], P = .04, respectively).

Interestingly, percentage of stroma showing reactivity for collagen I in bronchial biopsies was not related to the CT-derived variables assessing bronchial wall thickness, but to those evaluating airway lumen size, including RB1 lumen diameter and area (β = 0.15 [95%CI: 0.08-0.22], P = .04 and β = 0.18 [95%CI: 0.1-0.25], P = .01; respectively), RB10 lumen diameter and area (β = 0.2 [95%CI: 0.13-0.27], P = .01 and β = 0.23 [95%CI: 0.16-0.3], P = .02), LB1 + 2 lumen diameter and area (β = 0.16 [95%CI: 0.08-0.23], P = .03 and β = 0.19 [95%CI: 0.12-0.27], P = .01), and LB10 lumen diameter (β = 0.17 [95%CI: 0.1-0.25], P = .02). Moreover, percentage of stroma showing reactivity for collagen I remained in an inverse relationship with WAR and WTR of RB10 (β = −0.27 [95%CI: −0.35 to −0.2], P = .003

TABLE 3 Lung computed tomography indices

	Reversible bronchial obstruction n = 52	Fixed airflow limitation n = 53	*P*-value	*P*-value (adjusted)
Total lung volume, L	5.07 ± 1.04	5.55 ± 1.4	.08	.16
Low-attenuation lung area (threshold level of −950 Hounsfield units), %	6.78 (2.46-18.4)	19.07 (4.26-30.65)	.04	.03
The right upper lobe apical segmental bronchus (RB1)				
Lumen diameter, mm	4.23 ± 0.95	4.11 ± 1.1	.3	.02
Airway diameter, mm	7.98 ± 1.47	8.16 ± 1.9	.8	.22
Wall thickness, mm	1.8 (1.6-2.1)	1.9 (1.7-2.2)	.26	.7
Wall thickness ratio	23.5 (21.8-24.6)	24.8(22.9-26.9)	.04	.82
Lumen area, mm^2	12.9 (10-16.5)	12.5 (9.4-16)	.5	.06
Wall area, mm^2	34.9 (26.9-46.2)	34.8 (27.2-45.4)	.7	.53
Wall area ratio	72 (68.3-74.7)	75.1 (71.3-78.7)	.003	.004
The right lower lobe basal posterior bronchus (RB10)				
Lumen diameter, mm	4.4 (3.7-4.9)	3.7 (3.3-4.5)	.02	.004
Airway diameter, mm	7.9 (7.1-8.7)	7.7 (6.6-8.5)	.25	.03
Wall thickness, mm	1.8 (1.6-1.9)	1.8 (1.6-2.1)	.46	.73
Wall thickness ratio	22.9 (20.1-24.5)	24.1 (22.9-26.4)	.003	.001
Lumen area, mm^2	14.7 (10.3-18.9)	10.8 (8.7-15.8)	.03	.006
Wall area, mm^2	34.2 (26.7-43.5)	35.7 (24.9-42)	.76	.27
Wall area ratio	71 (64-73.9)	73.4 (70.3-77.9)	.007	.002
The left apicoposterior bronchus (LB1 + 2)				
Lumen diameter, mm	5.07 ± 1.08	5.05 ± 1.16	.6	.76
Airway diameter, mm	9.1 (8-10.3)	9.7 (8.3-10.35)	.2	.82
Wall thickness, mm	2 (1.7-2.2)	2.2 (1.9-2.4)	.04	.09
Wall thickness ratio	22.4 ± 2.7	23.5 ± 2.9	.09	.19
Lumen area, mm^2	19.7 (13.3-27.3)	20.95 (13.9-27.5)	.4	.73
Wall area, mm^2	46.2 ± 13.8	51.2 ± 16.3	.23	.48
Wall area ratio	69.3 ± 5.9	71.5 ± 6.3	.09	.18
The left lower lobe basal posterior bronchus (LB10)				
Lumen diameter, mm	4.3 (3.9-5.2)	4.2 (3.5-5)	.03	.002
Airway diameter, mm	8.35 ± 1.3	8.13 ± 1.4	.07	.02
Wall thickness, mm	2 (1.7-2.1)	1.9 (1.7-2.2)	.9	.75
Wall thickness ratio	23.1 (21.7-24.7)	24.3 (22-26.2)	.05	.01
Lumen area, mm^2	14.4 (11.7-20.1)	13.7 (9.9-19.5)	.09	.03
Wall area, mm^2	37.7 (30.6-47.1)	36.8 (29.7-46.6)	.2	.16
Wall area ratio	71.1 (68-74.7)	73.8 (68.5-77.2)	.04	.01
Arithmetic mean				
Lumen diameter, mm	4.4 (3.6-4.9)	3.9 (3.43-4.6)	.24	.008
Airway diameter, mm	8.15 (7-8.9)	7.85 (6.82-8.87)	.78	.24
Wall thickness, mm	1.85 (1.7-2)	1.9 (1.65-2.05)	.27	.28
Wall thickness ratio	22.32 (19.93-23.9)	22.95 (21.37-25.65)	.08	.01
Lumen area, mm^2	15.05 (10.8-19.03)	14.23 (10.6-17.3)	.53	.005
Wall area, mm^2	37.4 (30.56-43.95)	37.65 (29.15-45.2)	.59	.74
Wall area ratio	69.4 (63.98-72.95)	70.8 (67.3-76.2)	.046	.03

Note: Variables are presented as median and interquartile range, or mean and standard deviation, as appropriate.

TABLE 4 Results of histological investigations

	Reversible bronchial obstruction, n = 43	Fixed airflow limitation, n = 45	*P*-value	*P*-value (adjusted)
Collagen I, % of the stroma showing reactivity in bronchial mucosa section	30 (20-60)	30 (20-60)	.65	.59
Collagen I—strength of the staining	2 (1-2)	2 (1-2)	.45	.76
Collagen IV—strength of the staining	1 (1-2)	1 (1-2)	.69	.7
Thickness of the reticular basement membrane of bronchial mucosa, μm	6.76 (5.32-7.9)	6.2 (5.24-7.45)	.8	.56

Note: Variables are presented as median and interquartile range.

and β = −0.19 [95%CI: −0.27 to −0.12], *P* = .02), LB10 (β = −0.2 [95%CI: −0.27 to −0.13], *P* = .02 and β = −0.21 [95%CI: −0.28 to −0.13], *P* = .01), and LB1 + 2 (β = −0.17 [95%CI: −0.25 to −0.09], *P* = .02 and β = −0.15 [95%CI: −0.22 to −0.07], *P* = .047, respectively).

3.4.2 | Cluster analysis

Cluster characteristics are shown in Table 5. The most numerous was cluster 1 (n = 59, 57%), which included subjects with the smallest lumen area and the largest WAR. In turn, cluster 3 with the largest lumen area and the lowest WAR consisted of 14 representatives (13%). Patients with intermediate values of both CT-derived parameters were included into the cluster 2 (n = 31, 30%). All clusters were similar in age, asthma duration, and BMI. Women had increased OR to be classified in cluster 1 (1.9 [95%CI: 1.25-2.9]) and lower OR to be included into cluster 2 (OR 0.56 [95%CI: 0.36-0.86]). Interestingly, patients with very poorly controlled asthma had lower OR to be classified in the cluster 1 (0.32 [95%CI: 0.19-0.55]). As shown in Table 5, clusters 1 and 2 had fixed airflow limitation, clusters 2 and 3 had higher VC and TLC, while the cluster 2 was characterized by the highest level of circulating IL-17A and ADAM-33, increased BALF eosinophilia, as well as thicker RBM and raised LAA%, as compared to both remaining clusters. In turn, cluster 3 with normal spirometry had increased collagen I accumulation in mucosa.

4 | DISCUSSION

In the present study, we have shown that patients with fixed airflow limitation are characterized by lower lumen diameter and area, together with increased WTR and WAR in airway CT-metrics. This observation is in alignment with previous reports.[4,5] However, we also demonstrated that airway geometry indices of distal (smaller) bronchi correlate better with histological changes and lung function abnormalities than those obtained from proximal (larger) bronchi. This is an interesting finding that has not been described so far.

The wall thickening and luminal narrowing tend to be considered interchangeably in the literature.[4,5] Recently, however, Choi S et al[5] has demonstrated that separation of both these terms helps identify unique characteristics of four clusters in severe asthma patients. Indeed, that approach allowed us to identify individual clusters, comparable in age, asthma duration, and BMI, but different in inflammation, RBM thickening, and collagen I accumulation. Recent studies of large-scale focus on molecular, clinical, and genetic characteristics of asthma patients, defining specific phenotypes of this disease.[15] That clustering, however, triggers general inflammatory process and is different than proposed by us. We focused on airway structural changes identified in lung CT-metrics and histological investigations expanding our knowledge on asthma characteristics, this time with respect to airway remodelling.

Spirometric variables were related to the mean airway wall thickness measured in CT scans. Both of them were associated with classical T helper cell type 2 (Th_2) biomarkers, including IgE, blood and BAL eosinophilia and periostin,[15,16] but remained also in relationships with other laboratory variables, not directly related to the Th_2 pattern, such as white blood cell count, blood neutrophilia, circulating and BAL INFγ, hsCRP, fibrinogen, and serum ADAM33, reflecting complex inflammatory protein interactions in asthma remodelling. Particularly, a role of ADAM33 in that context deserves a comment. It has been demonstrated that this protein with a complex structure and function is involved in lung injury and repair.[17] Moreover, its gene polymorphism may be associated with decrease in FEV_1 in healthy population, but also in asthmatics.[18,19] Therefore, it seems that ADAM33 might be even a potential therapeutic target in asthma remodelling.

Surprisingly, RBM thickening and collagen I accumulation were not directly related to lung function abnormalities. However, important relationships were demonstrated between them and CT-derived indices, such as airway lumen area (for collagen I), airway wall thickening, and LAA% (for RBM). Again, many laboratory variables determined RBM thickening, including BALF periostin and INFγ, blood neutrophilia, HDL, and shortened aPTT.[16] One possible explanation of that variability is that all those laboratory parameters are independently driven by low-grade systemic inflammation,[20,21] or they might reflect asthma phenotype heterogeneity.[15] Neutrophilic asthma is related to the more severe disease, and thus, higher neutrophilia may influence airway remodelling.[15] In turn, HDL might increase inflammation by its protein cargo, including serum amyloid A1, a major apolipoprotein in HDL during inflammation.[22] Interestingly, recent large epidemiological study has demonstrated that higher HDL is associated with lower FEV_1/FVC ratio and greater percent of

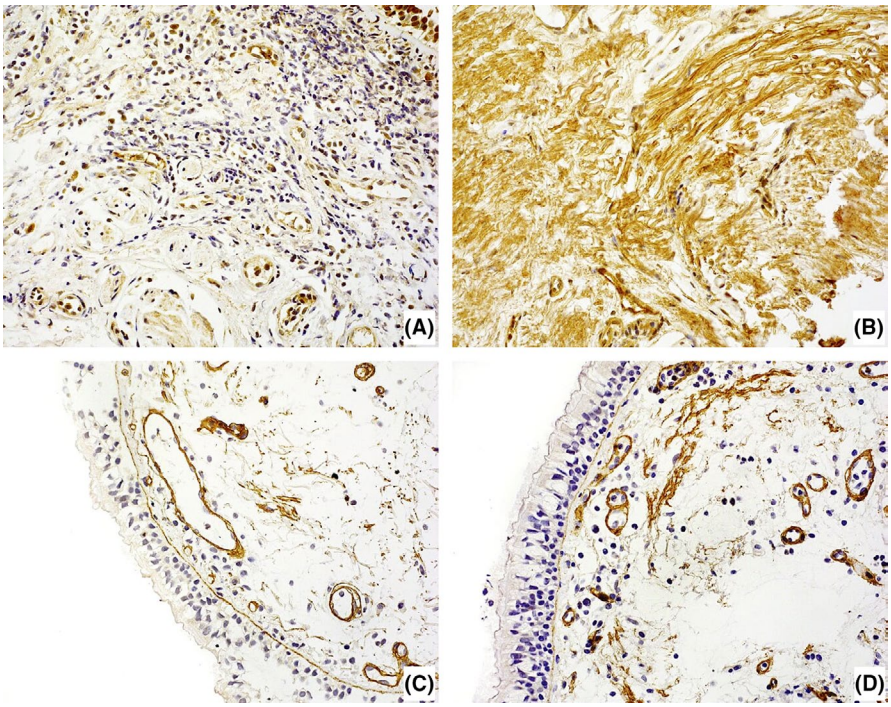

FIGURE 2 Immunochemistry of bronchial biopsies, original magnification 400×. A, Weak reaction to collagen I. B, Strong reaction to collagen I in lamina propria. C, Reaction to collagen IV of the surface epithelial basement membrane is weak, while vascular basement membranes are strongly immunopositive. D, All the basement membranes are strongly positive

emphysema in COPD.[23] Our report lines this observation, indicating HDL as a potent determinant of RBM thickening and higher LAA%. On the other hand, a negative association between RBM thickening and aPTT is an unexpected and intriguing finding. Shortened aPTT may lead to enhanced thrombin generation and increased thromboembolism risk.[24] Of note, recently, we have shown that asthma is related to the prothrombotic blood alteration ranging from enhanced thrombin generation to impaired fibrinolysis.[20] Moreover, those unfavourable abnormalities were associated with inflammation,[21] endothelial dysfunction,[25,26] and increased risk of severe exacerbation.[27] In the current study, we have expanded knowledge on that topic, demonstrating that shortened aPTT, likely reflecting prothrombotic tendency in asthmatics, is also related to the RBM thickening. The exact mechanism of that association remains unknown; however, aPTT as a global test is influenced by the combined levels and activity of many clotting factors. Some of them (eg fibrinogen and factor VIII) are increased in inflammation,[20] which may shorten aPTT and, at the same time influence airway remodelling.

Another interesting finding of our study refers to the positive relationship between RBM thickness and TSH. Thyroid hormones increase the basal metabolic rate affecting protein synthesis.[28] Therefore, a trend towards lower thyroid hormones and higher TSH might alter protein metabolism thickening the RBM, even if TSH is normal, like in our data.

Increased collagen I in bronchial mucosa indicates fibrosis.[29] Collagen I accumulation in our study was determined by BALF eosinophilia and periostin, both Th_2 biomarkers. However, a negative relationship between collagen I deposit and RBM thickening is an unexpected and novel finding, which deserves a comment. Previously, Saglani et al[30] have studied ultrastructure of the RBM in asthmatics and have demonstrated that although indeed, asthma is characterized by thicker RBM, the collagen fibres in its structure are thinner. Thus, their results line our observation, at least to some extent. Of note, in our study RBM thickness remained in a positive, while collagen I in a negative relationship with CT-derived airway wall thickness. On the other hand, collagen I accumulation was positively related to the airway lumen areas. These observations allow us to hypothesize that airway remodelling may proceed as a predominant RBM thickening, related to the thicker bronchial wall, or prevalent collagens accumulation leading to the impaired airway wall compliance, although large observational studies are needed to verify this hypothesis.

According to our knowledge, relationships between LAA% and asthma remodelling have not been comprehensively studied yet. LAA% was potently related to pulmonary function variables and RB10 WAR, but also to the numerous laboratory factors, including blood neutrophilia, RBC, serum HDL, IL-12p70, and IL-17A. Thus, that simple parameter, calculated automatically by the majority of CT software,[10] may be useful in asthma research.

The last issue that merits a comment in relation to our study is asthma-COPD overlap (ACO). At present, there is no clear definition of ACO, but only a clinical description. Some of our patients with fixed airflow limitation obviously met the ACO criteria, even if they had asthma at the very beginning. The main point of our study, however, was to characterize asthmatics, who after several years of asthma duration develop airway remodelling. Therefore, we believe that our study expands the knowledge also on that controversial topic.

Currently, there is no effective cure for airway remodelling in asthma, although reports on biological therapy bring an emerging hope.[31-34] The identification of simple and non-invasive imaging or laboratory biomarkers, however, is critical for its assessment.

TABLE 5 Characteristics of three different clusters obtained based on computed tomography-derived airway cross-sectional geometry of the right lower lobe basal posterior bronchus (RB10)

	Cluster 1 n = 59 (57%)	Cluster 2 n = 31 (30%)	Cluster 3 n = 14 (13%)	Cluster 1 vs 2 vs 3 *P*-value	Cluster 1 vs 2 *P*-value	Cluster 1 vs 3 *P*-value	Cluster 2 vs 3 *P*-value
Lumen area (RB10), mm^2	9.7 (8.84-10.56)	17.38 (16.18-18.56)	28.68 (24.1-33.26)	<.001	<.001	<.001	<.001
Wall area ratio (RB10)	75.29 (74.21-76.37	69.09 (67.67-70.48)	61.86 (60.59-63.13)	<.001	<.001	<.001	<.001
Male gender, number (%)	11 (19%)	14 (45%)	4 (29%)	.008	.02	.6	.5
FEV_1 before bronchodilator, % of the predicted value	81.74 (77.61-85.87)	79.48 (74.23-84.73)	104.06 (95.65-112.47)	<.001	.61	<.001	<.001
FEV_1 after bronchodilator, % of the predicted value	90.28 (86.49-94.06)	90.06 (84.74-95.37)	107.84 (99.11-116.56)	.007	.99	.003	.004
FEF50, % of the predicted value	46.76 (41.69-51.82)	45.92 (39.88-51.95)	72.6 (57-68-87.51)	<.001	.98	.002	.002
VC before bronchodilator, L	3.15 (3-3.3)	3.63 (3.4-3.86)	4 (3.57-4.42)	<.001	<.001	<.001	.08
VC before bronchodilator, % of the predicted value	99.98 (95.9-104.07)	100.29 (96.88-103.7)	113.35 (106-120.68)	.004	.99	.03	.04
VC after bronchodilator, L	3.28 (3.13-3.44)	3.8 (3.53-4.07)	3.98 (3.57-4.39)	.01	<.001	<.001	.51
Total lung capacity, L	4.7 (4.55-4.85)	5.21 (4.89-5.53)	5.5 (5.07-5.92)	.01	<.001	<.001	.23
Low-attenuation lung area (threshold level of −950 Hounsfield units), %	11.43 (9.35-13.52)	24.38 (19.81-28.95)	12.57 (7.22-17.92)	<.001	<.001	.93	<.001
Interleukin 17A, pg/mL (blood)	0.13 (0.07-0.18)	0.7 (0.2-1.21)	0.1 (0.02-0.19)	.016	.01	.99	.13
Interferon γ, pg/mL (blood)	0.68 (0.39-0.96)	0.09 (0.04-0.14)	1.68 (0.46-2.91)	<.001	.1	.06	<.001
A disintegrin and metalloproteinase domain-containing protein 33 (ADAM33), ng/mL (blood)	1.28 (1.07-1.5)	1.57 (1.24-1.9)	0.97 (0.54-1.4)	.01	.24	.21	.01
Periostin, ng/mL (blood)	0.33 (0.31-0.35)	0.34 (0.31-0.38)	0.22 (0.17-0.26)	<.001	.83	<.001	<.001
Periostin, ng/mL (BAL)	0.79 (0.77-0.81)	0.87 (0.83-0.91)	0.87 (0.83-0.91)	<.001	<.001	<.001	.99
Eosinophils in BAL (% of inflammatory cells)	1.06 (0.77-1.35	2.38 (1.43-3.32)	0.54 (0.35-0.74)	<.001	.006	.75	.003
Collagen I, % of the stroma showing reactivity in bronchial biopsies	33.6 (29.58-37.63)	42.29 (36.35-48.24)	51.5 (38.48-64.52)	.001	.1	.02	.34
Reticular basement membrane thickness in bronchial biopsies, μm	6.22 (5.94-6.49)	7.17 (6.51-7.79)	6.9 (6.31-7.48)	.005	.01	.32	.85

Note: Data are presented as mean and 95% confidence interval (CI) of the mean.

Abbreviations: BAL, bronchoalveolar lavage; FEF50%, 50% forced expiratory flow of forced vital capacity, for other abbreviations see Table 1; RB10, the right lower lobe basal posterior bronchus.

4.1 | Study Limitations

Our patients were relatively old and most of them were moderate to severe asthmatics. Subjects with fixed airflow limitation were older, although similar in asthma duration and severity. The biopsies were embedded and cut in a non-random fashion, as application of more sophisticated sampling methods would not be practical in small specimens. This may lead to less accurate and less unbiased assessment of RBM thickness, as it has been previously shown by Ferrando et al.[14] We did not analyse other histological airway remodelling traits, including smooth muscle layer, neovascularization, or other matrix components. We measured each laboratory variable at a single time-point, and thus, we cannot exclude their changes in time. Statistical associations reported here may not necessarily indicate cause-effect relationships. Particularly cluster analysis with a low number of individuals needs to be interpreted with caution. Finally, the clinical relevance of demonstrated associations requires further investigations.

5 | CONCLUSIONS

Fixed airflow limitation and histological changes of airway remodelling correlate better with CT-metrics of distal than proximal airways.

The CT low-attenuation lung area index might be useful in assessment of lung air trapping/ hyperinflation in asthmatics.

Airway remodelling is related to classical Th_2 profile biomarkers, including blood and airway eosinophilia and periostin, but also to the blood neutrophilia, serum ADAM33 and other inflammatory biomarkers, indicating complex regulation of this process.

Although large observational studies are needed to verify this hypothesis, it seems that airway remodelling in asthma may proceed as prevailing RBM thickening or dominant collagen I accumulation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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