

Non-asthmatic eosinophilic bronchitis is characterized by proximal airway eosinophilic inflammation as compared with classic asthma and cough variant asthma

To the Editor,

Non-asthmatic eosinophilic bronchitis (NAEB) is a common cause of chronic cough.¹ NAEB involves eosinophilic airway inflammation, which manifests as sputum eosinophilia similar to that in cough variant asthma (CVA), in which cough presents as the sole manifestation, or in classic asthma (CA) which is characterized by wheezing, dyspnoea and cough.¹ However, NAEB lacks the airway hyperresponsiveness associated with asthma. The mechanism underlying these different clinical features remains unclear. A few studies have investigated pathological changes in the airways of patients with NAEB.² A higher degree of mast cell infiltration was showed in the airway smooth muscle of asthmatic patients than that of NAEB,³ indicating that the site and degree of inflammatory cell infiltration may differ between NAEB and asthma. However, biopsies in previous studies were taken at a single site within the airway, and this may have prevented a full view of the differences in both the proximal airway and the periphery airway. Whether the degree of inflammation at different sites within the airway was different between NAEB and asthma remains unknown.

In this study, 24 patients with NAEB, 20 patients with CVA, 20 patients with CA and 8 healthy control (HC) subjects were recruited from the First Affiliated Hospital of Guangzhou Medical University. NAEB was preliminarily diagnosed when patients have chronic cough (lasting ≥ 8 weeks) as the sole symptom, sputum eosinophil count $\geq 2.5\%$, without airflow obstruction and airway hyperresponsiveness. CVA was initially diagnosed if patients present chronic cough as the sole symptom and evidence of airway hyperresponsiveness. A diagnosis of CA was made based on variable respiratory symptoms such as wheeze, shortness of breath, chest tightness and/or cough, and evidence of airway hyperresponsiveness. All of subjects were non-smokers, and none of them were taking corticosteroids or experiencing a respiratory tract infection within the previous 4 weeks. Each subject took two visits. During the first visit, demographic and clinical data were recorded. Serum total IgE, skin-prick tests to common allergens, spirometry, bronchial provocation tests and induced sputum tests for differential cell counts were performed. During the second visit, fiberoptic bronchoscopy was conducted with bronchoalveolar lavage fluid (BALF) collected. Bronchial mucosal biopsies of the carina of trachea, the left superior and inferior lingular segment carinae and the anterior basal segment of the right inferior lobe were

obtained from each patient. The final diagnosis of patients was confirmed if they respond to etiological treatment.

Each group of patients had participants of similar age and gender ratio (Table S1). There were no significant differences in the duration of disease, serum IgE, the proportion of atopy and the blood eosinophil counts between NAEB, CVA and CA, while subject with CA or CVA showed significant declines in FEV₁% and MMEF% relative to NAEB and HC.

Sputum eosinophil counts of NAEB ($13.9\% \pm 13.0\%$), CVA ($19.9\% \pm 17.5\%$) and CA ($35.8\% \pm 20.1\%$) were significantly higher than that of HC ($0.4\% \pm 0.3\%$, all $p < .05$), while the BALF eosinophil counts of NAEB ($2.8\% \pm 4.5\%$) were comparable to that of HC ($0.5\% \pm 0.5\%$) and were significantly lower than that of CVA ($14.0\% \pm 20.4\%$, $p < .05$) and CA ($11.7\% \pm 9.9\%$, $p < .05$).

The density of submucosal infiltrating eosinophils, mast cells and lymphocytes at the left lingular segment was significantly higher in the CA, CVA and NAEB than in the HC (Table 1, Figure S1). The density of eosinophils and lymphocytes at the inferior lobe submucosa of NAEB [0.0 (0.0 – 18.1) cells/mm², 145.5 (101.2 – 261.0) cells/mm²] was significantly lower than that in CVA [16.9 (0.0 – 136.3) cells/mm², 280.4 (182.1 – 320.4) cells/mm²] and CA [24.1 (1.6 – 78.4) cells/mm², 262.2 (147.0 – 371.9) cells/mm²]. No significant differences in the density of mast cells at the submucosa of carina, lingular segment or inferior lobe were found among NAEB, CVA and CA, while mast cell infiltration in the smooth muscle layer of the inferior lobe was more severe in CA than that in NAEB [51.4 (29.3 – 71.7) cells/mm² vs. 0 (0 – 21.2) cells/mm², $p < .05$]. (Figure 1, Figure S2). Regarding longitudinal comparison in NAEB, the density of infiltrating mast cells and lymphocytes at both the carina and lingular submucosa was significantly higher than that at the inferior lobe submucosa (Table 1).

Compared with CA or CVA, NAEB showed thinner basement membrane at the lingular segment [$(6.5 \pm 2.2 \mu\text{m}$ or $6.0 \pm 1.5 \mu\text{m})$ vs. $4.0 \pm 1.3 \mu\text{m}$, both $p < .05$] and at the inferior lobe [$(6.7 \pm 2.7 \mu\text{m}$ or $6.3 \pm 2.0 \mu\text{m})$ vs. $3.7 \pm 1.3 \mu\text{m}$, both $p < .05$]. Within the NAEB group, the basement membrane thickness at the carina of the trachea ($5.2 \pm 1.7 \mu\text{m}$) was significantly larger than that at the lingular segment and at the inferior lobe. There were no significant differences in the basement membrane thickness or the density of infiltrating eosinophils, mast cells or lymphocytes in the three parts of the airway either within or between the CA and CVA groups. (Table 1, Figure S3).

Eosinophil counts in sputum, BALF and submucosa were modest but significantly positively correlated with submucosal mast cell counts and basement membrane thickness (Figure S4). A significant positive correlation was also found between submucosal mast cell counts and basement membrane thickness. Negative correlations were observed between spirometry indices (FEV₁% predicted, MMEF% predicted) and the basement membrane thickness, the eosinophils in sputum, BALF and submucosa.

NAEB was generally diagnosed based on eosinophilia in induced sputum which reflected eosinophilia in the central airway. The inflammation in peripheral airways and alveolar airspace could well be justified by BALF. In our data, the BALF eosinophil counts in NAEB were significantly lower than that in CVA and CA, despite similar sputum eosinophil counts between NAEB and CVA, suggesting that the eosinophilic inflammation in the peripheral airways of NAEB seemed milder. In contrast, there was no significant difference in the BALF eosinophil counts between the NAEB and asthmatic patients in previous reports.² It is not clear what caused these conflicting results, which may be related to the different population selected or environmental factors. Our previous study found that traffic-related air pollution could induce non-allergic eosinophilic inflammation of airways.⁴

Like asthma, NAEB was shown to be co-involved with multiple inflammatory cells in the bronchial tissues, including eosinophils, mast cells and lymphocytes. It is well known that inflammation affects both proximal and peripheral airways in asthmatics. In this study, no significant difference was observed in the inflammatory cell infiltration in the airway submucosa between the carina of the trachea and the lingual segment in NAEB, while the inflammatory cell density was significantly decreased in the right inferior lobe submucosa, suggesting that the degree of airway inflammation in NAEB gradually decreased from the main bronchus to the peripheral airway. Mast cells could cause airway obstruction, bronchial

Key Messages

1. Non-asthmatic eosinophilic bronchitis and asthma have similar degree of eosinophilic inflammation in proximal airway.
2. Inflammation gradually decreased from the main bronchus to the peripheral airway in non-asthmatic eosinophilic bronchitis.
3. The milder peripheral airway eosinophilic inflammation and airway remodelling were showed in non-asthmatic eosinophilic bronchitis.

mucus production and airway mucosal edema.⁵ More mast cells were observed in the distal airways of asthmatic patients than in non-asthmatic controls.⁶ Mast cells were found at greater density within the smooth muscles and mucous glands in the distal airways. Our data showed that mast cells in the bundles of airway smooth muscles of the right inferior lobes of asthmatics were substantially more numerous than that in NAEB. These findings were consistent with a previous report from Brightling et al.³ However, no significant difference in the density of mast cells was found among NAEB, CVA or CA, in either the airway submucosa or the smooth muscle layer in the tracheal carina and the lingular segment. Significant positive correlations between airway mast cells and airway eosinophils were found in our data, which conformed to the concept of "allergic effector" unit formed by mast cells and eosinophils.⁷ The bidirectional interactions between mast cells and eosinophils play an important role in the pathogenesis of asthma. We found that the density of mast cells and eosinophils in the airway submucosa and the smooth muscle layer in the inferior lobe to be lowest in NAEB among these

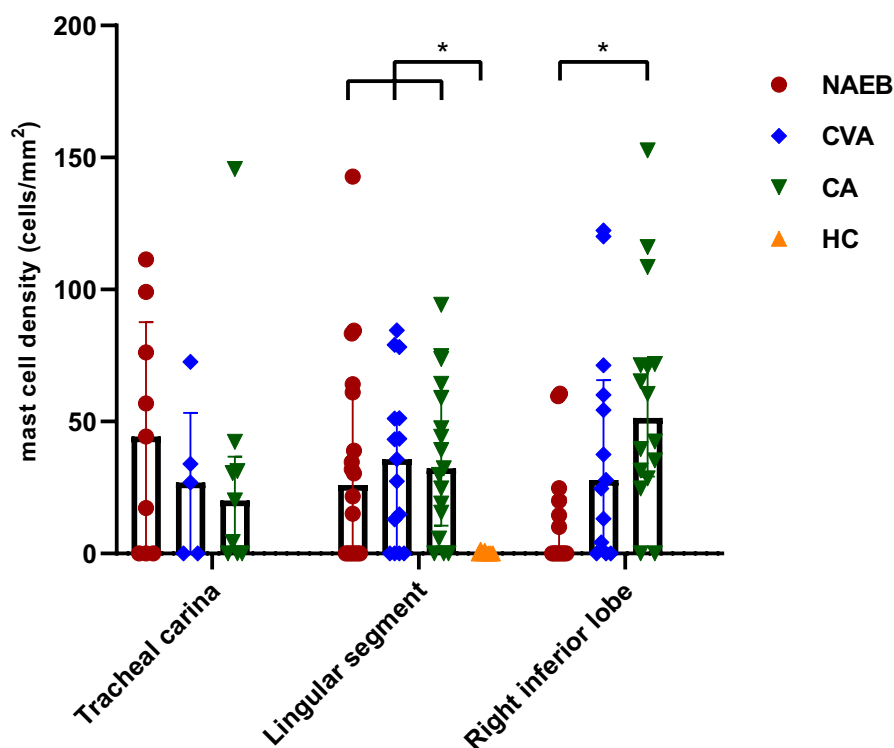
TABLE 1 Inflammatory cells in submucosa of carina, lingular segment and inferior lobe

	HC	NAEB	CVA	CA
Eosinophil				
Carina	NA	15.2 (1.8–44.1)a	65.5 (0.0–277.0)b	27.7 (8.9–80.0)a,b
Lingular segment	0.0 (0.0–1.9)a	16.0 (3.3–55.3)b	28.2 (4.0–80.9)b,c	80.3 (3.3–148.0)c
Inferior lobe	NA	0.0 (0.0–18.1)a	16.9 (0.0–136.3)b	24.1 (1.6–78.4)b
Mast Cell				
Carina	NA	45.0 (36.8–54.7)a*	38.5 (22.1–55.5)a	31.8 (21.6–43.4)a
Lingular segment	6.2 (4.1–9.2)a	43.5 (22.2–50.5)b*	32.4 (12.7–63.3)b	52.7 (33.9–70.5)b
Inferior lobe	NA	24.0 (13.3–41.3)a	35.3 (20.2–70.9)a	38.4 (22.1–58.0)a
Lymphocyte				
Carina	NA	285.4 (148.4–519.8)a*	256.8 (193.7–452.2)a	216.0 (172.5–317.0)a
Lingular segment	97.1 (75.7–143.7)a	269.8 (169.2–615.3)b*	288.3 (185.0–520.6)b	232.6 (163.8–374.0)b
Inferior lobe	NA	145.5 (101.2–261.0)a	280.4 (182.1–320.4)b	262.2 (147.0–371.9)b

Note: Data are presented as median (interquartile range) cells/mm². NA, not available; each alphabet denotes a subset of different disease categories whose column data do not differ significantly from each other at the .05 level.

*Compared with the anterior basal segment of right inferior lobe in the same disease categories, $p < .05$.

FIGURE 1 Mast cell counts (cells/mm²) in smooth muscle layer of biopsies taken from different sites of airway. Values are median (interquartile range). *refers to $p < .05$. CA, classic asthma; CVA, cough variant asthma; HC, healthy controls; NAEB, non-asthmatic eosinophilic bronchitis



diseases. Milder peripheral airway inflammation may account for the absence of bronchial hyperresponsiveness in NAEB.

NAEB showed a lower level of fibrin and basement membrane thickness at the lingular segment and at the inferior lobe than asthma. In addition, the basement membrane thickness was significantly correlated with the level of eosinophil counts in sputum, BALF and the bronchial submucosa, indicating that the thickness of basement membrane was likely to be related to eosinophilic inflammation. Airway remodelling in asthmatics is associated with airway eosinophilia.⁸ Activated eosinophils disrupt airway epithelium by synthesizing and releasing toxic proteins such as major basic proteins, eosinophil cationic proteins and neurotoxin. Activated eosinophils could also secrete TGF- β 1 and PDGF to promote fibroblast migration, proliferation and phenotypic differentiation, which play an important role in the process of tissue remodelling after injury.⁸ Imbalance between damage and repair, especially excess repair, could lead to remodelling of the airway structure, which is a risk factor of bronchial hyperresponsiveness.⁹

It is difficult to collect specimens from small airways through fiberoptic bronchoscopy. In the strictest sense, the three sampling sites of our data were all from the proximal airway rather than the peripheral airway. However, the trend of inflammation in the proximal and peripheral airways could be extrapolated from gradual and in-depth local changes. In addition, the different inflammatory cell patterns in BALF and sputum also indicated the differences between peripheral airway and proximal airway inflammation to some extent.

In conclusion, NAEB showed a degree of inflammation of the proximal airway similar to that of CVA and CA, while the degree of inflammatory cell infiltration and basement membrane thickness in the peripheral airway of NAEB gradually decreased. The milder degree of eosinophilic inflammation and the less marked airway

remodelling of the peripheral airway may account for the absence of bronchial hyperresponsiveness of NAEB.

KEYWORDS

airway inflammation, asthma, cough variant asthma, non-asthmatic eosinophilic bronchitis, peripheral airway

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors participated in manuscript writing and editing. Conception and design: Kefang Lai. Recruiting subjects and acquisition of data: Qingling Zhang, Wei Luo, Wenzhi Zhan, Jiaxing Xie, Hui Wang. Immunohistochemistry, image acquisition and processing: Qingling Zhang, Wei Luo, Wenzhi Zhan; Analysis and interpretation: Qingling Zhang, Wei Luo, Wenzhi Zhan. Critical review and editing: Kefang Lai, Qingling Zhang, Wei Luo, Wenzhi Zhan, Kian Fan Chung. All authors read and approved the final manuscript.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (no: IRB 02015027) and written informed consent was obtained from all subjects.

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DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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REFERENCES

1. Lai K, Chen R, Peng W, Zhan W. Non-asthmatic eosinophilic bronchitis and its relationship with asthma. *Pulm Pharmacol Ther.* 2017;47:66-71.
2. Brightling CE, Symon FA, Birring SS, Bradding P, Wardlaw AJ, Pavord ID. Comparison of airway immunopathology of eosinophilic bronchitis and asthma. *Thorax.* 2003;58:528-532.
3. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med.* 2002;346:1699-1705.
4. Fang Z, Huang C, Zhang JJ, et al. Traffic-related air pollution induces non-allergic eosinophilic airway inflammation and cough hypersensitivity in guinea-pigs. *Clin Exp Allergy.* 2019;49:366-377.
5. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med.* 2012;18:693-704.
6. Carroll NG, Mutavdzic S, James AL. Distribution and degranulation of airway mast cells in normal and asthmatic subjects. *Eur Respir J.* 2002;19:879-885.
7. Galdiero MR, Varricchi G, Seaf M, Marone G, Levi-Schaffer F, Marone G. Bidirectional mast cell-eosinophil interactions in inflammatory disorders and cancer. *Front Med (Lausanne).* 2017;4:103.
8. Kuo CS, Pavlidis S, Zhu J, et al. Contribution of airway eosinophils in airway wall remodeling in asthma: role of MMP-10 and MET. *Allergy.* 2019;74:1102-1112.
9. Bergeron C, Al-Ramli W, Hamid Q. Remodeling in asthma. *Proc Am Thorac Soc.* 2009;6:301-305.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

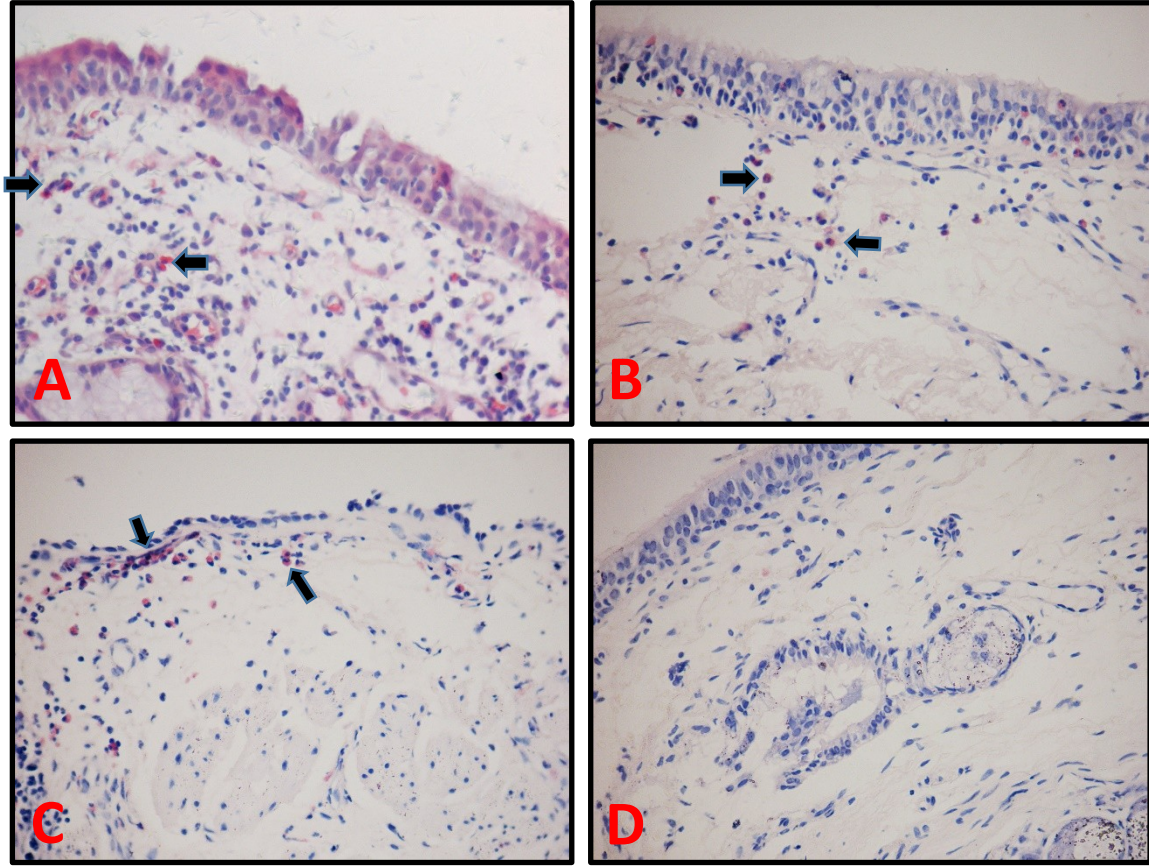


Figure S1 Pathological pictures of airway mucosa from the left superior and inferior lingular segment carinae in four groups (A-D). Hematoxylin eosin stain (200X). A: classic asthma, B: cough variant asthma, C: non-asthmatic eosinophilic bronchitis, D: healthy control. Eosinophils (black arrows).

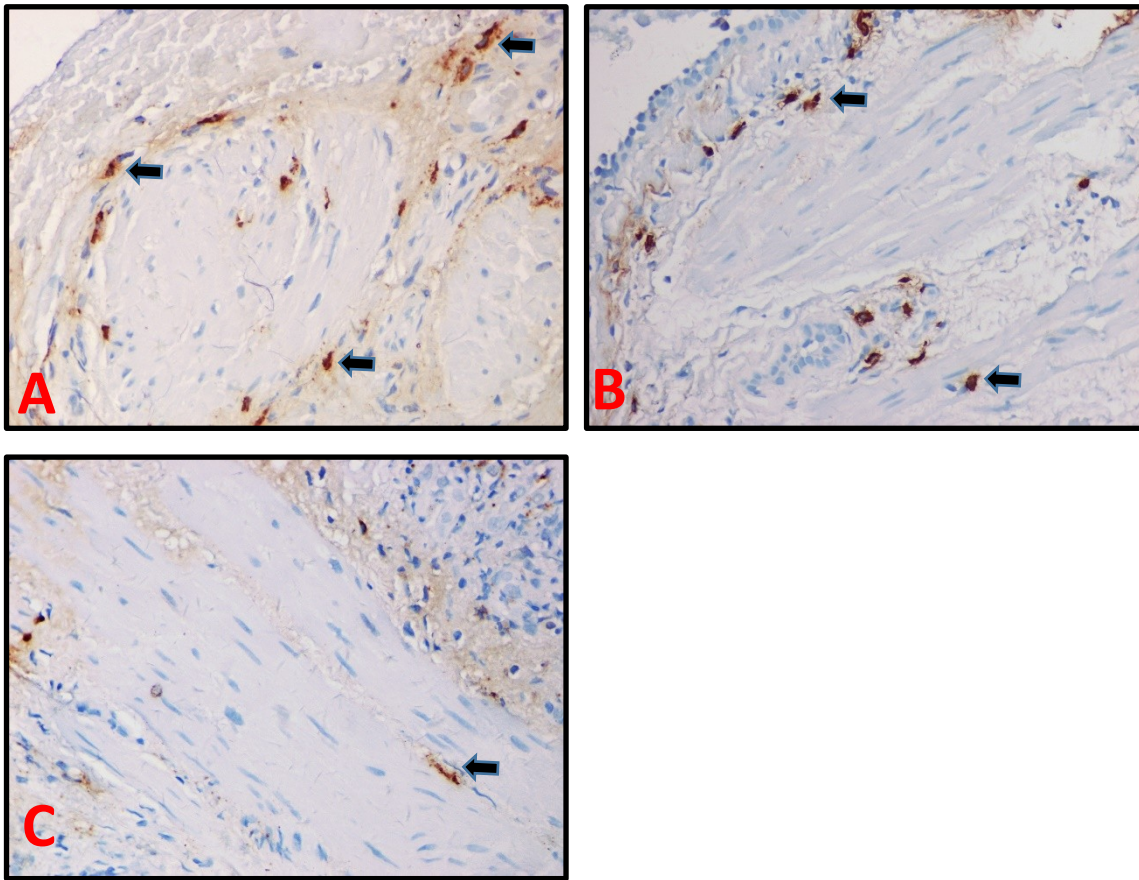


Figure S2. Mast cell in smooth muscle layer of biopsies taken from the anterior basal segment of the right inferior lobe (A-C). AA1(400X). A: classic asthma, B: cough variant asthma, C: non-asthmatic eosinophilic bronchitis. Mast cells (black arrows).

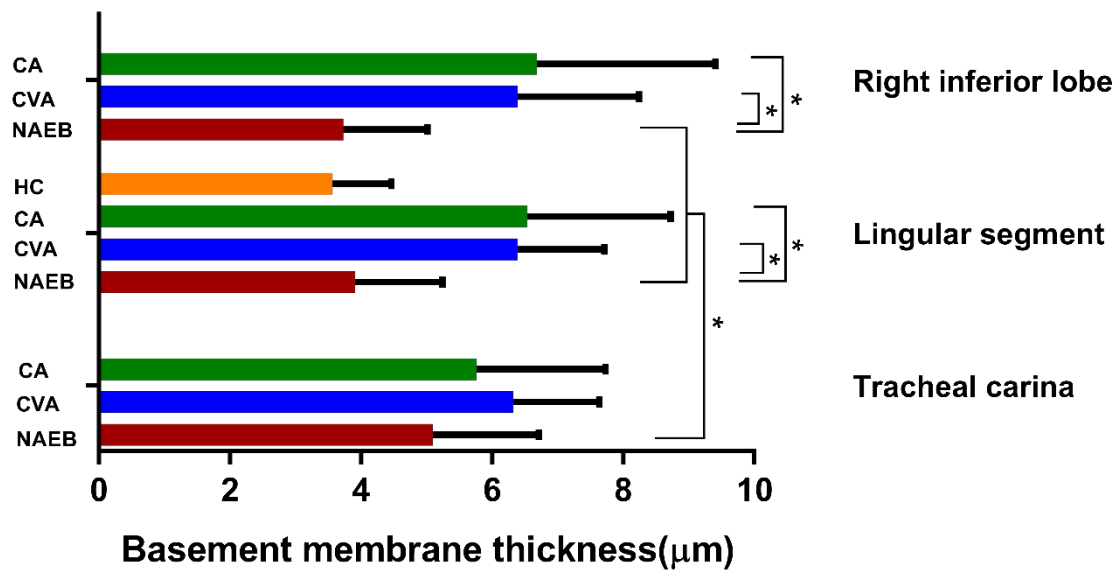


Figure S3. Basement membrane thickness (μm) measured in biopsies taken at the 3 different airway sites. Values are means $\pm$ (standard deviation); * refers to $P < 0.05$. NAEB: non-asthmatic eosinophilic bronchitis; CVA: cough variant asthma; CA: classic asthma; HC: healthy controls.

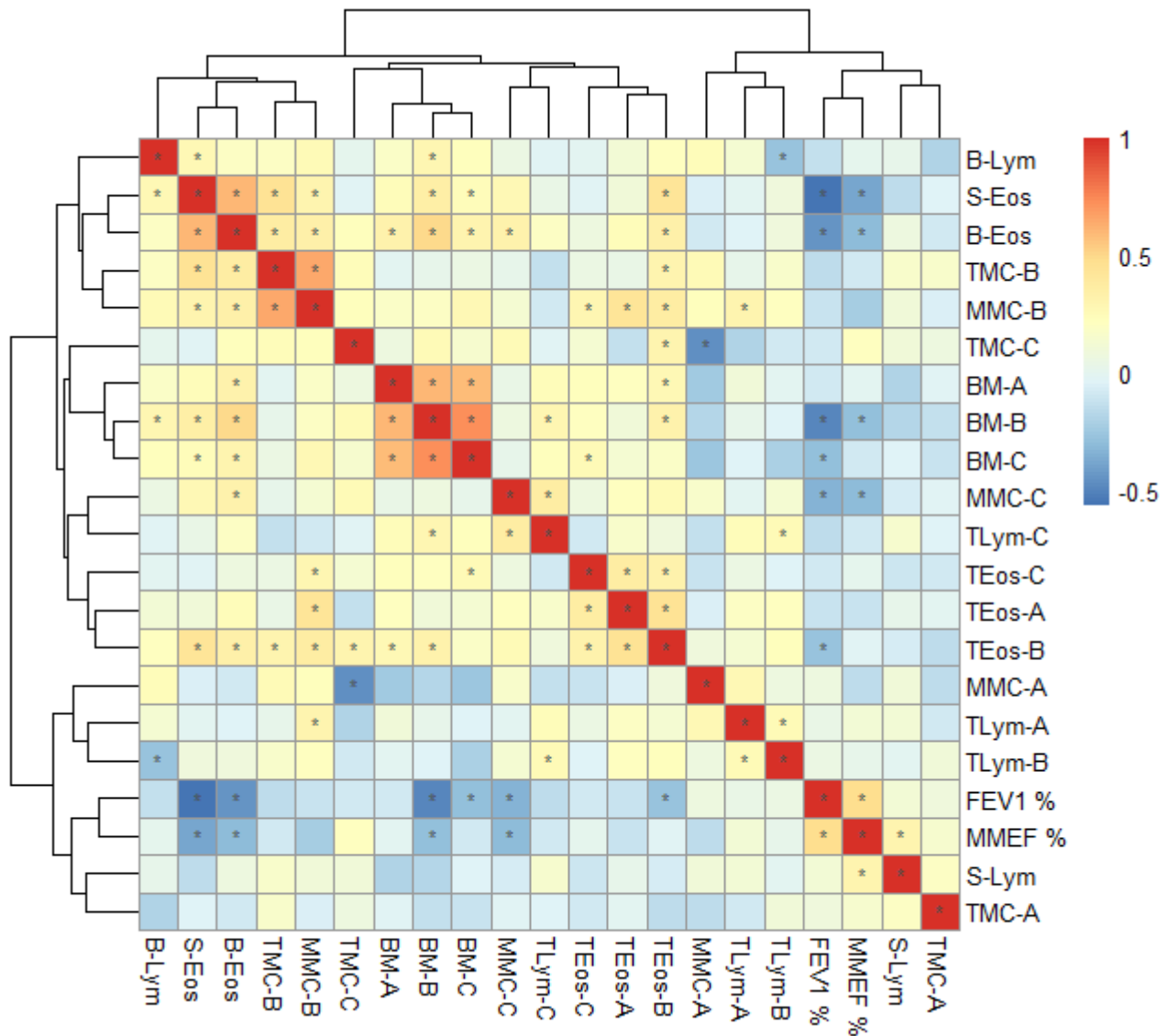


Figure S4. Correlation among clinical data, indices of inflammatory infiltration and mucosal remodeling in all subjects. The color depth represents correlation coefficient. The deeper color means the higher correlation coefficient; red color means positive correlation while blue color means negative correlation. * refers to P<0.05. B-Lym: BALF lymphocyte; S-Eos: sputum eosinophil; B-Eos: BALF eosinophil; TMC-B: submucosal infiltrating mast cell at lingular segment; MMC-B: smooth muscle layer infiltrating mast cell at lingular segment. TMC-C: submucosal infiltrating mast cell at right inferior lobe; BM-A: basement membrane at carina; BM-B: basement membrane at lingular segment; BM-C: basement membrane at right inferior lobe; MMC-C: smooth muscle layer infiltrating mast cell at right inferior lobe; TLym-C: submucosal infiltrating lymphocyte at right

inferior lobe; TEos-C: submucosal infiltrating eosinophil at right inferior lobe; TEos-A: submucosal infiltrating eosinophil at carina; TEos-B: submucosal infiltrating eosinophil at lingular segment; MMC-A: smooth muscle layer infiltrating mast cell at carina; TLym-A: submucosal infiltrating lymphocyte at carina; TLym-B: submucosal infiltrating lymphocyte at lingular segment; FEV1%: Forced expiratory volume in one second, % predicted; MMEF%: maximal mid-expiratory flow, % predicted; S-Lym: sputum lymphocyte; TMC-A: submucosal infiltrating mast cell at carina.

Table S1. Demographic and clinical data

	HC	NAEB	CVA	CA	<i>P</i> value
Number of subjects	8	24	20	20	
Age (year)	41.6±10.2	34.0±9.9	41.2±10.5	37.9±11.2	0.152
Male	3 (60.0%)	17 (70.8%)	7 (35%)	11 (55.0%)	0.091
Duration (months)	N/A	54.0 (27.0- 120.0) a	9.5 (4.0- 33.8) b	15.0 (8.3- 51.0) b	0.01
Atopy	0 (0) a	16 (66.7%) b	9 (45.0%) b	17 (85.0%) b	<0.001
Serum total IgE	32.0 (23.5- 44.9)	96.5 (32.5- 190.5) b	49.4 (34.1- 181.6)	129.5 (109.6-	0.002
(KU/L)	a		a,b	180.8) b	
Blood eosinophils	0.18 (0.14- 0.22)	0.20 (0.12- 0.39) a,b	0.20 (0.11- 0.33) a,b	0.32 (0.29- 0.50) b	0.015
(×10 ⁹ /L)	a				
FEV ₁ predicted, %	101.8±3.3 a	100.6±10.3 a	94.2±9.0 b	85.6±5.5 b	<0.001
FEV ₁ /FVC (%)	83.5±2.7 a	82.6±5.7 a	75.9±7.4 b	80.8±7.7 a,b	0.005
MMEF predicted, %	80.3±10.1 a	75.4±18.3 a	62.4±15.7 b	41.2±14.7 b	0.006

CA, classic asthma; CVA, cough-variant asthma; HC, healthy control; NAEB, nonasthmatic eosinophilic bronchitis. Data were shown as mean ± standard deviation, frequency (percentage) or median (interquartile range). Each subscript letter denotes a subset of different disease categories whose column data do not differ significantly from each other at the 0.05 level.