BMC Pulmonary Medicine

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Blood eosinophil count correlates with alveolar damage in emphysema-predominant COPD

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Abstract

Background Although blood eosinophil count is recognized as a useful biomarker for the management of chronic obstructive pulmonary disease (COPD), the impact of eosinophils in COPD has not been fully elucidated. Here we aimed to investigate the relationships between the blood eosinophil count and various clinical parameters including lung structural changes.

Methods Ninety-three COPD patients without concomitant asthma were prospectively enrolled in this study. Blood eosinophil count, serum IgE level, serum periostin level, and chest computed tomography (CT) scans were evaluated. Eosinophilic COPD was defined as COPD with a blood eosinophil count ≧ 300/μL. We examined the correlation between the blood eosinophil count and structural changes graded by chest CT, focusing specifically on thin airway wall (WT ^{thin}) and thick airway wall (WT ^{thick}) groups. In a separate cohort, the number of eosinophils in the peripheral lungs of COPD patients with low attenuation area (LAA) on chest CT was assessed using lung resection specimens.

Results The mean blood eosinophil count was 212.1/μL, and 18 patients (19.3%) were categorized as having eosinophilic COPD. In the whole group analysis, the blood eosinophil count correlated only with blood white blood cells, blood basophils, C-reactive protein level, and sputum eosinophils. However, the blood eosinophil count positively correlated with the percentage of LAA and negatively correlated with the diffusing capacity for carbon monoxide in the WT ^{thin} group. Lung specimen data showed an increased number of eosinophils in the peripheral lungs of COPD patients with LAA on chest CT scans compared to normal controls.

Conclusions Some COPD patients without concomitant asthma showed a phenotype of high blood eosinophils. Alveolar damage may be related to eosinophilic inflammation in patients with COPD without asthma and thickening of the central airway wall.

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Keywords COPD, Blood eosinophil count, Emphysema-predominant COPD, Asthma-COPD overlap, Periostin, Type-2 biomarker

Background

Blood eosinophil count has attracted attention as a potential biomarker for the treatment and management of chronic obstructive pulmonary disease (COPD). Eosinophilic inflammation has been observed in both stable COPD and exacerbated COPD [1, 2]. In addition, a relationship between blood eosinophil count and the efficacy of inhaled corticosteroids (ICS) in preventing exacerbation has been reported [3–5]. The 2023 Global Initiative for Chronic Obstructive Lung Disease (GOLD) report recommends the initiation of ICS treatment in combination with one or two long-acting bronchodilators based on the blood eosinophil count, along with the frequency of exacerbation, history of asthma and pneumonia, and mycobacterial infection. In the GOLD report, a blood eosinophil count of ≧300 cells/μL is the threshold for initiating strong support using ICS. However, a blood eosinophil count of <100 cells/μL advises against the use of ICS.

Asthma-COPD overlap (ACO) is a clinical condition with varying proportions of both asthma and COPD components [6]. Because eosinophilic inflammation is a hallmark of asthma, COPD patients with eosinophilic inflammation are often considered to have ACO. However, several large studies have revealed a certain group of COPD patients without asthma but with increased peripheral blood eosinophil counts [7, 8], and these patients are now referred to as those having eosinophilic COPD [9, 10]. Indeed, infiltration of eosinophils in the peripheral lung tissue has been reported in COPD [11, 12], and eosinophil-derived emphysematous changes have also been proposed in mouse models [13, 14]. However, the clinical features of eosinophilic COPD remain unclear.

Respiratory symptoms and chronic airflow limitation in COPD are caused by airway and/or alveolar abnormalities. The relative contribution of the small airways and parenchymal destruction varies widely from person to person and is thought to be related to individual clinical characteristics. Thus, to elucidate the morphologic heterogeneity and phenotype of COPD in a non-invasive manner, chest computed tomography (CT) scans are widely used in clinical and research settings. Emphysema, airway disease, and air trapping are commonly examined, and the impact of various structural changes on disease severity, symptoms, lung function, exacerbation rate, and mortality have been reported [15–17]. However, the relationship between these structural changes and eosinophilic inflammation has scarcely been investigated.

This study aimed to evaluate the impact of eosinophils on COPD and explore the characteristics of eosinophilic COPD, which is unlikely to be recognized as ACO in the clinic. We conducted a multicenter prospective study including patients with stable COPD without asthma. We analyzed the relationships between blood eosinophil counts and various clinical factors, including CT findings and type 2 inflammatory biomarkers. In addition, we quantified eosinophils in lung specimens to determine their localization.

Methods

Subjects

Ninety-three patients with stable COPD without asthma were recruited at Nagoya University Hospital, Ogaki Municipal Hospital, Tosei General Hospital, and Meijo Hospital from October 2013 to October 2017. The diagnosis of COPD was confirmed by pulmonologists based on the GOLD guidelines. The inclusion criteria were as follows: 40 years of age or older, smoking history of more than 10 packs per year, and post-bronchodilator FEV_1/FVC<70%. The exclusion criteria were (1) complications of bronchial asthma, interstitial pneumonia, collagen disease, atopic dermatitis, malignant tumor (eligible if 5 years had passed since radical treatment), myelofibrosis, or liver cirrhosis, (2) patients on dialysis, (3) patients who have proliferative diabetic retinopathy, (4) history of infection within two weeks, (5) history of fracture within three months, (6) patients who have diseases with hypereosinophilia, or (7) patients who take systemic steroids or immunosuppressive drugs. All patients underwent chest CT with pulmonary function and blood tests. Furthermore, some patients were subjected to sputum analysis and/or exhaled nitric oxide fraction (FeNO; NiOX MINO or NiOX VERO; Aerocrine, Solna, Sweden) measurements. Eosinophilic COPD was defined as COPD with a blood eosinophil count≧300/μL. All participants signed informed consent forms approved by the Ethics Committee of our institution (number: 2013-0075-2).

Chest CT data acquisition and analysis

Chest CT images were obtained using a 64-row multidetector CT scanner (Aquilion; Toshiba Medical, Tokyo, Japan). Patients underwent chest CT during deep inspiration and expiration, and the scans were obtained using the following scan parameters: X-ray tube voltage of 120 kVp; automatic tube-current; gantry rotation speed of 0.5 s; beam collimation of 64×0.5 mm. Axial thin-section CT images for 3D-CT were reconstructed as 0.5–1.0 mm thick slices using a high spatial-frequency reconstruction

algorithm. After the DICOM data for 3D-CT were transferred to a commercial 3D workstation (Synapse Vincent version 4.3; Fujifilm Medical Systems, Tokyo, Japan), emphysematous changes, airway wall thickening, and gas trapping were assessed. The amount of emphysema was assessed using the percentage of voxels below −950 Hounsfield unit on inspiratory CT and the Goddard scoring system [18]. The airway dimensions were measured as previously reported [19]. Briefly, the trachea or bronchi were extracted from each side of the lung, and a tracheobronchial tree was reconstructed automatically. We identified the upper apical segmental bronchus (B^1) and posterior basal segmental bronchus (B^{10}) in the right lung. After each bronchus point was selected on the axial image, three generations (third to fifth) of both the right B^1 and B^{10} were measured. Measurement of airway dimensions was performed semi-automatically by the workstation as wall thickness percentage (WT%), wall area percentage (WA%), luminal area (Al, mm^2), wall thickness (WT, mm), and wall area (WA, mm^2), which were measured with the full-width at half-maximum method. Air trapping was assessed by the expiratory to the inspiratory ratio of mean lung density (E/I ratio), which was calculated by dividing the expiratory mean lung density by the inspiratory mean lung density [20]. Thus, as air trapping increased, the E/I ratio became higher.

Pulmonary function test

Spirometry was performed using a FUDAC-77 (Fukuda Denshi Co., Ltd., Tokyo, Japan), CHESTAC-33, CHESTAC-55 V (CHEST M.I., Inc., Tokyo, Japan), or CHESTAC-8900 (CHEST M.I., Inc.) according to the American Thoracic Society (ATS) standards applied in hospitals.

Blood samples

Serum periostin and monomeric periostin levels were measured using an enzyme-linked immunosorbent assay (ELISA) at the Shino-Test Corporation (Kanagawa, Japan). Total serum IgE levels were measured using a commercial ELISA kit (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Other parameters were evaluated using data measured in the laboratories of each hospital.

Sputum samples

Sputum samples were obtained using an established protocol as previously reported [21]. After blocking with Human TruStain FcX™ (BioLegend, San Diego, CA, USA), cells were stained with the mAbs indicated in Table S1 and fixed. Cells were analyzed using FACS Canto II (Becton, Dickinson and Co., Franklin Lakes, NJ, USA), and data were analyzed using FlowJo software (FlowJo LLC, Ashland, OR, USA). Eosinophils were defined as $CD45^+$ $CD16^-$ $siglec8^+$ $CD66b^+$ $CCR3^{high}$ cells, and basophils were defined as $CD45^+$ $FcεRIα^{high}$ $SIRPα^{low}$ $HLA\text{-}DR^{low}$ $c\text{-}kit^-$ cells.

Lung tissues

Lung tissue samples were obtained from another group of patients who had undergone lung resection due to lung cancer at Nagoya University Hospital. An average of 2.05 g of lung pieces were collected from a peripheral site sufficiently distant from the lung cancer site in COPD patients with a visible low attenuation area (LAA) on chest CT or in never-smoker controls with normal lung function and no CT image abnormalities. The fragments were digested with collagenase D and DNase I (Roche Applied Science, Penzberg, Germany) and dissociated by a gentle MACS dissociator (Miltenyi Biotec, North Rhine-Westphalia, Germany). After blocking with Human TruStain FcX™ (BioLegend), cells were stained with the mAbs indicated in Table S1 and fixed [22]. Cells were analyzed using FACS Canto II (Becton, Dickinson and Co.), and data were analyzed using FlowJo software (FlowJo LLC). Eosinophils were defined as $CD45^+$ $CD16^-$ $siglec8^+$ $CD66b^+$ $CD14^{int}$ cells. For immunohistochemical staining, formalin-fixed, paraffin-embedded tissue samples were deparaffinized with xylene, immersed in 10mM pH6.0 citrate buffer, incubated in a warm bath at 95 degrees for 30 min to inactivate the antigen, and inactivated in 0.3% hydrogen peroxide/methanol for 30 min to remove endogenous peroxidase. Tissue sections were then incubated overnight with a 1:500 dilution of primary antibody to eosinophil cationic protein (anti-ECP (EG2); Diagnostics Development, Uppsala, Sweden), followed by incubation with polymer HRP-linked secondary antibody (En Vision™ Polymer anti-mouse HRP, Dako; Agilent, Santa Clara, CA, USA) for 1 h. Eosinophils were detected using Vina Green™ (Biocare Medical, Pacheco, CA, USA) [11]. All patients who underwent lung sample analysis signed informed consent forms approved by the Ethics Committee of our institution (number: 2012-0078).

Statistical analysis

Data were analyzed using SPSS version 27 (SPSS, Inc., Chicago, IL, USA). Results are expressed as mean values ± standard deviation (SD). Differences between the groups of categorical variables were analyzed using the chi-square test. Differences between groups in continuous variables were analyzed using unpaired Student's *t*-test or Mann-Whitney *U* test. Spearman's correlation analysis was used to estimate the relationships among clinical parameters. Significance was set at $P<0.05$.

Results

Patient characteristics

The baseline characteristics of all participants ($n=93$) are listed in Table 1. Eighty-three participants (89.2%) were men, and the mean age was 71.7 years old. The mean blood eosinophil count was 212.1/μL; Fig. 1 shows the distribution of blood eosinophils.

Blood eosinophils and clinical findings

Eighteen patients (19.4%) were categorized as having eosinophilic COPD with a blood eosinophil count ≧300/μL. There were no significant differences in the characteristics between patients with non-eosinophilic and eosinophilic COPD, except for the white blood cells (WBC) count, blood eosinophil count, and WA of B1 3rd generation (Table S2). Table 2 shows the relationships between the blood eosinophil count and various clinical variables. The blood eosinophil count correlated with the WBC count, blood basophil count, serum C-reactive protein (CRP) levels, and sputum eosinophil count. However, there were no significant correlations between blood eosinophils and other type 2 biomarkers, such as serum IgE, periostin, and FeNO, in the total group and in the eosinophilic COPD group (Table 2, Table S3).

Blood eosinophils and structural findings evaluated by chest CT

COPD is a heterogeneous disease with varying rates of structural changes in the airway and parenchyma. Thus, we examined the relationships between blood eosinophil count and structural changes graded by chest CT. Airway and emphysema components were evaluated by the total airway wall thickness of the 3rd to 5th generations of right B1 and B10 (total WT) and % LAA, respectively. Figure 2 shows the relationships between blood eosinophils and the structural features evaluated in each patient based on the total WT and % LAA. Patients with eosinophilic COPD (filled blue circles) showed various patterns of structural changes, suggesting morphological heterogeneity of eosinophilic COPD.

Blood eosinophils were associated with alveolar damage in the bronchial wall non-thickening group

Although we carefully excluded concomitant asthma by pulmonologists, it may be difficult to completely rule out ACO. As bronchial wall thickening determined by chest CT is one of the characteristic clinical manifestations of asthma or ACO as well as airway inflammation of COPD [23–27], we investigated the effect of blood eosinophils on clinical features in two groups divided based on the basis of total WT (median 6.31 mm), i.e., thin airway wall (WT ^{thin}) and thick airway wall (WT ^{thick}) groups. The diffusing capacity for carbon monoxide (% DLCO) was significantly lower in the WT ^{thin} group than in the WT ^{thick} group ($p=0.047$). However, the other clinical characteristics of the two groups were similar (Table S4). The relationships between blood eosinophils and various clinical parameters in the WT ^{thin} group are shown in Table 3 and Figure S1. There were significant positive associations with blood WBC, blood neutrophils, blood basophils, CRP level, sputum eosinophils, and % LAA, and significant negative associations with age and % DLCO. In contrast, there was a significant positive correlation between the blood eosinophils and CRP and WA of B1 5th generation, but there were no significant correlations between blood eosinophils and % LAA and % DLCO in the WT ^{thick} group (Table S5).

Eosinophils in peripheral lungs of patients with COPD

The effects of the relationship between blood eosinophils and alveolar damage on chest CT prompted us to examine the localization of eosinophils in COPD patients with LAA on chest CT. Peripheral tissue eosinophils from patients undergoing lung resection were evaluated using flow cytometry. The characteristics of the tissue eosinophil analysis group are shown in Table S6. As shown in Fig. 3, the number of eosinophils per gram of peripheral lung tissue was significantly higher in COPD patients with LAA on chest CT than in never-smoker controls ($p=0.043$). In addition, the percentage of eosinophils in $CD45^+$ cells in COPD lungs was significantly correlated with the number of blood eosinophils ($r=0.550$, $p=0.034$). Immunostaining of the lungs using an anti-ECP antibody revealed eosinophils in the alveolar walls of the peripheral lungs.

Discussion

In this study, we showed a positive correlation between the blood eosinophil count and % LAA in patients with COPD who did not have asthma or central airway wall thickening. In addition, the blood eosinophil count was negatively correlated with the %DLCO in the same group of patients. These results indicate a relationship between increased blood eosinophil count and alveolar damage in patients with emphysema-predominant COPD. Airway wall thickening has been observed in COPD and asthma [15, 28, 29]. In patients with asthma, airway wall thickening is associated with disease severity, lung function, and disease duration [23, 30]. In patients with COPD, airway wall thickening correlated with $FEV_{1.0}$ independently of emphysema and related to cough, sputum, or wheezing [26, 27], supporting the hypothesis that increased bronchial wall thickness is associated with bronchitis in COPD. Furthermore, patients with ACO have thicker airway walls than those with COPD [24, 25]. In our cohort, there was no difference in %LAA between the group of patients in the WT ^{thin} group and in the WT ^{thick} group, but the % DLCO was significantly higher in the WT ^{thick}

Table 1 Patient characteristics

	n **= 93**			
Age	71.7 ± 7.3	Emphysema		
Male sex, n (%)	83 (89.2)	%LAA (%)		39.1 ± 11.1
BMI (kg/m^2)	21.5 ± 3.5	Goddard score		12.7 ± 3.0
Pack-years smoking	64.7 ± 29.9			
		Air trapping		
GOLD 2017 A/ B/ C/ D	22 / 56 / 1 / 14	E/I MLD		0.95 ± 0.03
		Exp_{-856} (%)		60.0 ± 14.3
Stage 1/2/3/4	18 / 32 / 32 / 11			
		Airway dimensions		
Medication, n (%)		B^1	3rd	WT (mm)	1.32 ± 0.21
ICS	32 (34.4)			%WT (%)	36.2 ± 4.7
LABA	65 (69.9)			WA (mm^2)	26.5 ± 9.1
LAMA	67 (72.0)			%WA (%)	58.6 ± 5.9
				AI (mm^2)	19.5 ± 9.8
Blood values			4th	WT (mm)	1.02 ± 0.18
WBC (/μL)	6532 ± 1719			%WT (%)	38.5 ± 4.8
Neutrophils (/μL)	3963 ± 1364			WA (mm^2)	14.8 ± 5.2
Eosinophils (/μL)	212.1 ± 151.4			%WA (%)	61.7 ± 6.0
Basophils (/μL)	45.1 ± 22.6			AI (mm^2)	9.7 ± 5.3
CRP (mg/dL)	0.18 ± 0.30		5th	WT (mm)	0.80 ± 0.14
IgE (IU/mL)	481.6			%WT (%)	40.9 ± 4.2
	(239.2-1268.9)			WA (mm^2)	8.4 ± 2.9
Periostin (ng/mL)	104.1 ± 29.7			%WA (%)	64.6 ± 5.1
Periostin monomer (ng/mL)	10.1 ± 2.4			AI (mm^2)	4.7 ± 2.2
KL-6 (U/mL)	306.5 ± 113.1	B^{10}	3rd	WT (mm)	1.25 ± 0.18
SP-D (ng/mL)	59.7 ± 53.9			%WT (%)	36.3 ± 5.0
				WA (mm^2)	23.4 ± 5.7
Sputum sample				%WA (%)	58.9 ± 6.2
%Eosinophils (%)	7.2 ± 7.9			AI (mm^2)	16.8 ± 6.0
%Basophils (%)	0.19 ± 0.45		4th	WT (mm)	1.09 ± 0.18
				%WT (%)	38.1 ± 4.7
FeNO (ppb)	23.1 ± 15.0			WA (mm^2)	17.0 ± 5.6
				%WA (%)	61.2 ± 5.6
mMRC	1.6 ± 1.0			AI (mm^2)	11.2 ± 4.8
CAT	12.8 ± 7.9		5th	WT (mm)	0.91 ± 0.20
				%WT (%)	40.1 ± 5.3
Lung function tests				WA (mm^2)	11.3 ± 4.4
FEV1 (L)	1.35 ± 0.57			%WA (%)	63.7 ± 6.1
%FEV1 (%)	56.8 ± 24.3			AI (mm^2)	6.6 ± 3.0

Table 1 (continued)

	n = 93		
FEV1/FVC (%)	45.3 ± 12.6		
%DLCO (%)	76.6 ± 30.2	total WT (mm)	6.38 ± 0.76

Data presented as n (%) or mean ± standard deviation

IgE presented median (IQR)

BMI was evaluated in 76 patients

Basophils and KL-6 were evaluated in 92 patients

SP-D was evaluated in 87 patients

Eosinophils in sputum were evaluated in 38 patients

Basophils in sputum were evaluated in 37 patients

FeNO was evaluated in 34 patients

%DLCO was evaluated in 88 patients

%LAA, Goddard score, E/I MLD and Exp_{-856} were evaluated in 81 patients

Airway dimensions from the third to the fifth generations at B1 were evaluated in 80 patients

Airway dimensions from the third to the fifth generations at B10 were evaluated in79 patients

Total WT was evaluated in 78 patients

BMI, body mass index; GOLD, Global Initiative for Chronic Obstructive Lung Disease; ICS, inhaled corticosteroid; LABA, long-acting beta-agonist; LAMA, long-acting muscarinic antagonist; WBC, white blood cell; CRP, C-reactive protein; IgE, immunoglobulin E; KL-6, Krebs von den Lungen-6; SP-D, surfactant protein D; FeNO, fractional exhaled nitric oxide; mMRC, modified medical research council dyspnea scale; CAT, COPD assessment test; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; DLCO, carbon monoxide diffusing capacity; LAA, low attenuation area; E/I MLD, expiration/inspiration ratio of mean lung density; Exp_{-856}, percentage of lung voxels with attenuation below −856 HU on expiration CT; WT, airway wall thickness; WA, airway wall area; AI, airway luminal area; total WT, total airway wall thickness of the 3rd to 5th generations of right B1 and B10

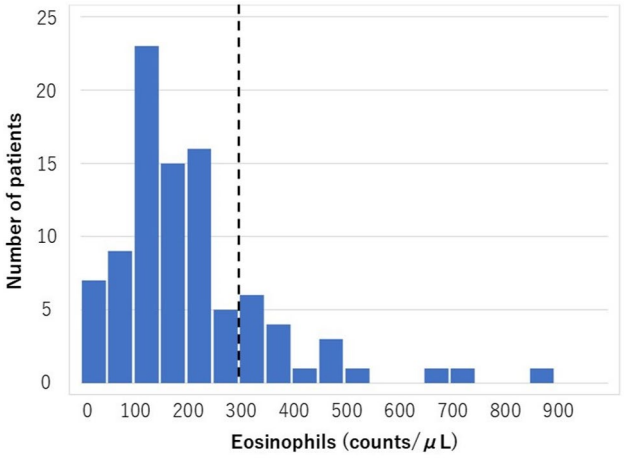

Fig. 1 The distribution of blood eosinophils. Blood eosinophil counts of all participants

group (Table S4). Therefore, the analysis of patients with unthickened bronchial walls could have excluded a certain number of patients with ACO, as well as patients with airway-disease-predominant and less parenchymal destruction COPD.

Increased eosinophil counts were confirmed in peripheral lung tissues of patients with COPD with LAA on chest CT using flow cytometry analysis. Jogdand et al. examined eosinophils and basophils in all anatomical compartments of COPD-affected lungs and showed that both cell types were increased in peripheral lung tissue, alveolar parenchyma, and peribronchial areas [11]. Additionally, the numbers of both eosinophils and basophils increased further in severe GOLD stages. Kolsum et al. showed increased eosinophils in bronchoalveolar lavage (BAL), sputum, and bronchial mucosa in patients with COPD with a high blood eosinophil count [12]. These data indicate the involvement of eosinophils in the peripheral lungs of patients with COPD and are consistent with our observations.

The blood eosinophil count correlated with the blood WBC count, blood basophil count, CRP level, and sputum eosinophil count. However, there were no significant correlations between blood eosinophils and other type 2 biomarkers, such as serum periostin, IgE, and FeNO, even in the group of patients with blood eosinophil count ≧ 300/μL in our study. Periostin is a matricellular protein involved in tissue remodeling and repair [31]. In patients with asthma, serum periostin levels are higher than those in normal controls and are positively correlated with blood eosinophil count, IgE, and FeNO [31, 32]. On the other hand, in agreement with our findings, Carpaij et al. reported that serum periostin levels were significantly higher in patients with COPD with no history of asthma than in healthy smokers, but there were no correlations between periostin and eosinophils in blood, sputum, or biopsy samples [33]. Konstantelou et al. also showed no correlation between the serum periostin level and blood eosinophil count in patients hospitalized for COPD exacerbations [34]. These observations suggest different mechanisms underlying eosinophilic inflammation in asthma and COPD.

In this study, 18 patients (19.4%) were classified as having eosinophilic COPD. On the other hand, previous studies estimated that approximately 30% of COPD patients had a blood eosinophil count higher than 300

Table 2 Relationship of blood eosinophils to variables

Variable	*r*	*P*-value	*N*	Variable	*r*	*P*-value	*N*
Age (years)	-0.103	0.324	93	FeNO (ppb)	0.315	0.069	34
Smoking index	0.055	0.599	93				
				FEV1 (L)	0.052	0.622	93
Blood samples				%FEV1 (%)	0.043	0.679	93
WBC (/μL)	**0.339**	**< 0.001**	**93**	%DLCO (%)	-0.056	0.607	88
Neutrophils (/μL)	0.175	0.094	93				
Basophils (/μL)	**0.305**	**0.003**	**92**	%LAA (%)	0.105	0.349	81
log IgE	0.067	0.529	91	B1-5th			
CRP (mg/dL)	**0.334**	**0.001**	**93**	WT (mm)	0.093	0.411	80
Periostin (ng/mL)	0.027	0.800	93	WA (mm^2)	0.141	0.212	80
				B10-5th			
Sputum samples				WT (mm)	-0.061	0.593	79
Eosinophils (%)	**0.480**	**0.002**	**38**	WA (mm^2)	-0.075	0.511	79
Basophils (%)	0.234	0.162	37				
				total WT (mm)	0.068	0.557	78

Spearman's correlation coefficient for each variable compared to blood eosinophils. Bold values indicate statistically significant correlations with $P<0.05$. WBC, white blood cell; IgE, immunoglobulin E; CRP, C-reactive protein; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in one second; DLCO, carbon monoxide diffusing capacity; LAA, Low attenuation area; WT, airway wall thickness; WA, airway wall area; total WT, total airway wall thickness of the 3rd to 5th generations of right B1 and B10

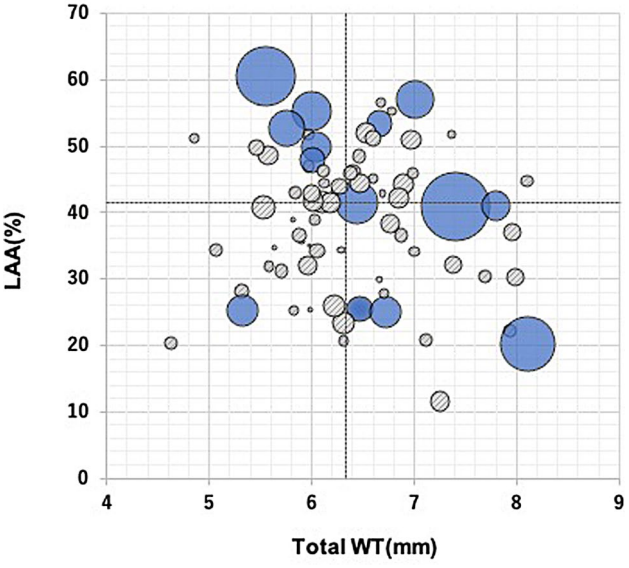

Fig. 2 CT-based structural change patterns in eosinophilic COPD. CT-based structural change patterns of each patient were determined by the percentage of low attenuation areas (% LAA) and the total wall thickness of the 3rd to 5th generations of right B1 and B10 (Total WT). The size of the bubbles indicates blood eosinophil counts. Filled blue circles indicate eosinophilic COPD patients (blood eosinophil counts ≧300/μL). LAA, low attenuation area; Total WT, total airway wall thickness of the 3rd to 5th generations of right B1 and B10

cells /μL [35]. However, in many cohorts, detailed patient characteristics are unknown and some patients may have a history of asthma or other allergic diseases. In the COPDGene cohort, 23.5% of participants had a blood eosinophil count≧300/μL and 19.9% had eosinophilic COPD without a prior diagnosis of asthma. In the ECLIPSE study, 27.5% of participants had a blood eosinophil count≧300/μL and 19.7% had eosinophilic COPD without a prior diagnosis of asthma [7]. In addition, Kolsum et al. used a research database of primary care COPD patients to carefully isolate eosinophilic COPD without a clinical history of asthma and reported that 20.9% of COPD patients corresponded to eosinophilic COPD [8]. In our study, COPD patients with concomitant asthma were carefully excluded to investigate the characteristics of eosinophilic COPD. As a result, the number of patients with a blood eosinophil count≧300/μL was small, but the percentage of these patients was equivalent to previous studies with a similar perspective.

The pathogenic role of eosinophils in peripheral lung tissue in COPD remains unclear [9, 10, 36]. The concept of eosinophil-driven emphysema has been proposed by several research groups. Eosinophil-derived interleukin 13 promoted matrix metalloprotease 12 (MMP-12) production via alveolar macrophages, causing airspace enlargement in a mouse model [13]. In an elastase-induced emphysema model, accumulated eosinophils promoted lung matrix destruction via cathepsin L, and these emphysematous changes were suppressed in eosinophil-deficient mice [14]. In the imaging analysis of the SPIROMICS cohort, patients with high sputum eosinophil count had higher emphysema indices, air trapping, and functional small airway disease, as assessed by parametric response mapping (PRM fSAD) [37]. In the COPDGene study cohort, the blood eosinophil count was associated with biomarkers of type IV collagen turnover [38]. However, recent studies have revealed non-pathogenic functions of eosinophils, such as contributing to maintaining homeostasis or tissue repair in the lung [39, 40]. Thus, the assembly of eosinophils may be the result

Table 3 Relationship of blood eosinophils to variables in thin airway wall group

Variable	*r*	*P*-value	*N*	Variable	*r*	*P*-value	*N*
Age (years)	**-0.355**	**0.026**	**39**	**%LAA (%)**	**0.342**	**0.035**	**38**
Smoking index	0.034	0.837	39	Goddard score	0.260	0.116	38
Blood samples				E/I MLD	0.131	0.431	38
WBC (/μL)	**0.530**	**< 0.001**	**39**	Exp_{-856} (%)	0.145	0.384	38
Neutrophils (/μL)	**0.342**	**0.033**	**39**				
Basophils (/μL)	**0.400**	**0.012**	**39**	B1-5th			
log IgE	0.064	0.704	38	WT (mm)	-0.123	0.455	39
CRP (mg/dL)	**0.353**	**0.027**	**39**	WA (mm^2)	-0.071	0.667	39
Periostin (ng/mL)	0.078	0.638	39	B10-5th			
				WT (mm)	-0.177	0.281	39
Sputum samples				WA (mm^2)	-0.157	0.339	39
Eosinophils (%)	**0.688**	**0.007**	**14**				
Basophils (%)	0.293	0.332	13	total WT (mm)	-0.047	0.777	39
FeNO (ppb)	0.060	0.888	8				
FEV1 (L)	-0.121	0.464	39				
%FEV1 (%)	-0.149	0.366	39				
%DLCO (%)	**-0.357**	**0.032**	**36**				

Spearman's correlation coefficient for each variable compared to blood eosinophils. Bold values indicate statistically significant correlations with $P < 0.05$. WBC, white blood cell; IgE, immunoglobulin E; CRP, C-reactive protein; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in one second; DLCO, carbon monoxide diffusing capacity; LAA, low attenuation area; E/I MLD, expiration/inspiration ratio of the mean lung density; Exp_{-856}, percentage of lung voxels with attenuation below −856 HU on expiration CT; WT, airway wall thickness; WA, airway wall area; total WT, total airway wall thickness of the 3rd to 5th generations of right B1 and B10

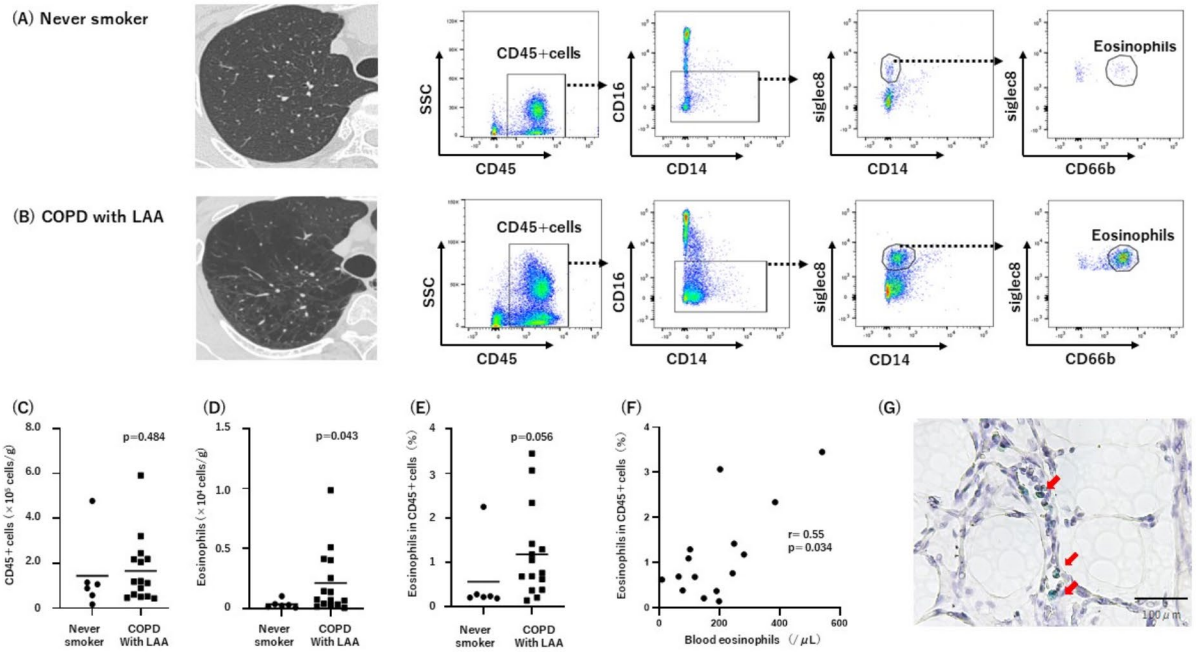

Fig. 3 Eosinophils were increased in peripheral lungs of COPD with low attenuation areas on chest CT. Eosinophils were defined as $CD45^+$ $CD16^-$ $CD14^-$ $siglec8^{high}$ $CD66b^+$ cells. (A) Representative data of a never-smoker. (B) Representative data of a COPD patient with a low attenuation area (LAA). Scattergrams showing (C) $CD45^+$ cell counts, (D) eosinophil counts, and (E) percentage of eosinophils in $CD45^+$ cells in the peripheral lung in the COPD patients with LAA on chest CT (n=15) and controls (n=6). (F) Correlation between percentage of lung eosinophils in $CD45^+$ cells and blood eosinophil count. Data were analyzed by flow cytometry (C-F). P-values were examined by the Mann-Whitney U test. Spearman's correlation coefficient for lung eosinophils to blood eosinophils. (G) Representative micrograph of stained eosinophils from a COPD patient with high eosinophil count (336.4 cells /μL) in the peripheral lung. Immunohistochemistry (IHC) of lung section stained for eosinophils (Vina green) (n=3). Arrows indicate positive cells. Scale bar: 100 μm

of lung damage. Further studies are needed to clarify the precise role of eosinophils in the peripheral lungs of COPD patients.

The efficacy of monoclonal antibody therapies targeting interleukin 5 (IL-5) signaling in COPD highlights the importance of eosinophil control in COPD management. Mepolizumab (anti-IL-5) reduced the annual rates of moderate or severe exacerbation in patients with COPD with an eosinophilic phenotype who had been receiving high-dose ICS, LABA, and LAMA [41]. In contrast, benralizumab (anti-IL-5Ra) did not significantly reduce COPD exacerbations compared to placebo in phase 3 trials [42]. However, post hoc analysis revealed subpopulations showing the greatest treatment effect with benralizumab, i.e., a high blood eosinophil count with a history of frequent exacerbation, low baseline lung function, and improvement in lung function with short-acting bronchodilators [43]. Although we could not determine the morphological phenotypes of the patients, these findings suggest the existence of several subpopulations of patients with eosinophilic COPD.

Our study had some limitations. First, despite our best efforts, we could not rule out the possibility of asthma or other allergic diseases entirely. In this study, we did not examine bronchodilator reversibility, airway response to histamine or acetylcholine, or allergy skin tests at the time of recruitment. These conditions can affect the number of eosinophils in the blood. However, the participants were carefully screened by experienced respiratory specialists, and we analyzed the data of patients with central airway non-thickening to exclude asthmatic components as much as possible. In addition, the levels of IgE and periostin were not elevated even in COPD patients with high blood eosinophils in our cohort (Table S2, Figure S1). Second, we only examined blood eosinophil counts at enrollment. Because the number of blood eosinophils fluctuates over time, we may have underestimated the effect of eosinophils in patients with COPD. Third, the number of patients who underwent FeNO and/or sputum analysis was small. In this study, we focused on emphysema-predominant COPD rather than on airway-disease–predominant COPD. Therefore, we believe that the number of tests does not significantly affect our observations. Fourth, lung tissue analysis was not a complete evaluation of emphysema-predominant COPD patients with high blood eosinophils. Because we used surplus lung specimens resected for cancer from an ethical standpoint, it was difficult to obtain a sufficient number of samples to meet our requirements. However, we used tissue from peripheral sites where visible LAA was identified on chest CT, thus we believe we were able to analyze peripheral alveolar regions with less central airway involvement. Indeed, an increase in tissue eosinophil counts was observed in the COPD group compared to the control group. In addition, the percentage of eosinophils in $CD45^+$ cells correlated with blood eosinophil counts. However, further studies are needed to elucidate the pathophysiology of eosinophilic inflammation in COPD.

Conclusions

Some COPD present without concomitant asthma showed a phenotype of high blood eosinophils. Alveolar damage may be associated with eosinophilic inflammation in patients with emphysema-predominant COPD. More clinical trials and basic studies are needed to understand the pathogenesis of COPD and to establish treatment strategies.

Abbreviations

ACO	Asthma-COPD Overlap
AI	Luminal Area
ATS	American Thoracic Society
BAL	Bronchoalveolar Lavage
CCR3	C-C Motif Chemokine Receptor 3
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-Reactive Protein
CT	Computed Tomography
DICOM	Digital Imaging And Communications In Medicine
%DLCO	Diffusing Capacity For Carbon Monoxide
ECP	Eosinophil Cationic Protein
E/I ratio	Expiratory to Inspiratory Ratio of Mean Lung Density
ELISA	Enzyme-Linked Immunosorbent Assay
FeNO	Exhaled Nitric Oxide Fraction
FEV1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GOLD	Global Initiative for Chronic Obstructive Lung Disease
ICS	Inhaled Corticosteroids
IL-5	Interleukin 5
LAA	Low Attenuation Area
mAb	Monoclonal Antibody
MMP-12	Matrix Metalloprotease 12
PRM fSAD	Functional Small Airway Disease as Assessed by Parametric Response Mapping
SD	Standard Deviation
SIRPa	Signal Regulatory Protein Alpha
total WT	Total Airway Wall Thickness of The 3rd to 5th Generation of Right B1 And B10
WA	Wall Area
WA%	Wall Area Percentage
WBC	White Blood Cells
WT	Wall Thickness
WT^{thick}	Thick Airway Wall
WT^{thin}	Thin Airway Wall
WT%	Wall Thickness Percentage
3D-CT	Three-Dimensional Computed Tomography

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12890-024-03320-2.

Supplementary Material 1: Figure 1. The distribution of blood eosinophils. Blood eosinophil counts of all participants. **Figure 2** CT-based structural change patterns in eosinophilic COPD: CT-based structural change patterns of each patient were determined by the percentage of low attenuation areas (% LAA) and the total wall thickness of the 3rd to 5th generations of right B1 and B10 (Total WT). The size of the bubbles indicates blood eosinophil counts. Filled blue circles indicate eosinophilic COPD patients (blood eosinophil counts ≧300/μL). LAA, low attenuation

area; Total WT, total airway wall thickness of the 3rd to 5th generations of right B1 and B10. **Figure 3** Eosinophils were increased in peripheral lungs of COPD with low attenuation areas on chest CT: Eosinophils were defined as $CD45^+$ $CD16^-$ $CD14^-$ $siglec8^{high}$ $CD66b^+$ cells. (A) Representative data of a never-smoker. (B) Representative data of a COPD patient with a low attenuation area (LAA). Scattergrams showing (C) CD45+ cell counts, (D) eosinophil counts, and (E) percentage of eosinophils in $CD45^+$ cells in the peripheral lung in the COPD patients with LAA on chest CT ($n = 15$) and controls ($n = 6$). (F) Correlation between % of lung eosinophils in $CD45^+$ cells and blood eosinophil count. Data were analyzed by flow cytometry (C-F). P-values were examined by the Mann–Whitney U test. Spearman's correlation coefficient for lung eosinophils to blood eosinophils. (G) Representative micrograph of stained eosinophils from a COPD patient with high eosinophil count (336.4 cells /μL) in the peripheral lung. Immunohistochemistry (IHC) of lung section stained for eosinophils (Vina green) ($n = 3$). Arrows indicate positive cells. Scale bar: 100 μm.

Supplementary Material 2: Table S1. Anti-human antibody. **Table S2.** Patient characteristics of eosinophilic COPD. **Table S3.** Relationship of blood eosinophils to variables in high eosinophil group (Eosinophil count ≧ 300/μL). **Table S4.** Patient characteristics sorted by airway wall thickness. **Table S5.** Relationship of eosinophils to variables in thick airway wall group. **Table S6.** Patient characteristics of the tissue eosinophil analysis group

Acknowledgements
We thank Mr. M. Tanaka for technical assistance in flow cytometry analysis, Dr. J Ono for measuring serum periostin levels, and Ms. Katherine Ono for her language assistance.

Author contributions
S.N., K.W., and S.I. designed the study; S.N., K.W., E.Y., E.F., R.O., T.Y., T.A., T.S., T.K., and K.I. collected clinical samples and analyzed samples; S.N., S.M., and S.I. evaluated CT; S.N., K.W., and F.K. analyzed the data and wrote the manuscript. N.H., M.I., and Y.H. supervised the study.

Funding
This work was supported by KAKEN (grant numbers: 18K08173, 21K08456).

Data availability
The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Nagoya University Graduate School of Medicine (number: 2013-0075-2) and written informed consent was obtained from all patients. Lung specimen analysis was approved by the Ethics Committee of Nagoya University Graduate School of Medicine (number: 2012-0078) and written informed consent was obtained from all patients.

Competing interests
The authors declare no competing interests.

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Received: 15 January 2024 / Accepted: 1 October 2024
Published online: 13 October 2024

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