Association between blood eosinophil count and small airway eosinophils in smokers with and without COPD

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Blood eosinophil count was associated with eosinophils observed in small airways of lung specimens in smokers with and without COPD. The blood eosinophil count may reflect eosinophilic inflammation in small airways. https://bit.ly/3rllhLg

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Abstract

Introduction Airway eosinophilic inflammation is a pathological feature in a subgroup of patients with COPD and in some smokers with a high COPD risk. Although blood eosinophil count is used to define eosinophilic COPD, the association between blood eosinophil count and airway eosinophilic inflammation remains controversial. This cross-sectional study tested this association in smokers with and without COPD while considering potential confounders, such as smoking status and comorbidities.

Methods Lung specimens were obtained from smokers with and without COPD and non-COPD never-smokers undergoing lung lobectomy. Those with any asthma history were excluded. The infiltration of eosinophils into the small airway wall was quantified on histological sections stained with major basic protein (MBP).

Results The number of airway MBP-positive cells was greater in smokers (n=60) than in never-smokers (n=14). Smokers with and without COPD (n=30 each) exhibited significant associations between blood eosinophil count and airway MBP-positive cells (ρ=0.45 and 0.71). When smokers were divided into the high and low airway MBP groups based on their median value, blood eosinophil count was higher in the high-MBP group, with no difference in age, smoking status, comorbidities, emphysema or coronary artery calcification on computed tomography, and inhaled corticosteroid (ICS) use. The association between greater blood eosinophil count and the high-MBP group was confirmed in multivariable models adjusted for smoking status, airflow limitation and ICS use.

Conclusion The blood eosinophil count may reflect eosinophilic inflammation in the small airways in smokers with and without COPD.

Introduction

COPD is diagnosed based on airflow limitation on spirometry, but its heterogeneous clinical presentation requires the establishment of a “treatable trait” for better personalised management [1, 2]. Blood eosinophil count is considered a biomarker to identify patients with eosinophilic COPD who may benefit from inhaled corticosteroid (ICS) therapy [3–5]. The validity of the use of the blood eosinophil count is built upon the assumption that the blood eosinophil count reflects eosinophilic airway inflammation [6–9]. However, there remains controversy, particularly regarding the association between the blood eosinophil

count and eosinophil infiltration of small airways [10, 11], although the small airway is the primary pathological site of COPD [12].

Smoking is associated with increased blood eosinophils [13]. A large cohort of non-COPD subjects without a history of asthma exhibit higher blood eosinophil counts associated with the future development of obstructive lung disease [14]. These findings suggest that the blood eosinophil count may also be used to identify smokers with airway eosinophilic inflammation who are at high risk of COPD, thereby providing an opportunity for early intervention to prevent the development of COPD. Moreover, factors other than smoking, for example, cardiovascular disease and obesity, may affect eosinophilic responses in the body [13]. Therefore, this study examined histological lung sections to test the hypothesis that blood eosinophil counts would be associated with eosinophilic inflammation in the small airways in smokers both before and after the onset of COPD while considering potential confounders such as cardiovascular comorbidities, smoking status and ICS use.

Methods

Study design and clinical information

This cross-sectional study used archived frozen lung specimens that were prospectively collected from never- and ever-smokers who underwent lung lobectomy for tumour resection at Kyoto University Hospital (Kyoto, Japan) between 2006 and 2010. The lung lobectomy was performed after ⩾3 weeks of smoking cessation. Those with a history of asthma were excluded. Prior to the surgery, blood tests, including differential leukocyte counts and post-bronchodilator spirometry, were performed. The diagnosis of COPD was based on forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7 [15]. Diffusing capacity of the lung for carbon monoxide (D_{LCO}) was measured in ever-smokers using the single-breath method with calculation of helium dilution rate and corrected by haemoglobin values [16, 17]. The predicted values for FEV_1, FVC and D_{LCO} were calculated using equations for the Japanese population [18, 19]. Global Initiative for Chronic Obstructive Lung Disease (GOLD) spirometry stages 1, 2, 3 and 4 were defined as FEV_1⩾80% predicted, 50% pred⩽FEV_1<80% pred, 30% pred⩽FEV_1<50% pred and FEV_1<30% pred, respectively. According to the Fleischner Society classification system [20], computed tomography (CT) images of the whole lung before surgery were visually evaluated by two CT-experienced pulmonologists (N. Tanabe and Y. Shiraishi) without knowledge of clinical information and the discordances were adjudicated by a chest radiologist with 16 years experience (R. Sakamoto). Centrilobular emphysema (CLE) was classified as trace, mild, moderate, confluent or advanced destructive, while paraseptal emphysema (PSE) was classified as mild or substantial. Weighted Cohen's κ for the visual assessment of CLE and PSE by N. Tanabe and Y. Shiraishi was 0.86 and 0.92, respectively. In this study, any CT finding of either at least trace CLE or at least mild PSE is considered as the presence of emphysema. The presence of pre-COPD was defined as FEV_1/FVC ⩾0.7 and either the presence of emphysema, D_{LCO} <80% pred or preserved ratio impaired spirometry (FEV_1/FVC ⩾0.7 and FEV_1 <80% pred) according to a recent article [21]. In the sensitivity analyses, another definition of the emphysema presence was used to redefine pre-COPD, where at least moderate CLE was considered as the presence of emphysema. The presence of coronary artery calcification was also visually identified. The ethics committee of Kyoto University (G155, G0620 and R1852) approved the study, and written informed consent was obtained from each subject.

Lung specimen preparation and assessment

Following surgical resection, a piece of lung tissue was obtained within 3 cm of the pleura for each lobe in a location distant from the tumour, inflated with Tissue-Tek OCT Compound (Sakura Finetek, Osaka, Japan), and immediately frozen in liquid nitrogen. The frozen pieces were cut into 8-µm-thick sections and stained with haematoxylin and eosin to identify the small airways, as reported previously [22]. Eosinophil was identified as major basic protein (MBP)-positive cells on immunohistochemistry, as reported previously [23, 24]. Each section was incubated with an anti-human MBP BMK-13 antibody (BIORAD MCA5751). The stained sections were digitalised, and as shown in figure 1, the number of MBP-positive eosinophils in the walls of small airways <2 mm in short-lumen diameter was manually counted and normalised by dividing this number by the basement membrane perimeter (Pbm) according to prior research [25].

Statistical analysis

Values are expressed as the median with interquartile range (IQR) unless indicated. Group comparison and correlation tests were performed using the Kruskal–Wallis rank-sum test and Spearman rank correlation test, because continuous variables were non-normally distributed. Intraclass correlation coefficient (ICC) of peripheral blood eosinophil count was calculated using cases with multiple blood tests (n=67). To explore factors associated with higher airway MBP-positive cells, smokers were divided into high and low airway MBP groups based on the median value of the number of MBP-positive cells/Pbm. Multivariable logistic

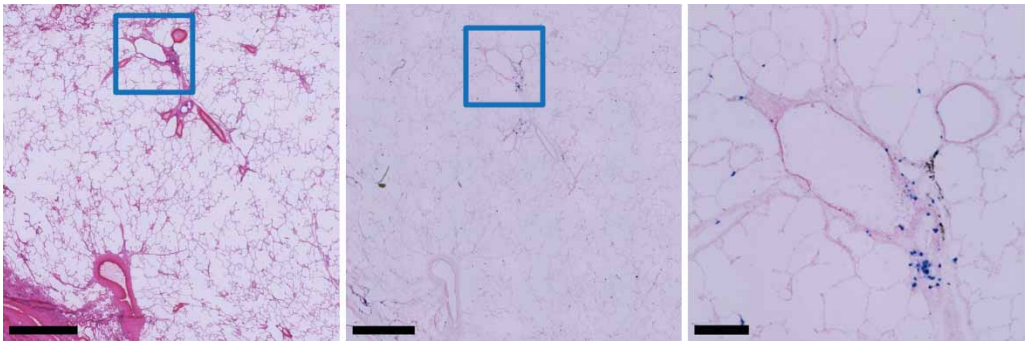

FIGURE 1 Representative histological sections for major basic protein-positive cells in the small airways. Representative image of haematoxylin and eosin staining and immunohistochemistry for major basic protein (MBP; eosinophil marker). The measurement of MBP-positive cells (blue) in the airways (<2 mm in short-lumen diameter) was performed to evaluate eosinophils. The number of MBP-positive cells was counted, and the basement membrane perimeter (Pbm) was measured manually. The number of MBP-positive cells normalisation was performed by dividing the number of MBP-positive cells by the Pbm.

regression models were constructed by including the high/low MBP group as a dependent variable and the blood eosinophil count, smoking status, smoking pack-years, FEV_1/FVC and use of ICS as independent variables. These variables were chosen based on previous reports of factors that could potentially affect eosinophilic inflammation in the blood or airways [10, 13, 26]. Statistics were performed using R statistical software 4.0.1.

Results

Subject characteristics

30 smokers with COPD, 30 smokers without COPD and 14 never-smokers were included in this study. None of the subjects had used oral corticosteroids. There were no never-smokers with airflow limitation. The median (IQR) interval between blood test and surgery was 6 days [3, 17]. ICC of blood eosinophil counts for the blood test used in the main analyses obtained just before the surgery and the second most recent blood test before the surgery was 0.86, and considered good. As shown in table 1, age, sex, body mass index (BMI), the rate of current smokers, smoking pack-years, D_{LCO} % pred, the presence of coronary artery calcification and hypertension did not differ between smokers with and without COPD. The use of ICS was observed only in smokers with COPD (23.3%). The proportion of pre-COPD was 96.7% among smokers without COPD. Furthermore, when smokers with and without COPD were combined, the ever-smokers were older, predominantly male, tall and weighed more than never-smokers, whereas the number of histologically measured airways and their Pbm did not differ. Supplementary table S1 shows that when dividing ever-smokers into four groups based on smoking status and COPD presence, age, sex, BMI, smoking pack-years and D_{LCO} % predicted did not differ. Figure 2 shows that blood eosinophil count and the number of airway MBP positive cells/Pbm did not differ between the four groups. In contrast, the number of airway MBP-positive cells/Pbm was significantly higher in ever-smokers than in never-smokers, whereas the blood eosinophil count did not differ.

Association between airway MBP-positive cells and blood eosinophil counts

Figure 1 presents a representative image of MBP-positive cells in the walls of the small airways. Figure 3 demonstrates that a higher blood eosinophil count was associated with a higher number of airway MBP-positive cells/Pbm in ever-smokers with and without COPD (Spearman's ρ=0.61, p<0.001), but not in never-smokers (ρ=0.32, p=0.27). The association between the blood eosinophil count and the number of airway MBP-positive cells/Pbm was observed when the analyses were performed in smokers with and without COPD separately (ρ=0.45, p=0.013, and ρ=0.71, p<0.001, respectively). Supplementary figure S1 shows no association between smoking pack-years and the number of airway MBP-positive cells/Pbm in ever-smokers. Moreover, supplementary figure S2 shows that the association was maintained in smokers with COPD who were not treated with ICS (n=23, ρ=0.56, p=0.007), smokers with COPD GOLD 1 and GOLD 2 (n=28, ρ=0.48, p=0.01) and smokers with pre-COPD (n=29, ρ=0.71, p<0.001). Furthermore, when using the other pre-COPD criteria by redefining the presence of emphysema as at least moderate CLE, the association was also maintained (n=19, ρ=0.59, p=0.008). Supplementary figure S3 shows that

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TABLE 1 Characteristics of ever- and never-smokers

	Smoker with COPD	Smoker without COPD	p-value	Ever-smoker	Never-smoker	p-value
Subjects	30	30		60	14	
Age years	72.0 (64.8–75.5)	70.0 (62.5–75.0)	0.64	72.0 (63.8–75.3)	62.5 (58.0–65.8)	0.027
Male	25 (83.3)	24 (80.0)	1	49 (81.7)	1 (7.1)	<0.001
Height m	1.64 (1.57–1.71)	1.65 (1.60–1.68)	0.85	1.64 (1.58–1.69)	1.52 (1.50–1.57)	<0.001
Weight kg	59.5 (55.5–65.1)	63.5 (58.0–67.0)	0.16	61.0 (57.0–67.0)	50.2 (47.1–55.5)	0.005
BMI kg·m^{-2}	22.9 (20.7–23.6)	23.1 (22.0–24.5)	0.3	23.0 (21.1–24.3)	22.1 (20.5–22.9)	0.27
Obesity (BMI ⩾30 kg·m^{-2})	0 (0.0)	0 (0.0)	NA	0 (0.0)	1 (7.1)	0.42
Current smoking	15 (50.0)	11 (36.7)	0.43	26 (43.3)	0 (0.0)	0.006
Pack-years	52.4 (45.2–69.4)	40.0 (25.5–79.5)	0.28	50.0 (33.3–75.0)	0.0 (0.0–0.0)	<0.001
FEV_1/FVC	0.63 (0.54–0.66)	0.77 (0.75–0.79)	<0.001	0.70 (0.63–0.76)	0.77 (0.73–0.80)	0.005
FEV_1 % pred	79.5 (67.2–88.9)	98.0 (87.3–108.4)	<0.001	87.4 (78.2–100.0)	95.5 (93.5–101.0)	0.073
D_{LCO} % pred	72.8 (60.1–87.8)	73.9 (59.1–87.4)	0.81	73.5 (59.6–87.9)	NA	
PRISm	0 (0.0)	3 (10.0)	0.24	3 (5.0)	0 (0.0)	0.92
CLE (⩾ trace)	28 (93.3)	25 (83.3)	0.42	53 (88.3)	1 (7.1)	<0.001
CLE (⩾ moderate)	15 (50.0)	5 (16.7)	0.014	20 (33.3)	0 (0.0)	0.028
PSE (⩾ mild)	20 (66.7)	18 (60.0)	0.79	38 (63.3)	0 (0.0)	<0.001
Any emphysema[#]	29 (96.7)	27 (90.0)	0.61	56 (93.3)	1 (7.1)	<0.001
Pre-COPD		29 (96.7)		29 (48.3)		
COPD	30 (100.0)			30 (50.0)		
Coronary artery calcification	12 (40.0)	12 (40.0)	1	24 (40.0)	1 (7.1)	0.043
Hypertension	10 (33.3)	17 (56.7)	0.12	27 (45.0)	2 (14.3)	0.069
Dyslipidaemia	1 (3.3)	6 (20.0)	0.11	7 (11.7)	1 (7.1)	0.99
ICS use	7 (23.3)	0 (0.0)	0.016	7 (11.7)	0 (0.0)	0.4
Leukocyte count cells·μL^{-1}	5650 (4825–7175)	6200 (4875–7500)	0.72	5750 (4800–7275)	5050 (4600–6075)	0.13
Blood eosinophil count cells·μL^{-1}	168.9 (100.8–260.3)	148.7 (54.2–220.7)	0.29	158.8 (90.2–247.1)	120.9 (54.9–189.1)	0.42
Blood eosinophils %	2.9 (2.1–4.0)	2.4 (1.2–3.3)	0.29	2.7 (1.8–3.6)	2.5 (1.1–4.3)	0.83
Number of measured airways	3.0 (2.0–4.8)	3.0 (2.0–5.0)	0.85	3.0 (2.0–5.0)	3.0 (2.0–4.0)	0.74
Average Pbm mm	2.50 (2.02–3.69)	2.51 (1.74–2.96)	0.43	2.51 (1.97–3.23)	2.07 (1.89–2.81)	0.3
Airway MBP/Pbm mm	1.48 (0.63–2.61)	0.87 (0.44–1.99)	0.086	1.03 (0.56–2.07)	0.33 (0.13–0.56)	0.003

Data are presented as n, median (interquartile range) or n (%), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; PRISm: preserved ratio impaired spirometry; CLE: centrilobular emphysema; PSE: paraseptal emphysema; ICS: inhaled corticosteroid; Pbm: perimeter of basement membrane; MBP: major basic protein; NA: not available. [#]: either at least trace CLE or at least mild PSE.

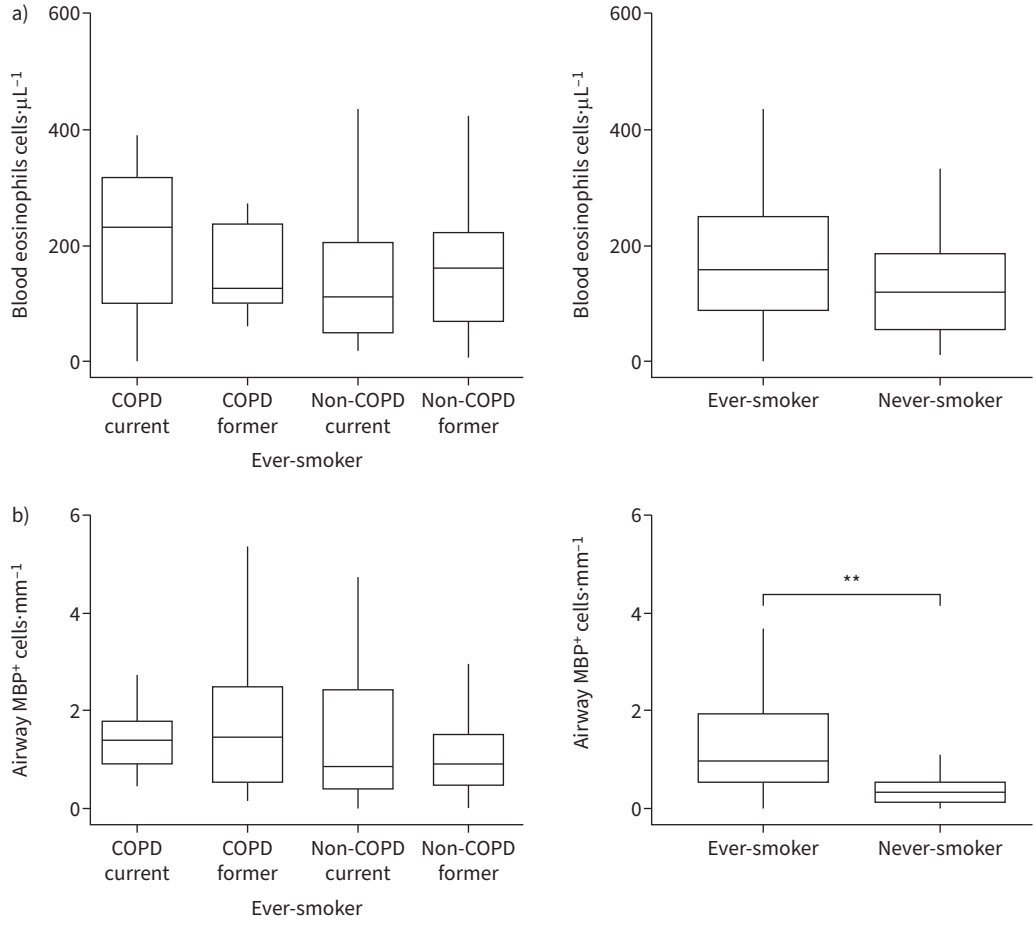

FIGURE 2 Blood eosinophil count and airway major basic protein (MBP)-positive cells in ever- and never-smokers. Blood eosinophil count and airway MBP (eosinophil marker)-positive cells did not differ between current and former smokers with and without COPD. Meanwhile, the number of airway MBP-positive cells/basement membrane perimeter was significantly higher in ever-smokers than in never-smokers, whereas the blood eosinophil count did not differ. **: p<0.01.

the association was maintained in current smokers with and without COPD (ρ=0.60, ρ=0.02 and ρ=0.85, p<0.001, respectively) and former smokers without COPD (ρ=0.63, p=0.004), but not in smokers without COPD (ρ=0.39, p=0.15).

Factors associated with a high number of airway MBP-positive cells in ever-smokers

As shown in table 2, ever-smokers were divided into high and low airway MBP groups based on the median value of the number of airway MBP-positive cells/Pbm (1.03 cells·mm^{-2}), and their characteristics were compared. Age, sex, body size, smoking history, FEV_1 % pred and D_{LCO} % pred, ICS use, the presence of CLE, paraseptal emphysema, coronary artery calcification on CT, the number of measured airways and Pbm did not differ between the two groups. In contrast, the blood eosinophil count and percentage blood eosinophils were significantly higher, and FEV_1/FVC was significantly lower in the high-MBP group than in the low-MBP group.

Moreover, as shown in table 3, multivariable logistic models revealed that a higher blood eosinophil count was associated with the high airway MBP group independent of FEV_1/FVC, current smoking, pack-years and ICS use. Supplementary table S2 shows that this association was also detected in the multivariable model using data of blood eosinophil count from the second most recent blood test before the surgery.

Discussion

This study demonstrated that the number of MBP-positive cells in the small airways of ever-smokers was higher than that in the small airways of never-smokers and that the blood eosinophil count was positively

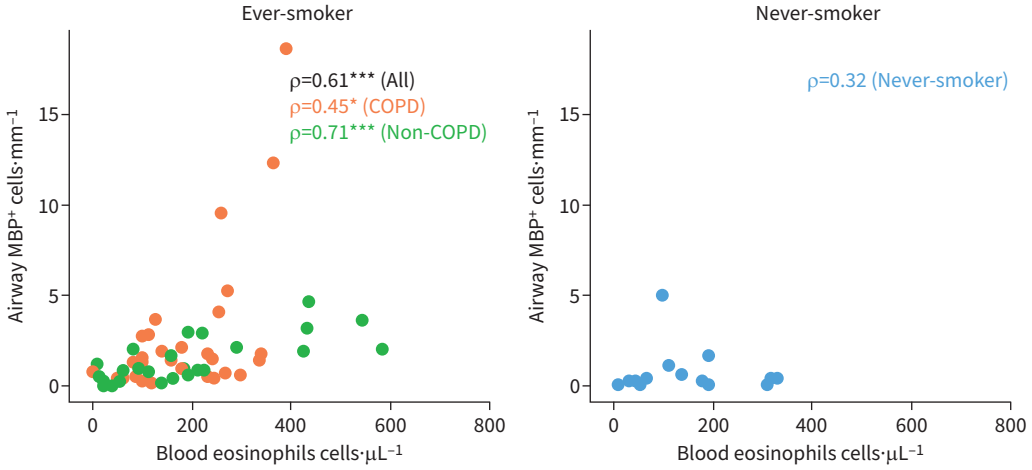

FIGURE 3 Associations between blood eosinophils and airway major basic protein-positive cells in ever- and never-smokers. A higher blood eosinophil count was associated with more airway major basic protein (MBP)-positive cells in ever-smokers with and without COPD, but not in never-smokers. Each correlation coefficient was described as Spearman's ρ. *: p<0.05; ***: p<0.001.

associated with the number of MBP-positive cells in the peripheral airway in smokers with and without COPD, but not in never-smokers. Moreover, the multivariable models demonstrated that the blood eosinophil count was associated with higher MBP-positive cells in ever-smokers independent of FEV_1/FVC, smoking history and ICS use. These findings support the clinical utility of blood eosinophil counts in smokers both before and after COPD development and may help further our understanding of the associations between smoking and eosinophilic inflammation in the small airways.

The observed higher numbers of MBP cells in the small airways of ever-smokers relative to never-smokers is consistent with a previous study by Lacoste *et al.* [27], who reported that the infiltration of eosinophils, but not neutrophils, on bronchial biopsy samples was greater in smokers, including patients with chronic bronchitis without airflow limitation and COPD, than in never-smokers. Moreover, this study showed no significant difference in airway MBP-positive cells/Pbm between smokers with and without COPD. Whether airway eosinophil infiltration is higher in smokers with COPD than in those without COPD remains unestablished [11, 26, 27]. Studies have demonstrated that eosinophilic inflammation is heterogeneous among patients with COPD [1, 28]. It is possible that eosinophilic inflammation in peripheral airways is a feature in subgroups of smokers, especially without COPD and/or with mild COPD.

Interestingly, pre-COPD was dominant in smokers without COPD in this study. This might cause an increase in airway MBP-positive cells in smokers without COPD to a level similar to that in smokers with COPD. Studies have suggested the involvement of eosinophilic inflammation in peripheral airway lesions in COPD: the associations between elevated eicosanoid and eosinophilic inflammation in the airways of lungs with COPD [29]; association between the T2 immune environment and patchy distribution of eosinophils in peripheral lung tissues with COPD [30]; association of sputum eosinophil and small airway dysfunction in mild COPD [31]. Taken together, we speculate that in addition to the direct response to cigarette smoke itself, the local inflammatory response against ongoing remodelling and destruction of the peripheral airways in lungs with COPD and pre-COPD may amplify airway eosinophilic inflammation.

Current smoking was not significantly associated with the high airway MBP. Martinez *et al.* [32] showed that eosinophils in bronchoalveolar lavage fluid is higher in current smokers with COPD than former smokers with COPD, but this difference was not found in smokers without COPD. Willemse *et al.* [33] showed that airway eosinophilic inflammation persists after smoking cessation in COPD. Therefore, the absence of the association between current smoking and the high airway MBP might be due to persistence of airway eosinophilic inflammation in former smokers.

Blood eosinophil count did not differ between smokers with and without COPD. This is consistent with a previous study [32] and contradicts with another study showing that COPD is associated with higher blood eosinophil count [13]. This study also showed no difference in blood eosinophil count between

TABLE 2 Comparison between ever-smokers with high and low numbers of airway major basic protein (MBP)-positive cells

	Low airway MBP	High airway MBP	p-value
Subjects	30	30	
Age years	71.0 (60.5–74.0)	72.0 (65.5–76.0)	0.28
Male	23 (76.7)	26 (86.7)	0.51
Height m	1.62 (1.57–1.67)	1.65 (1.61–1.71)	0.13
BMI kg·m^{-2}	23.1 (22.6–24.5)	22.6 (20.8–23.7)	0.16
Obesity (BMI ⩾30 kg·m^{-2})	0 (0.0)	0 (0.0)	NA
Current smoking	10 (33.3)	16 (53.3)	0.19
Smoking pack-years	47.9 (29.1–78.8)	51.4 (41.3–72.8)	0.47
FEV_1/FVC	0.74 (0.65–0.78)	0.66 (0.57–0.75)	0.026
FEV_1 % pred	91.9 (79.8–105.1)	86.1 (69.4–98.3)	0.18
D_{LCO} % pred	73.2 (60.1–88.1)	73.7 (57.4–86.5)	0.86
PRISm	3 (10.0)	0 (0.0)	0.24
CLE (⩾trace)	25 (83.3)	28 (93.3)	0.42
CLE (⩾moderate)	9 (30.0)	11 (36.7)	0.78
PSE (⩾mild)	16 (53.3)	22 (73.3)	0.18
Any emphysema[#]	27 (90.0)	29 (96.7)	0.61
Pre-COPD	18 (60.0)	11 (36.7)	0.12
COPD	11 (36.7)	19 (63.3)	0.071
Coronary artery calcification	10 (33.3)	14 (46.7)	0.43
Hypertension	13 (43.3)	14 (46.7)	1
Dyslipidaemia	4 (13.3)	3 (10.0)	1
ICS use	3 (10.0)	4 (13.3)	1
Leukocyte count cells·μL^{-1}	5600 (4700–7150)	5850 (5125–7675)	0.35
Blood eosinophil count cells·μL^{-1}	99.7 (50.0–189.0)	225.0 (113.8–339.7)	0.001
Blood eosinophils %	2.0 (1.1–3.1)	3.3 (2.4–4.8)	0.001
Number of measured airways	3.0 (2.3–5.0)	3.0 (2.0–4.8)	0.21
Average Pbm mm	2.34 (1.97–2.92)	2.66 (1.99–3.78)	0.26

Data are presented as n, median (interquartile range) or n (%), unless otherwise stated. Ever-smokers were divided into two groups by the median value of airway MBP-positive cells (1.03 cells·mm^{-2}). BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; D_{LCO}: diffusing capacity of the lung for carbon monoxide; PRISm: preserved ratio impaired spirometry; CLE: centrilobular emphysema; PSE: paraseptal emphysema; ICS: inhaled corticosteroid; Pbm: perimeter of basement membrane; NA: not applicable. [#]: either at least trace CLE or at least mild PSE.

never-smokers and ever-smokers. Considering the possible association between smoking and increased blood eosinophils [13], the lack of significant difference in blood eosinophil count between never-and ever-smokers might be due to the relatively small sample of never-smokers.

Both smokers with and without COPD exhibited significant associations between blood eosinophil counts and the infiltration of MBP cells into the wall of the peripheral airways. It is controversial whether the

TABLE 3 Multivariable logistic models to explore factors associated with high airway major basic protein (MBP)-positive cells in ever-smokers

	Model 1	Model 2
Blood eosinophil count (cells per 100 μL)	2.46 (1.47–4.70)**	2.50 (1.47–4.94)**
FEV_1/FVC (per 0.1)	0.62 (0.35–1.03)	0.59 (0.30–1.07)
Current smoking (yes)		2.13 (0.63–7.56)
Pack-years (per 1)		1.00 (0.98–1.02)
ICS use (yes)		0.50 (0.06 4.22)

Data are presented as odds ratios with 95% confidence intervals. Ever-smokers were divided into two groups according to the median value of airway MBP-positive cells (1.03 cells·mm^{-2}). Multivariable logistic models including high/low airway MBP-positive cells as a dependent variable were constructed. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; ICS: inhaled corticosteroid. **: p<0.01.

blood eosinophil count reliably reflects airway eosinophilic inflammation in patients with COPD. Studies by Kolsum *et al*. [7] and Hartjes *et al*. [34] demonstrated an association between blood eosinophils and airway eosinophils in patients with COPD. In contrast, Turato *et al*. [11] reported no such association in 36 smokers with and without COPD. This discrepancy might be due to the different demographics, particularly the prevalence of comorbidities, between the studies. A recent review by Benson *et al*. [13] suggested that higher blood eosinophil counts are associated with male sex, current smoking, comorbid allergic rhinitis, metabolic syndrome and adiposity.

Therefore, this study compared potential confounding factors between the high and low airway MBP groups. We observed no differences in age, sex, BMI, current smoking, emphysema or coronary artery calcification on CT between smokers with low and high airway MBP cells. Moreover, the multivariable models detected a significant association between the blood eosinophil count and high airway MBP cells even after adjusting for FEV_1/FVC, the proportion of current smokers, smoking pack-years and use of ICS. Furthermore, although blood eosinophil level might be variable over time [35], the additional analyses using data of the second most recent blood test before the surgery, instead of the blood test just before the surgery (used for the main analyses), confirmed the significant association between the blood eosinophil count and the higher airway MBP in the multivariable models. These results could increase the validity of the study and support the concept that the blood eosinophil count is a good biomarker for estimating airway eosinophil inflammation in smokers with and without COPD.

Notably, the association between blood eosinophil count and the infiltration of MBP cells into the small airways was also observed in smokers without COPD. This association was confirmed in a sub-analysis of non-COPD smokers with pre-COPD. To the best of our knowledge, this is the first study to demonstrate the association between blood eosinophil count and infiltration of eosinophils into the small airways in non-COPD smokers without a history of asthma. The concept of pre-COPD is increasingly recognised to identify smokers at high risk of COPD with the goal of providing interventions such as smoking cessation earlier and conducting interventional studies for more efficient drug discovery [21]. Together with previous studies demonstrating the association between higher baseline blood eosinophil counts and future development of airflow limitation in subjects without COPD [14, 36], we speculate that smokers with higher blood eosinophil counts may have higher peripheral airway eosinophil inflammation and represent a category of pre-COPD [21].

The observed greater association between blood and small airway eosinophils in smokers without COPD than in those with COPD is likely to reflect that the immune response may be more complex in COPD than non-COPD [37]. Although pre-COPD may share similar pathophysiological changes to COPD [38], COPD is characterised by more severe airway remodelling/destruction [39, 40], higher frequency of exacerbation and more complex alteration of microbiome [41, 42]. Therefore, these complex inflammatory factors might affect airway eosinophilic inflammation more intensely than eosinophilic blood count and lead to reduce the association between blood and airway eosinophils in patients with COPD.

The current criteria for pre-COPD include the presence of emphysema, but not specific emphysema subtypes [15, 21]. Because at least trace CLE has been shown to be associated with lower FEV_1 and skeletal muscle quantity as well as future greater decline in FEV_1 in smokers without COPD [43, 44], our main analyses defined the presence of emphysema as any CT finding of emphysema (either at least trace CLE or at least mild paraseptal emphysema) to identify smokers with pre-COPD. Meanwhile, a threshold of 5% is used for quantitative CT measurement to define subjects with clinically relevant emphysema [45, 46] and those with pre-COPD [21, 47]. However, the different CT scan conditions in this study did not allow for quantitative emphysema measurement. Alternatively, because the visual finding of moderate CLE is considered when >5% of lung fields are occupied by CLE [20], this study performed the sensitivity analysis based on the other pre-COPD criteria by redefining the presence of emphysema as at least moderate CLE, and confirmed the significant association between blood eosinophil count and airway MBP-positive cells. Considering that visual emphysema subtyping may provide additional clinical information, such as mortality, which quantitative emphysema measurement could not fully detect [48], further studies are needed to test whether pre-COPD should be defined based on quantitative emphysema measurement or visual emphysema subtyping, or a combination of both.

This study tested the association of blood eosinophil count with the airway MBP-positive cells on histological tissue samples from one lobe based on the assumption that the peripheral airway MBP positive cells from a part of lungs could represent airway eosinophilic inflammation in the whole lung. It is technically difficult to perform sampling of lung tissues from multiple lobes except in cases undergoing lung transplantation. This assumption is supported by Higham *et al*. [49] who showed good ICC of airway

submucosal eosinophil counts between different biopsy sites from the same bronchoscopy in patients with COPD. However, those authors also showed that the presence of higher levels of eosinophilic airway inflammation is more spatially heterogeneous than that of lower levels of eosinophilic airway inflammation in patients with COPD. Therefore, we should be aware that the regional variation of airway eosinophilic inflammation might affect the present findings particularly in smokers with the presence of higher levels of airway eosinophilic inflammation.

There are several limitations. First, the number of airways analysed was relatively small (median: three airways). However, this paper is superior in that the sample size is larger than in previously reported studies. Second, due to the small number of subjects without COPD or pre-COPD, it is unclear whether the association between blood eosinophil count and airway MBP-positive cells can be extrapolated to this population. Third, lung tissues were obtained by lung lobectomy for tumour resection. The presence of a tumour may have affected the results. Fourth, the number of never-smokers was small and the majority was female. The imbalance of sex distribution in ever- and never-smokers might affect the comparisons of eosinophil count in the blood and peripheral airways. Fifth, many smokers with COPD were categorised as GOLD 1 and GOLD 2. Because a previous study showed that eosinophilic infiltration on human lung tissue is greater in severe COPD than in mild COPD [30], the applicability of the present findings to smokers with severe COPD needs to be further tested. Lastly, the relatively smaller sample size might have caused a false positive for multiple testing. This point should be verified in future studies of large sample size.

In conclusion, the blood eosinophil count was associated with airway eosinophil infiltration in ever smokers even after adjusting for smoking history, pulmonary function and ICS use. The association between the blood eosinophil count and airway eosinophil infiltration in smokers with and without COPD suggests that the blood eosinophil count can be used in the management of patients with COPD and may also represent a method to identify smokers without COPD who may have airway eosinophilic inflammation and be at potential risk for future COPD development.

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