Exploring the roles of airway dipeptidyl peptidase 1 in obstructive airway disease

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Shareable abstract (@ERSpublications)
A greater sputum level of dipeptidyl peptidase 1 is associated with airway dilation without mucus plugging and altered microbiome, such as reduced colonisation of the phylum Firmicutes, in chronic obstructive airway disease with neutrophilic inflammation https://bit.ly/3C6ZMD4

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Abstract

Background Dipeptidyl peptidase 1 (DPP1) exacerbates airway neutrophilic inflammation in bronchiectasis, which is characterised by airway dysfunction and dilation and chronic bacterial infection. However, little is known about the pathogenetic roles of DPP1 in obstructive airway diseases, including COPD, asthma and asthma–COPD overlap (ACO). Here, we tested the hypothesis that airway DPP1 could enhance neutrophilic inflammation and affect mucus plugging, airway dilation and the airway microbiome in patients with these diseases.

Methods Sputum DPP1, cell differential count and microbiome were cross-sectionally evaluated in patients with COPD, asthma with airflow limitation and ACO. Sputum high mobility group box 1 (HMGB1) was measured to estimate airway epithelial damage. Chest computed tomography was also performed to visually assess mucus plugs and airway dilation with the Reiff score and quantify the total airway count and wall area percentage.

Results 68 patients were classified into high-DPP1/high-neutrophil (n=17), low-DPP1/high-neutrophil (n=37) and low-neutrophil (n=14) groups based on sputum DPP1 levels and neutrophil percentages. The rate of mucus plugging and the relative abundance of the phylum Firmicutes were significantly lower and the level of sputum HMGB1 was significantly greater in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil group. Moreover, airway dilation without mucus plugging was observed only in the high-DPP1/high-neutrophil group (prevalence 29%).

Conclusions High sputum DPP1 levels may reduce colonisation by the phylum Firmicutes and mucus plugging, but increase airway epithelial damage, which could induce airway dilation without mucus plugging in patients with obstructive airway disease with neutrophilic inflammation.

Introduction

Asthma, COPD and asthma–COPD overlap (ACO) are obstructive airway diseases with heterogeneous pathophysiological changes and clinical burdens [1–3]. While efforts have been made to assign patients with obstructive airway disease to a specific clinical diagnosis, improvements in their clinical outcomes are not fully satisfactory, and the importance of treatable traits is increasingly recognised [4]. Airway eosinophilic inflammation and type 2 inflammation are well-known treatable traits that can be efficiently managed by treatments with inhaled corticosteroids (ICSs) and biologics in patients with asthma or COPD [5, 6]. Airway neutrophilic inflammation is also observed in many patients with COPD and in a subgroup

of patients with asthma, although it is yet able to be controlled by targeted therapy [7]. A better understanding of the pathophysiological changes in chronic obstructive neutrophilic airway disease is critical to improve clinical outcomes.

Neutrophil serine proteases are critical for host defence against bacterial infection [8], but excessive release of neutrophil serine proteases causes airway and lung destruction *via* elastin degradation [9]. Neutrophil serine proteases are initially synthesised as inactive zymogens and are activated by dipeptidyl peptidase 1 (DPP1) during neutrophil differentiation in the bone marrow. This cascade has received much attention on the basis of studies showing that the DPP1 inhibitor brensocatib suppresses serine protease activity in neutrophils and reduces the exacerbation frequency in patients with bronchiectasis [9, 10]. Bronchiectasis appears to be driven by a "vicious vortex" involving chronic inflammation, airway dysfunction (*e.g.* ciliary dysfunction and mucus hypersecretion), chronic airway bacterial infection and structural alterations (*e.g.* airway dilation and lung destruction) [11]. Notably, bronchiectasis is observed in patients with COPD and asthma [12, 13], and a type 2-low inflammatory phenotype is associated with Gram-negative bacterial colonisation in patients with refractory asthma with bronchiectasis [14]. Moreover, DPP1 can be detected in bronchoalveolar lavage fluid from patients with asthma [15]. However, little is known about the pathophysiological roles of DPP1 in the airways of patients with asthma, COPD and ACO.

Transcriptomic, proteomic and multiomic analyses have revealed the variability of neutrophilic phenotypes in patients with asthma by identifying several neutrophilic clusters characterised by distinct antimicrobial capacities of neutrophils, the airway microbiome and host immune responses [16–18]. Therefore, it was hypothesised that the extent of DPP1 expression in airways may vary among patients with airway neutrophilic inflammation, and the activation of neutrophils through DPP1 upregulation in airways may affect airway structure, host immune responses, mucus plugging and the airway microbiome in chronic obstructive airway diseases. This study aimed to quantify DPP1 levels and neutrophil counts in the sputum, and to classify patients with ACO, COPD and asthma with airflow limitation into groups of those with higher DPP1 levels and neutrophil counts in the sputum (high-DPP1/high-neutrophil group), those with lower DPP1 levels and higher neutrophil counts (low-DPP1/high-neutrophil group) and those with low neutrophil counts (low-neutrophil group). The study compared the extent of airway dilation and mucus plugging on computed tomography (CT), the sputum microbiome, and the sputum levels of high mobility group box 1 (HMGB1) and secretory IgA (sIgA) representing epithelial damage and mucosal immunity, respectively [19, 20], in the high-DPP1/high-neutrophil group to those in the low-DPP1/high-neutrophil and low-neutrophil groups.

Methods

Detailed Methods are provided in the supplementary methods.

Study design and population

We used data from a completed cross-sectional study that prospectively enrolled stable patients with chronic obstructive airway disease, including ACO, asthma with airflow limitation and COPD, and examined the microbiome in sputum between 2019 and 2020 in Japan [21]. All patients had airflow limitation, *i.e.* forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) <0.7 on post-bronchodilator spirometry. Asthma and COPD were diagnosed based on the Global Initiative for Asthma and the Global Initiative for Chronic Obstructive Lung Disease reports [1, 2]. ACO was subsequently identified based on the management guideline for ACO published by the Japanese Respiratory Society [3]. Briefly, ACO was defined as the presence of chronic airflow limitation with a combination of COPD characteristics (either ⩾10 pack-years smoking history, emphysema on CT or impaired diffusion capacity) and asthma characteristics (two or more of the following factors: variable respiratory symptoms, history of asthma before age 40 years, fractional exhaled nitric oxide >35 ppb and multiple clinical asthma-like features). Among the originally enrolled patients, this study included patients whose DPP1 levels and differential cell counts in sputum were measured and whose chest CT was performed at Kyoto University Hospital (Kyoto, Japan). This study was approved by the Ethics Committee of Kyoto University (approval R1705-2) and registered with the University Hospital Medical Information Network (UMIN000013020). Each patient provided written informed consent.

Clinical assessments

Data on post-bronchodilator spirometry were normalised *via* a Japanese reference equation [22]. A history of exacerbations requiring the use of antibiotics and/or systemic corticosteroids in the previous year was confirmed, as reported [21].

Sputum cell differential count, DPP1 level and microbiome assessment

Differential cell counts were obtained from sputum cell samples stained *via* the May–Giemsa method. The sputum eosinophil (or neutrophil, macrophage) percentage was calculated as the percentage of the sputum eosinophil (or neutrophil, macrophage) count to the total cell count. Airway neutrophil inflammation was defined as a sputum neutrophil percentage ⩾61%, as previously reported [23]. ELISA kits for DPP1 (ab277434; Abcam, Cambridge, UK), HMGB1 (326078738; SHINO-TEST, Tokyo, Japan) and sIgA (K8870; Immunodiagnostik, Bensheim, Germany) were used to measure their sputum concentrations. We arbitrarily determined the 75th percentile of the sputum DPP1 concentration as the cut-off for high/low DPP1. Furthermore, bacterial DNA was extracted from induced or spontaneous sputum samples. We amplified the V3–V4 region of the 16S rRNA genes *via* a 16S Metagenomic Sequencing Library Preparation Kit (Illumina, San Diego, CA, USA), processed the sequencing reads *via* MiSeq and QIIME 1.9.1 [24], and assigned the taxonomy *via* Greengenes 13.8. The relative abundance of taxa was calculated as the percentage of the total read count within each phylum (or genus) in relation to the overall read count. As previously reported [25], we focused on *a priori* determined microbiome constituents, which included the phyla Firmicutes, Proteobacteria, Fusobacteria, Bacteroidetes and Actinobacteria, as well as the genera *Porphyromonas*, *Moraxella*, *Haemophilus*, *Streptococcus*, *Fusobacterium* and *Pseudomonas*, based on their reported impacts on asthma and/or COPD [21, 26, 27].

CT acquisition and analyses

Full-inspiratory chest CT data were obtained *via* an Aquilion Precision or PRIME scanner (Cannon Medical Systems, Otawara, Japan). Images with 0.5- and 1.0-mm slice thickness were reconstructed with FC51 sharp and FC13 soft kernels for analyses of airway and lung parenchyma, respectively. The extent of airway dilation was visually scored by two chest physicians experienced with CT using a modified Reiff score [28, 29]. The number of lobes (right upper, right middle, right lower, left upper, left lingula and left lower lobes) and the extent of dilation (tubular=1, varicose=2 and cystic=3) were assessed (scores ranging from 0 to 18). The intraclass correlation coefficient (ICC) for the two inspectors was 0.81 (95% CI 0.72–0.87). The scores of the two inspectors were averaged and the presence of airway dilation was defined as a Reiff score ⩾1. Moreover, we used mucus plug score, low attenuation volume percentage (LAV%) for emphysema severity, total airway count (TAC), and mean lumen area, wall area and wall area percentage (WA%) of segmental branches of the right apical, right lateral, right posterior basal, left apicoposterior and left anteromedial basal paths, all of which were obtained in our previous study [30]. Briefly, mucus plugging was visually scored based on bronchopulmonary segment anatomy [31] by two inspectors (ICC 0.85 (95% CI 0.78–0.90), as previously reported [30]). The scores were averaged. The presence of mucus plugs was defined as a mucus plug score ⩾1. LAV% and TAC values were computed *via* SYNAPSE VINCENT software (Fujifilm Medical, Tokyo, Japan).

Statistics

Patients were divided into three groups based on sputum neutrophil percentage and DPP1 level. Continuous variables were compared among the three groups *via* ANOVA followed by *post hoc* Tukey tests or Wilcoxon rank tests followed by *post hoc* Dunnett tests. Categorical variables were compared among the three groups *via* Fisher's exact test followed by multiple Fisher comparisons with Holm correction. In patients with higher sputum neutrophil percentages (⩾61%), multivariable logistic models were constructed by using the mucus score, presence of a Reiff score ⩾1, sputum HMGB1 level, sputum relative abundance of the phylum Firmicutes, percentage predicted FEV_1, sputum eosinophil percentage and clinical diagnosis (COPD, asthma or ACO) [32, 33]. Statistical analysis was performed using R version 4.2.3 (www.r-project.org). A p-value <0.05 was considered statistically significant.

Results

Patient characteristics stratified by sputum neutrophil count and DPP1 level

As shown in figure 1a, a total of 68 patients were divided into three groups, *i.e.* high-DPP1/high-neutrophil (n=17), low-DPP1/high-neutrophil (n=37) and (low-DPP1) low-neutrophil (n=14) groups, based on cut-off values of 61% for the sputum neutrophil percentage and the 75th percentile of the sputum DPP1 concentration (227.1 $ng \cdot mL^{-1}$). No patient had <61% of neutrophils in sputum and a sputum DPP1 concentration >75th percentile. As shown in figure 1b, the percentage of sputum eosinophils and macrophages in the high-DPP1/high-neutrophil group was comparable to that in the low-DPP1/high-neutrophil group but significantly lower than that in the low-DPP1/low-neutrophil group. Figure 1c shows no significant difference in the proportion of asthma/ACO/COPD patients among the three groups. The clinical characteristics of the patients in the three groups are summarised in table 1. There were no significant differences in age, sex, smoking history, FEV_1/FVC, percentage predicted FEV_1, or the use of ICS, oral corticosteroid or macrolide.

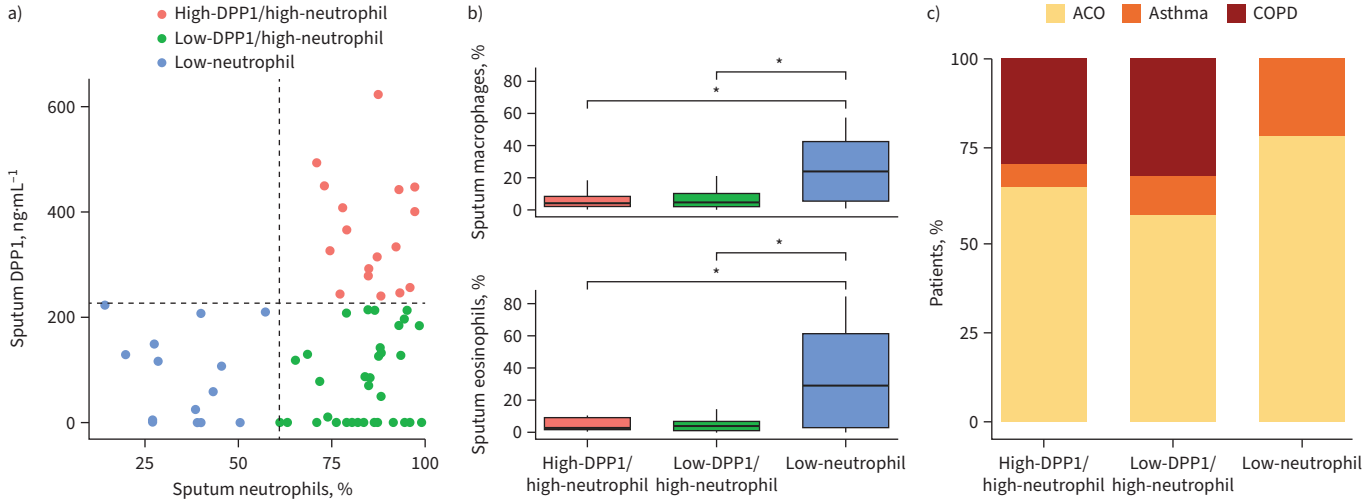

FIGURE 1 Patient stratification based on sputum dipeptidyl peptidase 1 (DPP1) levels and neutrophil counts and their associations with sputum eosinophil count and airway disease diagnosis. a) Distribution of sputum DPP1 levels and sputum neutrophil percentages. Patients were classified into three groups according to the sputum neutrophil percentage (61%) and the 75th percentile of the sputum DPP1 level (227.1 ng·mL^{-1}): high-DPP1/high-neutrophil, low-DPP1/high-neutrophil and low-neutrophil groups. b) The sputum eosinophil and macrophage counts were greater in the low-neutrophil group than in the other groups. c) The proportions of patients with asthma, COPD and asthma–COPD overlap (ACO) did not significantly differ among the three groups. *: p<0.05 based on the Wilcoxon rank test followed by the *post hoc* Dunnett test.

Mucus plugs and airway structure observed on CT in patients stratified by sputum neutrophil count and DPP1 level

Table 2 presents a summary of the CT findings for the three groups. Mucus plug scores in the high-DPP1/high-neutrophil group were significantly lower than those in the low-DPP1/high-neutrophil group and the low-neutrophil group. As shown in figure 2a and b, the rate of mucus plug presence (mucus plug score ⩾1) was significantly lower and the rate of airway dilation (Reiff score ⩾1) tended to be greater in the high-DPP1/high-neutrophil group than in the other groups. Moreover, as shown in figure 2c, airway dilation without mucus plugging (Reiff score ⩾1 and mucus plug score 0) was observed in 29% of patients in the high-DPP1/high-neutrophil group but not in the other groups (0%; p<0.01).

Sputum immune response mediators and the microbiome in patients stratified by sputum neutrophil count and DPP1 level

Table 3 presents a summary of the sputum mediators and the relative abundances of bacterial phyla and genera in the sputum of the three groups. While the sIgA level did not differ among the three groups, the HMGB1 concentration was significantly greater in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil group (figure 3a). Moreover, the relative abundance of the phylum Firmicutes was significantly lower in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil group (figure 3b). In contrast, the relative abundance of the genus *Haemophilus* did not differ between the high-DPP1/high-neutrophil group and the low-DPP1/high-neutrophil group (figure 3c). Moreover, analyses were performed separately in patients with ACO and COPD (supplementary tables S1–S4). As shown in supplementary figure S1, mucus plugging tended to be less frequent in the high-DPP1/high-neutrophil group for both ACO and COPD patients. Patients with COPD also showed significantly higher sputum HMGB1 concentration and relative abundance of the phylum Proteobacteria and significantly lower relative abundance of the phylum Firmicutes in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil group.

Subanalyses of patients with airway neutrophilic inflammation

To explore factors associated with high DPP1 levels in the context of airway neutrophilic inflammation, subanalyses were performed for patients whose sputum neutrophil count was ⩾61% (n=54). As shown in supplementary figure S2, a lower mucus plug score and higher HMGB1 level in the sputum were significantly associated with a higher DPP1 level in the sputum (mucus plug score: $\rho = -0.33$, p=0.01; HMGB1: $\rho = 0.45$, p=0.0006). Moreover, as shown in table 4, multivariable logistic models revealed that a lower mucus plug score and a lower relative abundance of the phylum Firmicutes were independently associated with high DPP1 concentrations (⩾227.1 ng·mL^{-1}) in the sputum. This association was detected

TABLE 1 Characteristics of patients with chronic obstructive airway disease stratified by sputum dipeptidyl peptidase 1 (DPP1) level and neutrophil count (n=68)

	All	High-DPP1/ high-neutrophil	Low-DPP1/ high-neutrophil	Low-neutrophil	p-value
Patients	68	17	37	14	
Age, years	70.2±9.5	71.7±8.4	69.9±9.9	69.1±10.2	0.74
Female	11 (16)	3 (18)	6 (16)	2 (14)	0.97
Height, m	1.64±0.08	1.64±0.07	1.64±0.08	1.63±0.09	0.96
BMI, kg·m^{-2}	24.0±3.3	24.3±3.0	23.6±2.8	24.8±4.6	0.50
Smoking status					0.94
Never-smoker	20 (29)	5 (29)	10 (27)	5 (36)	
Former-smoker	37 (54)	10 (59)	20 (54)	7 (50)	
Current smoker	11 (16)	2 (12)	7 (19)	2 (140)	
Smoking history, pack-years	25.9 (1.7–48.0)	31.0 (0.0–58.8)	28.5 (0.9–48.0)	10.4 (4.1–26.3)	0.55
FEV_1/FVC	0.56±0.09	0.54±0.09	0.56±0.10	0.59±0.08	0.33
FEV_1, % pred	71.3±18.8	72.0±19.7	70.7±19.9	72.4±15.6	0.95
Sputum eosinophil count, %	11.6±18.4	6.7±7.4	5.9±6.6	32.7±30.8*	<0.01
Sputum neutrophil count, %	74.7±22.5	85.6±8.7	84.5±10.2	35.6±12.0*	<0.01
Sputum macrophage count, %	10.8±14.0	6.3±6.5	7.2±7.7	25.8±22.1	<0.01
Blood eosinophils, µL^{-1}	259.5 (138.4–518.3)	258.8 (212.3–448.5)	220.0 (110.6–459.2)	501.9 (259.3–590.7)	0.09
IgE, IU·mL^{-1}	165.0 (53.3–625.0)	130.0 (69.0–200.0)	100.0 (44.0–620.0)	425.0 (170.0–620.0)	0.36
F_{ENO}, ppb	34.5 (24.0–58.5)	34.0 (31.0–58.0)	30.0 (21.0–52.0)	52.0 (34.5–64.8)	0.12
CAT score	9.0 (4.0–13.0)	8.0 (3.0–13.0)	8.0 (5.0–11.0)	10.5 (5.5–12.8)	0.94
CAT ⩾10	30 (44)	8 (47)	14 (38)	8 (57)	0.45
Chronic bronchitis#	10 (15)	1 (6)	7 (19)	2 (14)	0.45
ICS	55 (81)	13 (77)	28 (76)	14 (100)	0.12
Macrolide	9 (13)	2 (12)	5 (14)	2 (14)	0.98
OCS	8 (12)	2 (11)	3 (8)	3 (21)	0.42
Biologics	6 (9)	1 (6)	3 (8)	2 (14)	0.70
Diagnosis					0.14
ACO	43 (63)	11 (65)	21 (57)	11 (79)	
COPD	17 (25)	5 (29)	12 (32)	0 (0)	
Asthma	8 (12)	1 (6)	4 (11)	3 (21)	
Exacerbation antibiotics	7 (10)	0 (0)	5 (14)	2 (14)	0.27
Exacerbation steroid	12 (18)	3 (18)	6 (16)	3 (21)	0.91

Data are presented as n, n (%), mean±SD or median (interquartile range), unless otherwise stated. BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; F_{ENO}: fractional exhaled nitric oxide; CAT: COPD Assessment Test; ICS: inhaled corticosteroid; OCS: oral corticosteroid; ACO: asthma–COPD overlap. #: chronic bronchitis was defined as CAT1 (cough) score ⩾3 and CAT2 (sputum) score ⩾3. p-values were calculated via ANOVA, the Wilcoxon rank test or Fisher's exact test. *: p<0.05 compared with the low-DPP1/high-neutrophil group on the basis of *post hoc* Tukey multiple comparison.

even after adjusting for sputum eosinophil percentage, percentage predicted FEV_1 and clinical diagnosis (COPD, asthma or ACO). As shown in supplementary table S5, in sensitivity analyses that defined patients with airway neutrophilic inflammation as those with sputum neutrophil percentage ⩾70% (n=50) or ⩾80% (n=38), a lower mucus plug score and a lower relative abundance of the phylum Firmicutes were also independently associated with high sputum DPP1 levels.

Discussion

In this study, mucus plugging occurred less frequently in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil and low-neutrophil groups (table 2 and figure 2). Notably, airway dilation without mucus plugging was observed only in the high-DPP1/high-neutrophil group, with a 29% prevalence. Furthermore, the relative abundance of the phylum Firmicutes was lower in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil group (table 3 and figure 3). Although the role of DPP1 in the pathogenesis of bronchiectasis has been intensively investigated [9, 10], to the best of our knowledge this is the first study to show the associations of sputum DPP1 levels with airway dilation without mucus plugging and a lower relative abundance of the phylum Firmicutes in the ACO-enriched population without a prior diagnosis of definite bronchiectasis.

A lower mucus plugging rate and a lower relative abundance of Firmicutes were independently associated with high DPP1 levels in the sputum according to the multivariable models of patients with airway

TABLE 2 Computed tomography findings in patients with chronic obstructive airway disease stratified by sputum dipeptidyl peptidase 1 (DPP1) level and neutrophil count (n=68)

	High-DPP1/ high-neutrophil (n=17)	Low-DPP1/ high-neutrophil (n=37)	Low-neutrophil (n=14)	p-value
WA%, %	60.6±4.2	60.9±4.1	62.7±5.3	0.344
Lumen area, mm^2	21.4±5.2	20.8±5.1	20.7±7.7	0.94
Wall area, mm^2	33.0±5.5	31.5±5.3	35.4±8.5	0.13
TAC, n	346.1±88.5	366.8±118.5	298.4±109.5	0.148
Mucus plug score	0.0 (0.0–1.0)*	1.5 (0.0–4.5)	5.5 (2.4–8.6)*	0.004
Mucus plug score ⩾1	5 (29)*	24 (65)	11 (79)	0.012
Reiff score	0.00 (0.00–1.00)	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.395
Reiff score ⩾1	5 (29)	6 (16)	1 (7)	0.255
Reiff score ⩾1 without mucus plugs	5 (29)*	0 (0)	0 (0)	<0.01
LAV%, %	4.7 (2.5–14.4)	5.6 (2.9–9.9)	3.9 (1.1–6.8)	0.23

Data are presented as mean±SD, median (interquartile range) or n (%), unless otherwise stated. WA%: wall area percentage; TAC: total airway count; LAV%: low attenuation volume percentage. p-values were calculated via ANOVA, the Wilcoxon rank test or Fisher's exact test. *: p<0.05 compared with the low-DPP1/high-neutrophil group on the basis of the *post hoc* Dunnett multiple comparison test or multiple Fisher's exact tests with Holm correction.

neutrophilic inflammation (sputum neutrophil percentages ⩾61% in table 4; ⩾70% or ⩾80% in supplementary table S5). In a murine model, the neutrophil serine protease cathepsin G and neutrophil elastase are critical for neutrophil-mediated killing of *Streptococcus pneumoniae* [34], a member of the phylum Firmicutes. Although neutrophil elastase was not measured in this study, our data suggest that greater DPP1 may activate neutrophil elastase, which could reduce the colonisation of the phylum Firmicutes in the airways of this ACO-enriched population.

Patients with airway dilation without mucus plugging (Reiff score ⩾1 and mucus plug score 0) were identified only in the high-DPP1/high-neutrophil group. Moreover, sputum HMGB1 level was greater in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil group (table 3 and figure 3). HMGB1 is an immune response mediator belonging to the alarmin family that is passively released from damaged cells [20]. Sputum HMGB1 levels are greater in patients with asthma and COPD than in control subjects, and higher HMGB1 levels are associated with lower lung function and higher sputum neutrophil

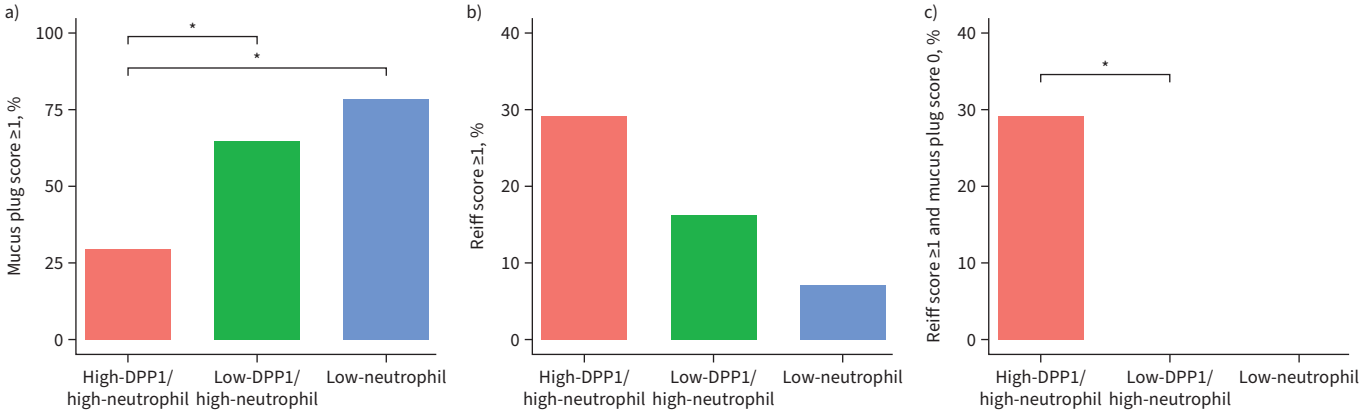

FIGURE 2 Computed tomography findings of mucus plugging and airway dilation in patients stratified by sputum dipeptidyl peptidase 1 (DPP1) concentration and neutrophil percentage. Patients were classified into three groups according to the sputum neutrophil (61%) percentage and the 75th percentile of the sputum DPP1 level (227.1 ng·mL^{-1}): high-DPP1/high-neutrophil, low-DPP1/high-neutrophil and low-neutrophil groups. a) The rate of mucus plugging, defined as a mucus plug score ⩾1, was significantly lower in the high-DPP1/high-neutrophil group than in the other groups. b) The rate of airway dilation, defined as a Reiff score ⩾1, tended to be greater in the high-DPP1/high-neutrophil group. c) Airway dilation without mucus plugging, defined as a combination of a Reiff score ⩾1 and a mucus plug score 0, was observed in the high-DPP1/high-neutrophil group (29%) but not in the other groups (0% and 0%). *: p<0.05 based on Fisher's exact test followed by multiple Fisher comparisons with Holm correction.

TABLE 3 Sputum mediators and relative bacterial abundance in patients with chronic obstructive airway disease stratified by sputum dipeptidyl peptidase 1 (DPP1) level and neutrophil count (n=68)

	High-DPP1/ high-neutrophil (n=17)	Low-DPP1/ high-neutrophil (n=37)	Low-neutrophil (n=14)	p-value
Sputum mediators				
sIgA, ng·mL^{-1}	10 455.1 (6446.6–12 422.2)	9356.5 (3530.7–12 023.0)	6854.8 (3169.7–10 486.9)	0.31
HMGB1, ng·mL^{-1}	378.3 (329.6–508.3)*	228.3 (118.5–325.2)	365.4 (199.8–581.6)	0.03
DPP1, ng·mL^{-1}	332.9 (278.5–441.6)	10.7 (0.0–129.8)	82.6 (1.3–143.9)	<0.001
Phylum				
Firmicutes, %	30.4 (24.5–32.2)*	33.9 (29.3–36.1)	31.1 (25.8–37.4)	0.04
Proteobacteria, %	23.0 (16.6–25.1)	17.6 (9.7–26.9)	18.8 (13.9–22.9)	0.48
Fusobacteria, %	8.1 (5.8–9.7)	7.4 (4.9–11.8)	7.1 (3.5–10.6)	0.94
Bacteroidetes, %	27.4 (12.6–30.9)	19.8 (13.0–26.4)	16.6 (8.9–25.1)	0.13
Actinobacteria, %	6.7 (5.3–8.7)	8.3 (6.0–10.6)	10.8 (5.7–13.1)	0.17
Genus				
Porphyromonas, %	5.1 (1.2–7.2)	2.4 (1.0–4.1)	2.0 (0.5–3.3)	0.27
Streptococcus, %	10.8 (9.6–13.8)	15.1 (10.7–17.9)	16.3 (13.4–18.4)	0.05
Fusobacterium, %	5.3 (2.7–6.8)	4.2 (2.3–5.8)	3.6 (1.5–4.4)	0.40
Moraxella, %	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.16
Haemophilus, %	3.3 (1.8–6.2)	4.3 (2.2–8.4)	1.7 (0.7–3.5)*	0.03
Pseudomonas, %	0.10 (0.02–0.23)	0.05 (0.02–0.15)	0.31 (0.14–0.67)*	0.04

Data are presented as median (interquartile range), unless otherwise stated. sIgA: secretory IgA; HMGB1: high mobility group box 1. p-values were calculated via the Wilcoxon rank test. *: p<0.05 compared with the low-DPP1/high-neutrophil group on the basis of the *post hoc* Dunnett multiple comparison test.

counts [35]. Mucus plugging is induced by hypersecretion of polymeric mucins such as MUC5AC due to multiple causes such as infection [36] and subsequent cross-linking and mucus gel stiffening due to oxidation from airway inflammation [37, 38]. Collectively, we speculate that in the ACO-enriched population with higher DPP1 levels and neutrophil counts in the sputum, alteration of the microbiome in the airways may be associated with lower production of polymeric mucins from the epithelial cells and lower frequency of mucus plug formation, while DPP1-induced activation of neutrophils causes airway

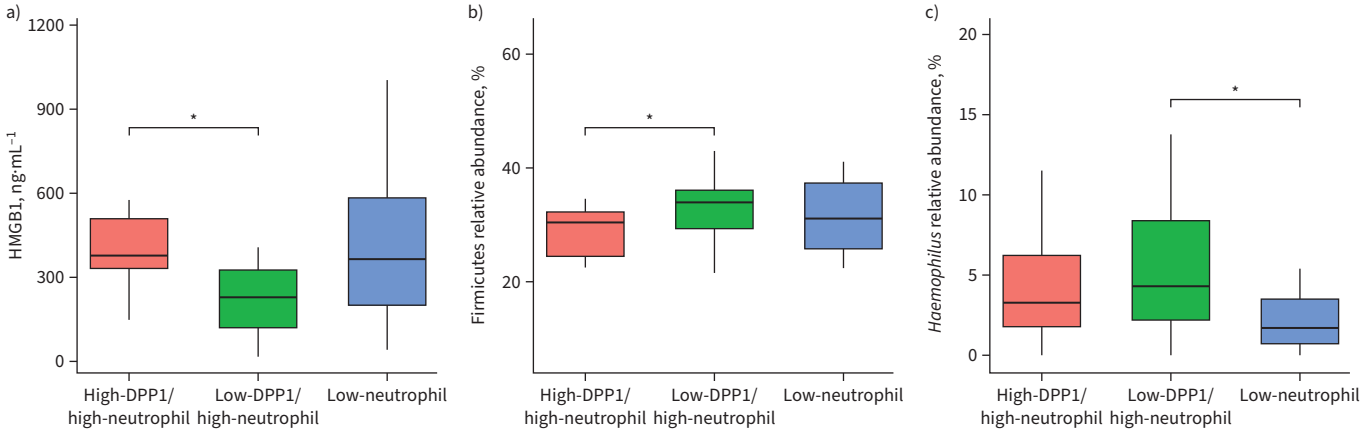

FIGURE 3 Sputum high mobility group box 1 (HMGB1) levels and the microbiome in patients stratified by sputum dipeptidyl peptidase 1 (DPP1) concentration and neutrophil percentage. Patients were classified into three groups according to the sputum neutrophil percentage (61%) and the 75th percentile of the sputum DPP1 level (227.1 ng·mL^{-1}): high-DPP1/high-neutrophil, low-DPP1/high-neutrophil and low-neutrophil groups. a) HMGB1 levels and b) the relative abundance of the phylum Firmicutes in the sputum were significantly higher and lower, respectively, in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil group. c) The relative abundance of the genus *Haemophilus* did not differ between the high-DPP1/high-neutrophil group and the low-DPP1/high-neutrophil group but was significantly lower in the low-neutrophil group. *: p<0.05 based on the Wilcoxon rank test followed by the *post hoc* Dunnett test.

TABLE 4 Multivariable models constructed to explore factors associated with high sputum dipeptidyl peptidase 1 (DPP1) levels in patients with airway neutrophilic inflammation (n=54)

	Model 1	Model 2	Model 3
Mucus score, per +1	0.69 (0.49–0.91)*	0.64 (0.41–0.88)*	0.59 (0.37–0.83)*
Reiff score ⩾1, yes	4.32 (0.78–30.26)	4.53 (0.77–30.49)	4.34 (0.73–3.46)
Sputum HMGB1, per +10	1.01 (0.99–1.04)	1.01 (0.99–1.04)	1.01 (0.99–1.04)
Sputum relative abundance of phylum Firmicutes, per +1	0.85 (0.74–0.95)*	0.82 (0.70–0.94)*	0.81 (0.69–0.93)*
FEV_1 % pred, per +1		0.98 (0.93–1.02)	0.97 (0.92–1.02)
Sputum eosinophil percentage		1.04 (0.93–1.16)	1.04 (0.94–1.17)
Diagnosis			
COPD			1 (reference)
Asthma			6.40 (0.14–388.61)
ACO			2.57 (0.50–1.52)

Multivariable models were constructed using mucus plug score and Reiff score on computed tomography, sputum high mobility group box 1 (HMGB1) level, and sputum relative abundance of the phylum Firmicutes as basic independent variables, and percentage predicted forced expiratory volume in 1 s (FEV_1) on spirometry, sputum eosinophil percentage and clinical diagnosis as additional independent variables. The presence of high DPP1 levels compared with low DPP1 levels was included as a dependent variable. ACO: asthma–COPD overlap. *: p<0.05.

epithelial damage and airway dilation by destroying elastin components in the airways. Therefore, DPP1 might be a therapeutic target in patients with chronic obstructive airway disease, mainly ACO, even when there is no clinical diagnosis of bronchiectasis.

Of note, because the original aim of this cohort was to examine the airway microbiome in an ACO-enriched population, the mean eosinophil percentages in the high-DPP1/high-neutrophil and the low-DPP1/high-neutrophil groups (6.7% and 5.9%, respectively) are higher than reported values in patients with COPD [39]. In separate analyses for patients with ACO and COPD (supplementary figure S3), the greater relative abundance of the phylum Proteobacteria in the high-DPP1/high-neutrophil group than in the low-DPP1/high-neutrophil groups was observed only in patients with COPD. Moreover, in contrast to mucus plugging, TAC and WA% in the high-DPP1/high-neutrophil group did not differ compared with the other two groups, and the association between mucus plug score and TAC ($\rho = -0.33$, $p=0.003$) (supplementary figure S3) was weaker than a reported association in patients with COPD [25]. Therefore, the pathophysiological roles of airway DPP1 in the ACO-enriched population might be different from those in patients with type 2-low chronic airway diseases such as (non-eosinophilic) COPD.

The mucus plug score, rate of mucus plugging and sputum eosinophil percentage were greater in the low-neutrophil group than in the other groups. This result is consistent with a previous finding that eosinophilic inflammation is associated with greater mucus plugging [31].

Macrolide treatment is effective against neutrophilic inflammation [40] and may affect the DPP1 concentration in the airways. However, because the rate of macrolide use did not differ among the three groups, macrolide use is less likely to affect the present findings.

There are several limitations in this study. First, the number of patients was relatively small. However, CT images were obtained under the same scanning conditions, and established methods for CT analysis were used. Second, sputum was obtained *via* spontaneous expectoration or sputum induction. The different methods used for sputum collection might have affected the present findings. Third, although the relative abundance of the phylum Firmicutes significantly differed between the high-DPP1/high-neutrophil and the low-DPP1/high-neutrophil groups, the extent of the difference was small. Whether this difference would have clinically relevant impacts should be further investigated.

In conclusion, greater sputum DPP1 levels accompanied by airway neutrophilic inflammation are associated with a greater rate of airway dilation without mucus plugging; greater airway inflammation, detected as higher HMGB1 levels; and a lower relative abundance of the phylum Firmicutes. These findings increase the understanding of the role of DPP1 in mucus plugging and suggest that increased sputum DPP1 levels might be involved in airway dilation in obstructive airway disease patients with neutrophilic inflammation. Since treatable traits remain unestablished in patients with chronic obstructive neutrophilic airway disease, the potential of airway DPP1 as a target should be further investigated in lung tissue samples from these patients.

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