

# Association of blood eosinophils and plasma periostin with FEV<sub>1</sub> response after 3-month inhaled corticosteroid and long-acting beta<sub>2</sub>-agonist treatment in stable COPD patients

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**Background:** COPD patients with increased airway eosinophilic inflammation show a favorable response to inhaled corticosteroids (ICS) in combination with a long-acting bronchodilator. Recent studies have demonstrated a significant correlation of sputum eosinophilia with blood eosinophils and periostin. We investigated whether high blood eosinophils and plasma periostin were associated with an improvement in forced expiratory volume in 1 second (FEV<sub>1</sub>) after 3-month treatment with ICS/long-acting beta<sub>2</sub>-agonist (LABA) in stable COPD patients.

**Patients and methods:** Blood eosinophils and plasma periostin levels were measured in 130 stable COPD subjects selected from the Korean Obstructive Lung Disease cohort. Subjects began a 3-month ICS/LABA treatment after washout period.

**Results:** High blood eosinophils (>260/μL, adjusted odds ratio =3.52, *P*=0.009) and high plasma periostin (>23 ng/mL, adjusted odds ratio =3.52, *P*=0.013) were significantly associated with FEV<sub>1</sub> responders (>12% and 200 mL increase in FEV<sub>1</sub> from baseline after treatment). Moreover, the addition of high blood eosinophils to age, baseline positive bronchodilator response, and FEV<sub>1</sub> <50% of the predicted value significantly increased the area under the curve for prediction of FEV<sub>1</sub> responders (from 0.700 to 0.771; *P*=0.045).

**Conclusion:** High blood eosinophils and high plasma periostin were associated with improved lung function after 3-month ICS/LABA treatment. In particular, high blood eosinophils, in combination with age and baseline lung function parameters, might be a possible biomarker for identification of COPD patients with favorable FEV<sub>1</sub> improvement in response to ICS/LABA treatment.

**Keywords:** eosinophils, periostin, COPD

## Introduction

COPD is a progressive inflammatory disorder that is characterized by persistent airflow limitation.<sup>1</sup> It affects approximately 10% of adults over 40 years of age and is the third leading cause of death worldwide.<sup>2</sup> Although most COPD guidelines advocate the use of inhaled corticosteroids (ICS) for only those with frequent exacerbations, in real clinical practice, they are widely used especially in combination with a long-acting beta<sub>2</sub>-agonist (LABA).<sup>3,4</sup> Because only a small fraction of COPD patients are responsive to ICS-based therapy,<sup>5,6</sup> identifying characteristics associated with ICS-responsiveness is crucial for clinicians to make therapeutic decisions. Nonetheless, little information is available on which clinical features might predict a favorable ICS response. Although neutrophils are thought to play a prominent role in the pathogenesis of COPD,<sup>7</sup>

Christenson et al<sup>8</sup> recently reported that eosinophilia and T helper type 2 (Th2) inflammation might also play a significant role in a subset of patients with COPD and that approximately 20% of smokers with COPD have a Th2-high signature. This result is consistent with previous findings that airway eosinophilic inflammation is not uncommon, but is present in approximately 20%–40% of COPD patients.<sup>9</sup> Furthermore, several studies showed that an increased sputum eosinophil count is related to improvement in lung function following treatment with ICS in COPD.<sup>10,11</sup> However, sputum induction is challenging in COPD, and the results are often difficult to interpret. A recent study suggested that blood eosinophils may be a good indicator for sputum eosinophils.<sup>12</sup> Another blood surrogate marker of eosinophilic airway inflammation may be serum periostin.<sup>13</sup> In this study, we determined whether increased levels of blood eosinophils and periostin are associated with a significant improvement in forced expiratory volume in 1 second (FEV<sub>1</sub>) after 3-month treatment with ICS/LABA in patients with stable COPD.

## Patients and methods

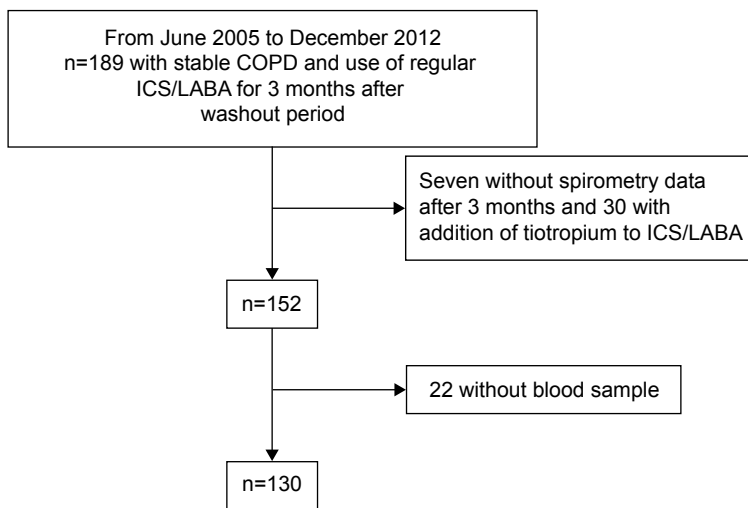
### Patients

All subjects were selected from the Korean Obstructive Lung Disease (KOLD) cohort, which prospectively recruited subjects with obstructive lung disease from the pulmonary clinics of 14 hospitals in Korea from June 2005 to December 2012. Details of the KOLD study have been published previously.<sup>14</sup> After enrollment in the KOLD cohort, some subjects underwent a 2-week washout period and then received treatment with a fixed-dose combination inhaler of ICS and LABA. However, subjects whose condition did not allow

cessation of medications did not undergo a washout period and maintained their original treatment. This decision was made at the discretion of the treating physicians. Among the subjects with a washout period, a total of 189 fulfilled the four inclusion criteria:  $\geq 40$  years, post-bronchodilator FEV<sub>1</sub>/forced vital capacity (FVC)  $< 0.7$ , more than 10 pack-years of smoking history, and no or minimal abnormality on chest radiography. Baseline clinical data were obtained after cessation of the following respiratory medications: an ICS for 2 weeks, an inhaled LABA, or long-acting muscarinic antagonist for 2 days, an inhaled short-acting  $\beta_2$ -agonist or inhaled short-acting anticholinergic for 12 hours. For the following 3 months, COPD was treated with a fixed-dose combination inhaler of ICS and LABA (50  $\mu$ g salmeterol/500  $\mu$ g fluticasone or 9  $\mu$ g formoterol/320  $\mu$ g budesonide, twice daily). Spirometry data were evaluated in 182 subjects after 3-month treatment with combined inhalation ICS and LABA. Excluding 30 subjects with addition of tiotropium to ICS and LABA and 22 subjects for whom blood samples were not collected, 130 subjects remained eligible for analysis in this study (Figure 1). The study protocol was approved by the Asan Medical Center Institutional Review Board (no 2005-0010). Written informed consent was obtained from all of the participating patients.

### Blood eosinophils and plasma periostin measurements

Blood samples were collected after 2-week washout of ICS and before the 3-month treatment, separated into their various components, aliquoted, transferred to the KOLD Data Coordinating Center on dry ice, and kept in  $-80^{\circ}\text{C}$  freezers until use in this study. Plasma samples were thawed



**Figure 1** Study flow diagram.

**Abbreviations:** ICS, inhaled corticosteroids; LABA, long-acting beta<sub>2</sub>-agonist.

for periostin and they were measured using a commercially available ELISA kit (Adipo Bioscience, Santa Clara, CA, USA) according to the manufacturer's instructions. The lower limit of detection of these assays was 2 ng/mL, and values that were below this level of detection were recorded as 1 ng/mL. Peripheral blood eosinophil counts were routinely obtained from the standard complete blood counts after 2-week washout of ICS and before the 3-month treatment. The reference range of blood eosinophils is 1%–5%.

## Pulmonary function test

Spirometry was performed according to the recommendations of the American Thoracic Society using a Vmax 22 (Sensor-Medics, Yorba Linda, CA, USA) and PFDX (MedGraphics, St Paul, MN, USA).<sup>15</sup> Absolute values of FVC and FEV<sub>1</sub> were obtained, and the percentage of the predicted (pred) values (% pred) for FEV<sub>1</sub> and FVC was calculated from equations obtained with a representative Korean sample.<sup>16</sup> A positive bronchodilator response (BDR) at baseline was defined as a post-bronchodilator increase in FEV<sub>1</sub> of at least 12% and 200 mL from baseline values.<sup>17</sup>

Spirometry was conducted at baseline and after 12 weeks of treatment. Subjects were classified as FEV<sub>1</sub> responders or FEV<sub>1</sub> non-responders based on FEV<sub>1</sub> improvement of at least 12% and 200 mL from baseline after 3 months of combined treatment with ICS/LABA.

## Statistical analysis

Data are presented as medians and interquartile ranges (IQRs) for continuous variables and as frequencies (percentages) for categorical variables. Data were compared using the Mann–Whitney *U*-test for continuous variables because of non-normality and Pearson's  $\chi^2$  test or Fisher's exact test for categorical variables. To determine the optimal cut-off value of blood eosinophils and plasma periostin for the prediction of FEV<sub>1</sub> responders, the Youden's index, defined as (sensitivity + specificity) – 1, was calculated at each cut-off. The cut-off point showing the highest Youden's index was considered the optimal cut-off value.<sup>18</sup> The optimal cut-off values of blood eosinophils and plasma periostin were 260/ $\mu$ L (sensitivity 60% and specificity 69% with Youden's index of 0.288) and 23 ng/mL (sensitivity 43% and specificity 76% with Youden's index of 0.190), respectively. A logistic regression model was used to assess the relationship between biomarkers and treatment response. Statistical adjustments were made for age, body mass index (BMI), smoking status, emphysema index, positive BDR, and baseline FEV<sub>1</sub> <50% pred as covariates. Sex was not included as a covariate, because 97.4% of the

population were male. We also determined the discriminatory properties of high blood eosinophils and plasma periostin in predicting FEV<sub>1</sub> responders by constructing a receiver operator characteristic curve and calculating the area under the curve (AUC) or *C*-statistic. To create the best model, we first conducted univariate analyses of the salient risk factors listed previously and identified the variables that were significant. Among them, the variable with the highest AUC was selected as the baseline covariate. To this baseline covariate, we successively added the variable with the second highest AUC and then the variable with the third-highest AUC score, and so forth until all the significant variables on the univariate analysis were exhausted. To the penultimate model, we added high blood eosinophils or plasma periostin to evaluate the incremental effects of high blood eosinophils or plasma periostin on AUC. All tests were two-sided, and a *P*-value <0.05 was considered to indicate statistical significance. Data were analyzed using IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY, USA) and Stata software, version 12 (StataCorp LP, College Station, TX, USA).

## Results

### Subjects

Baseline characteristics of the 130 COPD subjects are summarized in Table 1. There were 126 (97%) males and 4 (3%) females, with a median age of 67 years (IQR, 61–70 years). The median BMI was 23.4 kg/m<sup>2</sup> (IQR, 21.5–25.1 kg/m<sup>2</sup>). There were 53 (41%) current smokers and 77 (59%) ex-smokers, with a median smoking history of 45 pack-years. Also, 50 (39%) subjects had dyspnea with a modified Medical Research Council grade  $\geq 2$ . The most common comorbidity was chronic heart disease (42%), followed by diabetes mellitus (7%). The median FVC (L), FVC (% pred), FEV<sub>1</sub> (L), and FEV<sub>1</sub> (% pred) were 3.3 L (IQR, 2.6–3.7 L), 74.1% pred (IQR, 64.4%–87.1%), 1.5 L (IQR, 1.1–1.8 L), and 49.5% pred (IQR, 38.8%–60.8%), respectively. The median distance achieved on the 6-minute walk test was 445 m (IQR, 403–486 m), and a positive BDR was demonstrated in 47 (36%) subjects. Seventeen subjects reported 27 exacerbations during 3 months. Laboratory findings were as follows: median blood white blood cell count of 7,240/ $\mu$ L (IQR, 6,000–8,450/ $\mu$ L), median blood neutrophils 3,921/ $\mu$ L (IQR, 3,094–4,811/ $\mu$ L), median blood eosinophils 200.0/ $\mu$ L (IQR, 120.6–345.6/ $\mu$ L), and median C-reactive protein level 1.5 mg/L (IQR, 0.6–3.7 mg/L). The median plasma periostin level was 9.0 ng/mL (IQR, 1.0–41.3 ng/mL). There were 53 (41%) subjects with high blood eosinophilia (>260/ $\mu$ L)

**Table 1** Baseline characteristics of 130 COPD patients

Characteristics	Median (IQR) or no (%)
Age, years	67 (61–70)
Male	126 (96.9)
Body mass index, kg/m <sup>2</sup>	23.4 (21.5–25.1)
Body mass index $\geq$ 25 kg/m <sup>2</sup>	33 (25.4)
Smoking status	
Current smoker	53 (40.8)
Ex-smoker	77 (59.2)
Smoking pack-years	45 (35–59)
Comorbidities, overlapping	
Chronic heart disease	55 (42.3)
Diabetes	9 (7.0)
Malignant disease	1 (0.8)
Cerebrovascular disease	1 (0.8)
Chronic kidney disease	1 (0.8)
Dyspnea (mMRC $\geq$ 2)	50 (38.5)
Lung function test	
FVC (L)	3.3 (2.6–3.7)
FVC (% pred)	74.1 (64.4–87.1)
FEV <sub>1</sub> (L)	1.5 (1.1–1.8)
FEV <sub>1</sub> (% pred)	49.5 (38.8–60.8)
6-minute walk test (distance, m)	445 (403–486)
Positive bronchodilator response	47 (36.2)
Exacerbation (% of subjects)	17 (13.1)
Emphysema index	17.6 (8.0–30.9)
Laboratory findings	
White blood cell count (/ $\mu$ L)	7,240 (6,000–8,450)
Neutrophil count (/ $\mu$ L)	3,921 (3,094–4,811)
Eosinophil count (/ $\mu$ L)	200.0 (120.6–345.6)
C-reactive protein (mg/L) (n=63)	1.5 (0.6–3.7)
Plasma periostin (ng/mL)	9.0 (1.0–41.3)

**Abbreviations:** FEV<sub>1</sub>, forced expiratory volume in 1 second; FVC, forced vital capacity; IQR, interquartile range; mMRC, modified Medical Research Council; pred, predicted.

and 39 (30%) subjects with high plasma periostin level ( $>23$  ng/mL). Fifteen (12%) subjects had both high blood eosinophilia and high plasma periostin level.

## Comparison between FEV<sub>1</sub> responders and FEV<sub>1</sub> non-responders

After 3 months of treatment with ICS and LABA, 42 (32%) subjects were classified as FEV<sub>1</sub> responders. As shown in Table 2, there were no significant differences in sex, smoking history, BMI, dyspnea scale, distance on the 6-minute walk test, exacerbation, and emphysema index between FEV<sub>1</sub> responders and FEV<sub>1</sub> non-responders.

FEV<sub>1</sub> responders were more likely to have baseline FEV<sub>1</sub>  $<50\%$  pred ( $P=0.045$ ) and positive BDR at baseline ( $P=0.002$ ), whereas FEV<sub>1</sub> non-responders had a higher FEV<sub>1</sub> (L) and (% pred) and FVC (L) and (% pred) than FEV<sub>1</sub> responders (FEV<sub>1</sub> (L),  $P=0.036$ ; FEV<sub>1</sub> [% pred],  $P=0.005$ ; FVC (L),  $P=0.034$ ; and FVC [% pred],  $P=0.004$ ). The proportion of subjects with high blood eosinophils ( $>260/\mu$ L, 60% vs 32%;  $P=0.003$ ), high plasma periostin ( $>23$  ng/mL, 43% vs 24%;  $P=0.027$ ), and with high blood eosinophils and high plasma periostin (24% vs 6%;  $P=0.002$ ) were significantly higher in the FEV<sub>1</sub> responders than the FEV<sub>1</sub> non-responders (Figure 2).

## Blood eosinophils and plasma periostin levels in FEV<sub>1</sub> responders

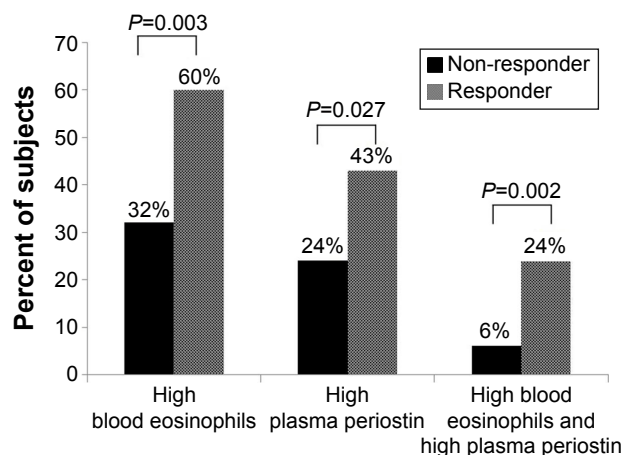
As shown in Table 3, blood eosinophils  $>260/\mu$ L, plasma periostin level  $>23$  ng/mL, positive BDR at baseline, and

**Table 2** Clinical findings of 130 COPD patients with and without ICS/LABA response

Characteristics of 130 COPD patients with and without ICS/LABA response	FEV <sub>1</sub> non-responders (n=88)	FEV <sub>1</sub> responders (n=42)	P-value
Age, years	68 (62–72)	65 (61–69)	0.046
Male	86 (97.7)	40 (95.2)	0.594
Smoking status			
Current smoker	33 (37.5)	20 (47.6)	0.272
Smoking pack-years	46 (30–58)	45 (40–61)	0.587
Body mass index, kg/m <sup>2</sup>	23.1 (20.5–25.3)	24.1 (22.7–25.0)	0.063
Dyspnea (mMRC $\geq$ 2)	33 (37.5)	17 (40.5)	0.744
Baseline FEV <sub>1</sub> (% pred)			0.045
FEV <sub>1</sub> 50 to 80% pred	48 (54.5)	15 (35.7)	
FEV <sub>1</sub> $<50\%$ pred	40 (45.5)	27 (64.3)	
Positive bronchodilator response	24 (27.3)	23 (54.8)	0.002
6-minute walk test (distance, m)	443 (404–487)	449 (401–483)	0.897
Chronic heart disease	34 (39.1)	21 (50.0)	0.240
Exacerbation (% of subjects)	14 (15.9)	3 (7.1)	0.166
Emphysema index	17.8 (8.4–30.3)	16.3 (7.3–32.2)	0.968

**Note:** The data are presented as numbers (%) or as medians and interquartile ranges.

**Abbreviations:** FEV<sub>1</sub>, forced expiratory volume in 1 second; ICS, inhaled corticosteroids; LABA, long-acting beta<sub>2</sub>-agonist; mMRC, modified Medical Research Council; pred, predicted.



**Figure 2** Comparison of high blood eosinophils and high plasma periostin between FEV<sub>1</sub> responders and FEV<sub>1</sub> non-responders.

**Abbreviation:** FEV<sub>1</sub>, forced expiratory volume in 1 second.

baseline FEV<sub>1</sub> <50% pred were positively associated with FEV<sub>1</sub> responders. Adjustment of age, BMI, smoking status, exacerbation, emphysema index, positive BDR at baseline, and baseline FEV<sub>1</sub> <50% pred showed that blood eosinophils >260/μL (adjusted odds ratio [aOR] =3.52; 95% confidence interval [CI] =1.37–8.99; *P*=0.009), plasma periostin level >23 ng/mL (aOR =3.52; 95% CI =1.31–9.49; *P*=0.013), and combined blood eosinophils >260/μL and plasma periostin level >23 ng/mL (aOR =6.40; 95% CI =1.60–25.55; *P*=0.009) were associated with FEV<sub>1</sub> responders.

Among the clinical variables, the most predictive variable for FEV<sub>1</sub> response after 3-month treatment with ICS and LABA was positive BDR at baseline, which had an AUC of 0.637 (95% CI =0.548–0.726). The next predictive

variable was age (AUC =0.608; 95% CI =0.509–0.707), followed by baseline FEV<sub>1</sub> <50% pred (AUC =0.594; 95% CI =0.504–0.684). The combination of positive BDR at baseline, age, and baseline FEV<sub>1</sub> <50% pred yielded an AUC of 0.700 (95% CI =0.602–0.800). The addition of blood eosinophils >260/μL increased the AUC of this model significantly to 0.771 (*P*=0.045; Table 4). However, addition of the variable of plasma periostin >23 ng/mL or combined high blood eosinophils >260/μL and high plasma periostin >23 ng/mL to the previous model did not yield a significant improvement in AUC (*P*=0.346 for plasma periostin >23 ng/mL and *P*=0.064 for high blood eosinophils >260/μL and high plasma periostin >23 ng/mL).

## Discussion

To the best of our knowledge, this is the first study that investigates the relationship of blood eosinophils and plasma periostin with lung function changes related to ICS and LABA combination treatment in stable COPD patients. We showed that high blood eosinophils (>260/μL) and high plasma periostin (>23 ng/mL) were significantly related to FEV<sub>1</sub> improvement after 12 weeks of treatment with ICS and LABA in COPD patients and found that the addition of high blood eosinophils (>260/μL) to baseline positive BDR, age, and baseline FEV<sub>1</sub> <50% pred significantly increased the prediction accuracy for treatment response.

Eosinophilic airway inflammation is increased during acute exacerbations of COPD, and peripheral blood eosinophil count has been reported as a promising biomarker to predict response to corticosteroid therapy during COPD

**Table 3** Adjusted odds ratio of blood eosinophils >260/μL and plasma periostin >23 ng/mL for FEV<sub>1</sub> responders

Variables	Adjusted state		
	OR	95% CI	P-value
<b>Model 1</b>			
Age	0.96	0.90–1.03	0.268
Blood eosinophils >260/μL	3.52	1.37–8.99	0.009
Positive bronchodilator response at baseline	2.85	1.15–7.10	0.024
Baseline FEV <sub>1</sub> <50% pred	3.12	1.18–8.27	0.022
<b>Model 2</b>			
Age	0.96	0.89–1.02	0.181
Plasma periostin >23 ng/mL	3.52	1.31–9.49	0.013
Positive bronchodilator response at baseline	2.91	1.17–7.21	0.021
Baseline FEV <sub>1</sub> <50% pred	2.74	1.05–7.15	0.040
<b>Model 3</b>			
Age	0.95	0.89–1.02	0.150
Combined high blood eosinophils >260/μL and high plasma periostin >23 ng/mL	6.40	1.60–25.55	0.009
Positive bronchodilator response at baseline	3.21	1.29–7.98	0.012
Baseline FEV <sub>1</sub> <50% pred	2.98	1.12–7.94	0.029

**Note:** Body mass index, smoking status, exacerbation, and emphysema index were entered into the logistic analysis in all models.

**Abbreviations:** CI, confidence interval; FEV<sub>1</sub>, forced expiratory volume in 1 second; OR, odds ratio; pred, predicted.

**Table 4** Area under the curve values from receiver operator characteristics generated for variables with blood eosinophils >260/ $\mu$ L and plasma periostin >23 ng/mL for the prediction of FEV<sub>1</sub> responders

Variables	AUC (95% CI)
Age	0.608 (0.509–0.707)
Baseline FEV <sub>1</sub> <50% pred	0.594 (0.504–0.684)
Positive bronchodilator response at baseline	0.637 (0.548–0.726)
High blood eosinophils >260/ $\mu$ L	0.639 (0.549–0.728)
High plasma periostin >23 ng/mL	0.595 (0.507–0.683)
Positive bronchodilator response at baseline	0.637 (0.548–0.726)
Positive bronchodilator response at baseline + age	0.685 (0.587–0.783) (P=0.093)*
Positive bronchodilator response at baseline + age + baseline FEV <sub>1</sub> <50% pred	0.700 (0.602–0.800) (P=0.527)*
Positive bronchodilator response at baseline + age + baseline FEV <sub>1</sub> <50% pred + high blood eosinophils >260/ $\mu$ L	0.771 (0.688–0.853) (P=0.045)*
Positive bronchodilator response at baseline + age + baseline FEV <sub>1</sub> <50% pred + high plasma periostin >23 ng/mL	0.729 (0.636–0.823) (P=0.346)‡
Positive bronchodilator response at baseline + age + baseline FEV <sub>1</sub> <50% pred + combined high blood eosinophils >260/ $\mu$ L and high plasma periostin >23 ng/mL	0.769 (0.682–0.856) (P=0.064)‡

**Notes:** \*P-value for comparison with model of previous step. †P-value for comparison with model of positive bronchodilator response at baseline + age + baseline FEV<sub>1</sub> <50% pred.

**Abbreviations:** AUC, area under the curve; CI, confidence interval; FEV<sub>1</sub>, forced expiratory volume in 1 second; pred, predicted.

exacerbations.<sup>19</sup> Stable COPD is classically thought to be driven by neutrophils and the T helper type 1 immune response;<sup>20</sup> however, COPD patients have elevated eosinophils in airway wall biopsies and increased activity of eosinophils in bronchoalveolar lavage fluid.<sup>21,22</sup> Moreover, Christenson et al showed a significant overlap of airway epithelial gene expression alterations in both asthma and COPD subset, and demonstrated that asthma-derived gene expression signatures of Th2 inflammation are associated with increased eosinophil counts and a favorable ICS response in COPD patients.<sup>8</sup> Our data extend these findings by demonstrating that high blood eosinophils were associated with treatment response (defined as an increase in FEV<sub>1</sub> of at least 12% and 200 mL from baseline) following ICS and LABA treatment. Furthermore, combining high blood eosinophils with age and lung function parameters at baseline (baseline positive BDR and baseline FEV<sub>1</sub> <50 [% pred]) increased AUC significantly, providing additional discrimination beyond that achieved by clinical variables. Thus, high blood eosinophils, together with lung function parameters at baseline, may identify an ICS-responsive phenotype in COPD.

Periostin is a disulfide-linked 90-kDa heparin-binding N-glycosylated protein containing four tandem fasciclin domains.<sup>23</sup> Periostin is secreted from bronchial epithelial cells in response to interleukin (IL)-13, a central mediator of allergic asthma.<sup>24,25</sup> Jia et al showed that the circulating levels of periostin are significantly higher in asthmatic patients with evidence of eosinophilic airway inflammation relative

to those with minimal eosinophilic airway inflammation, suggesting that periostin may serve as a good systemic indicator of eosinophilic airway inflammation.<sup>13</sup> Because ICS treatment substantially suppresses Th2-driven eosinophilic inflammation and reduces IL-13 levels,<sup>26</sup> periostin has received attention as a potential biomarker of treatment response in asthmatic patients. Indeed, lung function improvement following anti-IL-13 therapy in severe asthma was greater in patients with high periostin than in those with lower levels.<sup>27</sup> Our study analyzed the plasma periostin as another biomarker related to the Th2-related signature and showed that high plasma periostin levels were independently associated with treatment response after 3 months of treatment with ICS and LABA. However, in contrast to high blood eosinophils, high plasma periostin levels alone or combined with high blood eosinophils did not have an additive role to increase prediction accuracy for treatment response, suggesting limited utility as a biomarker of ICS-responsive phenotype in COPD. Our data agree with those of a previous study, which showed that the periostin gene (*POSTN*), one of the IL-13 inducible genes, was repressed by smoking, thus may not be a good Th2 inflammatory marker in smokers with COPD.<sup>8</sup>

In our study, a positive BDR at baseline and baseline FEV<sub>1</sub> were related to FEV<sub>1</sub> improvement after 3-month treatment with ICS and LABA in COPD patients, whereas the emphysema index was not significantly different between FEV<sub>1</sub> non-responders and FEV<sub>1</sub> responders. There have been conflicting data regarding the relationship between airway

reversibility and emphysema severity. Kitaguchi et al<sup>28</sup> reported that COPD patients without emphysema on chest high-resolution computed tomography were more likely to have bronchodilator responsiveness and the reversibility response to treatment with ICS, compared with those with emphysema. However, other studies found no significant differences in bronchodilator responsiveness among groups classified according to emphysema severity or presence of emphysema.<sup>29,30</sup> Our results are concordant with studies showing a lack of association between bronchodilator responsiveness and emphysema severity. However, a prospective study with treatment response according to emphysema severity is necessary to resolve conflicting data.

Our study had several limitations. First, as the KOLD prospective cohort was not originally designed to identify blood biomarkers for COPD, our study design and analysis had to be retrospective in nature. A prospective study with a larger sample size is needed to validate these findings. Second, we did not have serial measurements of blood eosinophil number or plasma periostin levels over 3 months. Third, airway inflammatory markers, such as eosinophils in sputum or bronchoalveolar lavage fluid, were not evaluated, thus mutual relationships between blood eosinophils and plasma periostin with eosinophil levels in the lung could not be investigated. Moreover, the cut-off values optimized for our study need to be replicated in other studies. Fourth, because ICS monotherapy is not recommended and a long-acting bronchodilator is a key treatment of COPD, a combination of ICS and LABA was administered to all subjects in this study. Thus, the FEV<sub>1</sub> function improvement after inhaled treatment cannot be explained by only the ICS effect on eosinophilic airway inflammation. Nevertheless, some COPD patients showed remarkable improvement in lung function following treatment with ICS and LABA. The American Thoracic Society/European Respiratory Society Task Force suggested a change of 100–140 mL or 5%–10% in FEV<sub>1</sub> from baseline as clinically important,<sup>31</sup> and a previous study reported an improvement in FEV<sub>1</sub> of approximately 200 mL with 26-week treatment of combination of long-acting muscarinic antagonist and LABA.<sup>32</sup> Thus, we employed the Global Initiative for Chronic Obstructive Lung Disease criteria, ie, an increase in FEV<sub>1</sub> of at least 12% and 200 mL from baseline after 3-month treatment, to define treatment response. Finally, further investigation is needed to determine whether high blood eosinophils are associated with long-term outcomes, such as lung function decline or mortality, in COPD.

In summary, the findings of this study showed that high blood eosinophils and high plasma periostin are significantly related to improved lung function with ICS and LABA treatment. However, only elevated blood eosinophils significantly improved prediction of treatment response beyond that achieved with age and traditional lung function parameters. These data raise the possibility of using blood eosinophils in concert with lung function parameters (baseline positive BDR and baseline FEV<sub>1</sub> <50% pred) to identify COPD patients who will benefit from ICS and LABA treatment. Additional studies are required to validate these preliminary findings.

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## Author contributions

Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data: HYP, HL, WK, SK, IJ, HK, TK, JWK, WJK, YO, DDS, SYL, and SL. Drafting the article or revising it critically for important intellectual content: HYP, HL, WK, SK, IJ, HK, TK, JWK, WJK, YO, DDS, SYL, and SL. Final approval of the version to be published: HYP, HL, WK, SK, IJ, HK, TK, JWK, WJK, YO, DDS, SYL, and SL. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: HYP, HL, WK, SK, IJ, HK, TK, JWK, WJK, YO, DDS, SYL, and SL.

## Disclosure

The authors report no conflicts of interest in this work.

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