



# Low Eosinophil Phenotype Predicts Noninvasive Mechanical Ventilation Use in Patients with Hospitalized Exacerbations of COPD

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**Rationale:** Eosinophilic inflammation is related to the progression and outcomes of acute exacerbation of chronic obstructive pulmonary disease (AECOPD). Till now, few studies have focused on low EOS in AECOPD.

**Objective:** To reveal the clinical characteristics, therapeutic responses and prognosis of patients hospitalized of AECOPD with low EOS.

**Methods:** The electronic database of Zhongshan Hospital, Fudan University was used. Cohort 1 included 608 patients with hospitalized AECOPD. Study population 2 consisted of 166 patients with AECOPD admission at least twice. Impact of low EOS on NIMV treatment, length of hospital stays and 12-month AECOPD-related readmission were analyzed with multivariable logistic regression model. Thirty-five hospitalized AECOPD patients were prospectively recruited as cohort 3 to explore the association between EOS and other immune cells using Spearman correlation coefficient for ranked data.

**Results:** EOS level was suppressed on admission in AECOPD patients, and significantly improved after hospitalized treatment ( $P < 0.05$ ). For inflammatory markers, leucocytes, neutrophils and lactate dehydrogenase levels were higher, while lymphocytes, monocytes and interleukin-6 levels were lower in the low-EOS group than those in the non-low EOS group ( $P < 0.05$ ). Low EOS (EOS  $< 50$  cells/ $\mu$ L) was an independent risk factor of NIMV use (OR = 1.86, 95% CI = 1.26 ~ 2.73). The EOS percentage was positively correlated with the T cell percentage ( $r = 0.46$ ,  $P < 0.05$ ) and negatively correlated with the natural killer cell percentage ( $r = -0.39$ ,  $P < 0.05$ ). The patients with low EOS had lower level of CD4<sup>+</sup> T cell ( $P < 0.05$ ) than that of patients with non-low EOS.

**Conclusion:** Low EOS might be a stable phenotype in patients with hospitalized AECOPD and could be used to inform NIMV management, hyperinflammatory state and impaired immunity situation.

**Keywords:** chronic obstructive pulmonary disease, acute exacerbation, eosinophilic inflammation, prognostic phenotype, noninvasive mechanical ventilation

## Background

Chronic obstructive pulmonary disease (COPD) is characterized by airflow obstruction or impaired lung function. The prevalence of spirometry-defined COPD in China was 13.7% among people aged 40 years or older.<sup>1</sup> Though classically mediated by neutrophilic and Th1 cells,<sup>2</sup> different COPD inflammatory profiles have recently gained more interest.<sup>3</sup> Studies demonstrated that a higher blood eosinophil (EOS) count was associated with higher risk of developing COPD in individuals from general population.<sup>4,5</sup> In patients with stable COPD, higher blood eosinophil count may predict increased risk of severe acute exacerbations of COPD (AECOPD). Severe AECOPD requiring hospitalization is associated with poorer outcomes, including accelerated lung function declining<sup>6</sup> and significant higher risk of mortality.<sup>7</sup> Therefore, adequately phenotyping eosinophilic inflammation in COPD is worthwhile.

Previous studies mainly focused on high EOS in AECOPD by different cut-off values of 150 to 300 cells/ $\mu$ L.<sup>8–10</sup> The results supported that patients with elevated blood eosinophil counts were at higher risk of acute exacerbations.<sup>11</sup> COPD-

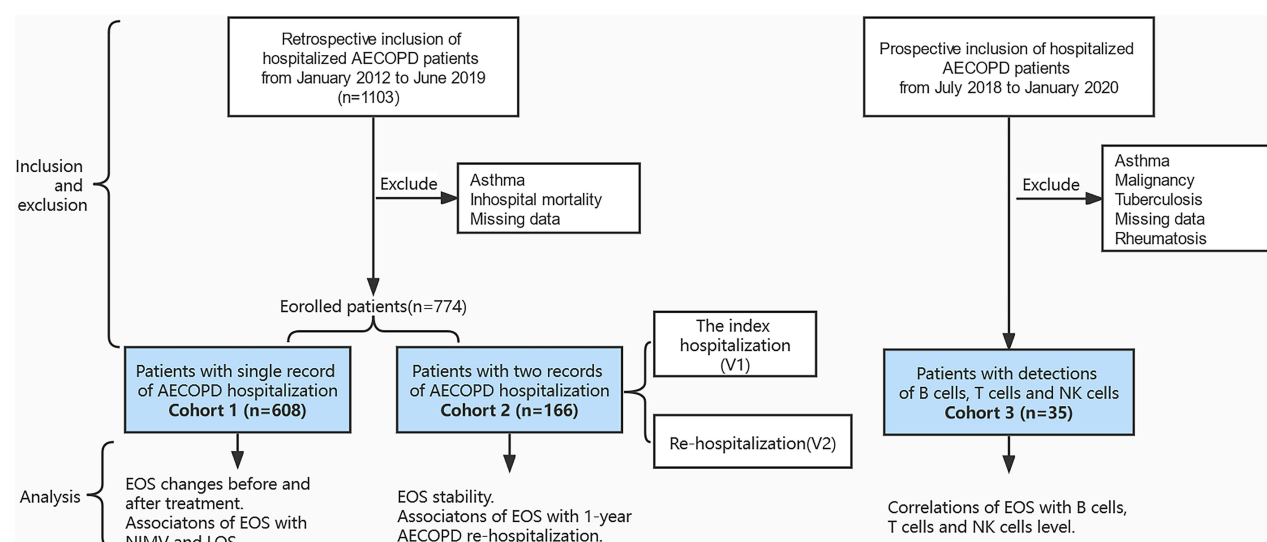
related readmission rates were significantly greater at 30, 60, and 90 days and 12 months after discharge for patients with eosinophil counts  $>300$  cells/ $\mu\text{L}$  (versus lesser) (OR range = 1.52–1.97).<sup>12</sup> It was also well established that patients with high eosinophilic exacerbations responded better to inhaled corticosteroids (ICS) therapy than patients with non-high eosinophilic exacerbations.<sup>13</sup> ICS combined with long-acting  $\beta_2$ -agonists (LABA) is already recommended for AECOPD treatment if EOS  $\geq 300$  cells/ $\mu\text{L}$ .<sup>14</sup> However, this treatable trait presented significant variability throughout the course of COPD, making it difficult to be a reliable predictor.<sup>15</sup> It was demonstrated that AECOPD patients with eosinophil counts  $<50$  cells/ $\mu\text{L}$  were more strongly associated with infection, longer median hospital stay and shorter 12-month survival than patients with eosinophil counts  $>150$  cells/ $\mu\text{L}$ .<sup>8</sup> However, a study from Utah suggested that a blood eosinophil count  $\leq 70$  cells/ $\mu\text{L}$  identified a patient group that was unlikely to require readmission.<sup>12</sup> Given that high EOS acts in AECOPD, low EOS might link to certain important clinical evidence. Studies on the clinical characteristics of patients with low EOS and outcomes of hospitalized AECOPD were scarce and need to be further investigated.

Thus, the objective of this study was to investigate whether AECOPD patients with low EOS had special clinical characteristics and to explore the associations of low EOS with inflammation and immunity.

## Materials and Methods

### Study Population and Design

The electronic database of Zhongshan Hospital, Fudan University was used for this study. From January 2012 to June 2019, there were altogether 1103 cases with AECOPD diagnosis<sup>16</sup> as shown in Figure 1. Three hundred and twenty-nine patients were excluded as they were diagnosed with asthma or had incomplete baseline characteristics. Finally, 608 patients with hospitalized AECOPD were included as study population 1. Besides, 166 patients with AECOPD admission at least twice were included as Study population 2. At the same time, 35 patients were prospectively recruited during consecutive hospital admission for AECOPD from July 2018 to January 2020 and were included as Study population 3. The Ethics Committee of Zhongshan Hospital, Fudan University approved the protocol (B2021-818R) and this study was conducted in accordance with the Declaration of Helsinki. As the data were anonymized, the institutional Ethics Committee gave their approval for the study with no need for informed consent from participants.



**Figure 1** Cohort diagram: Study population.

**Abbreviations:** AECOPD, acute exacerbation of chronic obstructive pulmonary disease; EOS, eosinophil; NK cells, natural killer cells; NIMV, noninvasive mechanical ventilation; LOS, length of stay.

## Data Collection

Patients' characteristics and comorbid diseases were collected from the hospital database. Hemogram parameters, C reactive protein (CRP) levels, biochemistry values on admission and discharge date, noninvasive ventilation (NIMV) usage, length of hospitalization (days), and readmissions were exported for the analysis. TNF- $\alpha$ , IL-2R, IL-6, IL-8 and CRP in serum were measured using multiplex bead sets from a variety of vendors (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. The percentages of B cells, T cells, cluster of differentiation (CD) 4, CD8 positive cells and natural killer (NK) cells were determined on a Coulter EPICS Profile flow cytometer (Coulter Cytometry, Hialeah, FL, USA) at Zhongshan Hospital, Fudan University. The normal range of the percentage of eosinophils in leucocytes used in Zhongshan hospital, Fudan University is 0.4–2.0%.

## Eosinophil Profiling and Outcomes

As for primary endpoints, treatment with NIMV and length of hospital stay were selected. Further analyses described long-term outcome of AECOPD (12-month AECOPD-related readmission within our hospital) according to blood EOS count strata (50 cells/ $\mu$ L<sup>8</sup> and 0.4% of leucocytes).

## Statistical Analyses

The study variables were compared between the low EOS and non-low EOS patients using the chi-2 test for categorical variables, and the *t*-test or the Wilcoxon rank test for continuous variables. The change of eosinophil levels is described as the EOS value at discharge subtracted from that on admission. Concordance was defined as blood eosinophil values persistently lower than or persistently higher than 50 cells/ $\mu$ L or 0.4%, respectively. Discordance was defined when the blood eosinophil values varied between two AECOPD hospitalization. For binary outcomes, associations of eosinophilia with outcomes were estimated using logistic regression with stepwise selection (variables were considered if they reached statistical significance). All statistical analyses were performed by SPSS for Windows (Version 26.0, Chicago, IL, USA). All *P* values were two-sided and a difference was considered statistically significant at *P* < 0.05.

## Results

### Study Population

Baseline demographics and clinical characteristics, including EOS counts for all cohorts, are presented in [Table S1](#). Study population 1 consisted of 80.9% men, with a median age (range) of 75 (68 to 82) years old. There were 215 subjects (35%) having diagnosis of concomitant pulmonary infection or pneumonia on admission and 251 subjects (41%) suffering from respiratory failure. For all subjects, either oral or systemic corticosteroids was administered in 49.5% patients and NIMV treatment was performed in 35.0% patients. The median (range) length of hospital stay was 10.5 days (7.5 to 15). Length of hospital stay equal or longer than 15 days was considered an extended stay.

The median (interquartile range) absolute peripheral eosinophil count and eosinophil count percentage of leucocytes was 35 cells/ $\mu$ L (0 to 150 cells/ $\mu$ L) and 0.4% (0.0 to 1.9), respectively. The low EOS phenotype defined as peripheral blood eosinophil count <0.4% of total leukocyte count occurred in 49.3% patients (*n* = 300). A peripheral blood eosinopenia less than 50 cells/ $\mu$ L occurred in 328 subjects (53.9%). There were no differences with respect to age, gender, complications, or comorbidities between subjects of low EOS and non-low EOS phenotype in all cohorts, except that more subjects with low EOS phenotype were admitted during winter than those with non-low EOS phenotype in cohort 1 (45.4% vs 30.7%, *P*<0.05) ([Tables 1](#), [S2](#) and [S3](#)).

### Stability of the Low EOS in AECOPD

The median blood eosinophil level for all subjects was 20 (0–130) cells/ $\mu$ L on admission, which was 90 (20–180) cells/ $\mu$ L at discharge ([Table 2](#)). The median EOS percentage of leucocytes on admission was also lower compared with that after hospitalization treatment, ranging from 0.3% (0–1.7) to 1% (0.2–2.7) ([Figure 2A](#)). In the low EOS subgroup, the level of EOS at discharge was significantly higher than that on admission, which remained stable between two measurements in the non-low EOS subgroup ([Figure 2B](#) and [C](#)). In patients with EOS values <50 cells/ $\mu$ L on the

**Table 1** Comparison of Clinical Characteristics Between AECOPD Patients with and without Low EOS in Cohort 1

Parameter	<b>EOS <math>\geq 0.4\%</math> (n=308)</b>	<b>EOS <math>&lt; 0.4\%</math> (n=300)</b>	<b>P<sup>a</sup></b>	<b>EOS <math>\geq 50/\mu\text{L}</math> (n=280)</b>	<b>EOS <math>&lt; 50/\mu\text{L}</math> (n=328)</b>	<b>P<sup>b</sup></b>
<b>Age (years)</b>	74.4 $\pm$ 9.7	74.1 $\pm$ 8.8	0.680	74.4 $\pm$ 9.7	74.1 $\pm$ 8.8	0.621
<b>Gender</b>						
<b>Male</b>	259 (84.1%)	233 (77.7%)	0.044*	233 (83.2%)	259 (79.0%)	0.184
<b>Female</b>	49 (15.9%)	67 (22.3%)		47 (16.8%)	69 (21.0%)	
<b>Admission season</b>						
Winter	95 (30.8%)	140 (46.7%)	$< 0.001^*$	86 (30.7%)	149 (45.4%)	$< 0.001^*$
Not winter	213 (69.2%)	160 (53.3%)		194 (69.3%)	179 (54.6%)	
<b>Pulmonary infection</b>						
Yes	106 (34.4%)	109 (36.3%)	0.621	100 (35.7%)	115 (35.1%)	0.867
No	202 (65.6%)	191 (63.7%)		180 (64.3%)	213 (64.9%)	
<b>Respiratory failure</b>						
Yes	115 (37.3%)	136 (45.3%)	0.045*	105 (37.5%)	146 (44.5%)	0.080
No	193 (62.7%)	164 (54.7%)		175 (62.5%)	182 (55.5%)	
<b>Hypertension</b>						
Yes	130 (42.2%)	133 (44.3%)	0.597	124 (44.3%)	139 (42.4%)	0.636
No	178 (57.8%)	167 (55.7%)		156 (55.7%)	189 (57.6%)	
<b>Pulmonary-heart disease</b>						
Yes	50 (16.2%)	57 (19.0%)	0.371	44 (15.7%)	63 (19.2%)	0.260
No	258 (83.8%)	243 (81.0%)		236 (84.3%)	265 (80.8%)	
<b>Diabetes mellitus</b>						
Yes	52 (16.9%)	45 (15.0%)	0.526	49 (17.5%)	48 (14.6%)	0.336
No	256 (83.1%)	255 (85.0%)		231 (82.5%)	280 (85.4%)	
<b>NIMV treatment</b>						
Yes	84 (27.3%)	129 (43.0%)	$< 0.001^*$	77 (27.5%)	136 (41.5%)	$< 0.001^*$
No	224 (72.7%)	171 (57.0%)		203 (72.5%)	192 (58.5%)	

**Notes:** <sup>a</sup>EOS  $< 4\%$  vs EOS  $\geq 4\%$ . <sup>b</sup>EOS  $< 50/\mu\text{L}$  vs EOS  $\geq 50/\mu\text{L}$ . \* $P < 0.05$ .

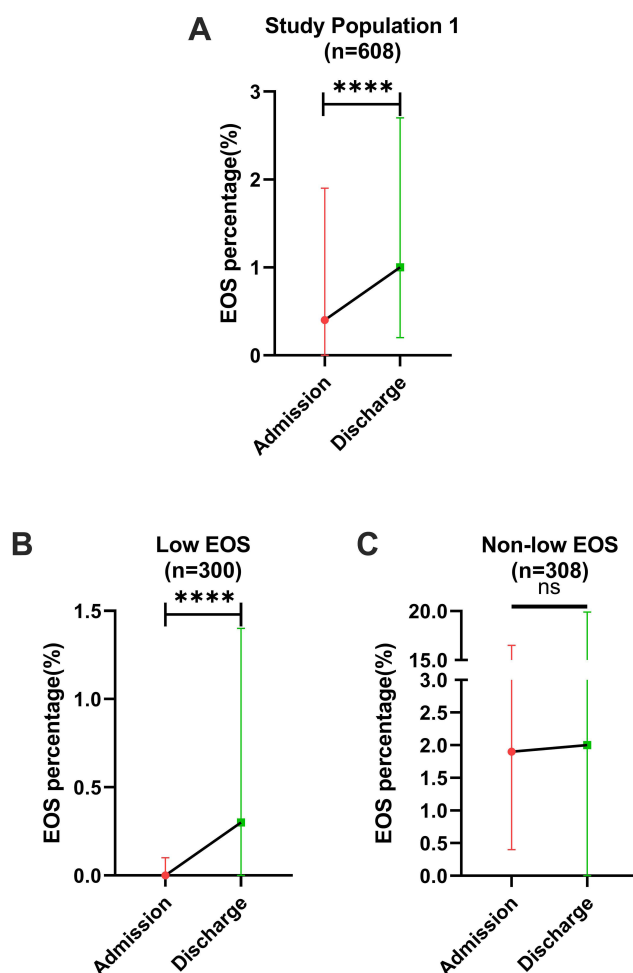
**Abbreviations:** AECOPD, acute exacerbation of chronic obstructive pulmonary disease; EOS, eosinophil; NIMV, noninvasive mechanical ventilation.

**Table 2** Longitudinal Change in Blood Cell Counts by AECOPD Admission or Discharge in Study Population I

	Admission (n=439)	Discharge (n=439)	P	Median Difference	Percentage Change in Difference
EOS ( $\times 10^9/L$ )	0.02 (0~0.13)	0.09 (0.02~0.18)	<0.001*	0.07	350%
EOS as a percentage of leukocytes (%)	0.3 (0~1.7)	1 (0.2~2.7)	<0.001*	0.7	233%
Basophils ( $\times 10^9/L$ )	0.01 (0.01~0.03)	0.02 (0.01~0.03)	<0.001*	0.01	100%
Basophils as a percentage of leukocytes (%)	0.2 (0.1~0.3)	0.2 (0.1~0.4)	<0.001*	0	0
Leucocytes ( $\times 10^9/L$ )	8.6 (6.63~11.45)	7.52 (5.94~9.98)	<0.001*	-1.08	13%
Neutrophils ( $\times 10^9/L$ )	6.7 (4.8~9.3)	5.4 (4~7.8)	<0.001*	-1.3	19%
Neutrophils as a percentage of leukocytes (%)	80.1 (72.3~87.1)	73.2 (65.2~81.6)	<0.001*	-6.9	9%
Lymphocytes ( $\times 10^9/L$ )	0.9 (0.6~1.3)	1.1 (0.8~1.6)	<0.001*	0.2	22%
Lymphocytes as a percentage of leukocytes (%)	11.1 (6.8~16.7)	15.9 (9.7~22.5)	<0.001*	4.8	43%
Monocytes ( $\times 10^9/L$ )	0.6 (0.4~0.81)	0.57 (0.42~0.79)	0.946	-	-
Monocytes as a percentage of leukocytes (%)	6.9 (4.6~9.4)	7.7 (5.9~9.8)	<0.001*	0.8	12%
CRP (mg/L)	22.5 (7~62.7)	7.8 (2.6~25.7)	<0.001*	-14.7	65%
PCT (ng/mL)	0.21 (0.15~0.27)	0.19 (0.11~0.27)	<0.001*	-0.02	10%

Note: \*P < 0.05.

Abbreviations: AECOPD, acute exacerbation of chronic obstructive pulmonary disease; EOS, eosinophil; CRP, C-reactive protein; PCT, procalcitonin.



**Figure 2** Longitudinal change of the median percentage of eosinophils by AECOPD admission or discharge.

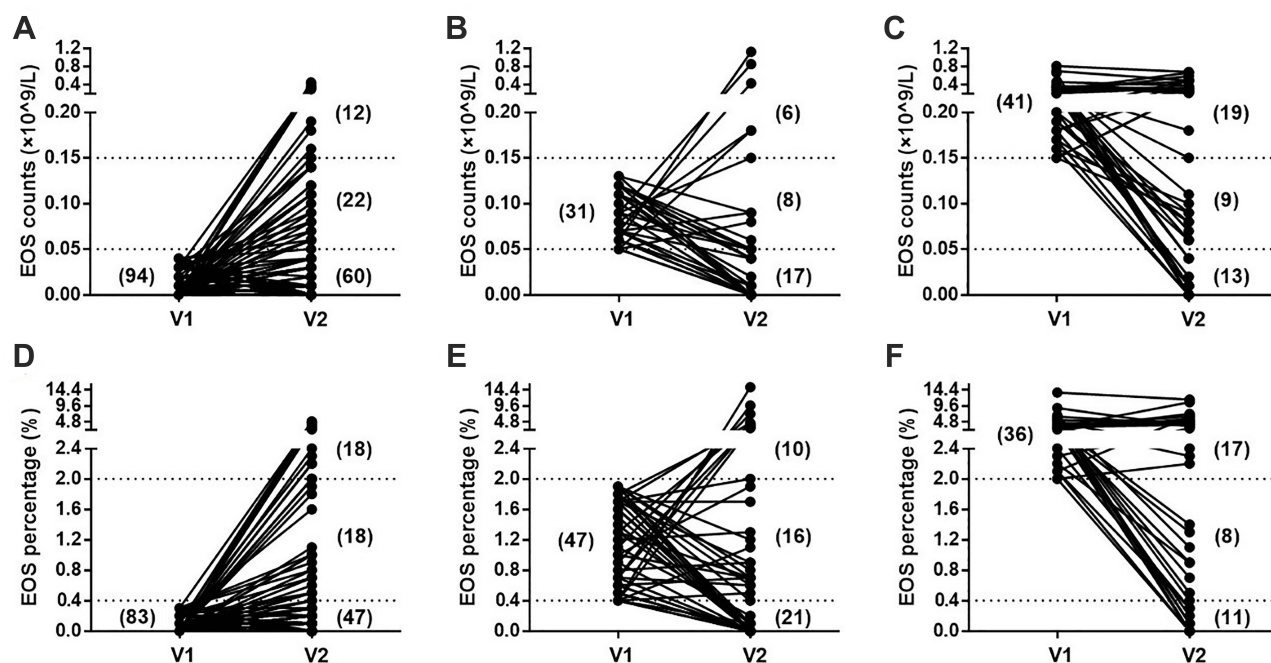
**Notes:** (A) The median percentage of EOS at baseline was lower compared with that after hospitalization in the whole population I, ranging from 0.3% (0–1.7) to 1% (0.2–2.7). (B) In the low EOS subgroup, the median percentage of EOS at discharge was significantly higher than that on admission. (C) The median percentage of EOS remained stable between two measurements in the non-low EOS subgroup. Data were presented as median with interquartile range. \*\*\*\* $P < 0.0001$ .

**Abbreviation:** EOS, eosinophil.

index date of hospitalized AECOPD, significantly higher proportion of patients were still in low EOS levels on the re-admission date for AECOPD compared with that of patients with  $\text{EOS} \geq 50$  cells/ $\mu\text{L}$  (63.8% vs 36.2%;  $P < 0.05$ ) (Figure 3A; Table 3A). The same difference was seen when stratifying low eosinophil level according to EOS percentage of leucocytes  $< 0.4\%$  (56.6% vs 43.4%;  $P < 0.05$ ) (Figure 3D; Table 3B). In patients without EOS values  $< 50$  cells/ $\mu\text{L}$  on the index date of hospitalized AECOPD, the levels of eosinophils on the second admission showed a greater discordance compared with that of the first admission (Figure 3B, C, E, F).

## Association of Low EOS with Infection

There was no difference with respect to the incidence of pulmonary infection between the low EOS and non-low EOS group (Table 1). As shown in Figure 4, the white blood cell (WBC) count, the neutrophil percentage of leucocytes and lactate dehydrogenase (LDH) concentration were significantly higher in patients with low EOS compared to those in patients with non-low EOS, whereas the lymphocyte and monocyte percentage of leucocytes and interleukin (IL)-6 concentration in the low EOS group were markedly lower compared with those in the non-low EOS group. Some other infectious parameters' levels were not statistically different between the low EOS and non-low EOS group, including tumor necrosis factor (TNF)- $\alpha$ , IL-2 receptor, IL-8, CRP and procalcitonin (PCT).



**Figure 3** Stability of the low EOS between two visits in cohort 2, ascertained from two cut-points. Eosinophil blood cell count strata was based on absolute numbers (A–C) or on a percentage of leukocytes (D–F), respectively.

**Notes:** (A) Of the 94 patients with the low EOS at V1, 60 patients were still subject to the low EOS subgroup at V2, and 34 patients were divided into the non-low EOS subgroup at V2. (B) Of the 31 patients with the median EOS at V1, 8 patients were still subject to the median EOS subgroup at V2, and 23 patients were not divided into the median EOS subgroup at V2. (C) Of the 41 patients with the high EOS at V1, 19 patients were still subject to the high EOS subgroup at V2, and 22 patients were divided into the non-high EOS subgroup at V2. (D) Of the 83 patients with the low EOS at V1, 47 patients were still subject to the low EOS subgroup at V2, and 36 patients were divided into the non-low EOS subgroup at V2. (E) Of the 47 patients with the median EOS at V1, 16 patients were still subject to the median EOS subgroup at V2, and 31 patients were not divided into the median EOS subgroup at V2. (F) Of the 36 patients with the high EOS at V1, 17 patients were still subject to the high EOS subgroup at V2, and 19 patients were divided into the non-high EOS subgroup at V2.

**Abbreviations:** EOS, eosinophil; V1, visit 1; V2, visit 2.

## Association of Low EOS with Outcomes of AECOPD

Patients with low EOS were more likely to be treated with NIMV than patients with non-low EOS (0.4%: 43.0% vs 27.3%; 50 cells/ $\mu$ L: 41.5% vs 27.5%;  $P < 0.001$ , Table 1). In a logistic regression analysis adjusting for confounders (hypertension, respiratory failure, the lymphocyte percentage of leucocytes and the level of PCT), patients with EOS  $< 50$

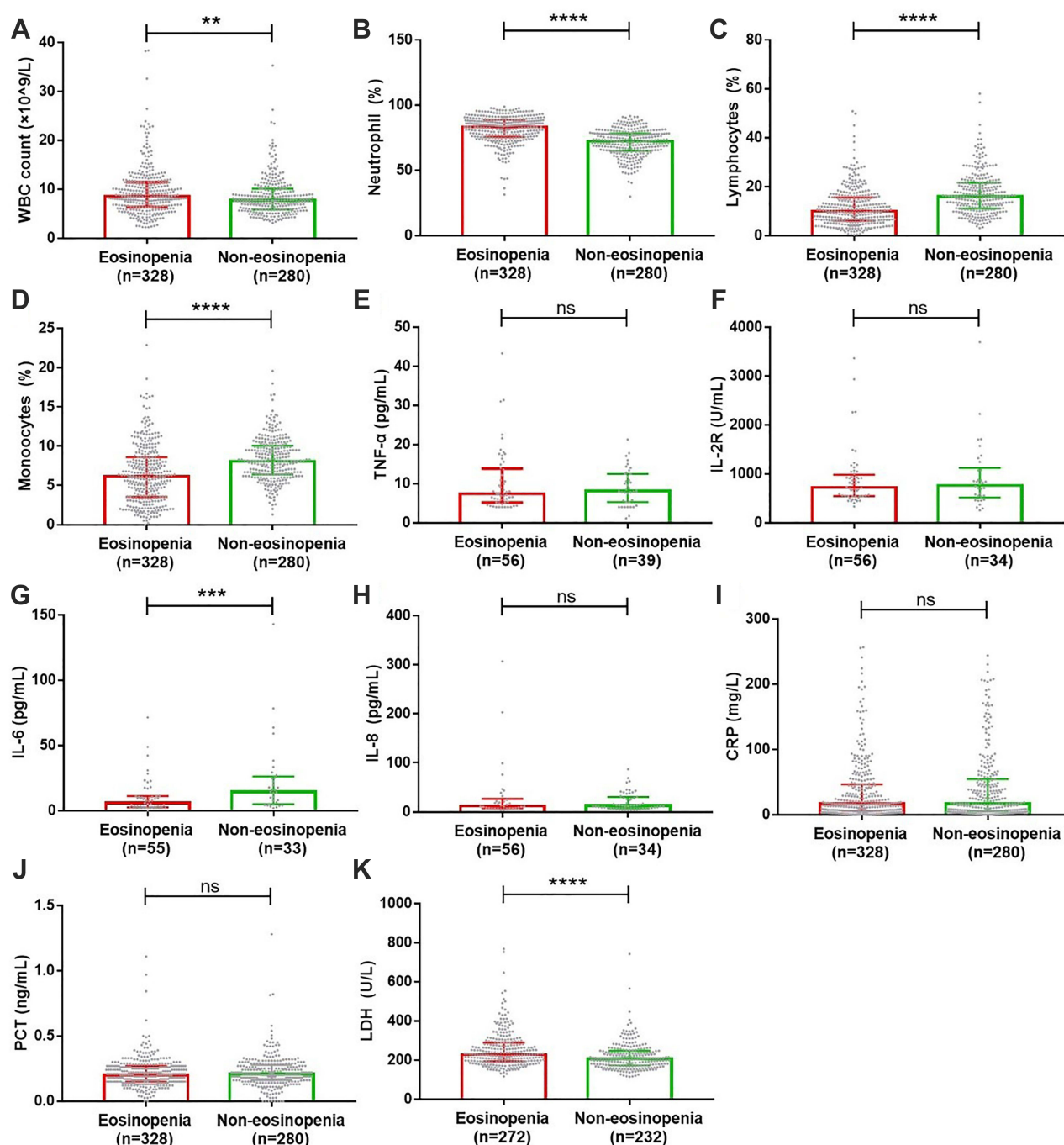
**Table 3** Stability and Variability of the Low EOS Between Two Hospitalizations, Stratified by EOS Values of 50 Cells/ $\mu$ L

A. Stability and Variability of the Low EOS Between Two Hospitalizations, Stratified by EOS Values of 50 Cells/μL.			
	Visit 2 non-low EOS	Visit 2 low EOS	P
Visit 1 low EOS (n=94)	34 (36.2%)	60 (63.8%)	0.003*
Visit 1 median EOS (n=31)	14 (45.2%)	17 (54.8%)	
Visit 1 high EOS (n=41)	28 (68.3%)	13 (31.7%)	
B. Stability and Variability of the low EOS between two hospitalizations, stratified by EOS percentage of 0.4%.			
	Visit 2 non-low EOS	Visit 2 low EOS	P
Visit 1 low EOS (n=83)	36 (43.4%)	47 (56.6%)	0.007*
Visit 1 median EOS (n=47)	31 (66.0%)	16 (34.0%)	
Visit 1 high EOS (n=36)	25 (69.4%)	11 (30.6%)	

**Notes:** (Bold figures) In patients with EOS values  $< 50$  cells/ $\mu$ L on the index date of hospitalized AECOPD, significantly higher proportion of patients were still in low EOS levels on the re-admission date for AECOPD compared with that of patients with EOS  $\geq 50$  cells/ $\mu$ L. The same difference was seen when stratifying low eosinophil level according to EOS percentage of leucocytes  $< 0.4\%$ . \* $P < 0.05$ .

**Abbreviation:** EOS, eosinophil.





**Figure 4** Comparison of inflammatory biomarkers between patients with eosinopenia and without in cohort I. The WBC count (A), the neutrophil percentage (B) and LDH concentration (K) were significantly higher in patients with eosinopenia compared to those in patients with non-eosinopenia, whereas the lymphocyte (C) and monocyte (D) percentage and IL-6 concentration (G) in eosinopenia group were markedly lower compared with those in the non-eosinopenia group. Some other infectious parameters' levels were not statistically different between eosinopenia and non-eosinopenia group, including TNF- $\alpha$  (E), IL-2 receptor (F), IL-8 (H), CRP (I) and PCT (J). **Notes:** Leucocytes, neutrophils and LDH levels were higher while lymphocytes, monocytes and IL-6 levels were lower in the low-EOS group than those in the non-low EOS group. \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .

**Abbreviations:** WBC, white blood cell; TNF, tumor necrosis factor; IL, interleukin; CRP, C-reactive protein; PCT, procalcitonin; LDH, lactate dehydrogenase.

cells/ $\mu L$  were more likely to be treated with NIMV than those with non-low EOS (OR = 1.86, 95% CI = 1.26~2.73; Table 4). However, no significant difference of length of hospital stay was observed between the low EOS and non-low EOS group (Table S4). In addition, patients with low EOS were prone to be re-admitted for AECOPD than patients with non-low EOS, although the difference was not statistically significant (0.4%: 55.6% vs 44.4%,  $P = 0.051$ ; 50 cells/ $\mu L$ :



**Table 4** Results from the Logistic Regression Model Showing NIMV Use for Low EOS Subgroups (<50 Cells/ $\mu$ L) Compared with the Reference Subgroup ( $\geq$ 50 Cells/ $\mu$ L) and Other Covariates (N = 608)

Predictor Variables	$\beta$ Coefficient	OR (95% CI)	P
Respiratory failure	2.117	8.31 (5.66~12.19)	<0.001*
EOS < 50 cells/ $\mu$ L	0.618	1.86 (1.26~2.73)	0.002*

Note: \* $P < 0.05$ .

Abbreviations: NIMV, noninvasive mechanical ventilation; EOS, eosinophil; OR, odds ratio; CI, confidence interval.

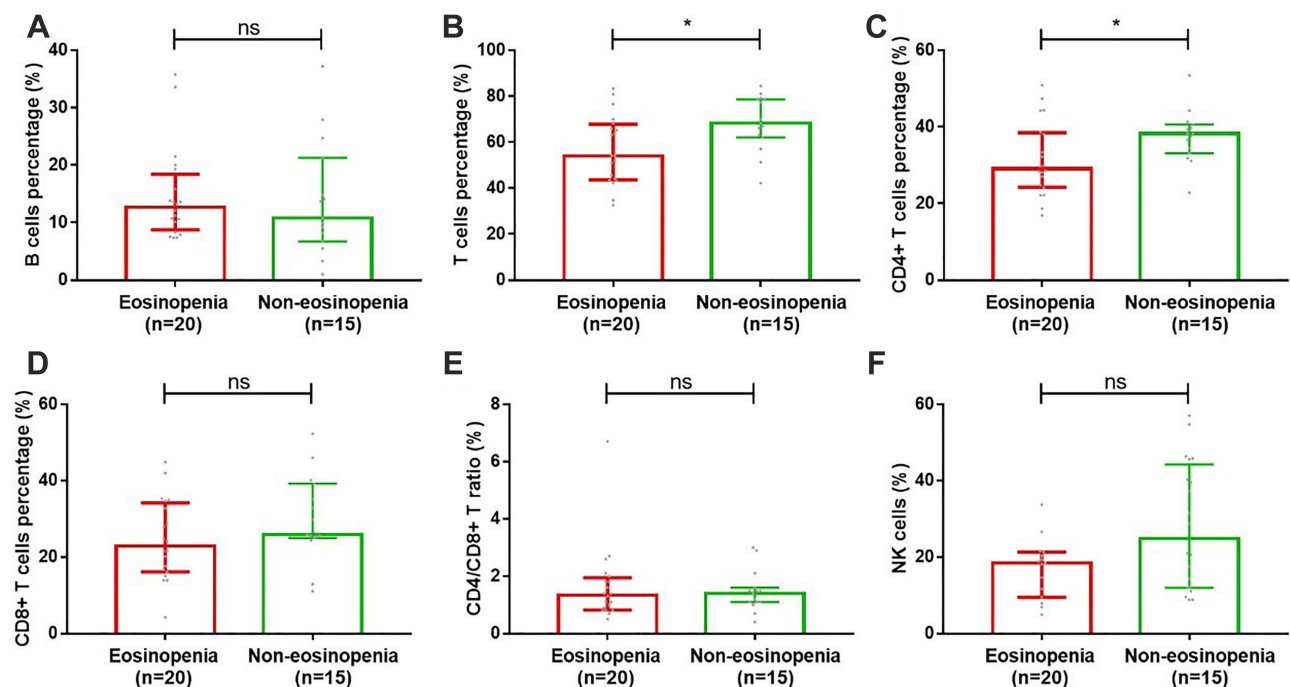
59.3% vs 40.7%,  $P = 0.350$ ; Table S5). The low EOS phenotype was not included in the risk factors for twelve-month AECOPD-related readmission, which consisted of hospitalization in winter (OR = 2.45, 95% CI = 1.17~5.15), ICS administration (OR = 2.90, 95% CI = 1.42~5.94) and non NIMV treatment (NIMV treatment vs non NIMV treatment, OR = 0.49, 95% CI = 0.25~0.97) (Figure S1).

## The Relationship Between EOS and Immune Cells

Total and CD4<sup>+</sup> T cells percentage were both markedly less in the low EOS group than those in the non-low EOS group (54.0% vs 85.7% for total T cells; 22.5% vs 43.4% for CD4<sup>+</sup> T cells;  $P < 0.05$ , Figure 5). However, differences of the percentage of B cells, CD8<sup>+</sup> T cells percentage, CD4<sup>+</sup>/CD8<sup>+</sup> ratio and NK cells account between the eosinopenia and the non-eosinopenia groups were not significant ( $P > 0.05$ ). Stronger positive associations between total T cells, CD4<sup>+</sup> T cells and the EOS percentage of leucocytes were observed in study population 3 ( $r = 0.46$  for total T cells;  $r = 0.40$  for CD4<sup>+</sup> T cells,  $P < 0.05$ ; Figure 6B and C). Blood EOS percentage correlated negatively with NK cell percentage ( $r = -0.39$ ,  $P < 0.05$ ; Figure 6E).

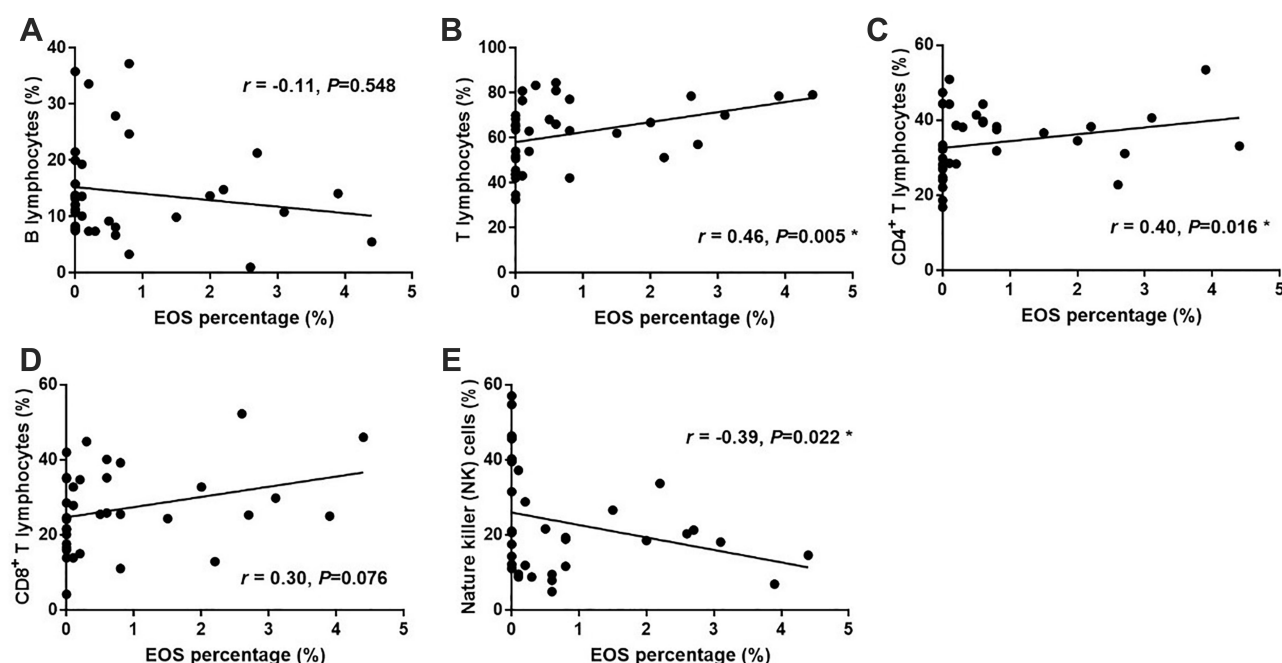
## Discussion

There has been a growing appreciation for the importance of blood eosinophil levels as a biomarker of future exacerbation risk and responsiveness to ICS in COPD patients.<sup>9,17</sup> This simple dichotomization of eosinophil counts



**Figure 5** Comparison of different immune parameters between patients with eosinopenia and without. Total (B) and CD4<sup>+</sup> T cells (C) percentage were both markedly less in the eosinopenia group than those in the non-eosinopenia group. Differences of the percentage of B cells (A), CD8<sup>+</sup> T cells percentage (D), CD4<sup>+</sup>/CD8<sup>+</sup> ratio (E) and NK cells account (F) between the eosinopenia and the non-eosinopenia groups were not significant.

Notes: Total and CD4<sup>+</sup> T cells percentages were both markedly less in the eosinopenia group than those in the non-eosinopenia group. \* $P < 0.05$ .



**Figure 6** Correlation between EOS percentage (%) and different immune cells.

**Notes:** No significant associations were found between total B cells (A), CD8+ T cells (D) and eosinophil percentage. Stronger positive associations between total T cells (B), CD4+ T cells (C) and eosinophil percentage was observed. Blood eosinophil percentage correlated negatively with NK cell percentage (E). \* $P < 0.05$ .

**Abbreviation:** EOS, eosinophil.

as “high” or “not high” may overlook important clinical evidence linked to low eosinophil counts. A recent study showed that a blood eosinophil threshold of 70 cells/ $\mu$ L showed the most discriminatory power to predict 30-day all-cause readmission (highest AUC) compared with that of 300 cells/ $\mu$ L.<sup>12</sup> However to date, there has been few data about the link of low eosinophil counts to the occurrence and prognosis of AECOPD. With an observational study design, we concluded that there existed low EOS phenotype and it was relatively stable between two hospitalizations for AECOPD. Low EOS was an independent risk factor for NIMV treatment in AECOPD. These exacerbations are usually associated with a decrease of CD4+ T cells, suggesting that the causative effect of CD4+ T cells on EOS can be explored.

The peripheral blood eosinopenia on admission of AECOPD, less than 50 cells/ $\mu$ L, occurred in 328 (53.9%) and 90 (54.2%) subjects among study population 1 and 2, suggestive of the prevalence of the low EOS phenotype. In this study, we also evaluated peripheral blood eosinophil count at discharge. Notably, the median percentage of EOS at discharge was significantly higher than that on admission in the low EOS subgroup. However, EOS count remained similar between two measurements in the non-low EOS subgroup. As for the stability of the low EOS during AECOPD, there was a concordance of 63.8% and 56.6% when using the 50 cells/ $\mu$ L and 0.4% cutoffs, respectively. Our results suggested that a low EOS phenotype might be a characteristic phenotype of AECOPD. Though we did not evaluate the stability of the low EOS between the stable state and acute exacerbations, Schumann et al demonstrated that the eosinophil counts between two exacerbations showed higher accordance than that between two measurements in stable state.<sup>15</sup> These study data suggest that the low EOS phenotype presents relatively stability during AECOPD, and attention should be paid to the influence of the low EOS on the clinical characteristics and outcomes of AECOPD.

Infection is the most common cause of AECOPD and the low eosinophilic group is predisposed to have clinical diagnosis of pulmonary infection than the non-low eosinophilic group.<sup>8,18</sup> Viral infection, especially rhinoviruses, is one of the major risk factors of acute exacerbations of COPD.<sup>19,20</sup> A case-control study showed viral respiratory pathogens were more often tested in sputum and nasal lavage of hospitalized patients with AECOPD than in patients with stable COPD (56% versus 19%).<sup>21</sup> Low EOS is a common feature of different types of viral infection.<sup>22</sup> Furthermore, Bafadhel et al reported that patients presenting to hospital with non-eosinophilic exacerbations of COPD had lower levels of CRP.<sup>17</sup> Contrary to their reports, no significant associations between low EOS with the diagnosis of clinical pulmonary

infection or the levels of CRP and PCT were observed in our study. Multiple studies reported that AECOPD due to viral infection is more common in the winter seasons.<sup>23,24</sup> Considering in our analysis more subjects with low EOS phenotype were admitted during winter than those with non-low EOS phenotype (45.4% vs 30.7%,  $P < 0.05$ ), it did hint that there might be some relationship of low EOS with viral infection in AECOPD. Hence, further prospective studies are needed.

Low EOS combined with the increased leukocytes has already been described as a risk factor for 30-day all-cause mortality in patients with cardiogenic shock complicating acute myocardial infarction.<sup>25</sup> It was also identified as a reliable marker of admission to medical intensive care units in patients with sepsis.<sup>26</sup> Both 30-day all-cause mortality and ICU admission mirror the severity of diseases, therefore we wondered whether low EOS was a potential predictor of AECOPD severity or not. In line with our hypothesis, we noted that eosinophil levels  $<50$  cells/ $\mu\text{L}$  or  $<0.4\%$  at admission for a severe AECOPD was associated with an almost double increase in the treatment with NIMV. The DECAF Score including “eosinopenia” was designed to identify those with higher risk of hospital mortality in exacerbations of COPD.<sup>27</sup> AECOPD patients could benefit from appropriate antibiotic and systemic corticosteroid therapy. In the first 72 hours of care, corticosteroids significantly improve dyspnea, lung function and ventilation-perfusion mismatch compared with placebo.<sup>28</sup> But recent studies reported that patients with low EOS did not respond to systemic corticosteroids as well as patients with non-low EOS.<sup>29</sup> In our analysis, the low eosinophilic group was prone to be suffered from respiratory failure than the non-low eosinophilic group. Respiratory failure is an indication for NIMV treatment in the clinical practice. More prospective trials are needed to verify this assertion. We aim to identify and prospectively validate biomarkers that allow the identification of patients in need of impending mechanical ventilation.

Although studies reported that patients with low EOS were accompanied with poor responsiveness to ICS therapy, elevated risk of infection<sup>13,17,30,31</sup> and we also showed high proportion of NIMV treatment in low EOS group, there was no difference in the length of hospital stay between the low and non-low EOS group in our study. MacDonald et al tentatively concluded that eosinophil count  $<50$  cells/ $\mu\text{L}$  was more strongly associated with longer median hospital stay than eosinophil count  $>150/\mu\text{L}$ . Careful attention to methodological differences revealed that an extended stay in their study was defined as 5 days or longer,<sup>8</sup> which is much shorter than our definition of 15 days. The confounding effect of medical insurance policy might mask the effect of low EOS on length of hospital stay. These findings require further confirmation in a larger prospective clinical trial by controlling for corticosteroid prescription and pre-defined discharge criteria. Additionally, we investigated whether long-term clinical outcomes, including readmission rate, differed by EOS level at the initial admission. In consistent with Bafadhel’s conclusion,<sup>17</sup> readmission rate over 12 months was not significantly different between the exacerbation phenotypes studied. First, patients might be admitted to another hospital when they were re-admitted with acute exacerbations. Second, the long-term outcomes of AECOPD or readmission rate might be more closely related to the underlying condition of the patient, such as comorbidities.<sup>32,33</sup> Pool-analysis with rigorously prospective design is required to explore the association of low EOS with 12-month AECOPD-related readmission.

Not only systemic inflammation, but also immune response are the major factors influencing the outcomes of AECOPD.<sup>34–36</sup> Our data indicated that less  $\text{CD4}^+$  T cells were frequently present in patients with eosinophil count  $<50$  cells/ $\mu\text{L}$ , with a stepwise decrease accompanied by gradual eosinophil count increment. It has been suggested that eosinophils are responsible for damage to bronchial epithelial cells by releasing toxic eosinophil granule proteins.<sup>37</sup> Our study showed that IL-6 level was lower in patients with low EOS than that in patients with non-low EOS. It was well established that elevated IL-6 levels could drive inflammation in a wide range of diseases, some of which were also characterized by enhanced T cell responses to IL-6.<sup>38</sup> Thus, we hypothesize that the level of IL-6 in the low EOS subgroup might be too low to enhance T cell differentiation, with the result of low  $\text{CD4}^+$  T cells percentage.

There are inevitable limitations in our study. We did not prove the causative effect of  $\text{CD4}^+$  T cells on EOS because it was an observational study. It has been proposed by us and others<sup>39</sup> that AECOPD patients with low EOS are associated with an increased risk of NIMV treatment and a decrease of  $\text{CD4}^+$  T cells. Furthermore, based on observations that accumulation of eosinophils can be mediated by activated  $\text{CD4}^+$  cells through numerous cytokines,<sup>40,41</sup> including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), we have hypothesized that low EOS was possibly due to a decrease of  $\text{CD4}^+$  T cells. AE-COPD would be associated with inappropriate immune activation, especially of the adaptive immune system<sup>42</sup> where  $\text{CD4}^+$  T cells have been shown to play a central role. Further experimental studies are needed to confirm the association of EOS and  $\text{CD4}^+$  T cells during AECOPD.

## Conclusion

To conclude, low EOS in AECOPD was a relatively stable phenotype. Low EOS was an independent risk factor for NIMV treatment in AECOPD. These exacerbations were usually accompanied with a decrease of CD4+ T cells, suggesting that the causative effect of CD4+ T cells on EOS might be explored.

## Funding

This work was supported, in part, by National Natural Science Foundation of China (project number 81970069; 82170091); Smart Healthcare Project of Zhongshan Hospital, Fudan University (project number 2020ZHXS18).

## Disclosure

The authors report no conflicts of interest in this work.

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