

Communication

Association between Serum Levels of Interleukin-25/Thymic Stromal Lymphopoietin and the Risk of Exacerbation of Chronic Obstructive Pulmonary Disease

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Citation: Choi, J.Y.; Kim, T.-H.; Kang, S.-Y.; Park, H.J.; Lim, S.Y.; Kim, S.H.; Jung, K.-S.; Yoo, K.H.; Yoon, H.K.; Rhee, C.K. Association between Serum Levels of Interleukin-25/Thymic Stromal Lymphopoietin and the Risk of Exacerbation of Chronic Obstructive Pulmonary Disease. *Biomolecules* **2023**, *13*, 564. <https://doi.org/10.3390/biom13030564>

Academic Editor: Kazuto Matsunaga

Received: 6 February 2023

Revised: 9 March 2023

Accepted: 17 March 2023

Published: 20 March 2023



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Abstract: Th2 inflammation is associated with various characteristics of patients with chronic obstructive pulmonary disease (COPD). In this study, we analyzed the COPD exacerbation risk associated with serum levels of interleukin (IL)-25/thymic stromal lymphopoietin (TSLP) and eosinophils. We studied the KOCOSS cohort, a multicenter COPD cohort created by 54 medical centers in South Korea. We extracted data collected between April 2012 and August 2020. We measured serum levels of TSLP and IL-25 in those who agreed to provide blood, and assessed exacerbation risk according to each. In all, 562 patients were enrolled. The IL-25-high group had a lower St. George's Respiratory Questionnaire score than others, and the TSLP-high group had a poorer exercise capacity than the TSLP-low group. There were no significant differences in the forced expiratory volume in 1 s (FEV₁), the levels of Th2 inflammatory biomarkers, or the exacerbation histories between the two groups. The 3-year decline in FEV₁ was not significantly affected by IL-25 or TSLP levels. In terms of 1-year exacerbation risk, individuals in the IL-25-high group were at lower risk for moderate-to-severe exacerbation than others. A high TSLP level was associated with a lower risk of severe exacerbation but only in the eosinophil-low group. Serum levels of IL-25 are negatively correlated with moderate-to-severe exacerbation risk in this cohort. A negative correlation between severe exacerbation risk and TSLP level was apparent only in the eosinophil-low group.

Keywords: alarmin; IL-25; TSLP; chronic obstructive pulmonary disease

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a heterogeneous condition characterized by persistent respiratory symptoms and fixed airway obstruction attributable to airway and/or alveolar damage caused by noxious particles or gases [1]. Most COPD patients evidence immunity of types 1 and 3 involving macrophages, neutrophils, CD8+ cytotoxic T cells, CD4+ TH1 cells, TH17 cells, ILC3 cells, and B cells. These reflect the mucosal inflammatory response to inhaled irritants [2]. However, 20–40% of COPD patients present with an eosinophilic phenotype associated with type 2 inflammation [3,4]. Such patients exhibit frequent exacerbations and higher mortality rates than non-eosinophilic patients [5–7]. Monoclonal antibodies targeting interleukin (IL)-5 or IL-5R α in eosinophilic COPD patients show promise in terms of reducing exacerbation [8–10].

Alarmins, including thymic stromal lymphopoietin (TSLP), IL-33, and IL-25, are cytokines secreted by bronchial epithelial cells in response to endogenous or exogenous danger signals. These cytokines are involved upstream of the Th2 inflammatory pathway [2]. Recently, high IL-33 levels were reported to be associated with chronic bronchitis and frequent exacerbation of COPD patients [11,12]; blockage of the IL-33 and ST2 pathways showed promise in terms of reducing COPD exacerbation [13,14]. The clinical outcomes associated with the levels of other alarmins (TSLP and IL-25) have been less thoroughly investigated. Particularly, because the anti-TSLP antibody tezepelumab showed promise in patients with severe uncontrolled asthma, biologic agents targeting the alarmins have become of increasing interest [15].

In this study, we explored the clinical characteristics of COPD according to IL-25 and TSLP levels. The exacerbation frequency over 1 year was prospectively determined by cytokine levels. As the cytokines act upstream of the Th2 inflammatory pathway, we stratified patients by high and low blood levels of eosinophils. We assessed differences in the rate of decline of the 3-year annual FEV1 according to each marker.

2. Materials and Methods

2.1. Study Population

The Korea COPD Subgroup Study (KOCOSS) cohort is a nationwide prospective group formed by 54 medical centers in South Korea, commencing in April 2012. Those aged >40 years with irreversible airflow obstructions (post-bronchodilator (BD) forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) \leq 70% of the normal predicted value) were included. Patients were evaluated at 6-month intervals after initial baseline examination. We extracted KOCOSS data from April 2012 to August 2020. We selected patients who gave blood samples. We excluded those with <10 pack-years of smoking history.

2.2. Clinical Characteristics

We collected baseline characteristics including age, sex, smoking history, and body mass index (BMI). Symptoms, health-related quality of life, and exercise capacity were evaluated using the modified Medical Research Council (mMRC) score, St. George's Respiratory Questionnaire (SGRQ), the COPD assessment test (CAT), and the 6 min walk test (6MWT). To explore asthma overlap and Th2 inflammation status, we evaluated asthma history and physician-diagnosed asthma–COPD overlap (ACO). We retrieved eosinophil counts, immunoglobulin (Ig)-E levels, and fractional exhaled nitric oxide (FeNO) values.

Pulmonary function tests (PFTs), including flow–volume curve construction, lung volume measurement, and assessment of the diffusion capacity of the lungs for carbon monoxide (DLCO) were performed at enrollment and annually thereafter. We extracted 3-year PFT results to derive lung function trajectories according to IL-25/TSLP levels. We recorded exacerbations in the year before baseline evaluation and during a 1-year follow-up. Exacerbation was defined as acute deterioration of respiratory symptoms requiring additional treatment such as antibiotics or corticosteroids. Exacerbations thus treated in outpatient clinics were defined as moderate; those that required hospitalization or an emergency room visit were defined as severe.

2.3. Measurement of IL-25 and TSLP

Plasma levels of IL-25 and TSLP were measured using Luminex-Multiplex kits (Komabiotech, Seoul, Korea). Blood samples were centrifuged for 10 min at $1000\times g$ within 30 min of collection in tubes with the anticoagulant sodium ethylenediaminetetraacetic acid (EDTA). Plasma was stored at $\leq -80\text{ }^{\circ}\text{C}$ and prepared for analysis in a 96-well plate employing a custom Human Cytokine/Chemokine Magnetic Bead Panel (Millipore Corp., Billerica, MA, USA). We followed the kit-specific protocol of the manufacturer of the Luminex 200 analyzer (Luminex, Austin, TX, USA) that ran MasterPlex QT software version 4 (MiraiBio, San Bruno, CA, USA). Standard curves for all analytes were generated using standards provided by the manufacturer. We distinguished high/low-IL-25 and high/low-TSLP groups according to median values. The median level of IL-25 was 1.33 ng/mL. We divided patients into IL-25-low and IL-25-high groups using this as the cutoff. The median TSLP level was 0.78 pg/mL; we formed TSLP-low and TSLP-high groups using this value as the cutoff.

2.4. Statistical Analysis

All statistical analyses were performed using R software (ver. 3.6.3; the R Foundation for Computing, Vienna, Austria). Continuous variables are shown as means \pm standard deviations and categorical variables are given as numbers (percentages). We compared the clinical parameters of each group, and performed subgroup analyses according to eosinophil levels (≥ 300 vs. < 300 cells/ mm^3). Groups were compared using the Pearson χ^2 test for categorical variables and the Student's t-test for continuous variables. Correlations between blood eosinophil counts and cytokine levels were evaluated by deriving Pearson correlation coefficients. The 3-year rates of decline in FEV1 were compared using a linear mixed model adjusted for age, sex, and BMI as covariates. The exacerbation risk for each group was analyzed using a binomial regression model after adjustment for age and smoking and exacerbation histories. A p -value < 0.05 was considered statistically significant.

3. Results

3.1. Baseline Characteristics

We included 562 patients; Table 1 summarizes their baseline characteristics. The mean age was 68.8 ± 7.6 years and most were male (96.8%). Of all patients, 29.5% were current smokers with mean BMI 23.2 ± 3.2 kg/ m^2 . The mean mMRC, SGRQ, and CAT scores were 1.1 ± 0.9 , 30.4 ± 18.7 , and 14.0 ± 7.9 , respectively, and the mean 6MWT distance was 409.6 ± 106.7 m. Of all patients, 23.6% had histories of asthma and 21.0% had been physician-diagnosed with ACO. The mean blood eosinophil count, IgE level, and FeNO values were 226.8 ± 250.2 cells/ mm^3 , 216.5 ± 247.0 mg/dL, and 26.8 ± 17.1 ppb, respectively. The mean post-BD FEV1% predicted and FEV1/FVC were $64.9 \pm 18.7\%$ and 52.4 ± 11.5 , respectively. Of all patients, 12.9% had a history of exacerbation in the year prior to enrollment.

Table 1. Baseline characteristics.

	Overall (n = 562)
Age	68.8 ± 7.6
Sex (male)	544 (96.8%)
Smoking history	
- Former smoker	396 (70.5%)
- Current smoker	166 (29.5%)
BMI (kg/ m^2)	23.2 ± 3.2
mMRC score	1.1 ± 0.9
SGRQ score	30.4 ± 18.7
CAT score	14.0 ± 7.9
6MWT (m)	409.6 ± 106.7

Table 1. *Cont.*

	Overall (n = 562)
Hx of asthma	131 (23.6%)
Physician-diagnosed ACO	97 (21.0%)
GOLD stage	
I	87 (15.5%)
II	333 (59.4%)
III	108 (19.3%)
IV	33 (5.9%)
FEV1 (L)	1.9 ± 0.6
FEV1 (%)	64.9 ± 18.7
FVC (L)	3.6 ± 0.7
FVC (%)	85.5 ± 15.1
FEV1/FVC	52.4 ± 11.5
DLCO (mL/mmHg/min)	64.9 ± 18.4
Blood eosinophil count (/mm ³)	226.8 ± 250.2
IgE (mg/dL)	216.5 ± 247.0
FeNO (ppb)	26.8 ± 17.1
Hx of exacerbation	71 (12.9%)
IL-25 (ng/mL)	1.3 ± 0.4
TSLP (pg/mL)	0.8 ± 0.7

Data are presented as *n* (%) or mean ± SD. BMI, body mass index; mMRC, modified Medical Research Council; SGRQ, St. George's Respiratory Questionnaire; CAT, COPD assessment test; 6MWT, 6 min walk test; Hx, history; ACO, asthma–COPD overlap; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusion capacity of lungs for carbon monoxide; IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide.

3.2. Clinical Characteristics of the IL-25-Low and IL-25-High Groups

The clinical parameters according to different levels of IL-25 are shown in Table 2. There were no significant differences in age, sex, smoking history, or BMI between the two groups. There were also no significant differences in symptoms or exercise capacity, except that the SGRQ score was higher in the low group. There were no between-group differences in lung function, the levels of Th2 inflammatory biomarkers, or the histories of prior exacerbations.

Table 2. Differences in the clinical characteristics between IL-25-low/high groups.

	IL-25-Low (n = 212)	IL-25-High (n = 350)	<i>p</i> -Value
Age	68.6 ± 7.8	68.9 ± 7.5	0.64
Sex (male)	256 (97.3%)	288 (96.3%)	0.66
Smoking history			0.48
- Former smoker	181 (68.8%)	215 (71.9%)	
- Current smoker	82 (31.2%)	84 (28.1%)	
BMI (kg/m ²)	23.2 ± 3.3	23.1 ± 3.1	0.77
mMRC score	1.1 ± 0.8	1.2 ± 0.9	0.37
SGRQ score	33.0 ± 19.6	28.1 ± 17.5	<0.01
CAT score	14.4 ± 8.0	13.5 ± 7.8	0.18
6MWT (m)	401.2 ± 102.6	417.2 ± 110.0	0.11
Hx of asthma	56 (21.6%)	75 (25.4%)	0.34
Physician-diagnosed ACO	48 (22.2%)	49 (19.9%)	0.62
GOLD stage			0.136
I	40 (15.2%)	47 (15.8%)	
II	151 (57.4%)	182 (61.1%)	
III	50 (19.0%)	58 (19.5%)	
IV	22 (8.4%)	11 (3.7%)	
FEV1 (L)	1.9 ± 0.6	1.9 ± 0.6	0.33
FEV1 (%)	64.2 ± 19.6	65.6 ± 17.9	0.39
FVC (L)	3.5 ± 0.8	3.6 ± 0.7	0.49
FVC (%)	85.0 ± 15.5	85.9 ± 14.8	0.48

Table 2. *Cont.*

	IL-25-Low (<i>n</i> = 212)	IL-25-High (<i>n</i> = 350)	<i>p</i> -Value
FEV1/FVC	51.6 ± 11.8	53.1 ± 11.2	0.11
DLCO (mL/mmHg/min)	65.1 ± 18.5	64.7 ± 18.4	0.83
Blood eosinophil count (/mm ³)	206.2 ± 175.3	245.0 ± 300.5	0.07
IgE (mg/dL)	221.3 ± 258.9	211.9 ± 235.4	0.69
FeNO (ppb)	26.1 ± 16.4	27.7 ± 18.0	0.62
Hx of exacerbation	28 (10.9%)	43 (14.7%)	0.23
IL-25 (ng/mL)	1.2 ± 0.1	1.5 ± 0.5	<0.01
TSLP (pg/mL)	0.8 ± 0.4	0.9 ± 0.8	0.08

Data are presented as *n* (%) or mean ± SD. BMI, body mass index; mMRC, modified Medical Research Council; SGRQ, St. George's Respiratory Questionnaire; CAT, COPD assessment test; 6MWT, 6 min walk test; Hx, history; ACO, asthma–COPD overlap; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusion capacity of lungs for carbon monoxide; IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide.

3.3. Clinical Characteristics of the TSLP-Low and TSLP-High Groups

The clinical parameters according to different levels of TSLP are shown in Table 3. The TSLP-low group was younger (67.8 ± 7.4 vs. 69.4 ± 7.7 years, $p = 0.02$) and individuals in the group were more likely to be current smokers (34.9% vs. 6.3%, $p = 0.04$). There were no differences in the symptom scores; however, the 6MWT exercise capacity was poorer in the TSLP-high group (422.7 ± 106.0 vs. 400.6 ± 106.5 m, $p = 0.03$). The FEV1 did not differ between the two groups; however, the FEV1/FVC and DLCO were lower in the TSLP-high group (53.6 ± 11.4 vs. 51.6 ± 11.6 , $p = 0.04$; 67.7 ± 18.0 vs. $63.1 \pm 18.4\%$, $p = 0.01$, respectively). There were no differences in the levels of Th2 inflammatory biomarkers or the numbers of prior exacerbations. Also, the differences in the clinical characteristics according to the breakdown of IL-25 and TSLP values are shown in Table S1.

Table 3. Differences in clinical characteristics between TSLP-low/high groups.

	TSLP-Low (<i>n</i> = 212)	TSLP-High (<i>n</i> = 350)	<i>p</i> -Value
Age	67.8 ± 7.4	69.4 ± 7.7	0.02
Sex (male)	203 (95.8%)	341 (97.4%)	0.40
Smoking history			0.04
- Former smoker	138 (65.1%)	258 (73.7%)	
- Current smoker	74 (34.9%)	92 (26.3%)	
BMI (kg/m ²)	23.5 ± 3.2	23.0 ± 3.2	0.08
mMRC score	1.1 ± 0.9	1.2 ± 0.9	0.35
SGRQ score	31.3 ± 19.2	29.8 ± 18.3	0.33
CAT score	14.2 ± 8.0	13.8 ± 7.9	0.60
6MWT (m)	422.7 ± 106.0	400.6 ± 106.5	0.03
Hx of asthma	49 (23.8%)	82 (23.6%)	1.00
Physician-diagnosed ACO	43 (23.5%)	54 (19.4%)	0.34
GOLD stage			0.681
I	31 (14.7%)	56 (16.0%)	
II	132 (62.6%)	201 (57.4%)	
III	37 (17.5%)	71 (20.3%)	
IV	11 (5.2%)	22 (6.3%)	
FEV1 (L)	1.9 ± 0.6	1.9 ± 0.6	0.23
FEV1 (%)	65.5 ± 18.2	64.5 ± 19.1	0.54
FVC (L)	3.6 ± 0.7	3.5 ± 0.8	0.81
FVC (%)	85.5 ± 15.2	85.4 ± 15.1	0.98
FEV1/FVC	53.6 ± 11.4	51.6 ± 11.6	0.04
DLCO (mL/mmHg/min)	67.7 ± 18.0	63.1 ± 18.4	0.01
Blood eosinophil count (/mm ³)	235.9 ± 278.4	221.4 ± 231.9	0.54
IgE (mg/dL)	213.2 ± 262.7	218.4 ± 238.	0.83
FeNO (ppb)	26.6 ± 17.2	27.0 ± 17.2	0.91

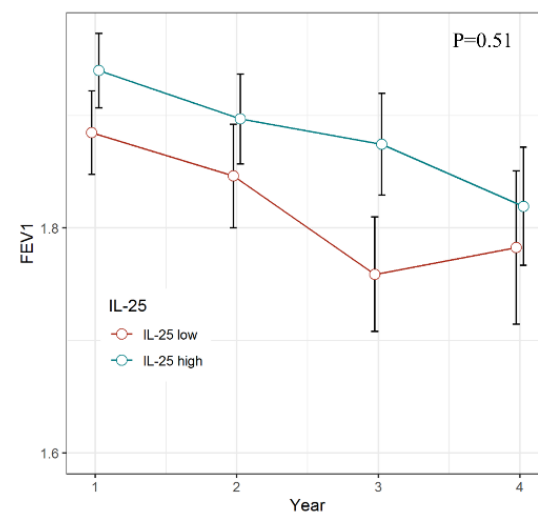
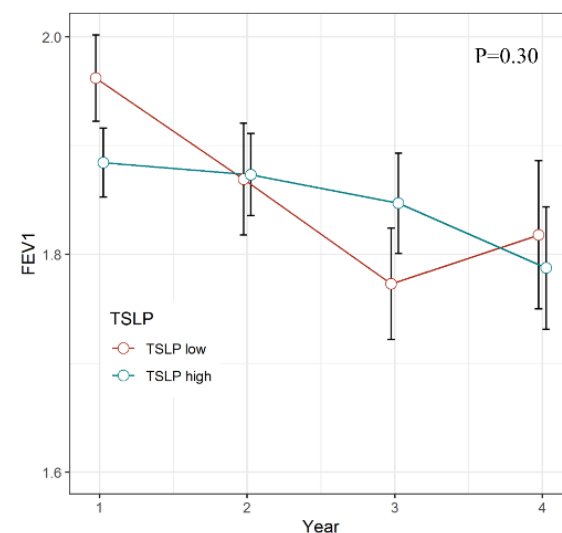
Table 3. *Cont.*

	TSLP-Low (<i>n</i> = 212)	TSLP-High (<i>n</i> = 350)	<i>p</i> -Value
Hx of exacerbation	27 (12.9%)	44 (12.9%)	1.00
IL-25 (ng/mL)	1.3 ± 0.2	1.4 ± 0.5	0.07
TSLP (pg/mL)	0.4 ± 0.2	1.0 ± 0.8	<0.01

Data are presented as *n* (%) or mean ± SD. BMI, body mass index; mMRC, modified Medical Research Council; SGRQ, St. George's Respiratory Questionnaire; CAT, COPD assessment test; 6MWT, 6 min walk test; Hx, history; ACO, asthma–COPD overlap; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; DLCO, diffusion capacity of lungs for carbon monoxide; IgE, immunoglobulin E; FeNO, fractional exhaled nitric oxide.

3.4. Differences in Decline Trajectories of FEV1

There were no significant differences in the rate of decline in FEV1 according to IL-25 (Figure 1) or TSLP level (Figure 2).

**Figure 1.** Differences in lung function trajectory between IL-25-low and IL-25-high groups.**Figure 2.** Differences in lung function trajectory between TSLP-low and TSLP-high groups.

3.5. Differences in Exacerbation Risk

Overall, high levels of IL-25 were associated with a lower risk of future moderate-to-severe exacerbation (OR = 0.69, 95% CI [0.49–0.98]); high TSLP levels had no such association (Table 4). In subgroup analyses according to eosinophil level, neither the

IL-25 level nor TSLP level significantly affected future exacerbation risk in the eosinophil-high group. However, in the eosinophil-low group, a high TSLP level was significantly associated with a lower risk of future severe exacerbation (OR = 0.32, 95% CI [0.11–0.92]). Moreover, risk of moderate-to-severe exacerbation were comparable between patients with IL-25 high & TSLP high group, compared to those with both low group (Table S2).

Table 4. Exacerbation risk according to IL-25 and TSLP.

	Moderate-to-Severe Exacerbation			Severe Exacerbation		
	OR	95% CI	<i>p</i> -Value	OR	95% CI	<i>p</i> -Value
Overall						
IL-25-high	0.69	0.49–0.98	0.04	0.08	0.20–1.12	0.08
TSLP-high	1.02	0.70–1.47	0.93	0.48	0.21–1.08	0.08
Eosinophil-high						
IL-25-high	0.64	0.34–1.19	0.16	0.65	0.20–2.10	0.46
TSLP-high	1.14	0.58–2.26	0.69	0.95	0.29–3.39	0.93
Eosinophil-low						
IL-25-high	0.66	0.43–1.00	0.05	0.41	0.00–1.18	0.11
TSLP-high	0.90	0.58–1.40	0.64	0.32	0.11–0.92	0.04

Adjusted covariates: age, smoking history, past exacerbation history. IL-25, interleukin-25; TSLP, thymic stromal lymphopoietin.

4. Discussion

We explored the clinical characteristics of COPD patients according to IL-25 and TSLP levels. Patients with higher IL-25 levels evidenced a better health-related quality of life; patients with higher TSLP levels exhibited poorer exercise capacity. There were no significant differences in lung function, the rate of decline in FEV1, or exacerbation history between the low- and high-cytokine groups. Although IL-25 and TSLP initiate Th2 inflammation in response to various antigens or allergens, the biomarkers of such inflammation (blood eosinophil count, as well as IgE and FeNO levels) were comparable among groups. However, and importantly, the risk of moderate-to-severe exacerbation was lower in the IL-25-high group and the risk of severe exacerbation was lower in TSLP-high patients with lower blood eosinophil counts.

IL-25, TSLP, and IL-33 are alarmins produced by bronchial epithelial cells in response to exogenous or endogenous danger signals [16]. These cytokines act upstream of the Th2 inflammatory cascade, thus initiating the Th2 response and eosinophilic inflammation caused by Th2 lymphocytes and type 2 innate lymphoid cells that produce cytokines including IL-4, IL-5, and IL-13 [2,17,18]. COPD is a heterogeneous disease; the principal pathophysiologies are immunity of types 1 and 3 [2]. However, in some COPD patients, Th2 airway inflammation is important; such patients include those with eosinophilic COPD or ACO [2,19,20].

About 20–40% of COPD patients show eosinophilic inflammation [3,4]. Clinical outcomes according to eosinophil levels have varied among studies. In one previous study, 49% of COPD patients were persistently eosinophilic and 47% were intermittently eosinophilic using a cutoff of 2% [21]. Those with persistent eosinophilia had a higher FEV1 and better health-related quality of life than the others. There were no significant differences in longitudinal changes in the FEV1 or COPD exacerbations. By contrast, another study reported that eosinophil levels > 340 cells/mm³ were associated with a 1.76-fold increased risk of severe exacerbation [7]; another study reported a 1.49-fold increased risk of exacerbation in eosinophilic COPD patients in the KOCOSS database and accelerated rate of decline in lung function in non-eosinophilic COPD patients [6]. However, one single-center retrospective study reported opposite results [22]. Finally, a large population-based study in the Netherlands reported that blood eosinophilia (≥ 275 cells/mm³) was associated with increased all-cause mortality [5].

TSLP is an innate cytokine that plays important roles in allergic and adaptive airway inflammation [23]. Its expression is elevated in asthmatic patients and is inversely correlated

with lung function [24,25]. It is also elevated in COPD patients, and negatively correlated with eosinophil levels in sputum. Thus, TSLP may also be associated with non-allergic immunity [16,26]. Higher expression of TSLP in certain COPD patients may be associated with cigarette smoke, which induces its expression in the epithelium [27,28]. The potential role of IL-25 in COPD has received less attention. IL-25 levels increase in COPD patients with high TSLP levels [29]. Its level can be used to distinguish COPD from asthma, being significantly higher in the latter [30]. Surprisingly, we found that the levels of both IL-25 and TSLP were negatively correlated with the risk of future exacerbation, but the blood levels of eosinophils and IL-33 were positively correlated in previous studies [5,11]. Further work on these different pathophysiologies is required, as is an exploration of whether biological therapies would usefully target certain cytokines.

IL-33, another alarmin, is a member of the IL-1 family that signals via the ST2 pathway to promote Th2 inflammation [12]. IL-33 and ST2 levels are increased in COPD patients, and their expression is induced by cigarette smoke [31]. A high IL-33 level is associated with a chronic bronchitis phenotype and increased risk of acute exacerbation [11,12]. Recently, blockage of this pathway has yielded promising results. Itepekimab, a double-blind phase 2a trial, treated former smokers with COPD with monoclonal antibodies targeting IL-33; treatment reduced the risk of exacerbation and increased lung function [13]. In another study, astegolimab, a selective ST2 IgG2 monoclonal antibody, improved the health-related quality of life of patients with moderate-to-very-severe COPD [14]. Recently, tezepelumab (an anti-TSLP antibody) was found to lower the exacerbation risk, increase the FEV1, and enhance asthma control and health-related quality of life in a phase 3 trial in patients with severe uncontrolled asthma [15]. An ongoing clinical trial (NCT04039113) is investigating a possibly useful role in COPD patients. However, the decreased exacerbation risk that we found in the TSLP-high eosinophil-low group may not reflect blockage of the IL-33 and ST2 pathways.

Our work has certain limitations. First, most patients were male. However, no previous report has described any sex differences in IL-25 or TSLP levels. Second, most patients were treated in tertiary hospitals; they do not represent the entire COPD population. Third, we only collected the drug history of patients at baseline, and therefore it is unknown whether treatment affected the levels of IL-25 and TSLP. The most novel finding of our study was the inverse correlations between IL-25/TSLP and exacerbation risk, which makes it surprising that another alarmin, IL-33, was shown to have a positive correlation. Our work is the first to analyze the differences of IL-25/TSLP and clinical characteristics (including exacerbation risks) in COPD patients.

5. Conclusions

We investigated the roles of alarmins in COPD patients. A high IL-25 level was associated with a favorable health-related quality of life, and a high TSLP level was associated with poor exercise capacity. Unlike IL-33 and eosinophil count, IL-25 and TSLP levels were inversely associated with exacerbation risk, although the association between TSLP level and reduced exacerbation risk was significant only in those with low eosinophil counts. Thus, IL-25 and TSLP are potential biomarkers of prognosis and future targets for use in biologic therapy.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/biom13030564/s1>, Table S1. Differences in clinical characteristics according to the breakdown of IL-25 and TSLP values; Table S2. Risk of moderate-to-severe exacerbations between IL-25 high/TSLP high and both low group.

Author Contributions: Conceptualization, J.Y.C. and C.K.R.; methodology, J.Y.C. and C.K.R.; software, J.Y.C. and C.K.R.; validation, J.Y.C., C.K.R. and S.H.K.; formal analysis, J.Y.C. and C.K.R.; investigation, J.Y.C., T.-H.K., S.-Y.K., H.J.P., S.Y.L., S.H.K., K.-S.J., K.H.Y., H.K.Y. and C.K.R.; resources, T.-H.K., S.-Y.K., H.J.P., S.Y.L., S.H.K., K.-S.J., K.H.Y., H.K.Y. and C.K.R.; data curation, J.Y.C. and C.K.R.; writing—original draft preparation, J.Y.C.; writing—review and editing, all authors; visualization, J.Y.C. and C.K.R.; supervision, C.K.R., K.H.Y., K.-S.J. and H.K.Y.; project administration, J.Y.C. and C.K.R.; funding acquisition, K.H.Y., K.-S.J. and C.K.R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by grants from the Korean Environment Industry and Technology Institute through the Core Technology Development Project for Environmental Disease Prevention and Management, funded by the Korean Ministry of Environment (Grant number 2022003310008). This research was also supported by funds (2016ER670100, 2016ER670101, 2016ER670102, 2018ER67100, 2018ER67101, 2018ER67102, 2021ER120500, 2021ER120501, 2021ER120502) from Research of Korea Centers for Disease Control and Prevention.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by Ethics Committees of participating medical centers. The names of the approving Ethics Committees are provided as follows: Gachon University Gil Medical Center, Hallym University Kangnam Sacred Heart Hospital, Gangnam Severance Hospital, Kyung Hee University Hospital at Gangdong, Hallym University Kangdong Sacred Heart Hospital, Kangbuk Samsung Hospital, Kangwon National University Hospital, Konkuk University Hospital, Konkuk University Chungju Hospital, Kyungpook National University Hospital, Gyeongsang National University Hospital, Korea University Guro Hospital, Korea University Anam Hospital, Seoul Eulji Hospital, Dongguk University Gyeongju Hospital, Dongguk University Ilsan Hospital, Keimyung University Dongsan Medical Center, Dong-A University Hospital, Hallym University Dongtan Sacred Heart Hospital, Pusan National University Hospital, Inje University Busan Paik Hospital, The Catholic University of Korea Bucheon St Mary's Hospital, Soonchunhyang University Hospital Bucheon, Seoul National University Bundang Hospital, Bundang CHA Hospital, Seoul Metropolitan Government Seoul National University Bora-mae Medical Center, Samsung Medical Center, Soonchunhyang University Hospital Seoul, The Catholic University of Korea Seoul St Mary's Hospital, The Catholic University of Korea St Paul's Hospital, The Catholic University of Korea St Vincent's Hospital, Severance Hospital, Asan Medical Center, Ajou University Hospital, The Catholic University of Korea Yeouido St Mary's Hospital, The Catholic University of Korea Uijeongbu St Mary's Hospital, Yeungnam University Medical Center, Ulsan University Hospital, Wonkwang University Sanbon Hospital, Wonju Severance Christian Hospital, Ewha Womans University Mokding Hospital, Incheon St Mary's Hospital, Inha University Hospital, Chonnam National University Hospital, Chonbuk National University Hospital, Jeju National University Hospital, Soonchunhyang University Hospital Cheonan, Hallym University Chuncheon Sacred Heart Hospital, Hallym University Sacred Heart Hospital, and Hanyang University Guri Hospital.

Informed Consent Statement: Written informed consent was received from all participants in this study. Confidentiality of all personal information was secured.

Data Availability Statement: The datasets supporting the conclusions of this article cannot be shared publicly because the data of the KOCOSS study are third party data. Data are available from the KOCOSS (www.kocoss.kr, accessed on 1 July 2022) for researchers who meet the criteria for access to confidential data.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guidelines, Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Lung Disease. 2022. Available online: <https://goldcopd.org/2022-gold-reports/> (accessed on 1 July 2022).
2. Barnes, P.J. Targeting cytokines to treat asthma and chronic obstructive pulmonary disease. *Nat. Rev. Immunol.* **2018**, *18*, 454–466. [[CrossRef](#)]
3. George, L.; Brightling, C.E. Eosinophilic airway inflammation: Role in asthma and chronic obstructive pulmonary disease. *Ther. Adv. Chronic Dis.* **2016**, *7*, 34–51. [[CrossRef](#)]

4. David, B.; Bafadhel, M.; Koenderman, L.; De Soyza, A. Eosinophilic inflammation in COPD: From an inflammatory marker to a treatable trait. *Thorax* **2021**, *76*, 188–195. [[CrossRef](#)]
5. Hoppers, J.J.; Schouten, J.P.; Weiss, S.T.; Postma, D.S.; Rijcken, B. Eosinophilia is associated with increased all-cause mortality after a follow-up of 30 years in a general population sample. *Epidemiology* **2000**, *11*, 261–268. [[CrossRef](#)]
6. Jo, Y.S.; Moon, J.Y.; Park, Y.B.; Kim, Y.H.; Um, S.J.; Kim, W.J.; Yoon, H.K.; Yoo, K.H.; Jung, K.-S.; Rhee, C.K. Longitudinal changes in forced expiratory volume in 1 s in patients with eosinophilic chronic obstructive pulmonary disease. *BMC Pulm. Med.* **2022**, *22*, 91. [[CrossRef](#)] [[PubMed](#)]
7. Vedel-Krogh, S.; Nielsen, S.F.; Lange, P.; Vestbo, J.; Nordestgaard, B.G. Blood eosinophils and exacerbations in ehronic obstructive pulmonary disease. The Copenhagen General Population Study. *Am. J. Respir. Crit. Care Med.* **2016**, *193*, 965–974. [[CrossRef](#)] [[PubMed](#)]
8. Pavord, I.D.; Chanez, P.; Criner, G.J.; Kerstjens, H.A.M.; Korn, S.; Lugogo, N.; Martinot, J.-B.; Sagara, H.; Albers, F.C.; Bradford, E.S.; et al. Mepolizumab for eosinophilic chronic obstructive pulmonary disease. *N. Engl. J. Med.* **2017**, *377*, 1613–1629. [[CrossRef](#)] [[PubMed](#)]
9. Pavord, I.D.; Chapman, K.R.; Bafadhel, M.; Sciurba, F.C.; Bradford, E.S.; Schweiker Harris, S.; Mayer, B.; Rubin, D.B.; Yancey, S.W.; Paggiaro, P. Mepolizumab for eosinophil-associated COPD: Analysis of METREX and METREO. *Int. J. Chronic Obstr. Pulm. Dis.* **2021**, *16*, 1755–1770. [[CrossRef](#)]
10. Zhang, C.; Wang, Y.; Zhang, M.; Su, X.; Lei, T.; Yu, H.; Liu, J. Monoclonal antibodies targeting IL-5 or IL-5R α in eosinophilic chronic obstructive pulmonary disease: A systematic review and meta-analysis. *Front. Pharmacol.* **2021**, *12*, 754268. [[CrossRef](#)]
11. Joo, H.; Park, S.J.; Min, K.H.; Rhee, C.K. Association between plasma interleukin-33 level and acute exacerbation of chronic obstructive pulmonary disease. *BMC Pulm. Med.* **2021**, *21*, 86.
12. Kim, S.W.; Rhee, C.K.; Kim, K.U.; Lee, S.H.; Hwang, H.G.; Kim, Y.I.; Kim, D.K.; Oh, Y.M.; Yoon, H.K. Factors associated with plasma IL-33 levels in patients with chronic obstructive pulmonary disease. *Int. J. Chronic Obstr. Pulm. Dis.* **2017**, *12*, 395–402. [[CrossRef](#)] [[PubMed](#)]
13. Rabe, K.F.; Celli, B.R.; Wechsler, M.E.; Abdulai, R.M.; Luo, X.; Boomsma, M.M.; Staudinger, H.; Horowitz, J.E.; Baras, A.; Ferreira, M.A.; et al. Safety and efficacy of itepekimab in patients with moderate-to-severe COPD: A genetic association study and randomised, double-blind, phase 2a trial. *Lancet Respir. Med.* **2021**, *9*, 1288–1298. [[CrossRef](#)] [[PubMed](#)]
14. Yousuf, A.J.; Mohammed, S.; Carr, L.; Yavari Ramsheh, M.; Micieli, C.; Mistry, V.; Haldar, K.; Wright, A.; Novotny, P.; Parker, S.; et al. Astegolimab, an anti-ST2, in chronic obstructive pulmonary disease (COPD-ST2OP): A phase 2a, placebo-controlled trial. *Lancet Respir. Med.* **2022**, *10*, 469–477. [[CrossRef](#)]
15. Menzies-Gow, A.; Corren, J.; Bourdin, A.; Chupp, G.; Israel, E.; Wechsler, M.E.; Brightling, C.E.; Griffiths, J.M.; Hellqvist, Å.; Bowen, K.; et al. Tezepelumab in adults and adolescents with severe, uncontrolled asthma. *N. Engl. J. Med.* **2021**, *384*, 1800–1809. [[CrossRef](#)]
16. Moermans, C.; Damas, K.; Guiot, J.; Njock, M.S.; Corhay, J.L.; Henket, M.; Schleich, F.; Louis, R. Sputum IL-25, IL-33 and TSLP, IL-23 and IL-36 in airway obstructive diseases. Reduced levels of IL-36 in eosinophilic phenotype. *Cytokine* **2021**, *140*, 155421. [[CrossRef](#)] [[PubMed](#)]
17. Brusselle, G.G.; Koppelman, G.H. Biologic therapies for severe asthma. *N. Engl. J. Med.* **2022**, *386*, 157–171. [[CrossRef](#)]
18. Israel, E.; Reddel, H.K. Severe and difficult-to-treat asthma in adults. *N. Engl. J. Med.* **2017**, *377*, 965–976. [[CrossRef](#)]
19. Mekov, E.; Nuñez, A.; Sin, D.D.; Ichinose, M.; Rhee, C.K.; Maselli, D.J.; Coté, A.; Ulrik, C.S.; Maltais, F.; Anzueto, A.; et al. Update on Asthma-COPD Overlap (ACO): A narrative review. *Int. J. Chronic Obstr. Pulm. Dis.* **2021**, *16*, 1783–1799. [[CrossRef](#)]
20. Hiles, S.A.; Gibson, P.G.; McDonald, V.M. Disease burden of eosinophilic airway disease: Comparing severe asthma, COPD and asthma-COPD overlap. *Respirology* **2021**, *26*, 52–61. [[CrossRef](#)]
21. Singh, D.; Kolsum, U.; Brightling, C.E.; Locantore, N.; Agusti, A.; Tal-Singer, R. Eosinophilic inflammation in COPD: Prevalence and clinical characteristics. *Eur. Respir. J.* **2014**, *44*, 1697–1700. [[CrossRef](#)]
22. Rogliani, P.; Puxeddu, E.; Ciapriani, C.; Ora, J.; Onorato, A.; Pezzuto, G.; Calzetta, L.; Cazzola, M. The time course of pulmonary function tests in COPD Patients with different levels of blood eosinophils. *BioMed Res. Int.* **2016**, *2016*, 4547953. [[CrossRef](#)]
23. Ziegler, S.F.; Artis, D. Sensing the outside world: TSLP regulates barrier immunity. *Nat. Immunol.* **2010**, *11*, 289–293. [[CrossRef](#)]
24. Li, Y.; Wang, W.; Lv, Z.; Li, Y.; Chen, Y.; Huang, K.; Corrigan, C.J.; Ying, S. Elevated expression of IL-33 and TSLP in the airways of human asthmatics in vivo: A Potential biomarker of severe refractory disease. *J. Immunol.* **2018**, *200*, 2253–2262. [[CrossRef](#)]
25. Ying, S.; O'Connor, B.; Ratoff, J.; Meng, Q.; Mallett, K.; Cousins, D.; Robinson, D.; Zhang, G.; Zhao, J.; Lee, T.H.; et al. Thymic stromal lymphopoietin expression is increased in asthmatic airways and correlates with expression of Th2-attracting chemokines and disease severity. *J. Immunol.* **2005**, *174*, 8183–8190. [[CrossRef](#)]
26. Ying, S.; O'Connor, B.; Ratoff, J.; Meng, Q.; Fang, C.; Cousins, D.; Zhang, G.; Gu, S.; Gao, Z.; Shamji, B.; et al. Expression and cellular provenance of thymic stromal lymphopoietin and chemokines in patients with severe asthma and chronic obstructive pulmonary disease. *J. Immunol.* **2008**, *181*, 2790–2798. [[CrossRef](#)]
27. Hizawa, N.; Masuko, H.; Sakamoto, T.; Kaneko, Y.; Iijima, H.; Naito, T.; Noguchi, E.; Hirota, T.; Tamari, M. Lower FEV1 in non-COPD, nonasthmatic subjects: Association with smoking, annual decline in FEV1, total IgE levels, and TSLP genotypes. *Int. J. Chronic Obstr. Pulm. Dis.* **2011**, *6*, 181–189. [[CrossRef](#)] [[PubMed](#)]
28. Fang, C.; Siew, L.Q.; Corrigan, C.J.; Ying, S. The role of thymic stromal lymphopoietin in allergic inflammation and chronic obstructive pulmonary disease. *Arch. Immunol. Ther. Exp.* **2010**, *58*, 81–90. [[CrossRef](#)] [[PubMed](#)]

29. Wu, L.; Fang, L.; Xu, X.; Pei, D.; Zhou, W.; Wan, H. Effect of TSLP on the function of platelets and IL-25 in chronic obstructive pulmonary disease. *Int. J. Clin. Exp. Med.* **2019**, *12*, 4942–4948.
30. Katoh, S.; Ikeda, M.; Shirai, R.; Abe, M.; Ohue, Y.; Kobashi, Y.; Oka, M. Biomarkers for differentiation of patients with asthma and chronic obstructive pulmonary disease. *J. Asthma* **2018**, *55*, 1052–1058. [[CrossRef](#)]
31. Gabryelska, A.; Kuna, P.; Antczak, A.; Białasiewicz, P.; Panek, M. IL-33 mediated inflammation in chronic respiratory diseases: Understanding the role of the member of IL-1 superfamily. *Front. Immunol.* **2019**, *10*, 692. [[CrossRef](#)]

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