

Interleukin-25 in sputum as a marker of airway inflammation in children asthma

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Dear Editor,

Interleukin-25 (IL-25, also called IL-17E) is an important IL-17 family member that helps initialize and regulate type 2 immunity, exerting an important influence on the pathogenesis of asthma (Corrigan et al., 2011; Barlow et al., 2012). Furthermore, it plays a vital role in initiating type 2 innate lymphoid cell activation and maintenance (Tong and Li, 2018) and inducing Th2-type cytokine-mediated allergic airway eosinophilia (Morita et al., 2015). IL-25 expression increases in patients with asthma in different types of lung cells, including lung structural cells (such as endothelium and airway epithelium), eosinophils, T-cells, and mast cells. Following allergen exposure, cells in the bronchial submucosa increase and secrete or respond to IL-25 (Corrigan et al., 2011). Plasma IL-25 levels increase in patients with asthma and correlate with lung function (Cheng et al., 2014). The association between IL-25⁺ cells and pulmonary function indicates that IL-25 could be used to monitor asthma severity. In some mouse models, epithelial IL-25 cytokines are closely related with a range of airway inflammation modalities, including type 2 cytokine production, airway eosinophil inflammation, mucinous epithelial metaplasia, and airway hyperresponsiveness (AHR) (Suzukawa et al., 2012).

The sputum induction is a relatively safe and noninvasive method that can be used to assess airway inflammation by analyzing cellular composition and soluble inflammatory mediator levels. Sputum cytometry may be related to different asthma phenotypes (James and Hedlin, 2016). Recent studies concluded that the analysis of induced sputum can identify different “cytokine-high” patterns associated with lung function parameters, asthma control, and body mass index (BMI) (Seys et al., 2013; Marijsse et al., 2014). Remarkably, IL-25 levels in the sputum of children with bronchial asthma have rarely been studied. Therefore, this study aims to assess the association between IL-25 levels in induced sputum and clinical parameters in children with asthma with few confounding factors.

We enrolled 80 children with mild-to-moderate asthma according to the Global Initiative for Asthma guidelines and 18 age-matched healthy controls. Children with asthma were categorized into groups A and B. Group A comprised patients ($n=32$) with uncontrolled asthma having a history of several mild-to-moderate attacks during the previous year; none of them received corticosteroids, except bronchodilators. They did not use any antiasthmatic drugs, such as inhaled corticosteroids (ICSs), leukotriene receptor antagonists, or theophylline, and had normal lung auscultation. These patients had no acute attacks of bronchial asthma in the previous 4 weeks before they enrolled in the study, with forced expiratory volume (FEV) 1% predicted to be

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$\geq 75\%$ before using a bronchodilator and an Asthma Control Questionnaire (ACQ) score < 1.5 . Group B comprised patients ($n=30$) with well-controlled asthma due to the use of antiasthmatic drugs. They were symptom free for > 3 months with regular use of antiasthmatic drugs, including ICSs and/or leukotriene receptor antagonists. These patients were allowed to continue their usual drug treatment, ensuring that no acute asthma attack occurred during the previous 4 weeks; they had a similar FEV₁ condition to that observed in group A, with an ACQ score < 0.75 . This study was approved by the medical ethics committee of Shandong University Medical Academy, and written informed consent was obtained from parents and children before they enrolled in the study.

Sixteen patients (20%) of children with asthma and three healthy volunteers (16.6%) were excluded from our study due to poor-quality sputum production and adverse reactions. The mild adverse reactions in 8 cases were as follows: cough ($n=3$), vomiting ($n=2$), wheezing ($n=2$), and transient decrease in oxygen saturation ($n=1$). Finally, 15 healthy volunteers and 46 patients with asthma (group A, $n=24$; group B, $n=22$) were enrolled (Figure 1A). No statistical difference was observed between patients with asthma and healthy controls in terms of age, sex, and BMI.

IL-25 levels in the supernatant of induced sputum (SpIL-25) and serum were determined via enzyme-linked immunosorbent assay using a commercially available kit (R&D systems, USA). We found that IL-25 levels in sputum and plasma were elevated in group A compared with the control ($P<0.001$) and group B ($P<0.05$). The median SpIL-25 value was considerably higher in group A (47.16 ± 13.50) than in group B (39.14 ± 9.03) or the control (36.76 ± 6.48) (Figure 1B), indicating a significant correlation between them in children with asthma (group A, $r=0.669$, $P<0.01$; group B, $r=0.576$, $P<0.05$) (Figure 1C). Patients with well-controlled asthma had lower IL-25 levels than those with uncontrolled asthma without antiasthmatic drugs. In addition, the ratios of IL-25 protein in the induced sputum significantly increased in both groups A and B, signifying that IL-25 protein levels increased in children with asthma; therefore, a significant difference was found between groups A and B ($P<0.001$) (Figures 1D). Moreover, IL-25 protein levels were increased in children with asthma, particularly in those who did not receive any antiasthmatic treatment (Figure 1D), which is consistent with the findings of previous studies in which IL-25 levels were elevated in induced sputum samples of adults with asthma (Marijsse et al., 2014; Salter et al., 2016), indicating that IL-25 can reflect airway inflammation in patients with asthma. IL-25 levels produced by patients with asthma are associated with the clinical status and control level of bronchial asthma. These findings may provide additional support for the reduction of airway IL-25 levels as a therapeutic approach for asthma.

Through sputum induction, we obtained qualified sputum

samples, in which the percentage of squamous cells with standard was < 25 squamous epithelial cells and > 25 leukocytes per low-power field ($10\times$ lens objective). In our study, the induced sputum of group A had a significantly lower number and percentage of neutrophils but had a higher number and percentage of eosinophils (both $P<0.001$) than that in group B and healthy controls. The percentages of eosinophils and neutrophils positively correlated with the SpIL-25 levels in group A ($r=0.886$, $P<0.01$ and $r=0.655$, $P<0.01$, respectively), reflecting the influence of IL-25 on neutrophils and eosinophils in induced sputum. More recently, similar findings about the association between sputum eosinophil and IL-25 were demonstrated (Seys et al., 2013). This finding may further support the association of IL-25 with the activation and recruitment of granulocytes and eosinophils in patients with asthma.

To assess AHR, the methacholine inhalation test was conducted in all patients according to the guidelines of the American Thoracic Society. By completing the pulmonary function and methacholine bronchial provocation tests, a positive correlation was observed between SpIL-25 levels and FEV₁ (% pred) ($r=0.956$, $P<0.01$) and PC20 (mg mL^{-1}) ($r=0.549$, $P<0.05$). A similar correlation was also observed in group B ($r=0.539$, $P<0.05$), indicating that SpIL-25 may reflect a higher AHR in children with uncontrolled asthma than in those with well-controlled asthma or healthy controls. These data may supply a potential link between IL-25 and airway inflammation in patients with asthma.

We also determined fractional exhaled nitric oxide (FENO) levels and found that SpIL-25 levels significantly correlated with FENO in group A ($r=-0.77$, $P<0.01$). A correlation between SpIL-25 levels and eosinophils in sputum in group B was also observed ($r=0.405$, $P<0.05$). In addition, SpIL-25 levels correlated significantly with IL-25 levels in the serum, FENO, and eosinophils (%) in induced sputum in groups A and B (Figures 1E). These data may certify a powerful association between airway inflammatory state and elevated IL-25 levels in induced sputum in patients with asthma.

Other asthma characteristics, including FEV₁/FVC% predicted, FEF25%–75% (forced expiratory flow between 25% and 75%) predicted, macrophages (%) and epithelial cells in sputum, C-reactive protein (CRP), and serum eosinophil counts, did not correlate with SpIL-25 levels. Total immunoglobulin E levels in the serum were significantly increased in groups A and B, but their correlation with SpIL-25 was only observed in group A ($r=-0.431$, $P<0.05$). Although blood eosinophil counts significantly increased in group A ($P<0.001$), no correlation was observed between SpIL-25 and blood eosinophil counts.

In conclusion, IL-25 levels in induced sputum might be an important biomarker in the assessment of airway inflammation and asthma-control status in children with

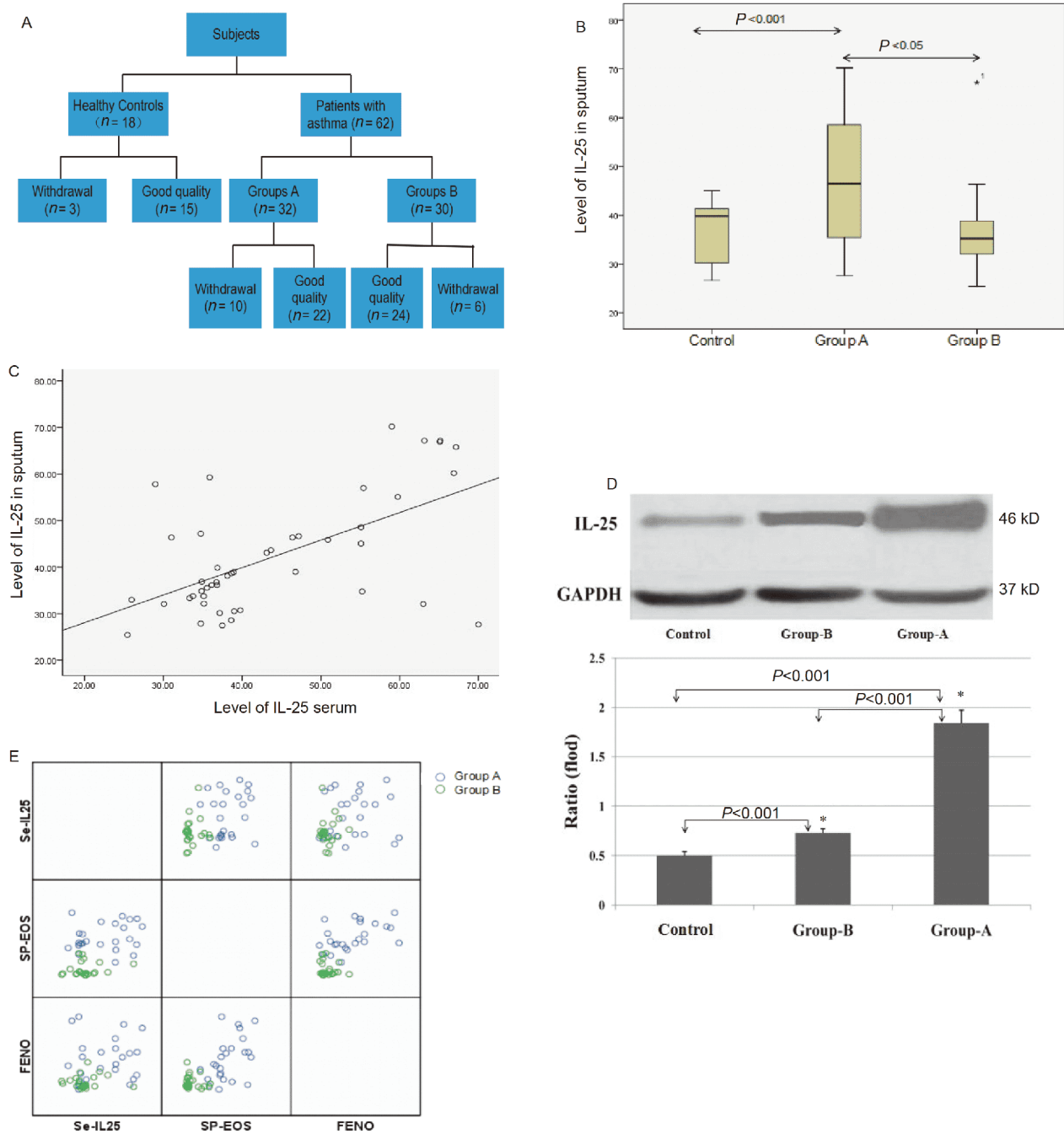


Figure 1 Study of sputum interleukin-25 as a marker of airway inflammation in children with asthma ($P < 0.05$ indicates a statistical significance). **A**, Flowchart of the study. (group A: patients with uncontrolled asthma without antiasthmatic drug treatment; group B: patients with controlled asthma treated with antiasthmatic drugs). **B**, IL-25 concentration in induced sputum in the study population. IL-25 levels in the supernatant of induced sputum were significantly increased in group A compared with the control ($P < 0.001$) and group B ($P < 0.05$). **C**, Relationships between IL-25 levels in induced sputum and serum in patients with asthma. **D**, The protein expression levels of IL-25 in induced sputum were analyzed by Western blotting. *, $P < 0.05$ versus the control group. **E**, The correlation between IL-25 levels in induced sputum and that in serum, FENO, and eosinophils in induced sputum samples in groups A and B. A correlation was observed between the SpIL-25 levels and IL-25 levels in serum, FENO, and eosinophils (%) in induced sputum in groups A and B.

asthma and might provide a potential clue regarding the therapeutic potential of targeting IL-25 for asthma. However, these results require further confirmation from in-depth sample analysis with large sample sizes.

Compliance and ethics The author(s) declare that they have no conflict of interest.

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SUPPORTING INFORMATION

Table S1 Clinical characteristics of the study population ($n=61$)

Table S2 Comparison of induced sputum findings between patients with asthma and healthy controls

Table S3 Correlations between sputum IL-25 levels and independent variables in patients with asthma ($n=46$)

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1 **Table S1.** Clinical characteristics of the study population (n = 61).

Variable	Control (n =15)	Asthmatic patients (n = 46)		p-value
		Group A (n = 24)	Group B (n = 22)	
Age(yr)	(7-14) 9.32	(6-17) 10.15	(6-14) 9.41	NO
Sex (M/F)	6/9	10/14	12/10	NO
BMI kg/m ²	19.22±2.2	18.22±2.91	18.58±2.75	NO
Total IgE IU/mL	65.13±2.29	673.22±521.63 ^{Δ†}	401.1±327.0 [‡]	<0.001
CRP, mg/L	1.47±0.65	6.09±4.73 ^{Δ†}	3.21±2.08	<0.001
FEV1 (% pred)	93.74±3.97	87.43±4.7 ^{Δ*}	88.07±5.53 ^{&}	<0.001
FEV1%FVC	90.49±3.7	84.07±4.17 ^Δ	86.39±4.69 ^{&}	<0.001
FEF25-75% predicted	88.68±3.04	76.43±7.76 ^Δ	81.04±7.0 [‡]	<0.001
FENO, ppb	8.86±4.08	37.54±20.77 ^{Δ†}	18.44±6.75 [‡]	<0.001
DFEV1after salbutamol (% pred)	6.11±3.0	16.33±4.3 ^{Δ#}	8.59±3.9	<0.001
PC20, mg/mL	19.24±9.86 [#]	7.07±3.29	13.82±5.41	<0.001
B Eos count, cells/L	1.14±1.08	6.42±4.57 ^{Δ#}	2.47±2.46	<0.001
SpIL-25	36.76±6.48	47.16±13.50 ^{Δ*}	39.14±9.03	<0.01
SeIL-25	37.85±7.31	59.04±13.53 ^{†&}	38.41±8.36	<0.01

2 Values are given as median (interquartile range) or mean SD unless indicated otherwise.

3 M-males, F-females;

4 BMI=body mass index;

5 IgE=immunoglobulin E;

6 CRP=C-reactive protein

7 FEV1 (% pred)=forced expiratory volume in 1s (percentage of predicted value);

8 FEV1%FVC=the ratio of FEV1 to FVC;

9 FEF25-75%=forced expiratory flow during the middle half of the FVC maneuver;

10 FENO=fractional exhaled nitric oxide;

11 DFEV1 after salbutamol (% pred)=difference in forced expiratory volume in first second in 15 min
12 after delivering 200 mg of salbutamol expressed as percentage of predicted value;

13 PC20=provocational concentration of methacholine that produced a 20% decrease in FEV1;

14 B Eos count=Blood eosinophils count;

15 SpIL-25=level of IL-25 in sputum;

16 SeIL-25=level of IL-25 in serum.

17 * $p < 0.05$, † $p < 0.01$, # $p < 0.001$ (vs. Group B).

18 ‡ $p < 0.05$, & $p < 0.01$, Δ $p < 0.001$ (vs. Control subjects).

19 $p < 0.001$ (vs. Group A)

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35 **Table S2.** Comparison of induced sputum findings between asthmatic patients and healthy controls.

Group	Control	Group A	Group B	p-value
Volume(ml)	2.2	2.0	3.0	NO
Total cells (x106/g of sputum)	2.14±1.44	2.65±1.88	2.49±1.68	NO
Macrophages (%)	30.73±10.51	33.21±11.91	34.05±9.27‡	<0.01
Neutrophils (%)	40.4±11.67	36.53±15.6 # Δ	50.62±10.98 &	<0.001
Eosinophils (%)	0.57±0.42	20.73±6.58 # Δ	5.09±3.39	<0.001
Lymphocytes (%)	17.51±4.01	3.67±2.74	3.23±2.75	NO
Epithelial cells (%)	8.05±2.85	7.07±3.63	7.15±3.96	NO

Data are expressed as medians and ranges. * $p < 0.05$, † $p < 0.01$, # $p < 0.001$ (vs. Group B); ‡ $p < 0.05$, & $p < 0.01$, Δ $p < 0.001$ (vs. control subjects).

Table S3. Correlations between sputum IL-25 levels and independent variables in asthmatic patients (n = 46).

Group	Group A	Group B
Se IL-25	0.669 **	0.576*
Total IgE	-0.431*	0.142
C-reactive protein	0.071	-0.026
FEV1 (% pred)	1.027* *	0.539*
FEV1%FVC	0.078	-0.130
FEF25-75% predicted	-0.112	-0.286
FENO	-0.77**	-0.483*
DFFV1after salbutamol (% pred)	-0.112	-0.286
PC20	0.546*	0.09
B Eos count	-0.112	0.079
Macrophages (%)	0.261	-0.087
Neutrophils (%)	0.886* *	-0.008

Eosinophils (%)	0.655**	0.405*
Lymphocytes (%)	0.725*	0.162
Epithelial cells (%)	-0.308	0.256

Data are expressed as medians and ranges. * $p < 0.05$, ** $p < 0.01$ for differences between the four groups.