ORIGINAL RESEARCH

Serum IL-1β and IL-17 levels in patients with COPD: associations with clinical parameters

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Abstract: COPD is a chronic airway inflammatory disease characterized mainly by neutrophil airway infiltrations. Interleukin (IL)-1β and IL-17 are the key mediators of neutrophilic airway inflammation in COPD. This study was undertaken to evaluate the serum IL-1β and IL-17 levels and associations between these two key mediators with clinical parameters in COPD patients. Serum samples were collected from 60 COPD subjects during the acute exacerbation of COPD, 60 subjects with stable COPD and 40 healthy control subjects. Commercial enzyme-linked immunosorbent assay kits were used to measure the serum IL-1β and IL-17 concentrations. The association between serum IL-1β and IL-17 with $FEV_1\%$ predicted, C-reactive protein, neutrophil percentage and smoking status (pack-years) was assessed in the COPD patients. We found that serum IL-1β and IL-17 levels in acute exacerbation of COPD subjects were significantly higher than that in stable COPD or control subjects and were positively correlated to serum C-reactive protein levels, neutrophil % and smoking status (pack-years) but negatively correlated with $FEV_1\%$ predicted in COPD patients. More importantly, serum IL-1β levels were markedly positively associated with serum IL-17 levels in patients with COPD (P=0.741, P<0.001). In conclusion, elevated serum IL-1β and IL-17 levels may be used as a biomarker for indicating persistent neutrophilic airway inflammation and potential ongoing exacerbation of COPD.

Keywords: neutrophilic airway inflammation, cigarette smoking, exacerbation

Introduction

COPD, which features chronic airway inflammation and incompletely reversible airflow obstruction, is mainly caused by chronic cigarette smoking (CS) and exposure to various noxious gases or particles.[1] Although the pathogenesis of COPD is not clear, as is known to all, cigarette smoke-induced chronic inflammatory reactions in the airways and lung parenchyma are the leading causes of COPD.[2,3] Toxic chemicals in cigarette smoke give rise to abnormal airway inflammation, which triggers the release of chemokines, promoting the infiltration of neutrophils and other inflammatory cells into airways.[4,5] Particularly, neutrophils are the key players in the development of COPD, as demonstrated by the prominent increase in neutrophil count and percentage observed in sputum from COPD patients.[6] Accumulated neutrophils can produce and release a variety of proinflammatory mediators and enzymes including neutrophil elastase (NE) and matrix metalloproteinases, which work together to promote the development of chronic bronchitis and emphysema.[7–9]

Neutrophil airway inflammation, one of the prominent features of COPD, is mediated by proinflammatory cytokines. Interleukin (IL)-1β, a typical innate immune cytokine that has been correlated to COPD, plays an important role in initiating and maintaining

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airway inflammation.[10,11] IL-1β secretion is increased in stable and exacerbating COPD.[12,13] Many studies in the COPD model in mice demonstrated that IL-1β was pivotal in contributing to the development of airway inflammation and emphysema.[12,14–16] IL-1β is a key driver of neutrophil airway inflammation in COPD, mainly involving the following mechanisms: on the one hand, IL-1β is a potent inducer of IL-8 and IL-6 in normal human bronchial epithelial cells, two key cytokines promoting neutrophil recruitment and activation in airway;[17] on the other hand, IL-1β mediates increase in the number of T-lymphocytes and dendritic cells in bronchoalveolar lavage fluid (BLF) and lung tissues in CS-induced pulmonary inflammation model, and these two types of cells promote the release of IL-6 and IL-17, increasing the recruitment of neutrophils into airway.[12,18] A recent study reported that IL-1β-driven neutrophilia was mediated by IL-17 in the early stage, rather than in the peak phase of viral replication, in a chronic lung inflammation model in mice induced by lipopolysaccharide and elastase.[19]

Another important cytokine implicated in the pathogenesis of COPD is IL-17 (previously known as IL-17A). Increased levels of IL-17 and enhanced number of IL-17+cells have been detected in the bronchial mucosa and sputum of COPD patients.[20–23] IL-17, which is primarily produced by the T-lymphocytes, can mobilize the neutrophils to infiltrate into airways through the induction of release of IL-8 or rat macrophage inflammatory protein-2 (rMIP-2) in airway inflammation models in mice.[24–26]

Apart from ongoing local airway inflammation, it is increasingly recognized that systemic inflammation, which is possibly derived from a spillover from the lung into the systemic circulation, may be a key link between COPD and comorbidities.[27,28] Thus, the aim of this study was to evaluate serum IL-1β and IL-17 levels and associations between these two cytokines and clinical parameters (C-reactive protein [CRP], FEV_1% predicted, neutrophil percentage [Neu%] and smoking status [pack-years]) in patients with COPD.

Methods
Recruitment of subjects
In total, 60 subjects with stable COPD (S-COPD), 60 subjects with acute exacerbation of COPD (AE-COPD) and 40 healthy control subjects were recruited from Xiangya hospital, Central South University between April and November 2016. Lung function tests were performed by skilled technicians in all subjects. In order to meet the inclusion criteria, COPD subjects were recruited on the basis of clinical history of

chronic respiratory symptoms (ie, coughing, sputum or both, dyspnea), physical examination, chest radiography and a post-bronchodilator FEV_1/forced vital capacity (FVC) ratio <70% according to the Global Initiative for Chronic Obstructive Lung Disease guidelines.[1] Adults with normal pulmonary function (FEV_1/FVC >70% and FEV_1 >80% of predicted) can be included as healthy control subjects. Exclusion criteria are listed as follows: asthma or atopy, a history of coronary heart disease or malignancy or a systemic infection or an inflammatory process that could be associated with abnormal IL-1β and IL-17 levels.

An acute exacerbation was defined as a change in the symptoms of cough, expectoration and dyspnea that was beyond the daily variation and required changes in therapy in patients with COPD. All exacerbation visits occurred within 7 days after its onset. S-COPD was defined as having no symptoms correlated with exacerbation over at least 6 weeks.[29] Serum CRP was measured and routine blood examination conducted, and baseline characteristics, including sex, age, body mass index and smoking history (pack-years), were collected in all subjects.

Specimen collection
Blood samples were drawn in vacuum blood tube and centrifuged (1,500 × g for 20 minutes) at room temperature within 60 minutes of collection. The supernatants were frozen at −80°C until analysis. This study was approved by the Ethics Committee of Xiangya Hospital, Central South University, and all subjects signed informed consent to participate in this study.

Measurements of serum IL-1β and IL-17
Serum IL-1β and IL-17 concentrations were measured using commercial enzyme-linked immunosorbent assay kits in accordance with the manufacturer's protocols (R&D Systems, Minneapolis, MN, USA). All samples were measured in duplicate.

Statistical analysis
All statistical analyses were performed with SPSS version 19.0 (IBM Corporation, Armonk, NY, USA). Data were presented as the mean ± standard deviation (SD). Baseline characteristics of the subjects were compared by the independent *t*-test for continuous variables and the chi-square test for categorical variables. Differences between subject groups were analyzed by one-way analysis of variance with post hoc analysis carried out using Tamhane's test. Correlations between serum IL-1β and IL-17 levels and FEV_1% predicted, CRP, Neu% and

smoking status (pack-years) were performed using Spearman or Pearson methods, depending on the normality of the data distribution. A two-tailed $P < 0.05$ was considered significant.

Results

Study population

The demographic, clinical and lung function characteristics of the study subjects are presented in Table 1. As expected, the COPD patients had higher smoking exposure (pack-years), obvious airflow obstruction and higher serum CRP and Neu% than the controls. Patients with COPD and healthy control subjects were of similar age, sex and body mass index ($P > 0.05$).

IL-1β and IL-17 levels in serum

Serum IL-1β and IL-17 concentrations of the three subject groups are listed in Table 2. Figure 1 shows significantly higher serum IL-1β (12.73 ± 3.16 vs 6.19 ± 2.44 vs 2.66 ± 0.52 pg/mL, $P < 0.001$) and IL-17 (63.10 ± 10.78 vs 50.47 ± 8.94 vs 32.50 ± 5.40 pg/mL, $P < 0.001$) levels in the AE-COPD group than in the S-COPD or control group.

Relationship between serum IL-1β and IL-17 levels in patients with COPD

In patients with COPD, serum IL-1β levels showed a notable positive correlation with the levels of IL-17 ($r = 0.741$, $P < 0.001$; Figure 2A). Further regression analysis showed that IL-1β might be an important factor contributing to elevation of IL-17 in patients with COPD ($R^2 = 0.55$, regression coefficient $= 2.01$; Figure 2B).

Association of serum IL-1β and IL-17 levels with pulmonary function, CRP, Neu% and smoking status

There was significant inverse correlation between serum IL-1β ($r = -0.634$, $P < 0.001$; Figure 3A) or IL-17 ($r = -0.562$,

Table 2 Statistical comparison of serum IL-1β and IL-17 levels in patients with COPD and controls

Variables	Control (n=40)	S-COPD (n=60)	AE-COPD (n=60)	P-value
IL-1β (pg/mL)	2.66 (0.52)	6.19 (2.44)	12.73 (3.16)	<0.001
IL-17 (pg/mL)	32.50 (5.40)	50.47 (8.94)	63.10 (10.78)	<0.001

Abbreviations: AE-COPD, acute exacerbation of COPD; IL, interleukin; S-COPD, stable COPD.

$P < 0.001$; Figure 3B) levels and FEV$_1$% predicted. Serum IL-1β levels showed a significant positive correlation with the levels of CRP ($r = 0.751$, $P < 0.001$; Figure 4A), Neu% ($r = 0.497$, $P < 0.001$; Figure 5A) and smoking status ($r = 0.628$, $P < 0.001$; Figure 6A). Serum IL-17 levels also showed a significant positive correlation with the levels of CRP ($r = 0.506$, $P < 0.001$; Figure 4B), Neu% ($r = 0.252$, $P = 0.005$; Figure 5B) and smoking status ($r = 0.403$, $P < 0.001$; Figure 6B).

Discussion

There are several important findings of this study, which are 1) serum IL-1β and IL-17 levels were significantly higher in patients with COPD than those in the healthy control group; 2) levels of these two inflammatory mediators in the serum were associated with the important clinical parameters in COPD, including degree of airflow limitation, smoking status, CRP and Neu% in serum; and 3) serum IL-1β levels were correlated positively with IL-17, and more importantly, IL-1β might be an important factor contributing to elevation of IL-17 in patients with COPD. Therefore, serum IL-1β or IL-17 may

Table 1 Basic characteristics of patients with COPD and controls

Variables	Control (n=40)	Stable COPD (n=60)	AE-COPD (n=60)	P-value
	M=28/F=12	M=42/F=18	M=39/F=21	
Age (years)	64.3 (8.8)	62.8 (10.8)	63.9 (9.7)	NS
BMI (kg/m²)	21.7 (3.9)	22.2 (3.5)	21.0 (3.3)	NS
Pack-years	29 (6)	84 (21)	109 (26)	<0.001
FEV$_1$/FVC (%)	84.8 (4.3)	52.3 (6.5)	NA	<0.001
FEV$_1$% predicted	105.8 (12.9)	55.0 (10.1)	NA	<0.001
CRP (mg/L)	5.23 (1.16)	31.68 (14.28)	70.76 (24.98)	<0.001
Neu%	61.01 (5.90)	75.65 (6.63)	81.66 (9.88)	<0.001

Abbreviations: BMI, body mass index; CRP, C-reactive protein; FVC, forced vital capacity; Neu%, neutrophil percentage; NA, not applicable; NS, not significant.
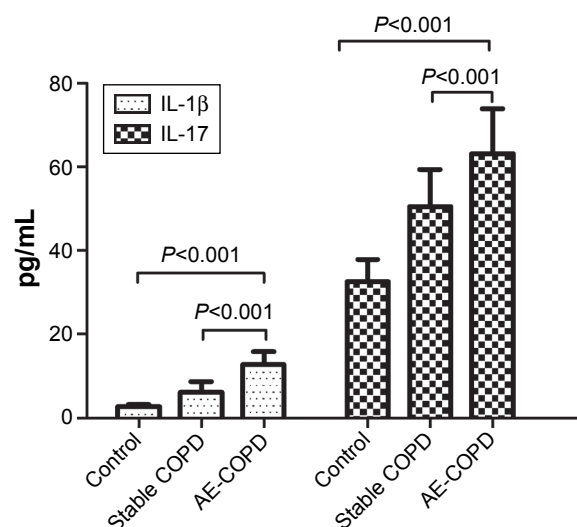

Figure 1 Serum IL-1β and IL-17 levels in patients with COPD and healthy control subjects.
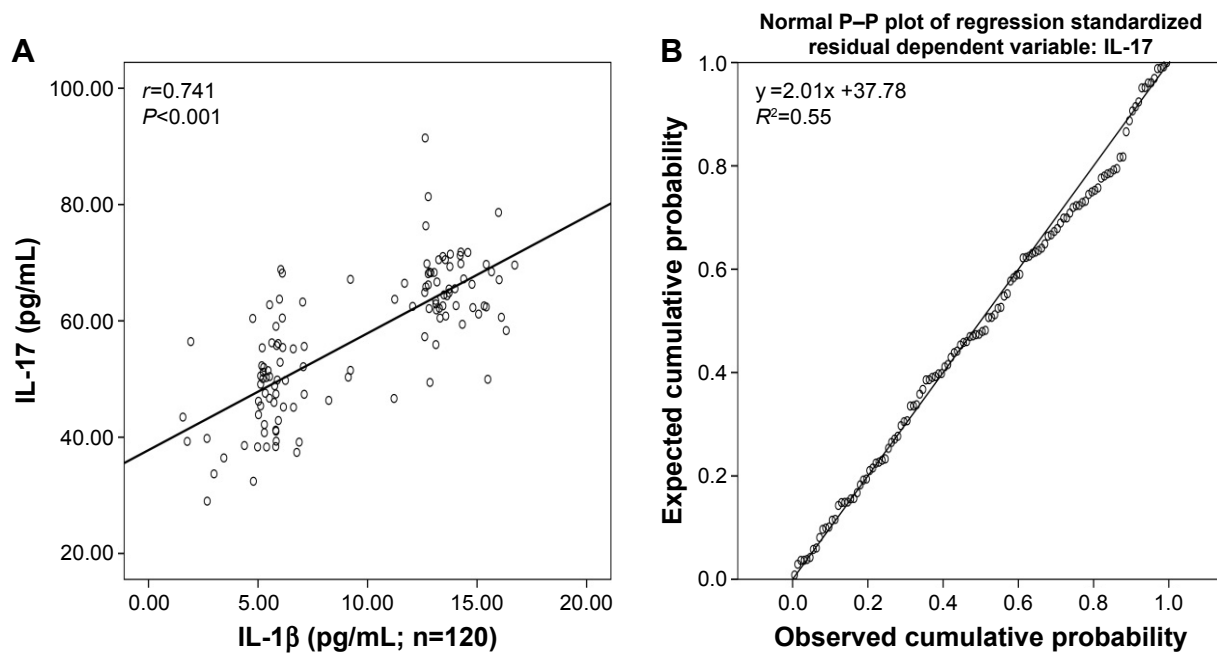**Abbreviations:** AE-COPD, acute exacerbation of COPD; IL, interleukin.

Figure 2 Relationship between serum IL-1β (**A**) and IL-17 (**B**) levels in patients with COPD.
Abbreviation: IL, interleukin.

be an important biomarker for distinguishing patients with COPD from healthy subjects, which helps in evaluating the severity of COPD and predicting the clinical outcomes.

COPD is characterized by anomalous and persistent inflammation, and neutrophils are the most prominent inflammatory cells infiltrating into the airway.[6,30] Moreover, in a CS-induced pulmonary inflammation model in mice, neutrophils infiltrated first and increased with time in the BLF and lung tissue.[18] Accumulated neutrophils can produce reactive oxygen species and release a variety of proinflammatory mediators and enzymes
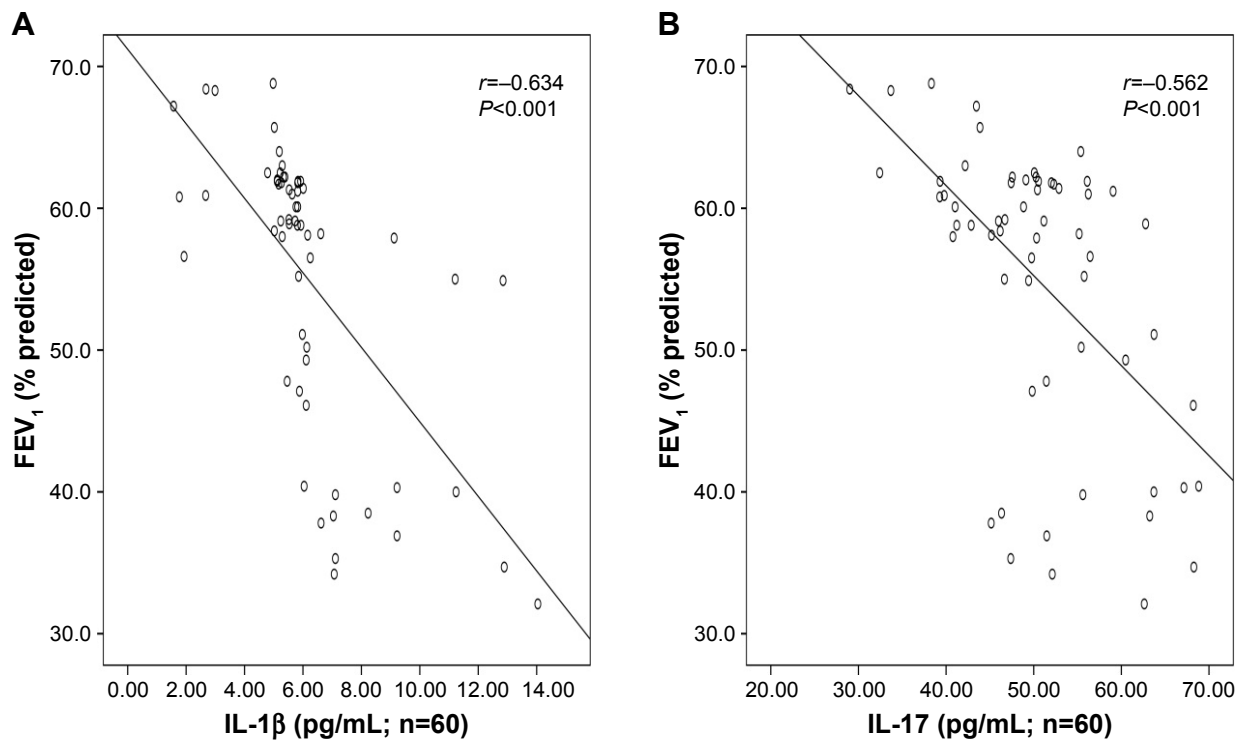
Figure 3 Relationship of serum IL-1β (**A**) and IL-17 (**B**) levels with FEV₁% predicted in patients with COPD.
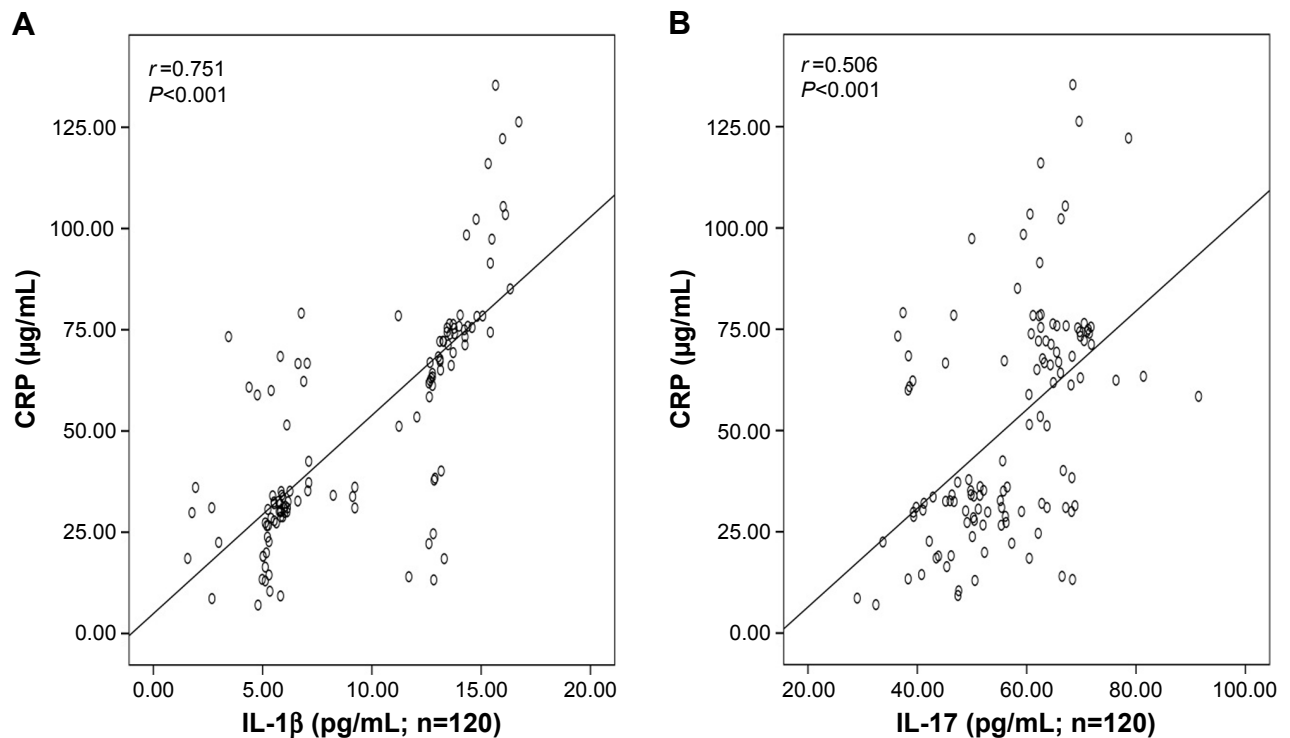**Abbreviation:** IL, interleukin.

Figure 4 Relationship of serum IL-1β (**A**) and IL-17 (**B**) levels with CRP in patients with COPD exacerbations.
Abbreviations: CRP, C-reactive protein; IL, interleukin.

including NE and myeloperoxidase, promoting the occurrence and development of chronic bronchitis and emphysema.[7–9]

In addition, neutrophils can produce and release neutrophil extracellular traps (NETs) in patients with COPD.[31–33] NETs are web-like structures containing DNA, histones, NE and myeloperoxidase,[34] and they can prime macrophages to produce a precursor form of the inflammatory cytokine IL-1β (pro-IL-1β).[35] NETs collaborate with another activation signal, such as cholesterol crystals or heat shock protein, promoting the release of IL-1β together.[35,36] This theory
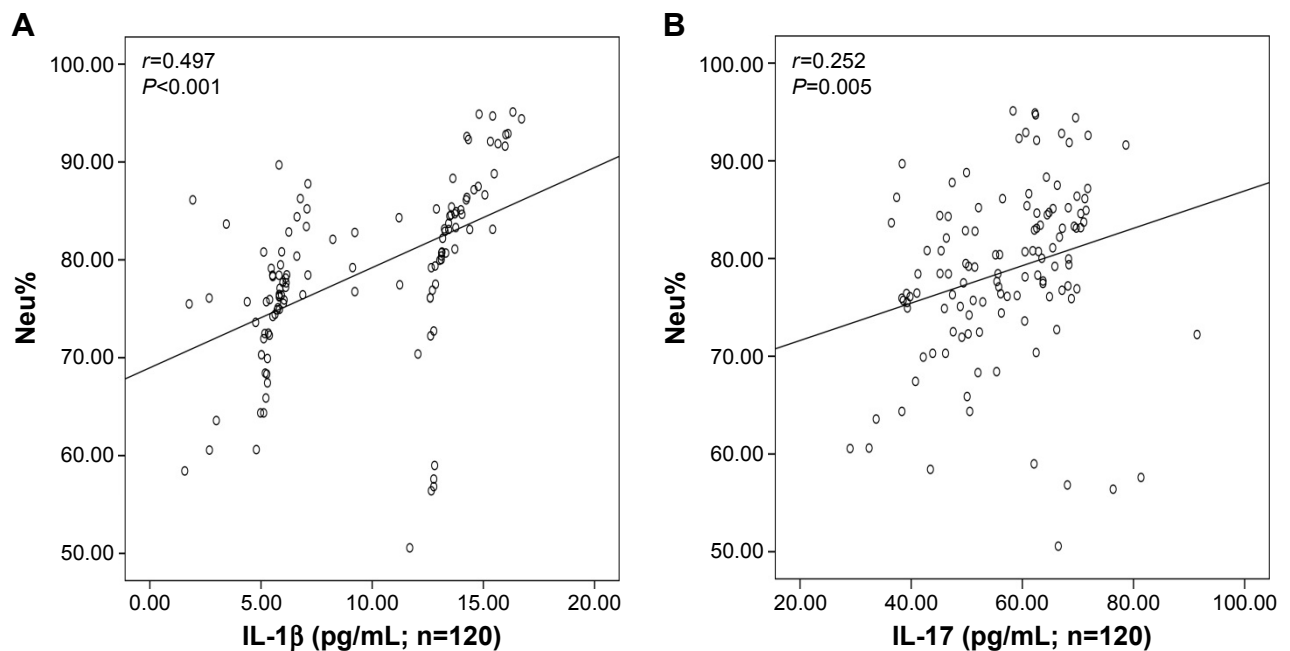

Figure 5 Relationship of serum IL-1β (**A**) and IL-17 (**B**) levels with Neu% in patients with COPD.
Abbreviations: IL, interleukin; Neu%, neutrophil percentage.
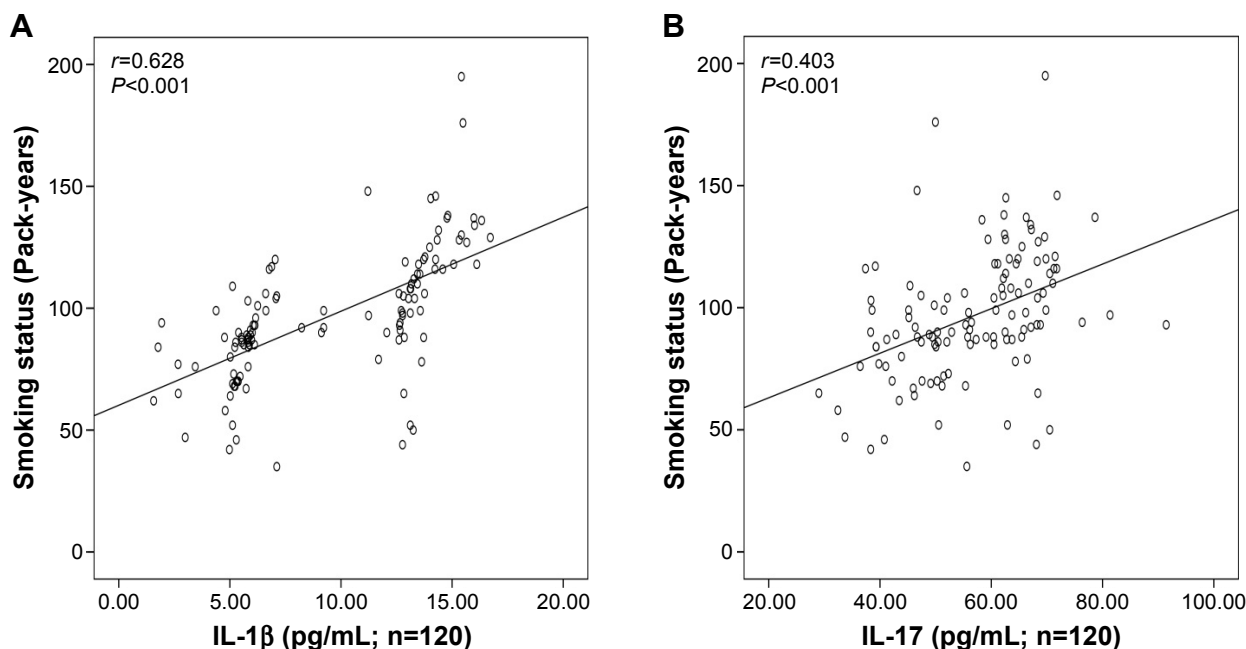
Figure 6 Relationship of serum IL-1β (**A**) and IL-17 (**B**) levels with smoking status (pack-years) in patients with COPD.
Abbreviation: IL, interleukin.

supports our results that serum IL-1β was increased and associated with Neu% in patients with COPD. Previous studies also confirmed that IL-1β secretion was increased in S-COPD and exacerbating COPD.[12,13] In return, IL-1β initiated and maintained neutrophil airway inflammation in COPD, mainly involving the following two mechanisms: first, IL-1β stimulated normal human bronchial epithelial cells to produce many inflammatory cytokines, such as IL-6 and IL-8, two putative cytokines promoting neutrophil recruitment and activation;[17] second, IL-1β could promote the expression of IL-17 in the lung by increasing the number of IL-17–producing T lymphocytes (αβ T cells and γδ T cells),[18,35] and IL-17 was recognized as a key regulator of neutrophils. Therefore, neutrophils mediate the formation of IL-1β that facilitates the neutrophil recruitment into airways, creating a vicious circle of neutrophil airway inflammation and contributing to the progressive development of COPD.

IL-17 expression and the number of IL-17+cells were increased in the bronchial mucosa and sputum of COPD patients in previous studies.[20–23] Furthermore, Zhang et al recently reported that serum IL-17 was higher in COPD compared to that in healthy subjects and positively correlated with CRP and negatively correlated with FEV_1% predicted,[37] which is in accordance with our results. Another study showed that the serum concentrations of IL-17 in the patients with S-COPD were lower than those in the healthy controls, but serum IL-17 was higher in AE-COPD than

that in S-COPD or healthy nonsmokers.[38] These differences between studies may be attributed to different medications for COPD patients and COPD stages and severity. Moreover, IL-17 is mainly secreted by IL-17–producing T-lymphocytes including αβ T cells and γδ T cells, and these two kinds of cells can be induced by IL-1β in the lung tissue and BLF in patients with COPD.[18,35] Consequently, it is proposed that IL-1β may be an important factor leading to increased expression of IL-17 in patients with COPD, and this was consistent with our result of the regression analysis.

This study has limitations. First, its relatively small size affected the power to detect associations between systemic markers and clinical parameters. Second, this study was carried out as a cross-sectional evaluation, and thus, alterations in serum inflammatory cytokines are correlative, but not predictive. Above all, the medications for patients with COPD were not investigated, and the influence of inhaled corticosteroid and bronchodilators on the systemic levels of these two cytokines could not be evaluated.

Conclusion

Our data indicate that concomitant measurements of IL-1β and IL-17 in serum may provide a novel biomarker of COPD. Serum IL-1β and IL-17 levels may be valuable as a marker of neutrophilic inflammation, chronic airflow obstruction and an acute exacerbation. Blockade of IL-1β or IL-17 could be a valid strategy for the prevention and control of COPD.

Acknowledgments

Disclosure

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