

Diagnostic Value of Serum Levels of IL-22, IL-23, and IL-17 for Idiopathic Pulmonary Fibrosis Associated with Lung Cancer

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Background: Correlations between idiopathic pulmonary fibrosis (IPF) and lung cancer have been discussed in many previous studies. In the present work, we evaluated the potential diagnostic value of serum levels of Th17 cell-related cytokines for the detection of lung cancer-associated IPF.

Methods: Fifty-six patients who had been diagnosed with lung cancer-associated IPF were enrolled, and 59 patients with lung cancer but without IPF were also enrolled, and 60 healthy volunteers were served as the control group. The expression of IL-22, IL-23, and IL-17 was evaluated by enzyme-linked immunosorbent assay (ELISA) kits.

Results: We observed that IL-22, IL-23 as well as IL-17 were significantly increased in the serum of lung cancer patients associated IPF; moreover, the results of ROC curve showed that the expression of IL-22, IL-23 and IL-17 can distinguish the lung cancer patients with lung cancer-associated IPF group; finally, the expression of IL-22, IL-23 and IL-17 was positively correlated with the degree of differentiation and metathesis of the tumor.

Conclusion: In conclusion, we first reported that IL-22, IL-23 and IL-17 were elevated in the serum of patients with lung cancer-associated IPF, and our data may provide novel evidence for the prevention and treatment of lung cancer-associated IPF.

Keywords: idiopathic pulmonary fibrosis, diagnosis, IL-22, IL-23, IL-17, lung cancer

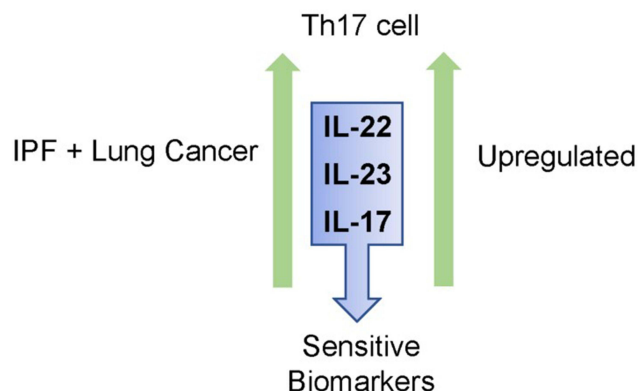
Introduction

Idiopathic pulmonary fibrosis (IPF) is known as an inflammation-associated lung disease. It has been characterized by the long-term inflammatory condition and infiltration of immune cells, as well as the aberrant collagen deposition, which leads to the remodeling of the lung.¹⁻³ Although the pathogenesis of IPF remains unclear, the results of previous studies suggested that IPF may initiate from a lesion that locates at the alveolus, and the long-term inflammatory condition could further lead to damages on the structure within the alveolar, including aberrant apoptosis of type I epithelial cells, over-proliferation of the type II cells, as well as the thickening of small airways and the artery walls.⁴⁻⁶ It is believed that damage to the alveolar parenchymal tissue could consequentially induce the fibrotic condition in the alveolar walls and remodeling of the lung tissue.

Based on the results of previous studies, idiopathic pulmonary fibrosis was considered as an important risk factor for the progression of lung cancer.⁷⁻⁹ It has been estimated that the incidence rate of lung cancer associated IPF patients ranges from about 10% to 40%. Unfortunately, lung cancer has also become a major contributor to IPF related-death among the above patients¹⁰⁻¹² and the prognosis for patients with IPF associated lung cancer remains poor.

CD4+ T helper cells (CD4+ T cells) were known as regulators of many immune responses. CD4+ T cells were first divided into two subgroups, Th1 and Th2, based on the cytokines they expressed. In recent years, the investigation of Th17, a new subgroup of CD4+ T cells, has become a hot field. Th17 cells were known to produce interleukin-17, as well as other cytokines, for example, IL-22 and IL-21.^{13,14} IL-17 is known as a cytokine that participates in many immune

Graphical Abstract



diseases, for example, autoimmune diseases. The secretion of IL-17 is affected by several factors, for example, interleukin-23 (IL-23). IL-23 is mainly produced by dendritic cells as well as macrophages.¹⁵ Previous studies have suggested that IL-23 and IL-17 may play important roles in different types of cancers, including lung cancer. On the other hand, the aberrant expressions of the above cytokines were also found in patients with idiopathic pulmonary fibrosis; however, whether Th-17 related cytokines may serve as a biomarker for the early diagnosis of patients with combined idiopathic pulmonary fibrosis and lung cancer remains unclear.

This study was designed to test the hypothesis that one or more early serum biomarkers for idiopathic pulmonary fibrosis combined with lung cancer in the general Chinese population, recruited patients who signed informed consent while staying in hospital and was carried out according to the Declaration of Helsinki guidelines, and authorized by the ethics committee. Our data may provide novel evidence for the prevention and treatment of lung cancer patients with idiopathic pulmonary fibrosis.

Materials and Methods

Patients and Clinical Information

In this study, we enrolled 59 patients who had been diagnosed with lung cancer with idiopathic pulmonary fibrosis. Fifty-six patients with lung cancer but without idiopathic pulmonary fibrosis were also enrolled, and 60 healthy volunteers were served as the control group. The clinical information of the patients is shown in [Table 1](#). The inclusion and exclusion criteria were as follows:

Inclusion Criteria

- 1) Outpatients or inpatients aged 30 ~ 80 years, who meet the diagnostic criteria of lung cancer complicated with pulmonary interstitial fibrosis and sign informed consent.
- 2) No serious α 1-antitrypsin deficient patients;
- 3) Patients who did not take hormones and other immunosuppressants within one month and did not receive desensitization treatment.

Exclusion Criteria

- 1) Patients with severe respiratory failure ($\text{PaO}_2 < 40$ mmHg, with or without $\text{PaO}_2 > 90$ mmHg), pulmonary encephalopathy or artificial mechanical ventilation (noninvasive or invasive) who cannot cooperate with the information collection;
- 2) Patients with other serious chronic respiratory diseases, such as lung cancer, active tuberculosis and pulmonary fibrosis;

- 3) Patients with malignant tumors; acute or persistent chronic infectious diseases of any part; autoimmune diseases; hypertension, diabetes, heart failure and other chronic diseases that may affect the results;
- 4) Psychiatric patients and patients who cannot provide accurate medical history.

For each patient, the serum was collected and stored in -80°C until needed. This study has been approved by the ethics committee of The Traditional Chinese Medicine Hospital Affiliated to Xinjiang Medical University (No. 2021XE0184-1).

Enzyme-Linked Immunosorbent Assay

The serum levels of IL-22, IL-23, and IL-17 were measured using enzyme-linked immunosorbent assay (ELISA) kit (all purchased from Biocalvin, Suzhou, China) according to the manufacturer's protocol.

Statistical Analysis

Statistical analysis was performed using SPSS 19.0. The qualitative data were compared with a χ^2 test, and if the quantitative data fit the normal distribution, Student's *t*-test was performed; if the quantitative data that did not fit the normal distribution, the Mann-Whitney test or Kruskal-Wallis test was performed. The Spearman and Pearson analysis were performed to investigate the correlation between the two variables. $p < 0.05$ has been considered to be statistically significant.

Results

Clinical Information of the Patients

Fifty-nine non-small cell lung cancer patients and 56 patients with combined lung cancer and idiopathic pulmonary fibrosis, as well as 60 healthy controls were included in the study. The clinical information of the patients is shown in Table 1.

Table 1 Clinical Information of the Patients

| | Number | IPF | | p value |
|---------------------------|--------|------------|-----------|----------|
| | | Yes (n=59) | No (n=56) | |
| Gender | | | | |
| Male | 69 | 39 | 30 | 0.1704 |
| Female | 46 | 20 | 26 | |
| Ages | | | | |
| ≤65 | 40 | 21 | 19 | 0.8514 |
| >65 | 75 | 38 | 37 | |
| Metastasis | | | | |
| Yes | 46 | 24 | 22 | 0.8789 |
| No | 69 | 35 | 34 | |
| TNM stage | | | | |
| I-II | 35 | 12 | 23 | 0.0157** |
| III-IV | 80 | 47 | 33 | |
| Degree of differentiation | | | | |
| Well-Moderate | 52 | 31 | 25 | 0.3969 |
| Poor | 63 | 28 | 31 | |
| Smoking history | | | | |
| Yes | 78 | 42 | 36 | 0.4285 |
| No | 37 | 17 | 20 | |

Note: ** $p < 0.01$.

Comparison of the Serum Levels of IL-17, IL-22, and IL-23 Among Different Groups

Next, we compared the serum levels of healthy controls, lung cancer patients, and lung cancer patients with idiopathic pulmonary fibrosis using ELISA methods. As shown in Figure 1, the serum level of IL-17 was significantly increased in the lung cancer group in comparison with the control and lung cancer group ($p < 0.01$), and on the other hand, the serum levels of IL-17 were markedly increased in lung cancer combined with idiopathic pulmonary fibrosis group compared with the lung cancer group ($p < 0.01$). Furthermore, both the serum levels of IL-22 (Figure 2, $p < 0.01$) and IL-23 (Figure 3, $p < 0.01$) were elevated in lung cancer patients compared with the controls, and the serum levels of both IL-22 (Figure 2, $p < 0.01$) and IL-23 (Figure 3, $p < 0.01$) were significantly up-regulated in lung cancer combined with idiopathic pulmonary fibrosis group compared with the lung cancer group.

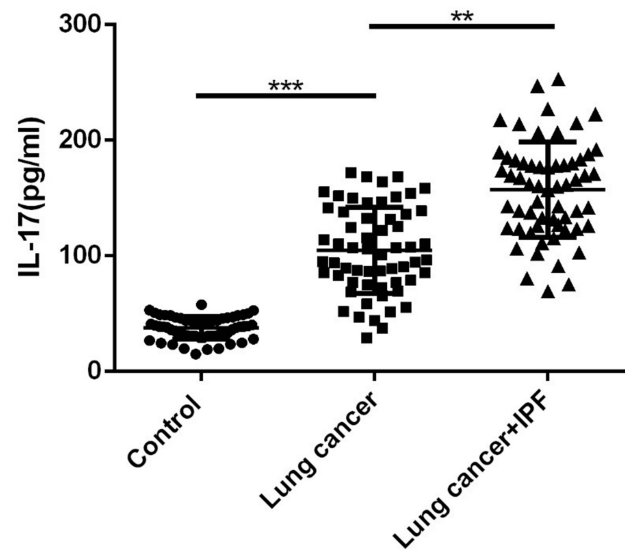


Figure 1 Comparison of the levels of IL-17 between control, lung cancer patients and lung cancer-associated IPF. ** $P < 0.01$, *** $P < 0.001$.

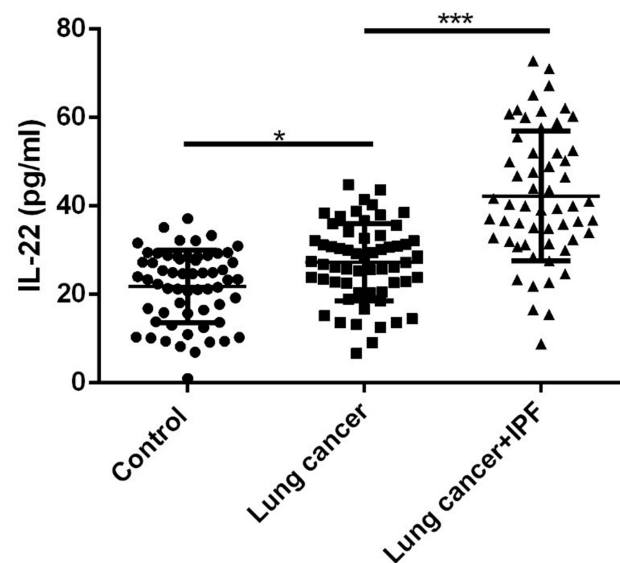


Figure 2 Comparison of the levels of IL-22 between control, lung cancer patients and lung cancer-associated IPF. * $P < 0.05$, *** $P < 0.001$.

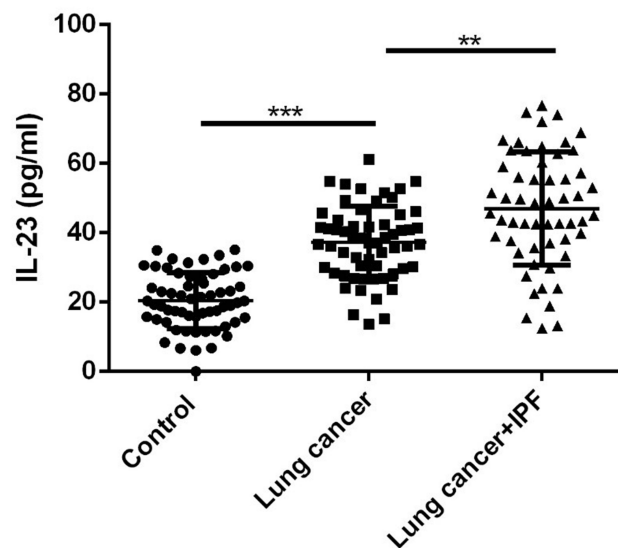


Figure 3 Comparison of the levels of IL-23 between control, lung cancer patients and lung cancer associated IPF. ** $P < 0.01$, *** $P < 0.001$.

Serum Levels of IL-17, IL-22, and IL-23 as Diagnostic Markers in Patients with Combined Lung Cancer and Idiopathic Pulmonary Fibrosis

Finally, the receiver-operating characteristic curve (ROC) has been drawn to show the sensitivity and specificity of IL-17, IL-22, and IL-23 to distinguish patients with combined lung cancer and idiopathic pulmonary fibrosis from lung cancer patients. As shown in Figures 4–6, the area under the curve (AUC) of IL-17 was 0.8229 (95% confidence interval (CI), 0.7502 to 0.8955), the IL-22 was 0.8081 (95% CI, 0.7275 to 0.8887), and the IL-23 was 0.7001 (95% CI, 0.6016 to 0.7986).

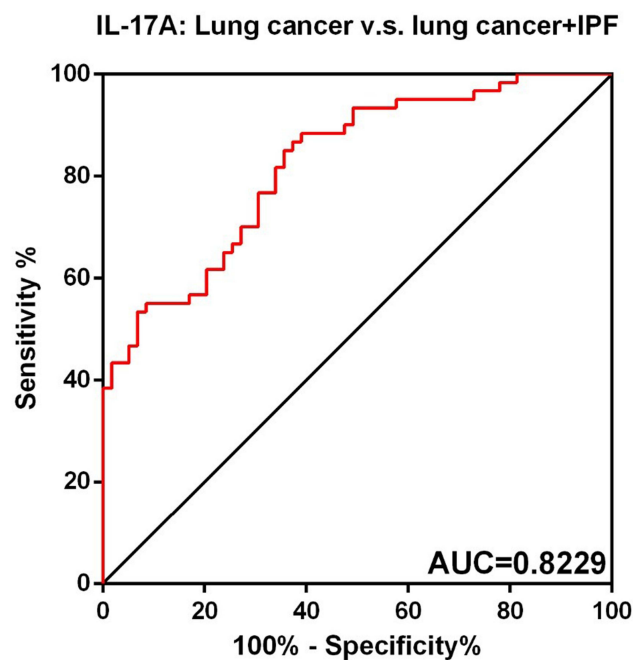


Figure 4 ROC of IL-17.

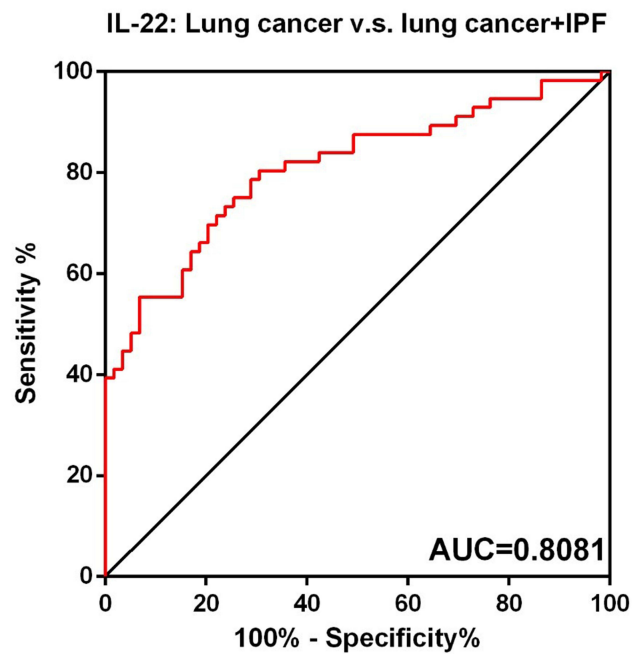


Figure 5 ROC of IL-22.

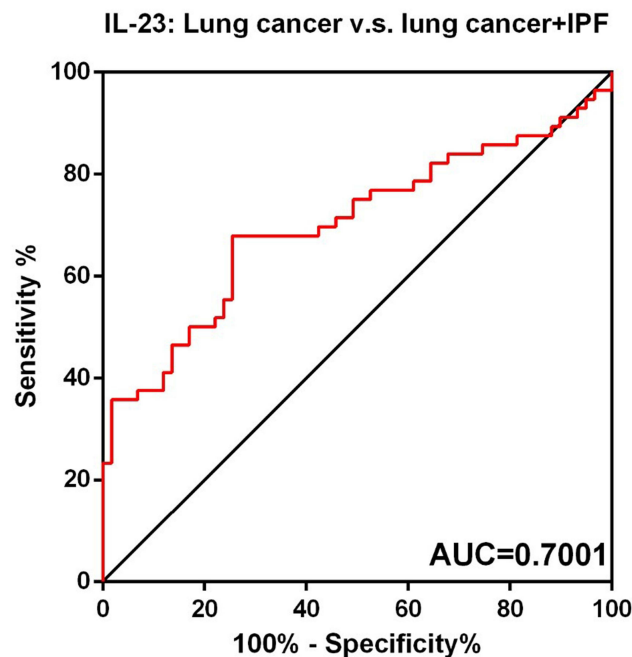


Figure 6 ROC of IL-23.

Discussion

Idiopathic pulmonary fibrosis (IPF) represents a group of heterogeneous disorders. The burden of IPF is increasing worldwide and is associated with acute and chronic complications, leading to significant morbidity and mortality. Similar to IPF, the prevalence of lung cancer has also been increasing worldwide. However, the relationship between IPF and cancer has long been debated. Epidemiologic studies have demonstrated an increased risk of lung cancer in patients with IPF.^{9,16,17} The relevance of this finding is complicated by the fact that both IPF and cancer have similar risk factors (for

example, age, family history, and smoking history), which might confound the results of these studies.^{18,19} Furthermore, some somatic alterations were shared by IPF-associated lung cancer and fibrosing lung tissue, for example, KRAS or TP53 mutations, were found to be higher in lung tissue samples among patients with IPF associated with lung cancer than those with IPF but without lung cancer.^{18–20} However, the relationship between IPF and lung cancer remains to be further investigated.

New-onset lung cancer in IPF is likely a paraneoplastic phenomenon, mediated by tumor secreted product(s).^{7,8,21} Since IPF has a high prevalence in lung cancer patients and develops at a time when the cancer may not even be visualized on imaging, patients with new-onset IPF offer a group of high-risk patients who could be screened for lung cancer. Further studies to discover the mediators of lung-associated IPF will not only improve our understanding of the pathophysiology of this phenomenon but could also offer potential biomarkers to screen patients for lung cancer.^{10,11,17}

There are studies about whether IL-17 could serve as biomarkers for cancer; however, the results have been controversial. In the field of lung cancer studies, it has been reported that the expression level of IL-17 is elevated in the serum of patients, and the level of IL-17 in the serum of the patients may function as a prognostic marker,^{15,22–24} while on the other hand, some other studies had shown that the levels of IL-17 did not show differences between the patients and healthy controls. In the present study, we observed that the serum level of IL-17 was significantly upregulated in the serum of the patients in comparison with the healthy control group. Interestingly, we first observed that the serum level of IL-17 was significantly upregulated in patients with lung cancer compared with lung cancer patients with idiopathic pulmonary fibrosis, and the curve (AUC) of IL-17 was 0.8066 (95% confidence interval (CI), 0.7220 to 0.8912), suggesting that lung cancer patients with high serum level of IL-17 may have the risk of idiopathic pulmonary fibrosis.

In addition, the roles of Th17 cells-related cytokines IL-22 and IL-23 in tumors have also been discussed. In the case of lung cancer, Bi et al proved that Interleukin-22 promotes lung cancer cell proliferation and migration via the IL-22R1/STAT3 and IL-22R1/AKT signaling pathways.²⁵ Tufman et al proved that interleukin-22 is elevated in lavage from patients with lung cancer and other pulmonary diseases.²⁶ Baird et al proved that IL-23 is pro-proliferative, epigenetically regulated, and modulated by chemotherapy in non-small cell lung cancer.²⁷ Li et al observed that interleukin 23 regulates the proliferation of lung cancer cells in a concentration-dependent way in association with the interleukin-23 receptor.²⁸ In the present study, we observed that both IL-22 and IL-23 were upregulated in the serum of patients with lung cancer compared with healthy controls, which were consistent with previous findings. Moreover, we first reported that the expression of both IL-22 and IL-23 were markedly increased in the serum of patients with combined lung cancer and idiopathic pulmonary fibrosis, compared with the lung cancer group, the AUC of IL-17 was 0.8066 (95% confidence interval (CI), 0.7220 to 0.8912), the IL-22 was 0.7818 (95% CI, 0.6952 to 0.8684), and the IL-23 was 0.7324 (95% CI, 0.6419 to 0.8230), suggesting that IL-22 and IL-23 are effective biomarkers to distinguish patients with combined lung cancer and idiopathic pulmonary fibrosis with the lung cancer patients.

Our studies have limitations. First, the results should be verified with larger sample size in future studies. In this study, dozens of samples represent part of the experimental results and lots of assumptions is still remained to be confirmed. We would like to calculate the sample-size under a single sample clinical diagnostic test as $\alpha=0.05$, type of two-sided test, $1-\beta=0.8$, and produce a result with the minimum required number of sample data is 62 cases.²⁹ However, the study of biomarkers follows the rules of multiplex analysis, multi-biomarker and multi-validation, as well as a rule of thumb, we would like to take at least 134 cases for the future experimental verification process.

Second, this study was based on Chinese Han population, so whether IL-17, IL-22, and IL-23 have diagnostic value for patients with combined lung cancer and idiopathic pulmonary fibrosis in other races still needs to be validated.

In conclusion, we proved for the first time that IL-17, IL-22, and IL-23 were significantly upregulated in patients with combined lung cancer and idiopathic pulmonary fibrosis, and our results provided novel evidence that IL-17, IL-22 and IL-23 were sensitive biomarkers for the early diagnosis of patients with combined lung cancer and idiopathic pulmonary fibrosis.

Ethics Statement

This study has been approved by the ethics committee of The Traditional Chinese Medicine Hospital Affiliated to Xinjiang Medical University (No. 2021XE0184-1).

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Disclosure

The authors report no conflicts of interest in this work.

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