Research Square

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Serum SP-A and KL-6 levels can predict the improvement and deterioration of patients with interstitial pneumonia with autoimmune features

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Abstract

Background Some patients with Interstitial pneumonia with autoimmune features (IPAF) showed a progressive course despite therapy. Furthermore, it is difficult to predict poor patient outcomes. This study aimed to evaluate whether serial changes in serum levels of surfactant protein-A (SP-A) and Krebs von den Lungen-6 (KL-6) can predict disease progression.

Methods 64 patients with IPAF and 50 healthy people served as normal controls. Based on long-term changes in lung function, 36 IPAF patients who were followed up for more than 3 months were divided into a progressive group (9), improvement group (13), and stable group (14). Serum KL-6 and SP-A levels were measured. The sensitivity, specificity, cut off value, and area under the curve (AUC) value for each of the indices were determined using the receiver operating characteristic (ROC) curve analysis. The expression differences of these biomarkers and their correlation with the severity of the disease were analyzed.

Results Compared with healthy controls, serum SP-A and KL-6 levels in IPAF patients increased significantly [SP-A: ($p < 0.001$); KL-6: ($p < 0.001$)] and were negatively correlated with DLCO (SP-A: $r_S = -0.323$, $p = 0.018$; KL-6: $r_S = -0.348$, $p = 0.0011$). In the patients with progressive disease, the posttreatment serum SP-A and KL-6 levels increased significantly compared with pretreatment levels [SP-A: ($p = 0.021$); KL-6: ($p = 0.008$)]. In the patients showing improvement, the levels decreased significantly SP-A ($p = 0.007$) and KL-6 ($p = 0.002$). Changes in serum biomarkers (Delta SP-A and Delta KL-6) were significantly negatively correlated with changes in lung function (Delta FVC, Delta DLCO and Delta FEV1) ($r_S = 0.482$, $p < 0.05$). A significant positive correlation was found between Delta SP-A and Delta KL-6 ($r_S = 0.482$, $p < 0.001$).

Conclusions Serum SP-A and KL-6 offers high sensitivity and specificity for the diagnosis of IPAF. The decrease of serum SP-A and / or KL-6 levels in patients with IPAF is related to the improvement of pulmonary function. SP-A and KL-6 may be important biomarkers to evaluate the prognosis of patients with IPAF.

Background

Interstitial pneumonia with autoimmune features (IPAF) is a new term that was proposed by the joint research statement of the European Respiratory Society and American Thoracic Society (ERS/ATS) [1]. IPAF specifically refers to idiopathic interstitial pneumonia (IIP) that has some characteristics of connective tissue disease (CTD) but cannot be diagnosed as a certain type of CTD. IPAF is clinically located in the cross-domain of IIP and CTD-interstitial lung disease (CTD-ILD). Presently, the diagnosis of IPAF is mainly based on clinical manifestations, pulmonary imaging and lung histopathology [2]. ILD is the main manifestation among patients, and there are some serological autoantibody-positive or multisystem extrapulmonary manifestations, such as morning stiffness, Raynaud's phenomenon, and dry symptoms [3]. Lung function test is extremely sensitive to the early changes of IPAF, and the sensitivity is

even higher than that of high-resolution computed tomography (HRCT) [2]. The changes in lung function can also reflect the progression of IPAF as well as determine the effect of treatment and prognosis. However, it is not suitable for critically ill patients. Therefore, finding a more convenient, reliable, and accurate diagnostic method is of great significance for the screening, treatment, and dynamic evaluation of IPAF.

Surfactant protein-A (SP-A) and Krebs von den Lungen-6 (KL-6) are proteins expressed in type Ⅱ alveolar epithelial cells and are related to the pathogenesis of pulmonary fibrosis [4, 5]. The expression level of KL-6 significantly increases in alveolar tissue affected by interstitial pneumonia and it enters the blood circulation through the damaged alveoli [6]. Alveolar surface protein SP-A, which is synthesized and secreted by airway and alveolar epithelial cells, is an important marker of alveolar injury [7, 8]. In Japan [9, 10], serum KL-6, SP-A and SP-D are widely used as useful biomarkers of idiopathic pulmonary fibrosis to diagnose ILD and predict prognosis. While KL-6 has high specificity and sensitivity in the diagnosis of interstitial lung diseases, but SP-A can well distinguish IPF from other ILDs [11]. In IPF, serum levels of KL-6 and SP-A levels are associated with disease severity at the time of measurement and with long-term outcomes [12, 13]. However, few studies have examined the correlation between these biomarkers in IPAF and disease severity.

Previous clinical trials or observational studies on patients with ILD have usually defined ILD disease progression as a decline in forced vital capacity (FVC), typically by 10% of the predicted value [14]. Lee et al. [15] defined disease improvement or progression of ILD as changes in FVC ≥ 10% and/or changes in diffusing capacity for carbon monoxide (DLCO) ≥ 15%. Jiang et al. [16] defined progression as mortality or the reduction of FVC by > 10% and/or DLCO by > 15%. According to the abovementioned criteria, 36 patients with IPAF who were followed up for > 3 months were divided into three groups: progressive group (9 cases), improvement group (13 cases) and stable group (14 cases).

In the present study, we determined the serum SP-A and KL-6 levels in patients with IPAF, analyzed the correlation between their expression levels and lung function indicators, and explored further changes in the abovementioned marker levels during disease progression, providing guidance for early diagnosis, condition monitoring, and prognostic assessment.

Methods

Study design

This study includes two parts. The purpose of the first part was to compare the serum SP-A and KL-6 levels between patients diagnosed with IPAF and healthy individuals and study the diagnostic value. The purpose of the second part was to compare serum SP-A and KL-6 levels before and after treatment and evaluate their prognostic value. The research scheme has been approved by the institutional ethics committee of the First Affiliated Hospital of Guangzhou Medical University (ethics approval no. Gyfyy-2016-73).

Patient Enrollment Criteria

We retrospectively investigated 64 patients with autoimmune ILDs diagnosed in the First Affiliated Hospital of Guangzhou Medical University from October 2015 to February 2019 according to the diagnostic criteria of IPAF established by the ERS/ATS in 2015. Pregnant women, patients with malignant tumors or other autoimmune diseases or co-infections, and patients aged < 18 years were excluded from the study. Healthy individuals (50) were included as normal controls. The study also followed the 36 patients with IPAF treated for > 3 months. The following data were collected from the patients' medical records: gender, age, body mass index (BMI), smoking history, and lung function.

Lung Function Measurements

According to the recommendations of the ERS/ATS, lung function tests were performed on a computerized spirometer (MasterScreen, Leibnizstrasse, Hoechberg, Germany). The examination parameters included FVC, forced expiratory volume of 1 s (FEV1), and DLCO.

Measurement Of Serum Sp-a And Kl-6 Levels

Serum SP-A and KL-6 levels were measured by a fully automatic immunoanalyzer, the HISCL-5000 (Sysmex Corp., Hyogo, Japan), according to the manufacturer's instructions. The detection range for SP-A level was 1–1000 ng/mL and that for KL-6 was 10–6000 U/mL. Results higher than the upper detection limit were excluded from the analysis. SP-A and KL-6 assay kits were provided by Sysmex Corporation.

Definitions Of Disease Progression, Improvement, And Stable

Disease progression was defined as a decrease in FVC ≥ 10% and/or DLCO ≥ 15%. Disease improvement was defined as an increase in FVC by ≥ 10% and/or DLCO by ≥ 15%. Stable condition was defined as change in FVC by < 10% and DLCO by < 15%.

Statistical analysis

The normality of continuous variables was tested by Shapiro-Wilk, and the data are expressed as mean ± standard deviation or as median plus inter quartile range (25th–75th percentiles) according to their normal or non-normal distribution. Dichotomous data are presented as frequencies and percentages. Chi-squared test or fisher's exact test was used to analyze the differences of categorical data. Differences the levels of the various serum markers between subject groups were analyzed using Kruskal-Wallis H test and Wilcoxon's rank sum test. Correlation analyses were performed using the Spearman's rank correlation. Receiver-operating characteristic (ROC) curve was prepared to analyze the specificity and

sensitivity for SP-A and KL-6 for disease activity. All statistical analyses were performed using the SPSS statistical software package for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). P values < 0.05 were considered significant.

Results

Clinical data of subjects

The study included 64 IPAF patients (35 females and 29 males) with an average age of 51.5 ± 13.15 and an average BMI of 24.33 ± 3.29 kg/m^2, 18 (28.13%) smokers. 50 healthy individuals (24 females and 26 males), the average age was 54.7 ± 11.55 years old, and the average BMI was 25.49 ± 3.46 kg/m^2, 12 (24%) smokers (Table 1). The results showed that there were no significant differences in age, gender, BMI, and smoking among patients with IPAF compared with healthy controls.

Table 1
Baseline characteristics in patients with IPAF

	IPAF	Health	P value
Number, n	64	50	—
Age, year	54.5 ± 13.15	54.7 ± 11.55	0.394
Female, n (%)	35 (54.69%)	24 (48%)	0.478
BMI (kg/m2)	24.33 ± 3.29	25.49 ± 3.46	0.829
Smoker,n (%)	18 (28.13%)	12 (24%)	0.620
DLCO (%Pred)	55.05 ± 12.9	—	—
FVC (%Pred)	70.11 ± 17.75	—	—
FEV1 (%Pred)	73.15 ± 16.6	—	—

The data are presented as means ± standard deviation. Other variables are presented as numbers (percent). IPAF, Interstitial pneumonia with autoimmune features; BMI, body mass index; DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiatory volume in 1 s; %Pred, percent predicted.

Comparison of serum KL-6 and SP-A levels between healthy individuals and patients with IPAF

The serum SP-A levels in IPAF patients was 46.6 (32.38–72.58) ng/mL, which was significantly higher than that 19.65 (15.03–23.3) ng/mL in healthy individuals ($p < 0.001$). Similarly, serum KL-6 levels of 1315.5 (848.75-2416.75) U/mL in IPAF patients was significantly higher than that 188 (153.75–266) U/mL ($p < 0.001$) in healthy individuals (Fig. 1). We also used ROC analysis to evaluate the sensitivity and

specificity of serum SP-A and KL-6 concentrations as biomarkers for the diagnosis of IPAF (Fig. 2). Based on the area under the ROC curve, When the cut-off level for SP-A to distinguish IPAF was 27.6 ng/mL, the sensitivity and specificity was 87.5% and 92%, respectively (AUC = 0.947, 95% CI = 0.908–0.986). When the cut-off level for KL-6 to distinguish IPAF was 344 U/mL, the sensitivity and specificity was 98.4% and 96%, respectively (AUC = 0.996, 95% CI = 0.990-1.000).

Correlations Between Biomarkers And Pulmonary Function

Both biomarkers SP-A and KL-6 showed significantly negative correlations with DLCO (%Pred) (SP-A: rS = -0.323, $p = 0.018$; KL-6: rS = -0.348, $p = 0.0011$) (Fig. 3A, 3B). However, there was no significant correlation between SP-A, KL-6 levels and FVC (SP-A: rS = -0.098, $p = 0.454$; KL-6: rS = -0.15, $p = 0.25$) (Fig. 3C, 3D). Similarly, SP-A and KL-6 did not show significant correlation with FEV1) (SP-A: rS = -0.093, $p = 0.477$; KL-6: rS = -0.225, $p = 0.081$) (Fig. 3E, 3F).

Analysis of serum SP-A and KL-6 levels before and after treatment

To determine the value of serum SP-A and KL-6 levels in the evaluation of therapeutic efficacy in patients with IPAF, patients with IPAF who were followed up for > 3 months were divided into the progressive group (n = 9), improvement group (n = 13) and stable group (n = 14) according to the changes in pulmonary function. The patients' clinical baseline characteristics are shown in Table 2. There were no significant differences in any of the parameters among the three groups. Kruskal-Wallis H test was used to compare the serum SP-A and KL-6 levels of the three groups of patients before treatment, and the results were not statistically different ($p > 0.05$) (Fig. 4). Subsequently, we compared the levels of SP-A and KL-6 in patients with IPAF before and after treatment by Wilcoxon's rank sum test (Fig. 5). In the progressive group, the levels of serum SP-A [35.3 (31.35–90.4) ng/mL versus 50.3 (31.35-125.75) ng/mL, ($p = 0.021$)] and KL-6 [738 (584–1471) U/mL versus 1143 (676.5–3888) U/mL, ($p = 0.008$)] increased significantly after treatment. Compared with before treatment, the levels of serum SP-A [42.9 (34.85–71.2) ng/mL versus 36.5 (20.25–54.25) ng/mL, ($p = 0.007$)] and KL-6 [1440 (1039.5–2478) U/mL versus 635 (407-1379.5) U/mL, ($p = 0.002$)] in the improvement group decreased significantly after treatment. In the stable group, serum SP-A [41.75 (27.475–48.125) ng/mL versus 29.65 (18.6-46.95) ng/mL, ($p > 0.05$)] and KL-6 [979.5 (777.75 -1430.25) U/mL versus 949 (523.5-1347.25) U/mL, ($p > 0.05$)] levels did not change significantly compared with those before treatment.

Table 2
Baseline characteristics of the three groups of patients with IPAF

	Progressive	Improved	Stable	P value
Number, n	9	13	14	—
Age, year	53.78 ± 17.18	53.38 ± 10.85	53.44 ± 12.16	0.996
Female, n (%)	5 (55.55%)	9 (69.23%)	8 (57.14%)	0.753
BMI (kg/m^2)	24.36 ± 3.05	24.13 ± 4.49	24.38 ± 2.19	0.979
Smoker, n (%)	2 (22.22%)	2 (15.38%)	5 (35.71%)	0.379
DLCO (% Pred)	62.37 ± 14.43	54.05 ± 12.8	53.85 ± 13.54	0.355
FVC (% Pred)	68.59 ± 11.92	69.76 ± 10.82	76.25 ± 19.82	0.220
FEV1 (% Pred)	73.74 ± 14.49	70.44 ± 10.85	80.6 ± 18	0.422

IPAF, Interstitial pneumonia with autoimmune features; BMI, body mass index; DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6; %Pred, percent predicted. The data are presented as median with interquartile range or as number (percentage).

Correlations between changes in Delta KL-6 and Delta SP-A and changes in pulmonary function

We also used Spearman's correlation test to study the correlation between changes in serum biomarkers (Delta SP-A and Delta KL-6) and changes in lung function (Delta FVC, Delta DLCO and Delta FEV1) before and after treatment. The results show that the serum levels of Delta SP-A did inverse correlate significantly with Delta FVC, Delta DLCO and Delta FEV1 (FVC: r_S = -0.564,p < 0.001; DLCO: r_S = -0.422, p = 0.01; FEV1: r_S = -0.387, p = 0.02) (Fig. 6A, 6C and 6E). Delta KL-6 also did inverse correlate significantly with Delta FVC, Delta DLCO and Delta FEV1 (FVC: r_S = -0.626, p < 0.001; DLCO: r_S = -0.664, p < 0.001; FEV1: r_S = -0.439, p = 0.007) (Fig. 6B, 6D and 6F). We also tested the correlation between Delta SP-A and Delta KL-6. This showed a significant positive correlation (r_S = 0.616, p < 0.001; Fig. 7).

Discussion

Because there is no international standard for the diagnosis, nomenclature and classification of IPAF, studies on IPAF are limited. The diagnosis of IPAF is based on laboratory indicators, chest HRCT, pathological biopsy and other clinical manifestations, which is more complex and less practical. Therefore, the identification of biomarkers of IPAF can not only improve the level of diagnosis of IPAF, but also help to understand the pathophysiological mechanism of the occurrence and development of IPAF. So far, there are few studies on IPAF biomarkers.

KL-6 is a MUC-1 mucin, commonly found in regenerative type Ⅱ alveolar epithelial cells [17, 18]. Interstitial pneumonia will proliferate type Ⅱ alveolar epithelial cells, resulting in an increase in KL-6 concentration and due to the damage leads to an increase in vascular permeability, allowing KL-6 to enter the bloodstream, so the concentration of KL-6 in the serum of patients with ILD increases [19, 20]. SP-A is a member of the water-soluble C-type lectin family and is an important part of the lung's innate immune system [21]. The pathogenesis of IPF may be related to abnormal endoplasmic reticulum processing of lung surfactant proteins [22]. The expression of SP-A1 gene is up-regulated by genetic analysis of lung biopsy in IPF patients, and SP-A2 gene defects are also associated with familial IPF pathogenesis [23, 24]. In Japan, serum SP-A and KL-6 levels are widely used as biomarkers for diagnosis, severity assessment and prognosis prediction of ILD patients [9]. These findings collectively indicate that serum SP-A and KL-6 can act as a surrogate marker for the active process of disease progression [25] [26]. However, it is not known whether the changes of SP-A and KL-6 levels, especially in the serum of patients with IPAF, can reflect the correlation between the changes and the progression of IPAF patients.

Our study found that compared with the healthy control group, the serum levels of SP-A and KL-6 in IPAF patients were significantly increased ($P < 0.01$). Our findings are consistent with the results from the report by Xue et al [27]. When a cut-off value of 27.6 ng/mL was used, the sensitivity and specific of using serum SP-A levels as a diagnostic biomarker was 87.5% and 92%, respectively. When a cut-off value of 344 U/mL was used, the sensitivity and specific of using serum KL-6 levels as a diagnostic biomarker was 98.4% and 96%, respectively. Indicating that serum SP-A and KL-6 might be a promising biomarker for diagnosis of IPAF patients. In IPF patients, cut-off values were set as the levels that resulted in the optimal diagnostic accuracy for SP-A and KL-6: 476 U/mL for KL-6 and 44.0 ng/mL for SP-A [9]. It is suggested that different levels of criticality may be required in ILD patients of different subtypes.

In IPAF, the serum SPA, KL-6 and %DLCO were significantly negatively correlated ($P < 0.05$) but not significantly correlated with FVC and FEV1($P > 0.05$). Interestingly, an IPF cohort study by Sokai et al also reported a similar correlation between KL-6 and pulmonary function parameters such as FVC and DLCO [28], so it is possible that the severity of the disease before treatment may be more accurately reflected by DLCO than FVC in IPAF.

Among the follow-up patients, the levels of serum SP-A and KL-6 in patients with progressive disease were significantly higher after treatment, while the levels of serum SP-A and KL-6 were significantly lower in patients with improved condition, suggesting that the levels of serum SP-A and KL-6 may be effective serum biomarkers for monitoring IPAF activity. Arai et al [24] believe that the periodic measurement of KL-6 and SP-D levels will be useful in the evaluation of disease behavior and treatment response of idiopathic fibrotic nonspecific interstitial pneumonia.

Herein, we used Spearman's correlation test to study the correlation between Delta SP-A, Delta KL-6 and changes of lung function parameters (Delta DLCO, Delta FVC and Delta FEV1) to further explore the role of SP-A and KL-6 in the monitoring of prognoses in patients with IPAF. The results showed that Delta SP-A and Delta KL-6 were significantly negatively correlated with Delta DLCO, Delta FVC, and Delta FEV1 (P <

0.01). However, it is worth noting that in this study, serum SP-A and KL-6 levels before treatment were not related to FVC. Previous report also indicates that IPAF patient's serum levels of both KL-6 and SP-D at baseline showed a negative correlation with %DLCO, but not with FVC [29]. However, the change in FVC is still a reliable, effective, and responsive clinical status indicator that can be used as the primary endpoint of ILD crucial treatment studies. In the report of Lee et al. [15], serum SP-A and KL-6 levels in CTD-ILD were significantly negatively correlated with FVC and DLCO. This study has reported similar negative correlations between KL-6 and respiratory parameters [30]. These studies also confirmed the relationship between SP-A and KL-6 with disease activity, suggesting that SP-A can also be used as an indicator to judge the prognosis of ILD. Therefore, we confirmed that the serum level of SPA and KL-6 reflected the severity of IPAF in terms of pulmonary function deterioration.

At present, the diagnosis of ILD mainly depends on HRCT or invasive transbronchial lung biopsy, which cloud be affected by various factors or cause great pain to patients [31]. Although lung function test is known to be non-invasive, it is highly dependent on patient cooperation. Particularly, many elderly patients are usually unable to cooperate with the implementation of pulmonary function tests. Throughout the patient's disease, serum SP-A and KL-6 testing are easier to perform than frequent repeated lung function tests, x-rays, and invasive transbronchial lung biopsies [30]. The correlation analysis of this study suggests that when the pulmonary function tests is difficult, we can make a preliminary evaluation and prediction of the patient's condition according to the expression level of the above two markers.

Our results also showed that although there was a significant correlation between Delta SP-A and Delta KL-6, the correlation coefficient was not high, suggesting that each marker may represent a different pathophysiological mechanism.

Our research had some limitations. First of all, due to the retrospective nature of the study, data related to symptom initiation and partial examination were missing. In addition, there was no analysis of chest HRCT manifestations, clinical symptoms and drug treatment. Second, the sample size of this study is small. Future studies with larger sample sizes are needed to verify this finding. Third, retrospective analysis of the course of disease does not allow changes in treatment and follow-up time, so it is difficult to avoid the impact of other confounding factors.

To sum up, this study showed that the levels of serum SP-A and KL-6 in patients with IPAF were significantly increased, which was negatively correlated with diffusion function. It was found that the levels of SP-A and KL-6 increased with the aggravation of the disease and decreased with the remission of the disease. As far as we know, we are the first to report the changes of serum SP-A and KL-6 levels with disease in patients with IPAF.

Conclusions

To sum up, IPAF patients have higher serum SP-A and KL-6 levels and are significantly related to disease activity. We believe that regular measurement of KL-6 and SP-A levels will help in the evaluation of

disease behavior and treatment response of IPAF.

Abbreviations

IPAF：Interstitial pneumonia with autoimmune features; SP-A：Surfactant protein-A; KL-6：Krebs von den Lungen-6; AUC：Area under the curve; ROC：Receiver operating characteristic; ERS/ATS：European Respiratory Society and American Thoracic Society; IIP：Idiopathic interstitial pneumonia; CTD：Connective tissue disease; CTD-ILD：Connective Tissue Disease-Interstitial lung disease; HRCT; High-resolution computed tomography; FVC：Forced vital capacity; DLCO：Diffusing capacity for carbon monoxide; FEV1：Forced expiratory volume of 1 s; BMI：Body mass index.

Declarations

Ethics approval and consent to participate

Informed written consent was obtained from all participants and the present study was approved by the institutional ethics committee of the First Affiliated Hospital of Guangzhou Medical University (ethics approval no. Gyfyy-2016-73).

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ Contributions

Conception and design: BQS, NSZ; Administrative support: BQS, NSZ; Provision of study materials or patients: JXW, PYZ, ZFH; Collection and assembly of data: JXW, PYZ, ZFH, HMH, CXL; Data analysis: JXW, PYZ, ZFH, HMH, MSX; Manuscript writing: All authors; Final approval of manuscript: All authors.

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References

1. A F, KM A, KK B, J C, TJ C, RM dB, JS L, KO L, DA L, EL M *et al*: **An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features**. *The European respiratory journal* 2015, **46**(4):976-987.
2. M S, G C, L DP, S C, G DC, C V, F L, C S, A M: **Interstitial pneumonia with autoimmune features: A single center prospective follow-up study**. *Autoimmunity reviews* 2020, **19**(2):102451.
3. Y L, Z Z, Q H, Z L, R X, R Z, B Z, P Z: **IPAF should receive early treatment for sharing similar clinical characteristics as CTD-ILD: a report from 273 Chinese patients**. *Clinical rheumatology* 2020.
4. Sun AP, Ohtsuki Y, Fujita J, Ishida T, Kohno N: **KL-6, a human MUC1 mucin, is expressed early in premature lung**. *Respir Med* 2003, **97**(8):964-969.
5. Zhang JH, Zhi-Wei YU, Chen HM, Lin XL, Liang-Xia WU: **Effects of Budesonide and Bacillus Calmette-Guerin on Damages of Type Ⅱ Alveolar Epithelial Cell in Asthmatic Mice Model**. *Journal of Applied Clinical Pediatrics* 2011, **26**(16):1250-1249.
6. HR C, TE K, BB B, JS V, MI S, KK B: **Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis**. *American journal of respiratory and critical care medicine* 2003, **168**(5):538-542.
7. Collard HR, King TE, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK: **Changes in Clinical and Physiologic Variables Predict Survival in Idiopathic Pulmonary Fibrosis**. *American Journal of Respiratory & Critical Care Medicine* 2003, **168**(5):538-542.
8. Sokai A, Tanizawa K, Handa T, Kanatani K, Kubo T, Ikezoe K, Nakatsuka Y, Tokuda S, Oga T, Hirai T: **Importance of serial changes in biomarkers in idiopathic pulmonary fibrosis**. *Erj Open Research* 2017, **3**(3):00019-02016.
9. K H, H I, N I, Y H, T M, S M, T N, S O, K F, H H *et al*: **Comparative Study of Circulating MMP-7, CCL18, KL-6, SP-A, and SP-D as Disease Markers of Idiopathic Pulmonary Fibrosis**. *Disease markers* 2016, **2016**:4759040.

10. H H, X P, J N: **Advances in the study of biomarkers of idiopathic pulmonary fibrosis in Japan**. *Bioscience trends* 2013, **7**(4):172-177.
11. T K, S Y, Y I, T H, S N, S H, N S, H K, M M, Y M *et al*: **Serum heat shock protein 47 levels are elevated in acute exacerbation of idiopathic pulmonary fibrosis**. *Cell stress & chaperones* 2013, **18**(5):581-590.
12. Greene KE, King TE, Kuroki Y, Bucher-Bartelson B, Hunninghake GW, Newman LS, Nagae H, Mason RJ: **Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis**. *European Respiratory Journal* 2002, **19**(3):439-446.
13. N I, N H, A Y, N K: **Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases**. *Respiratory investigation* 2012, **50**(1):3-13.
14. V C, L W, A F, M Q, S S, S H: **Fibrosing interstitial lung diseases: knowns and unknowns**. *European respiratory review : an official journal of the European Respiratory Society* 2019, **28**(151).
15. YS L, HC K, BY L, CK L, MY K, SJ J, HS L, J M, TV C, DS K: **The Value of Biomarkers as Predictors of Outcome in Patients with Rheumatoid Arthritis-Associated Usual Interstitial Pneumonia**. *Sarcoidosis, vasculitis, and diffuse lung diseases : official journal of WASOG* 2016, **33**(3):216-223.
16. Y J, Q L, Q H, J H, Y O, M C, Y W, SS M, K D, R C: **Sequential changes of serum KL-6 predict the progression of interstitial lung disease**. *Journal of thoracic disease* 2018, **10**(8):4705-4714.
17. Ishikawa N, Hattori N, Yokoyama A, Kohno N: **Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases**. *Respiratory Investigation* 2012, **50**(1):3-13.
18. Kohno N: **Serum marker KL-6/MUC1 for the diagnosis and management of interstitial pneumonitis**. *Journal of Medical Investigation Jmi* 1999, **46**(3-4):151-158.
19. N K, S K, Y A, H F, M Y, M A: **New serum indicator of interstitial pneumonitis activity. Sialylated carbohydrate antigen KL-6**. *Chest* 1989, **96**(1):68-73.
20. H N, M H, M H, M H, H T, F S, N K, S K: **KL-6 as a novel serum marker for interstitial pneumonia associated with collagen diseases**. *The Journal of rheumatology* 2000, **27**(5):1164-1170.
21. E S, Z C, K R, V V, KF H, F H, S N, F S, M S, B G *et al*: **Staphylococcus aureus Lung Infection Results in Down-Regulation of Surfactant Protein-A Mainly Caused by Pro-Inflammatory Macrophages**. *Microorganisms* 2020, **8**(4).
22. CW A, G S, I G-A: **Alveolar lipids in pulmonary disease. A review**. *Lipids in health and disease* 2020, **19**(1):122.
23. JW S, KH D, SJ J, TV C, S H, DS K: **Blood biomarkers MMP-7 and SP-A: predictors of outcome in idiopathic pulmonary fibrosis**. *Chest* 2013, **143**(5):1422-1429.
24. Arai S, Kurasawa K, Maezawa R, Owada T, Okada H, Fukuda T: **Marked increase in serum KL-6 and surfactant protein D levels during the first 4 weeks after treatment predicts poor prognosis in patients with active interstitial pneumonia associated with polymyositis/dermatomyositis**. *Modern Rheumatology* 2013, **23**(5):872-883.
25. T Y, M O, H C, K I, Y M, Y U, H N, K K, H T: **Surfactant protein A as a biomarker of outcomes of anti-fibrotic drug therapy in patients with idiopathic pulmonary fibrosis**. *BMC pulmonary medicine* 2020,

20(1):27.

26. S K, M S, T O, K S, M S, K K, Y H, Y M, M N: **Clinical Course of Histologically Proven Multifocal Micronodular Pneumocyte Hyperplasia in Tuberous Sclerosis Complex: A Case Series and Comparison with Lymphangiomyomatosis**. *Respiration; international review of thoracic diseases* 2018, **95**(5):310-316.
27. M X, Z G, C C, B S, H W: **Evaluation of the Diagnostic Efficacies of Serological Markers KL-6, SP-A, SP-D, CCL2, and CXCL13 in Idiopathic Interstitial Pneumonia**. *Respiration; international review of thoracic diseases* 2019, **98**(6):534-545.
28. A S, T H, K T, T O, K U, T T, T K, K I, Y N, K T *et al*: **Matrix metalloproteinase-10: a novel biomarker for idiopathic pulmonary fibrosis**. *Respiratory research* 2015, **16**:120.
29. Yamakawa H, Hagiwara E, Ikeda S, Iwasawa T, Otoshi R, Tabata E, Okuda R, Sekine A, Baba T, Iso S: **Evaluation of changes in the serum levels of Krebs von den Lungen-6 and surfactant protein-D over time as important biomarkers in idiopathic fibrotic nonspecific interstitial pneumonia**. *Respiratory Investigation* 2019.
30. C H, C W, E Y, H H, D X, Y H, J Z, M L, Z X, X Z *et al*: **Serum KL-6 is associated with the severity of interstitial lung disease in Chinese patients with polymyositis and dermatomyositis**. *Clinical rheumatology* 2019, **38**(8):2181-2187.
31. Hu C, Wu C, Yang E, Huang H, Wang Q: **Serum KL-6 is associated with the severity of interstitial lung disease in Chinese patients with polymyositis and dermatomyositis**. *Clinical Rheumatology* 2019, **38**(8):1-7.

Figures

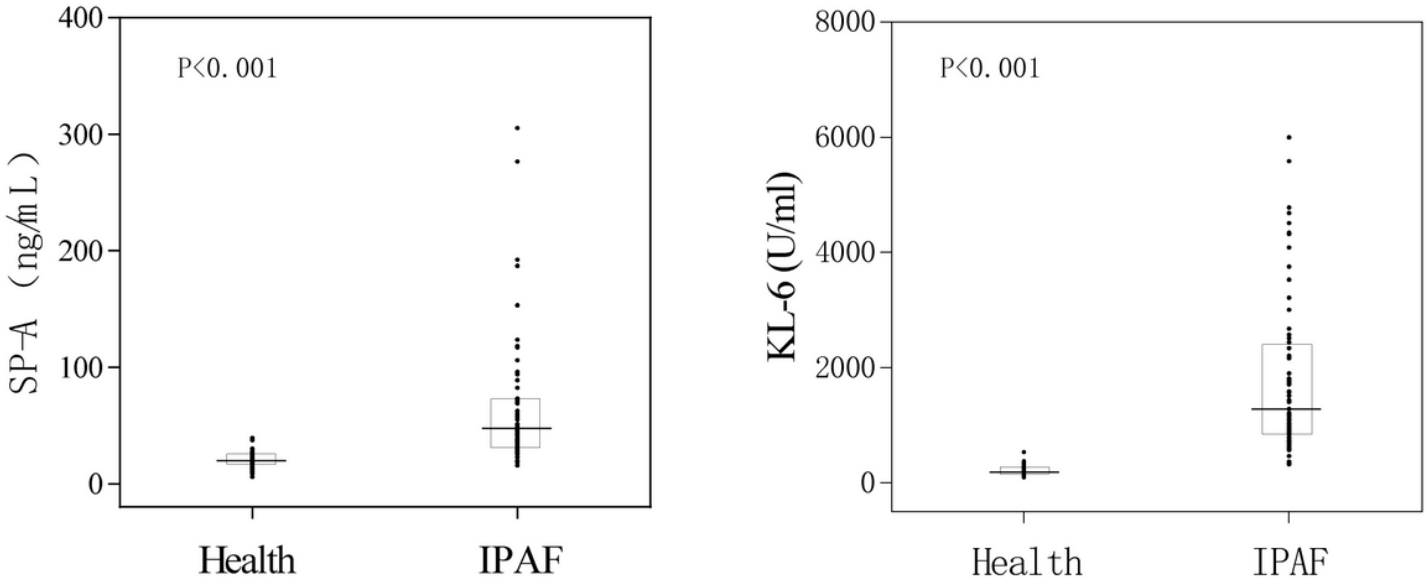

Figure 1

Comparison of serum SP-A and KL-6 levels in healthy individuals and IPAF patients. IPAF, Interstitial pneumonia with autoimmune features; SP-A, Surfactant protein-A; KL-6, Krebs von den Lungen-6. The data was presented as median with interquartile range.

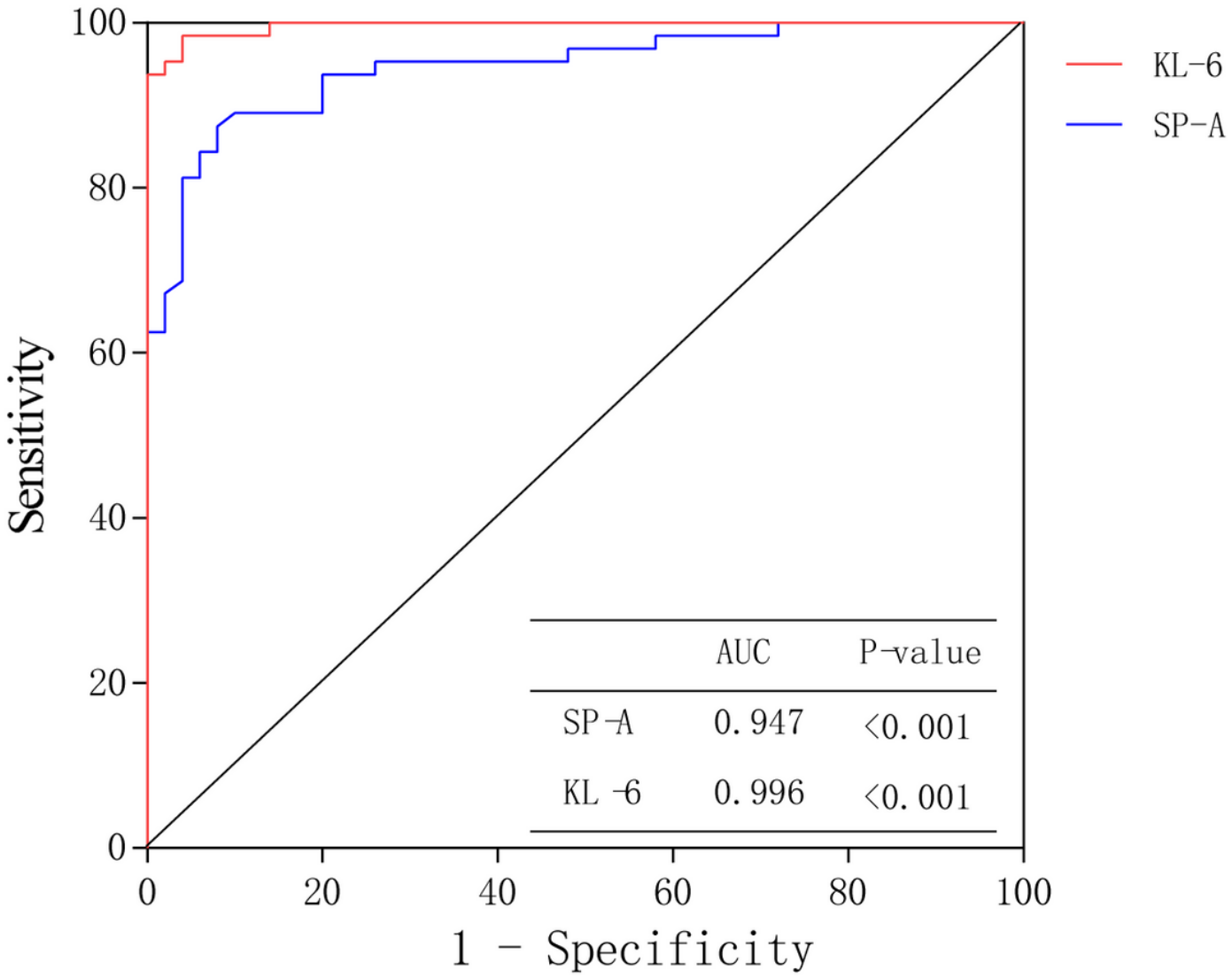

Figure 2

Receiver-operating characteristic (ROC) curve according to the specificity and sensitivity of serum SP-A and KL-6 levels. SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6.

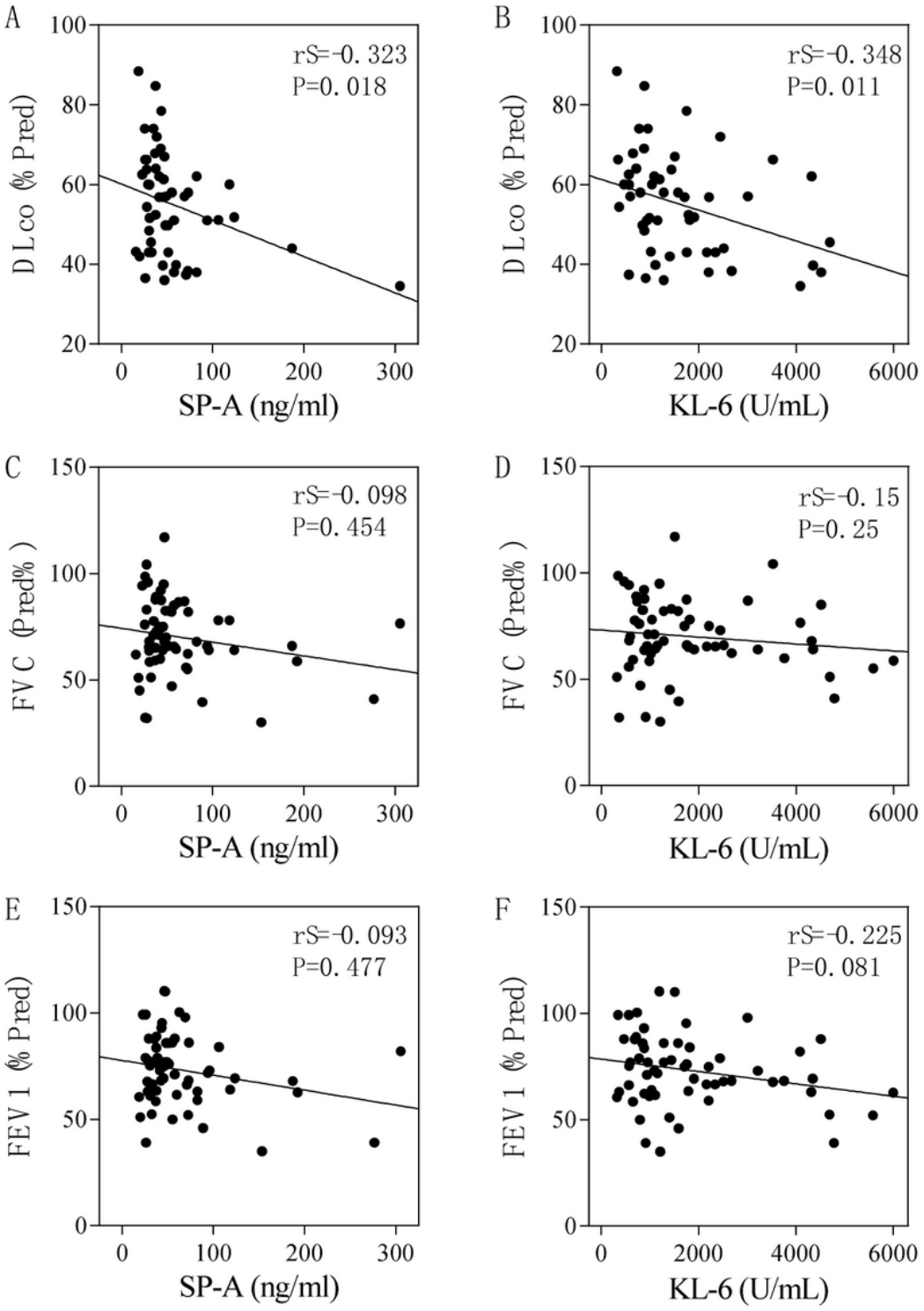

Figure 3

Correlation between serum SP-A and KL-6 concentrations and pulmonary function test parameters in IPAF patients using Spearman correlation test. SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6; DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s.

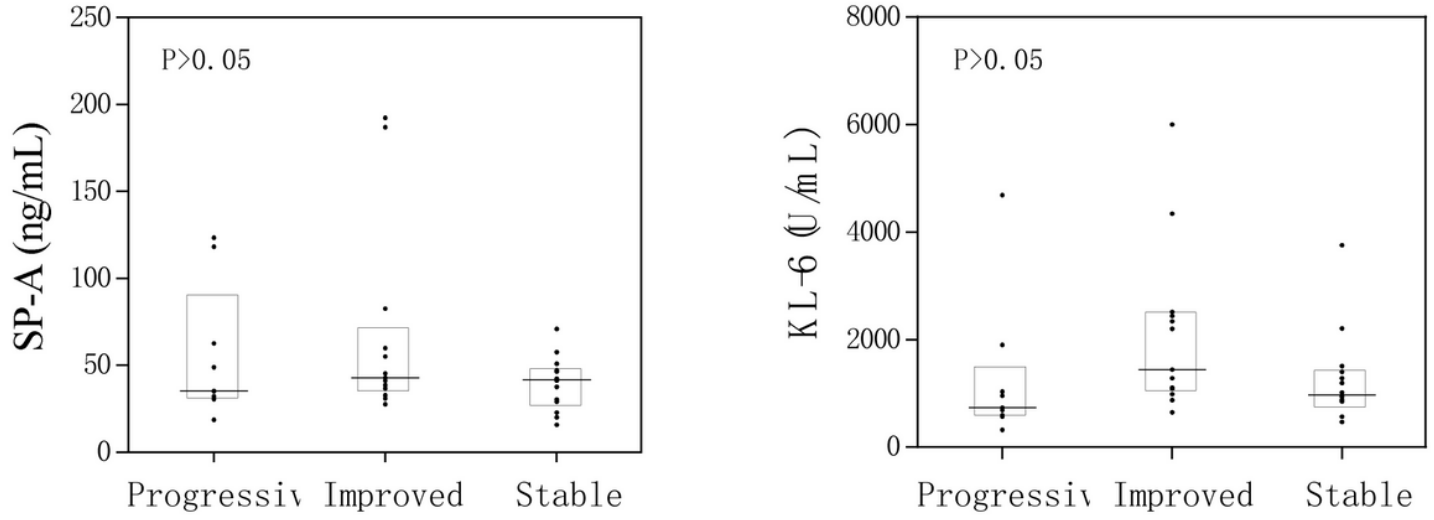

Figure 4

Comparison of serum SP-A and KL-6 levels in the progress, improve and stable groups before treatment. SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6. The data was presented as median with interquartile range.

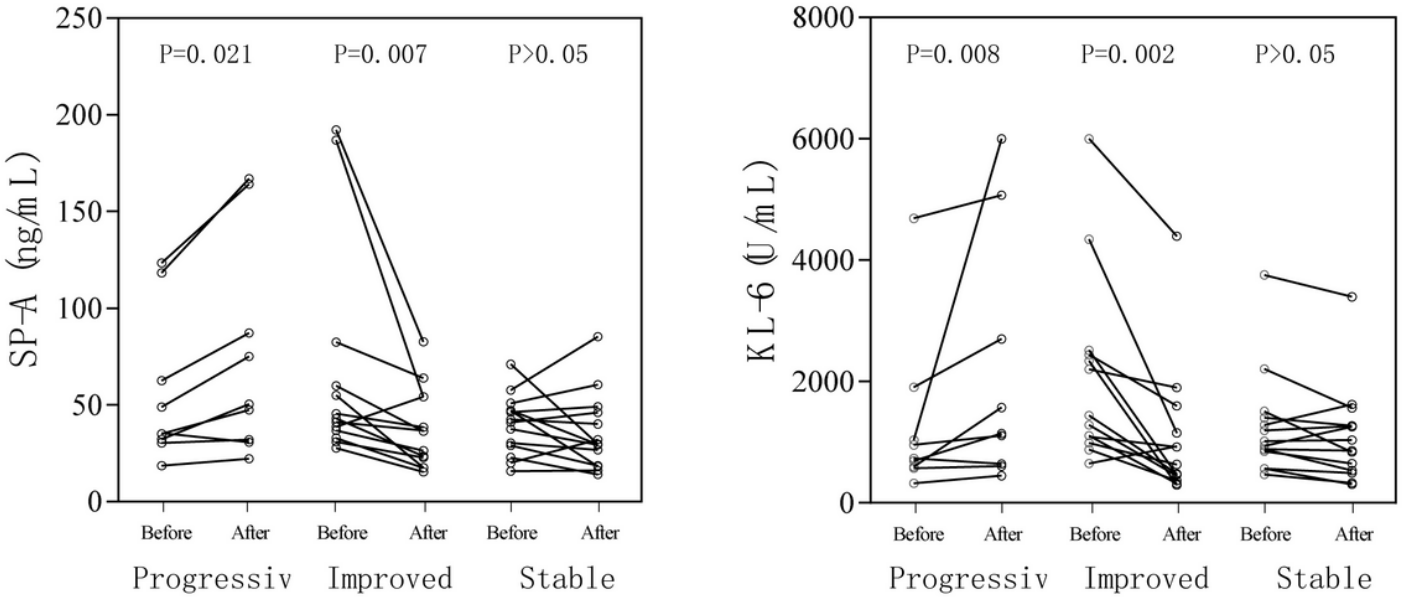

Figure 5

Pretreatment and posttreatment serum SP-A and KL-6 levels compared between the three groups. SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6.

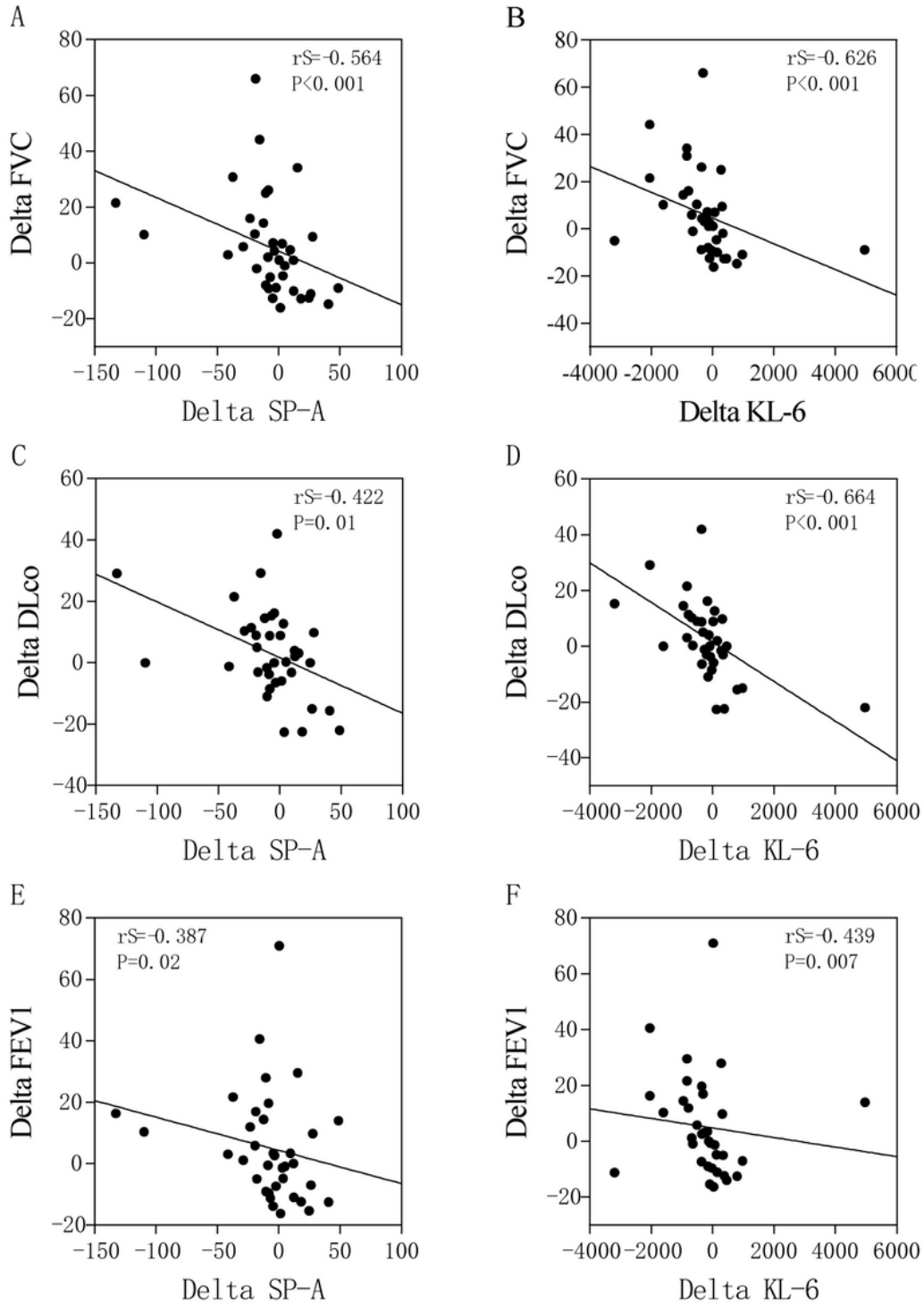

Figure 6

Correlations between Delta SP-A and Delta KL-6 and changes in pulmonary function test parameters. SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6; DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s.

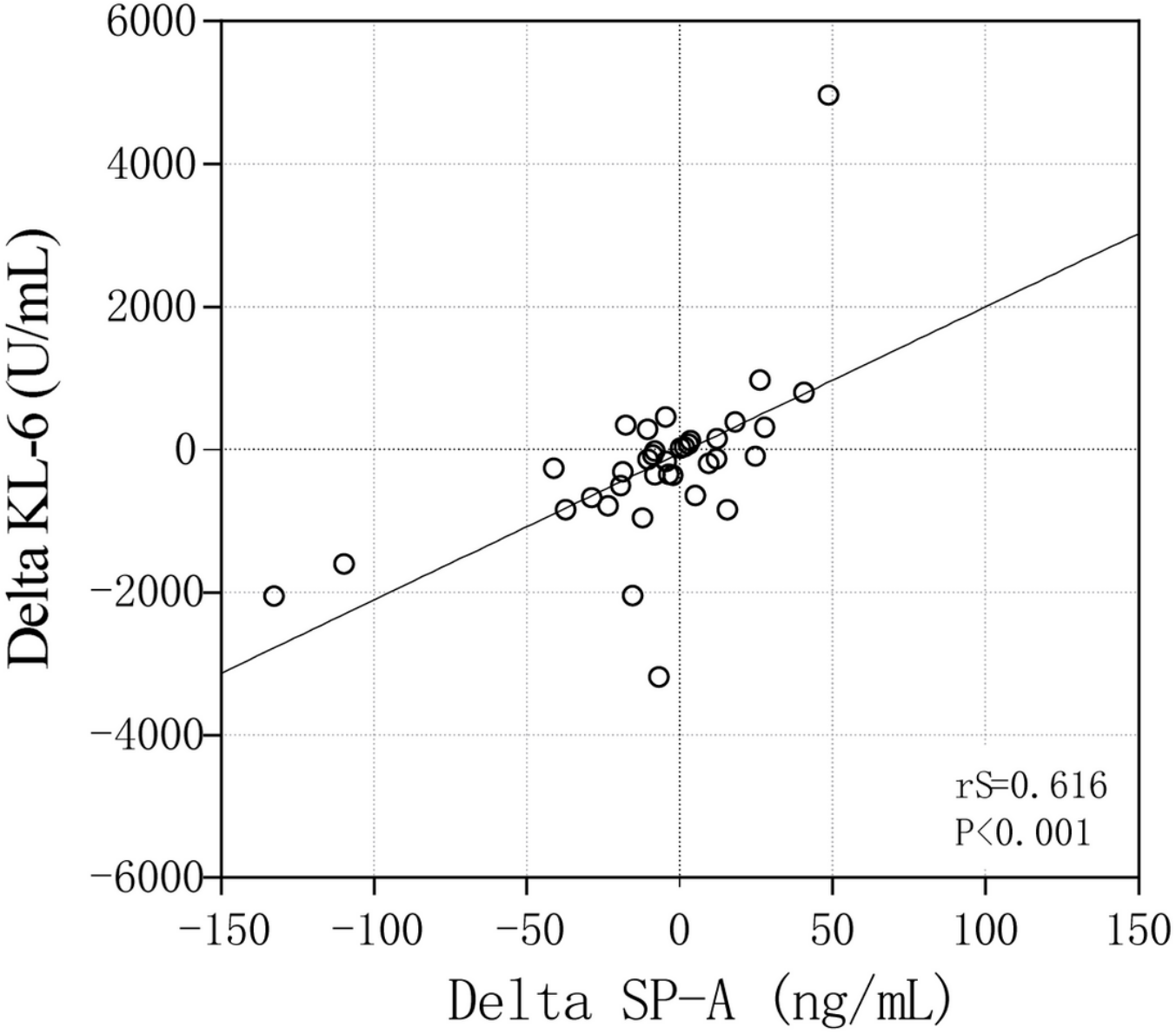

Figure 7

Correlation between serum Delta KL-6 and Delta SP-A values. SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6.