

The Difference Between SP-A and KL-6 Levels Responses for Treatment of Interstitial Lung Disease

Peiyan Zheng

Guangzhou Medical University

Xiaomao Zheng

Guangzhou Medical University

Hasegawa Takehiro

Sysmex Europe GmbH

Jingxian Wang

Guizhou Medical University

Mingshan Xue

Guangzhou Medical University

Quanming Lin

Guangzhou Medical University

Zhifeng Huang

Guangzhou Medical University

Huimin Huang

Guangzhou Medical University

Chenxi Liao

Guangzhou Medical University

Baoqing Sun (✉ sunbaoqing@vip.163.com)

First Affiliated Hospital of Guangzhou Medical University <https://orcid.org/0000-0002-1671-0723>

Research

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Abstract

Background

The highly variable clinical course of interstitial lung disease (ILD) makes it difficult to determine patients' prognoses. Serum surfactant protein-A (SP-A) and Krebs von den Lungen-6 (KL-6) were known biomarkers as a monitor of the prognoses. However, the clinical or pathophysiological differences of those biomarkers are not well evaluated. Therefore, through the comparison of the changes of SP-A and KL-6 levels before and after treatment, we investigated the clinical or pathophysiological differences which are embodied by those markers.

Methods

This study included retrospective data for 71 patients treated for ILD at the First Affiliated Hospital of Guangzhou Medical University between August 2015 and September 2019. Serum SP-A and KL-6 levels were measured before and after treatment. The patients were followed for at least 3 months.

Results

Changes in the serum biomarkers (Delta SP-A and Delta KL-6) were significantly correlated ($r_s = 0.482$, $P < 0.001$); Delta SP-A and Delta KL-6 were inversely correlated with changes in pulmonary function ($P < 0.05$). In a cluster analysis of delta SP-A and KL-6 levels, patients were classified into three groups. In the cluster analysis, in the group in which only SP-A levels decreased after treatment, 50.0% of patients recovered respiratory function and had a significant reduction of serum LDH levels.

Conclusions

Reduced serum SP-A and/or KL-6 levels were associated with improved lung function in patients with ILD. However, there were patients who showed only a reduction of SP-A levels after treatment. Thus, for proper disease monitoring, measuring both markers are important.

Introduction

Interstitial lung disease (ILD) comprises a group of acute and chronic lung diseases characterized by diffuse pulmonary parenchyma, alveolar inflammation, and interstitial fibrosis. ILD includes both common and rare clinical diseases. In most cases, the causes remain unclear. ILD can be life-threatening; its overall mortality rate is as high as 52% [1]. Appropriate diagnosis and treatment are essential for patients with ILD.

The present ILD diagnostic methods depend on pulmonary function tests (PFT), radiological and histological examinations, and high-resolution computed tomography (HRCT) [2]. These methods provide important information for treating ILD. However, ILD's pathophysiology is complex. Various biomarkers are used to support the diagnosis of ILD and prognosis evaluation, and these could potentially help

identify vulnerable patients. Of these, the noninvasive serum biomarkers surfactant protein-A (SP-A) and Krebs von den Lungen-6 (KL-6) have been found to provide particularly important reference values for diagnosing ILD, monitoring disease activity, and evaluating prognoses [3-5].

SP-A and KL-6 are produced in the alveoli and released into the bloodstream when the alveoli are destroyed; therefore, they increase as the disease worsens and decrease as the patient recovers [4, 6-8]. It has been reported that serum levels of SP-A and KL-6 vary with different disease types, and that they show different correlations with the ground-glass opacity grade and honeycomb lung grade derived from HRCT imaging [4, 9-14]. SP-A and KL-6 are also used as blood biomarkers to predict the prognosis of idiopathic pulmonary fibrosis (IPF) patients [15, 16], and SP-A has been identified as a biomarker for various lung diseases, including acute respiratory distress syndrome, chronic obstructive pulmonary disease, and progressive systemic sclerosis [17]. Measuring and monitoring serum KL-6 concentration have been reported to be useful screening tools for ILD and its severity [18-21]. In Japan, serum SP-A and KL-6 levels are widely used as biomarkers for ILD diagnosis, severity assessment, and prediction of clinical outcomes. A cut-off value for serum KL-6 of 500 U/mL has been used in clinical practice to distinguish patients with ILD from healthy individuals or non-ILD patients with pulmonary disease [3, 4].

Despite the increasing number of publications about SP-A and KL-6 in the context of ILD, there has been no detailed study on the differences between the two biomarkers. Therefore, this study investigated the characteristics of serum SP-A and KL-6 levels in patients with ILD, comparing levels before and after treatment, to explore the monitoring value of these biomarkers for predicting ILD prognoses in Chinese patients.

Methods

Subjects

This retrospective study included data for 71 patients with ILD who were treated at the First Affiliated Hospital of Guangzhou Medical University between August 2015 and September 2019. The Ethical Committee of the First Affiliated Hospital of Guangzhou Medical University approved the study (ethics approval no. gyfyy-2016-73). All procedures were performed in accordance with the Ethics Committee's relevant guidelines and regulations. All the patients provided written informed consent.

Idiopathic interstitial pneumonia (IIP) was classified according to the 2013 American Thoracic Society/European Respiratory Society (ATS/ERS) consensus [22], and interstitial pneumonia, with autoimmune features, was diagnosed according to the 2015 ATS/ERS consensus [23]. The diagnostic criteria for connective tissue disease associated with ILD (CTD-ILD), IPF, hypersensitivity pneumonitis (HP), and other types of ILD were based on the latest internationally accepted guidelines. The patients in this study included those with CTD-ILD, IIP, interstitial pneumonia with autoimmune features (IPAF), HP, and unclassifiable ILD. CTD-ILD included rheumatoid arthritis, systemic sclerosis, primary Sjogren's syndrome, polymyositis, dermatomyositis, systemic lupus erythematosus, and mixed connective tissue disease. IIP included IPF, nonspecific interstitial pneumonia, desquamative interstitial pneumonia,

cryptogenic organizing pneumonia, and acute interstitial pneumonia. The patients were treated with immunosuppressants or corticosteroids according to the internationally accepted guidelines and their clinical symptoms. Patients with other connective tissue diseases, autoimmune diseases, malignancies, infectious diseases, or drug-induced ILD were excluded, as were pregnant and lactating women and patients aged younger than 18 years.

Collection of clinical information and serum samples

Blood samples were collected before and after treatment to measure serum levels of SP-A and KL-6. The serum was allocated and stored at -80°C until analysis. All the patients were followed for at least 3 months. The following data were collected from the patients' medical records: sex, age, body mass index (BMI), smoking history, white blood cell (WBC) count, neutrophil (NEUTP), monocyte (MONO), eosinophil (EOP), and basophil (BASOP) ratios, C-reactive protein (CRP) and lactate dehydrogenase (LDH) levels, therapeutic drugs used, and lung function.

Pulmonary function test (PFT)

Where possible, lung function parameters, including forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), and diffusing capacity for carbon monoxide (DLCO), were measured in accordance with the Standardization of Spirometry guidelines. Some patients with severe ILD were unable to undergo the PFT.

Measurement of serum SP-A and KL-6 levels

Serum levels of SP-A and KL-6 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 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. The cut-off concentrations were 43.8 ng/mL and 500 U/mL, respectively. SP-A and KL-6 assay kits were provided by Sysmex Corporation.

Definitions of disease progressive, improvement, and no change

Disease progressive was defined as a decline in FVC $\geq 10\%$ and/or a decrease in DLCO $\geq 15\%$. Disease improvement was defined as an increase in FVC by $\geq 10\%$ and/or an increase in DLCO by $\geq 15\%$. No change in condition was defined as when the FVC changed by $< 10\%$ and DLCO changed by $< 15\%$.

Statistical analyses

The normality of the continuous variables distributions was tested by the Shapiro–Wilk test, and the data are expressed as mean \pm standard deviation for normal distributions or as median with interquartile range for non-normal distributions. Dichotomous variables are presented as frequencies and percentages. The chi-squared test was used to analyze the differences in categorical data. Differences in the serum marker levels between the patient groups were analyzed using the Wilcoxon signed-rank test and Fisher's exact test. Other differences between the three patient groups were analyzed using the

Kruskal-Wallis test, steel-dwass test or Fisher's exact test. Correlation analyses were performed using Spearman's rank correlation analysis. The calculated values were standardized, and an unsupervised hierarchical cluster analysis was performed using Cluster 3.0 (University of Tokyo Human Genome Center). The statistical analyses were performed using SPSS for Windows version 22.0 (IBM Corp., Armonk, NY, USA) and Cluster 3.0 (University of Tokyo Human Genome Center). P-values <0.05 were considered significant.

Results

Patient characteristics

Of the 71 patients with ILD included in this study, 36 were diagnosed with CTD-ILD, 20 with IIP, 2 with IPAF, 4 with HP, and 9 with unclassified ILD. Figure S1 shows a flowchart of the allocation of the patients to the Progressive, Improved, and Unchanged groups, according to the change in pulmonary function before and after treatment. After treatment, 21 (30%) of the cases were classified as Progressive, 29 (41%) as Improved, and 21 (30%) as Unchanged groups.

The patients' clinical baseline characteristics are shown in Table 1. The patients ranged in age from 19 to 80 years, with a median age of 54 (46–64) years; 34 (48%) were male and 17 (24%) were smokers. The median BMI was 24 (22.02–26.29) kg/m². The median follow-up time was 8.5 months. The median values for the lung function parameters (% predicted values for FVC, FEV1, and DLCO) were lower than the normal range. There were no significant differences in any of the parameters between the three groups.

Serum SP-A and KL-6 levels before and after treatment

Pretreatment serum levels of SP-A and KL-6 were not significantly different among the Progressive, Improved, and Unchanged group (Table 1).

Comparing SP-A and KL-6 levels before and after treatment, in the Progressive group, there was a significant increase in median serum SP-A levels from 39.6 (33.4–81.4) ng/mL to 75.1 (42.4–95.2) ng/mL ($P < 0.05$) and in median serum KL-6 levels from 1329 (980–2138) U/mL to 2409 (1174–4909) U/mL ($P < 0.05$). Serum levels of SP-A and KL-6 were above the cutoff level in 47.6% and 95.2% of patients before treatment; those ratios became 76.2% and 100.0% after treatment (Table 1 and Figure 1 A, B).

Conversely, in the Improved group, there was a significant decrease in median serum SP-A levels, from 46.3 (32.5–66.2) ng/mL to 36.7 ng/mL (24.1–50.3) ng/mL ($P < 0.001$) and in median serum KL-6 levels from 1085 (696.5–3948.5) U/mL to 662 (409.5–1394.5) U/mL ($P < 0.05$). Serum levels of SP-A and KL-6 were above the cutoff level in 51.7 % and 89.7 % of patients before treatment; those ratios became 33.5% and 69.0% after treatment (Table 1 and Figure 1 A, B).

In the Unchanged group, there were no significant changes in median serum SP-A levels [46.3 (39.9–59.6) ng/mL vs. 46.7 (30.7–62.5) ng/mL; $P > 0.05$] or in median serum KL-6 levels [1226 (793–2260) U/mL vs. 1266 (639.5–2292.5) U/mL; $P > 0.05$]. Serum levels of SP-A and KL-6 were above the cutoff level in 52.4% and 90.5% of patients before treatment; those ratios became 57.1% and 81.0% after treatment (Table 1 and Figure 1 A, B).

Clinical and laboratory parameters before and after treatment

Summarizing the pretreatment and post-treatment values in the three groups of WBC count, NEUTP ratio, MONO ratio, EOP ratio, CRP, LDH, and the PFT parameters (Figure 1), MONO and EOP ratios showed a significant decrease in the Improved group ($P=0.024$ and $P = 0.005$, respectively; Figure 1E and F, respectively). The three pulmonary function parameters (% predicted values of DLCO, FVC, and FEV1) significantly improved in the Improved group, and DLCO and FVC significantly reduced in Progressive group. These deteriorations and improvements were not statistically significant in the Unchanged group. ($P > 0.05$) (Figure 1 I, J, K).

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

Figure 2 shows the results of the correlation analysis of the relationships between pretreatment and post-treatment changes in the serum biomarker levels (Delta SP-A and Delta KL-6) and predicted PFT parameters (Delta DLCO, Delta FVC, and Delta FEV1). Delta SP-A showed significant inverse correlations with Delta DLCO ($r_S = -0.491$, $P < 0.001$), Delta FVC ($r_S = -0.441$, $P < 0.001$), and Delta FEV1 ($r_S = -0.354$, $P = 0.003$). Similarly, Delta KL-6 showed significant inverse correlations with Delta DLCO ($r_S = -0.520$, $P < 0.001$), Delta FVC ($r_S = -0.592$, $P < 0.001$), and Delta FEV1 ($r_S = -0.610$, $P < 0.001$). We also tested the correlation between Delta SP-A and Delta KL-6. This showed a significant positive correlation ($r_S = 0.482$, $P < 0.001$; Figure 3). However, in Improved patients, cases with decreased SP-A and KL-6 were inconsistent.

Cluster analysis based on the serum levels of SP-A and KL-6

To explore the pathophysiological differences between KL-6 and SP-A, we classified the patients by cluster analysis and compared pathophysiological characteristics between the clusters (Figure 4). The patients were classified into three groups based on the changes in KL-6 and SP-A. In G3 and G1, the levels of SP-A and KL-6 changed in the same direction: G3, SP-A, and KL-6 all decreased; G1, SP-A, and KL-6 all increased. On the other hand, only SP-A levels were reduced by treatment in G2. (Figure 5A, B). In G1, 56% of patients progressed disease activity. In G3, pretreatment KL-6 levels were significantly higher than G2. Post-treatment FVC and FEV1 were significantly improved, and 77.8% of patients improved disease activity. In G2, FVC was significantly improved, and half of patients improved disease activity (Figure 5C-H). Immune suppressive treatment ratios did not differ significantly among the groups (Figure 5I). Serum LDH levels and EOP ratios were significantly reduced in G2 (Figure 5J, K). In G3, pretreatment EOP ratios tended to be higher than other groups but were not statistically significant (Figure 5L).

Discussion

KL-6 is a mucin-like, high molecular weight glycoprotein, strongly expressed on type II alveolar pneumocytes cells. It was discovered in 1985 by Kohno et al. [24], and several studies have reported that it is a serum marker of ILD. SP-A is a lung-specific protein produced by two types of epithelial cells in the peripheral airway: alveolar type II cells and Clara cells within the lung [21]. Type II lung cells are alveolar wall cells that proliferate during lung injury repair [7]. Elevated serum levels of SP-A and KL-6 reflect increased type II pneumocyte activity in the injured lung, with resultant back-leak into the blood [6, 9, 18-20]. Serum KL-6 levels are known as a biomarker in the diagnosis, severity assessment, and prediction of outcomes for patients with ILD, and have been used in Japan [3, 4, 18-20, 25]. In patients with IPF, SP-A is a useful predictor of mortality and disease progression [15]. It was suggested that serum SP-A has a potential as a biomarker of the therapeutic outcomes of anti-fibrotic drugs [26]. However, although there are increasing reports about KL-6 and SP-A in the ILD context, there has been little investigation of the difference between SP-A and KL-6 as biomarkers. Therefore, this study investigated the characteristics of SP-A and KL-6 based on treatment responses, and we evaluated their value for disease monitoring.

We categorized a decline in FVC $\geq 10\%$ or in DLCO $\geq 15\%$ as an indicator of disease progression and an increase in FVC $\geq 10\%$ or DLCO $\geq 15\%$ as disease improvement. In light of previous classifications, our criteria can be considered reasonable [27, 28].

In this study EOP ratios showed a significant reduction after treatment in the Improved group patients. Eosinophil is one of the Type 2 immune components. As previously reported, T2 immunity is activated in some ILD patients [30, 31]. Among the patients in this study, EOP ratios were within normal ranges both before and after treatment. However, there is a possibility that T2 inflammation of ILD-induced low-level eosinophilic inflammation was improved by the immune suppressive treatment of the Improved group patients.

In pretreatment, 92% of patients showed higher serum KL-6 levels than the previously determined cutoff levels, whereas in SP-A, only 51% of patients showed higher than the cutoff levels. The cut-off value of SP-A was defined by IPF patients, and sensitivity was reported as 78.8% [30]. In the present study, the patients were not limited to IPF, which may affect the sensitivity of SP-A.

In the present study, pretreatment serum levels of SP-A and KL-6 did not relate to disease prognosis. It was reported that pretreatment KL-6 levels was significantly different depending on the response of pirfenidone therapy for IPF [32]. In the present study, pirfenidone was used on only 6 patients; the difference of treatment may affect this discrepancy. On the other hand, our study indicated the changes in serum levels of SP-A and KL-6 correlated significantly with changes in respiratory function, which reflects disease activity. These results are also consistent with other reports [6, 10, 11, 26, 28, 32, 33, 35-38]. Because of the difficulty of respiratory function tests for patients of ILD, this evidence strongly supports the value of biomarkers in monitoring the activity of ILDs.

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 pathophysiology. Ishii et al. [4] reported that serum SP-A levels were higher in usual interstitial pneumonitis and lower in nonspecific interstitial pneumonia, but KL-6 levels were higher in both. Yoshikawa et al. also indicated that changes in serum SP-A levels reflected more strongly the outcomes of anti-fibrotic drug therapy than KL-6 levels [26]. SP-A is produced mainly in Clara cells and type II alveolar cells, whereas KL-6 is produced only in type II alveolar cells [10, 14, 24]. SP-A is a C-type lectin with a molecular weight of 26–38 kDa, whereas KL-6 is a mucin-like glycoprotein with a large molecular weight of 200 kDa [14, 39]. Biological and biochemical differences between SP-A and KL-6 are expected to be associated with different pathological changes in ILD.

To elucidate the pathophysiological differences between SP-A and KL-6, the patients were divided into three groups (G1, G2, and G3), by cluster analysis, according to the values of Delta SP-A and Delta KL-6. Pathophysiological characteristics were compared between clusters. Both SP-A and KL-6 were elevated in the G1 group. In this group, 80% of the patients were categorized as in the Progressive or Unchanged groups. Despite the higher immune suppressive treatment ratio of this group, respiratory function decreased. This suggests that non-inflammatory mechanisms, such as fibrosis, may contribute to pathophysiology.

In the G3 group, the levels of both SP-A and KL-6 decreased, and the patients showed improved respiratory function. Interestingly, the pretreatment EOP ratio tended to be higher in the G3 patients than in the other groups. Around 77.8% of the patients overall were treated with immune suppressive treatment. Although not significantly different due to the small number of patients, the EOP ratio decreased in 7 of 9 samples. These patients might be sensitive to immune suppressive treatment due to dependence on Th2 inflammatory pathophysiology. In the G2 group, only SP-A levels decreased; this also corresponded to improvement of respiratory function. Interestingly, the LDH and EOP ratios of G2 were significantly reduced after treatment (Figure 5J), despite the LDH levels of G3 not being changed. Previous reports suggested the serum levels of LDH have value monitoring disease activity and progression. It is considered to reflect pulmonary cell damage [40]. In the acute phase of ARDS that is caused by COVID-19 infection, it was reported that SP-A levels elevated from a relatively early stage of the pneumonia [41].

Reportedly, serum SP-A levels increased with acute exacerbations of ILD, whereas KL-6 levels were elevated in drug-induced pneumonia or CTD-ILD [13]. In addition, SP-A expression was reported to correlate negatively with fibrosis score [42]. Takahashi et al. [10] reported that SP-A levels were associated with reversible ground-glass opacity levels, but not with indicators of the end stage of fibrotic changes, such as honeycombing. In addition, they reported that SP-A levels were significantly lower in parenchymal collapse opacity-dominant type patients than in ground-glass opacity-dominant type patients [10]. Studies have shown that serum SP-A rises faster than KL-6 in the progressive group [43]. This suggests the G2 patients were not at the end stage of the disease, which might explain why their respiratory function improved with immune suppressive treatment. Compared to KL-6, the lower

molecular weight of SP-A may provide an advantage in detecting it in blood serum at an earlier stage of disease.

In summary, although both SP-A and KL-6 are pathological markers that reflect the disease course with treatment of patients with ILD; in some patients, only SP-A levels responded to an improvement of the disease. It is, therefore, important to measure both SP-A and KL-6 levels simultaneously for the pathophysiological monitoring of patients with ILD.

This has several limitations. First, we included patients with definite usual interstitial pneumonitis patterns on HRCT but without surgical lung biopsies; therefore, misclassification was possible. Second, this was a retrospective study, and the observation period differed among the patients. Finally, we were unable to compare the results of serum biomarkers to histopathological patterns because of the lack of surgical lung biopsies. In the future, we intend to investigate these biomarkers in a prospective study setting.

Conclusions

In conclusion, our results demonstrated that serum levels SP-A and KL-6 in patients with ILD significantly decreased in patients who showed disease improvement and significantly increased in patients who showed disease progression. However, the response of both markers was different according to their pathophysiological or biological characteristics; measuring both markers are important for understanding a patient's pathophysiological condition.

Abbreviations

ILD: interstitial lung disease; SP-A: surfactant protein-A; KL-6: Krebs von den Lungen-6; PFT: pulmonary function tests; HRCT: High-resolution computed tomography; IPF: idiopathic pulmonary fibrosis; IIP: idiopathic interstitial pneumonia; ERS/ATS: European Respiratory Society and American Thoracic Society; CTD-ILD: connective tissue disease associated with interstitial lung disease; HP: hypersensitivity pneumonitis; IPAF: interstitial pneumonia with autoimmune features; BMI: body mass index; WBC: white blood cell count; NEUTP: neutrophil ratio; EOP: eosinophil ratio; BASOP: basophil ratio; CRP: C-reactive protein; LDH: lactate dehydrogenase; DLCO: diffusing capacity for carbon monoxide; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s.

Declarations

Ethics approval and consent to participate

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University (approval number: GYFYY-2016-73).

The use of human serum samples was in accordance with relevant legislation in China and the wishes of donors, their legal guardians, or next of kin, where applicable, who had offered written informed consent to using the serum samples for future unspecified research purposes.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

(I) Conception and design: B Sun; (II) Administrative support: B Sun and P Zheng; (III) Provision of study materials or patients: P Zheng, X Zheng, J Wang; (IV) Collection and assembly of data: X Zheng; (V) Data analysis and interpretation: H Takehiro, X Zheng, P Zheng, Z Huang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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References

- [1] Huapaya JA, Wilfong EM, Harden CT, Brower RG, Danoff SK. Risk factors for mortality and mortality rates in interstitial lung disease patients in the intensive care unit. *Eur Respir Rev.* 2018; **27**.
- [2] Meyer KC. Diagnosis and management of interstitial lung disease. *Transl Respir Med.* 2014; **2**: 4.
- [3] Ishikawa N, Hattori N, Yokoyama A, Kohno N. Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases. *Respir Investig.* 2012; **50**: 3-13.
- [4] Ishii H, Mukae H, Kadota J, Kaida H, Nagata T, Abe K, Matsukura S, Kohno S. High serum concentrations of surfactant protein A in usual interstitial pneumonia compared with non-specific

interstitial pneumonia. *Thorax*. 2003; **58**: 52-7.

[5] Hamai K, Iwamoto H, Ishikawa N, Horimasu Y, Masuda T, Miyamoto S, Nakashima T, Ohshimo S, Fujitaka K, Hamada H, Hattori N, Kohno N. Comparative Study of Circulating MMP-7, CCL18, KL-6, SP-A, and SP-D as Disease Markers of Idiopathic Pulmonary Fibrosis. *Dis Markers*. 2016.

[6] Ohnishi H, Yokoyama A, Kondo K, Hamada H, Abe M, Nishimura K, Hiwada K, Kohno N. Comparative study of KL-6, surfactant protein-A, surfactant protein-D, and monocyte chemoattractant protein-1 as serum markers for interstitial lung diseases. *Am J Respir Crit Care Med*. 2002; **165**: 378-81.

[7] Chiba H, Otsuka M, Takahashi H. Significance of molecular biomarkers in idiopathic pulmonary fibrosis: A mini review. *Respir Investig*. 2018; **56**: 384-91.

[8] Samukawa T, Hamada T, Uto H, Yanagi M, Tsukuya G, Nosaki T, Maeda M, Hirano T, Tsubouchi H, Inoue H. The elevation of serum napsin A in idiopathic pulmonary fibrosis, compared with KL-6, surfactant protein-A and surfactant protein-D. *BMC Pulm Med*. 2012; **12**: 55.

[9] Cho EJ, Park KJ, Ko DH, Koo HJ, Lee SM, Song JW, Lee W, Lee HK, Do KH, Chun S, Min WK. Analytical and Clinical Performance of the Nanopia Krebs von den Lungen 6 Assay in Korean Patients With Interstitial Lung Diseases. *Ann Lab Med*. 2019; **39**: 245-51.

[10] Takahashi H, Fujishima T, Koba H, Murakami S, Kurokawa K, Shibuya Y, Shiratori M, Kuroki Y, Abe S. Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. *Am J Respir Crit Care Med*. 2000; **162**: 1109-14.

[11] Nakamura M, Okamoto M, Fujimoto K, Ebata T, Tominaga M, Nouno T, Zaizen Y, Kaieda S, Tsuda T, Kawayama T, Hoshino T. A retrospective study of the tolerability of nintedanib for severe idiopathic pulmonary fibrosis in the real world. *Ann Transl Med*. 2019; **7**: 262.

[12] Kakugawa T, Yokota S, Ishimatsu Y, Hayashi T, Nakashima S, Hara S, Sakamoto N, Matsuoka Y, Kubota H, Mine M, Mukae H, Nagata K, Kohno S. Serum heat shock protein 47 levels in patients with drug-induced lung disease. *Respir Res*. 2013; **14**: 133.

[13] Kitajima H, Takahashi H, Harada K, Kanai A, Inomata S, Taniguchi H, Saikai T, Abe S. Gefitinib-induced interstitial lung disease showing improvement after cessation: disassociation of serum markers. *Respirology*. 2006; **11**: 217-20.

[14] Kuroki Y, Takahashi H, Chiba H, Akino T. Surfactant proteins A and D: disease markers. *Biochim Biophys Acta*. 1998; **1408**: 334-45.

[15] Song JW, Do KH, Jang SJ, Colby TV, Han S, Kim DS. Blood biomarkers MMP-7 and SP-A: predictors of outcome in idiopathic pulmonary fibrosis. *Chest*. 2013; **143**: 1422-9.

- [16] Xue M, Guo Z, Cai C, Sun B, Wang H. Evaluation of the Diagnostic Efficacies of Serological Markers KL-6, SP-A, SP-D, CCL2, and CXCL13 in Idiopathic Interstitial Pneumonia. *Respiration*. 2019; **98**: 534-45.
- [17] Zhang Y, Kaminski N. Biomarkers in idiopathic pulmonary fibrosis. *Curr Opin Pulm Med*. 2012; **18**: 441-6.
- [18] Bonella F, Volpe A, Caramaschi P, Nava C, Ferrari P, Schenk K, Ohshimo S, Costabel U, Ferrari M. Surfactant protein D and KL-6 serum levels in systemic sclerosis: correlation with lung and systemic involvement. *Sarcoidosis Vasc Diffuse Lung Dis*. 2011; **28**: 27-33.
- [19] Hant FN, Ludwicka-Bradley A, Wang HJ, Li N, Elashoff R, Tashkin DP, Silver RM, Scleroderma Lung Study Research G. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. *J Rheumatol*. 2009; **36**: 773-80.
- [20] Benyamine A, Heim X, Resseguier N, Bertin D, Gomez C, Ebbo M, Harle JR, Kaplanski G, Rossi P, Bardin N, Granel B. Elevated serum Krebs von den Lungen-6 in systemic sclerosis: a marker of lung fibrosis and severity of the disease. *Rheumatol Int*. 2018; **38**: 813-9.
- [21] Wang K, Ju Q, Cao J, Tang W, Zhang J. Impact of serum SP-A and SP-D levels on comparison and prognosis of idiopathic pulmonary fibrosis: A systematic review and meta-analysis. *Medicine (Baltimore)*. 2017; **96**: e7083.
- [22] Zheng P, Liu X, Huang H, Guo Z, Wu G, Hu H, Cai C, Luo W, Wei N, Han Q, Sun B. Diagnostic value of KL-6 in idiopathic interstitial pneumonia. *J Thorac Dis*. 2018; **10**: 4724-32.
- [23] Fischer A, Antoniou KM, Brown KK, Cadranet J, Corte TJ, du Bois RM, Lee JS, Leslie KO, Lynch DA, Matteson EL, Mosca M, Noth I, Richeldi L, Strek ME, Swigris JJ, Wells AU, West SG, Collard HR, Cottin V, CTD-ILD EATFoUfo. An official European Respiratory Society/American Thoracic Society research statement: interstitial pneumonia with autoimmune features. *Eur Respir J*. 2015; **46**: 976-87.
- [24] Kohno N, Kyoizumi S, Awaya Y, Fukuhara H, Yamakido M, Akiyama M. New serum indicator of interstitial pneumonitis activity. Sialylated carbohydrate antigen KL-6. *Chest*. 1989; **96**: 68-73.
- [25] 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. *Mod Rheumatol*. 2013; **23**: 872-83.
- [26] Yoshikawa T, Otsuka M, Chiba H, Ikeda K, Mori Y, Umeda Y, Nishikiori H, Kuronuma K, Takahashi H. Surfactant protein A as a biomarker of outcomes of anti-fibrotic drug therapy in patients with idiopathic pulmonary fibrosis. *BMC Pulm Med*. 2020 Jan 31; **20(1)**:27
- [27] Cottin V, Wollin L, Fischer A, Quaresma M, Stowasser S, Harari S. Fibrosing interstitial lung diseases: knowns and unknowns. *Eur Respir Rev*. 2019; **28**.

- [28] Lee YS, Kim HC, Lee BY, Lee CK, Kim MY, Jang SJ, Lee HS, Moon J, Colby TV, Kim DS. The Value of Biomarkers as Predictors of Outcome in Patients with Rheumatoid Arthritis-Associated Usual Interstitial Pneumonia. *Sarcoidosis Vasc Dif.* 2016; **33**: 216-23.
- [29] Jiang Y, Luo Q, Han Q, Huang JT, Ou Y, Chen M, Wen Y, Mosha SS, Deng KM, Chen RC. Sequential changes of serum KL-6 predict the progression of interstitial lung disease. *Journal of Thoracic Disease.* 2018; **10**.
- [30] Kishi M, Miyazaki Y, Jinta T, Furusawa H, Ohtani Y, Inase N, Yoshizawa Y. Pathogenesis of cBFL in common with IPF? Correlation of IP-10/TARC ratio with histological patterns. *Thorax.* 2008 Sep;63(9):810-6.
- [31] Nukui Y, Yamana T, Masuo M, Tateishi T, Kishino M, Tateishi U, Tomita M, Hasegawa T, Aritsu T, Miyazaki Y. Serum CXCL9 and CCL17 as biomarkers of declining pulmonary function in chronic bird-related hypersensitivity pneumonitis. *PLoS One.* 2019 Aug 1;14(8):e0220462.
- [32] Ikeda K, Shiratori M, Chiba H, Nishikiori H, Yokoo K, Saito A, Hasegawa Y, Kuronuma K, Otsuka M, Yamada G, Takahashi H. Serum surfactant protein D predicts the outcome of patients with idiopathic pulmonary fibrosis treated with pirfenidone. *Respir Med.* 2017 Oct;131:184-191
- [33] Hu Y, Wang LS, Jin YP, Du SS, Du YK, He X, Weng D, Zhou Y, Li QH, Shen L, Zhang F, Su YL, Sun XL, Ding JJ, Zhang WH, Cai HR, Dai HP, Dai JH, Li HP. Serum Krebs von den Lungen-6 level as a diagnostic biomarker for interstitial lung disease in Chinese patients. *Clin Respir J.* 2017; **11**: 337-45.
- [34] Hanaoka M, Katsumata Y, Kawasumi H, Kawaguchi Y, Yamanaka H. KL-6 is a long-term disease-activity biomarker for interstitial lung disease associated with polymyositis/dermatomyositis, but is not a short-term disease-activity biomarker. *Mod Rheumatol.* 2019; **29**: 625-32.
- [35] Hu C, Wu C, Yang E, Huang H, Xu D, Hou Y, Zhao J, Li M, Xu Z, Zeng X, Wang Q. Serum KL-6 is associated with the severity of interstitial lung disease in Chinese patients with polymyositis and dermatomyositis. *Clin Rheumatol.* 2019; **38**: 2181-7.
- [36] Cao XY, Hu SS, Xu D, Li MT, Wang Q, Hou Y, Zeng XF. Serum levels of Krebs von den Lungen-6 as a promising marker for predicting occurrence and deterioration of systemic sclerosis-associated interstitial lung disease from a Chinese cohort. *Int J Rheum Dis.* 2019; **22**: 108-15.
- [37] Lee JS, Lee EY, Ha YJ, Kang EH, Lee YJ, Song YW. Serum KL-6 levels reflect the severity of interstitial lung disease associated with connective tissue disease. *Arthritis Res Ther.* 2019; **21**: 58.
- [38] Yamakawa H, Hagiwara E, Kitamura H, Yamanaka Y, Ikeda S, Sekine A, Baba T, Okudela K, Iwasawa T, Takemura T, Kuwano K, Ogura T. Serum KL-6 and surfactant protein-D as monitoring and predictive markers of interstitial lung disease in patients with systemic sclerosis and mixed connective tissue disease. *J Thorac Dis.* 2017; **9**: 362-71.

- [39] Brayman M, Thathiah A, Carson DD. MUC1: a multifunctional cell surface component of reproductive tissue epithelia. *Reprod Biol Endocrinol*. 2004; **2**: 4.
- [40] Tzouvelekis A, Kouliatsis G, Anevlavis S, Bouros D. Serum biomarkers in interstitial lung diseases. *Respir Res*. 2005;6(1):78
- [41] Saito A, KURONUMA K, Moniwa K, et al. Serum surfactant protein A and D may be novel biomarkers of COVID-19 pneumonia severity. *Research Square*; 2020. DOI: 10.21203/rs.3.rs-29567/v1.
- [42] Nagata N, Kitasato Y, Wakamatsu K, Kawabata M, Fukushima K, Kajiki A, Kitahara Y, Watanabe K. Prognostic value of immunohistochemical surfactant protein A expression in regenerative/hyperplastic alveolar epithelial cells in idiopathic interstitial pneumonias. *Diagn Pathol*. 2011; **6**: 25.
- [43] Yoshikawa T, Otsuka M, Chiba H, Ikeda K, Mori Y, Umeda Y, Nishikiori H, Kuronuma K, Takahashi H. Correction to: Surfactant protein A as a biomarker of outcomes of anti-fibrotic drug therapy in patients with idiopathic pulmonary fibrosis. *BMC Pulm Med*. 2020; **20**: 131.

Tables

Table 1. Baseline characteristics of the three groups of patients with ILD

Variables	Progression Group	Unchanged Group	Improvement Group	P-value
Number (%)	21 (29.6%)	21 (29.6%)	29 (40.8%)	-
Follow-up (months)	9.9 (7.3–12.1) ^a	6.4 (4.0–10.6) ^a	7.5 (4.2–14.4) ^a	0.100 ^c
Age (years)	53 (48.8–63.3) ^a	54 (45.8–68.0) ^a	55 (44.5–61.3) ^a	0.642 ^c
Male (%)	7 (33.3%)	12 (57.1%)	15 (51.7%)	0.262 ^d
BMI (kg/m ²)	24.5 (22.7–27.8) ^a	24.4 (22.7–36.3) ^a	22.7 (21.4–24.8) ^a	0.161 ^c
Smoker, n (%)	4 (19.0)	5 (23.8)	8 (27.6)	0.836
WBC (10 ⁹ /L)	8.9 (7.1–10) ^a	7 (5.3–10.2) ^a	8.1 (6.6–9.7) ^a	0.307 ^c
NEUTP ratio (%)	61.4 (53.8–71.7) ^a	65.6 (60.7–77.6) ^a	64 (56.9–71.9) ^a	0.312 ^c
MONO ratio (%)	6.7 (5.4–8.5) ^a	7.6 (5.9–9.5) ^a	9.5 (6.9–11.3) ^a	0.477 ^c
EOP ratio (%)	1 (0.4–3.6) ^a	1.8 (0.9–2.6) ^a	3.1 (1–4.6) ^a	0.162 ^c
BASOP ratio (%)	0.4 (0.3–0.5) ^a	0.4 (0.3–0.6) ^a	0.4 (0.3–0.6) ^a	0.828 ^c
CRP (ml/dL)	0.4 (0.1–1.1) ^a	0.2 (0.1–0.5) ^a	0.2 (0.1–0.5) ^a	0.254 ^c
LDH (U/L)	229 (197.2–260.0) ^a	196.7 (179.9–252.1) ^a	203.5 (169.2–216.7) ^a	0.212 ^c
DLCO (% predicted)	58.9 (51.2–69.5) ^a	48.8 (38.5–60.5) ^a	56 (43.5–68.9) ^a	0.161 ^c
FVC (% predicted)	70.2 (62.2–78.4) ^a	68 (54.67–80.37) ^a	69.5 (55.9–75.4) ^a	0.852 ^c
FEV1 (% predicted)	77 (58.3–79.5) ^a	74.9 (55.5–87.3) ^a	70.9 (58.9–80.5) ^a	0.789 ^c
Pretreatment SP-A (ng/mL)	39.6 (33.4–81.4) ^a	46.3 (39.9–59.6) ^a	46.3 (32.5–66.2) ^a	0.993 ^c
Post-treatment SP-A (ng/mL)	75.1 (42.4–95.2) ^a	46.7 (30.7–62.5) ^a	36.7 (24.1–50.3) ^a	<0.001 ^c
Pretreatment KL-6 (U/mL)	1329 (980–2138) ^a	1226 (793–2260) ^a	1085 (696–3948) ^a	0.428 ^c
Post-treatment KL-6 (U/mL)	2409 (1174–4909) ^a	1266 (640–2293) ^a	662 (410–1395) ^a	<0.001 ^c
SP-A (>43.8ng/mL)				1.00 ^d
Pretreatment	47.6% (26.3%–69.0%) ^b	52.4% (31.0%–73.7%) ^b	51.7% (33.5%–69.9%) ^b	

Post-treatment	76.2% (58.0%-94.4%) ^b	57.1% (36.0%-78.3%) ^b	33.5% (17.2%-51.8%) ^b	0.013 ^d
KL-6 (>500U/mL)				
Pretreatment	95.2% (86.1%-100.0%)	90.5% (77.9%-100.0%)	89.7% (78.6%-100.0%)	0.875 ^d
Post-treatment	100.0%	81.0% (64.2%-97.7%)	69% (52.1%-85.8%)	0.010 ^d

Figures

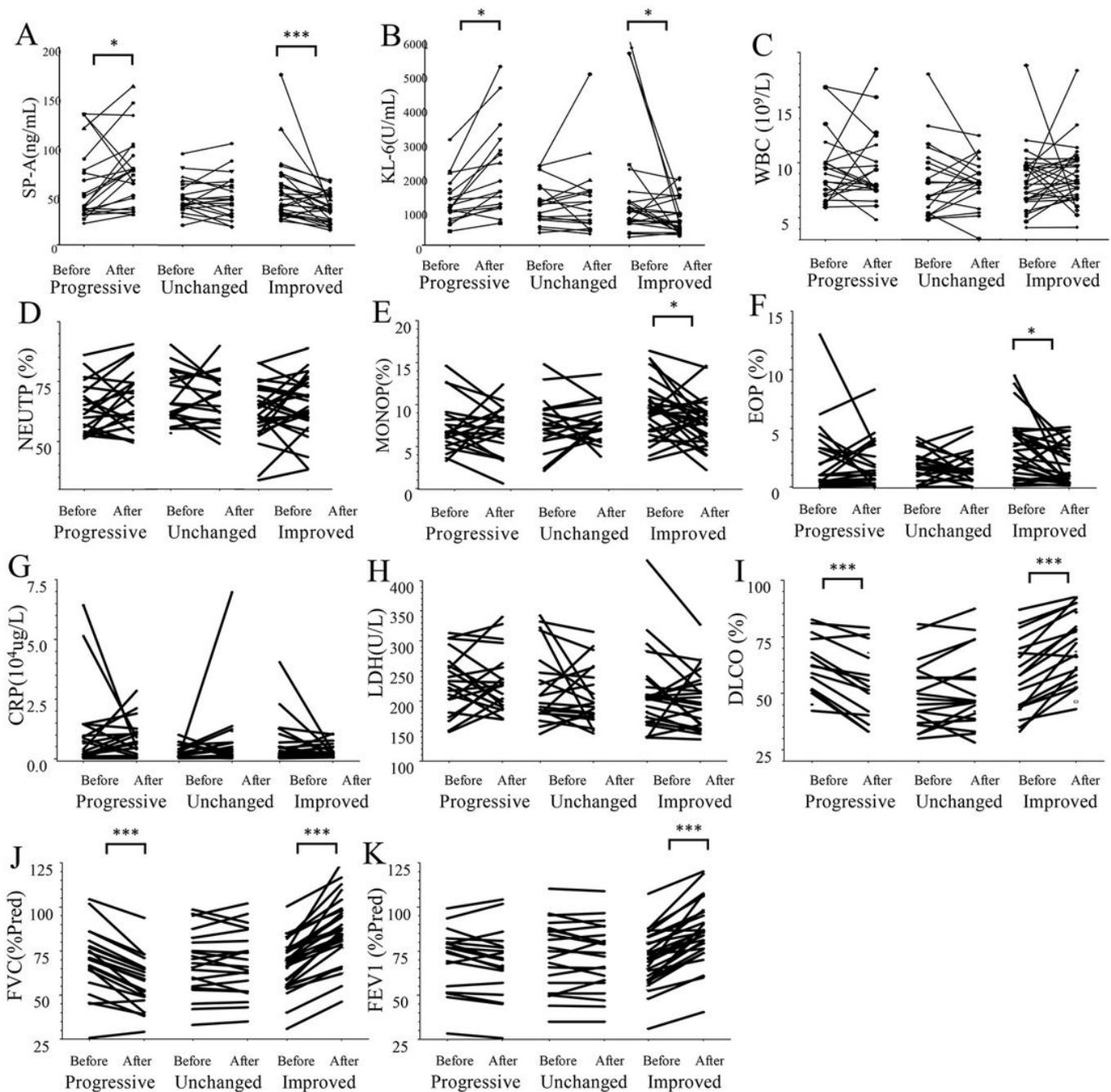


Figure 1

Comparison of clinical and laboratory parameters between pretreatment and post-treatment. The patients were allocated to the Progressive, Unchanged, and Improved groups according to the difference between pretreatment and post-treatment lung function. A: Serum surfactant protein-A (SP-A) levels. B: Serum Krebs von den Lungen-6 (KL-6) levels. C: White blood cell (WBC) count. D: Neutrophil ratio (NEUTP). E: Monocyte ratio (MONOP). F: Eosinophil ratio (EOP). G, C-reactive protein (CRP) levels. H: Lactate dehydrogenase (LDH) levels. I: Percent-predicted diffusing capacity for carbon monoxide (DLCO). J: Percent-predicted forced vital capacity (FVC). K: Percent-predicted forced expiratory volume in 1s (FEV1); P-values were calculated by Wilcoxon signed-rank U test. *: P < 0.05, **: P < 0.005, ***: P < 0.001

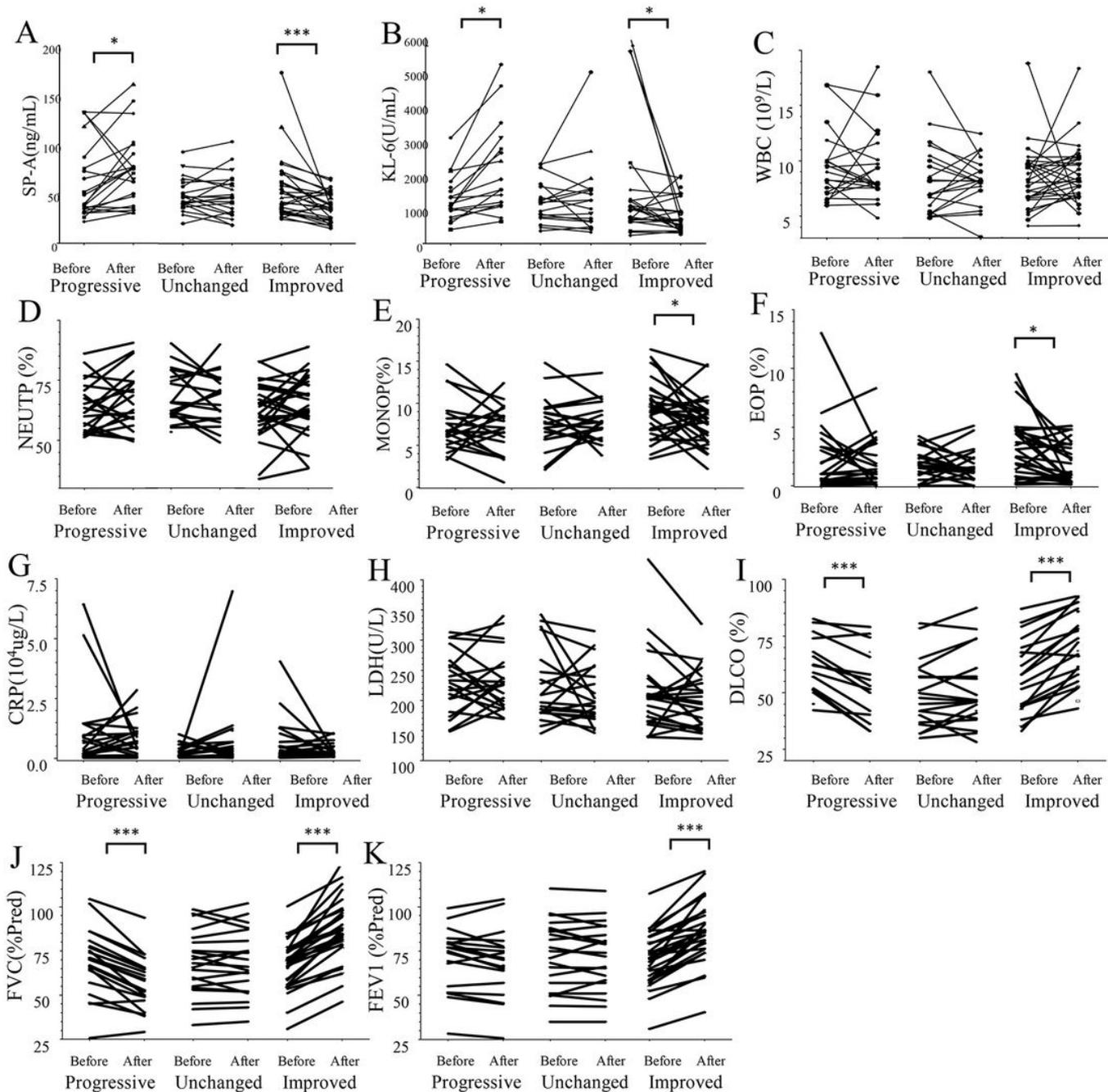


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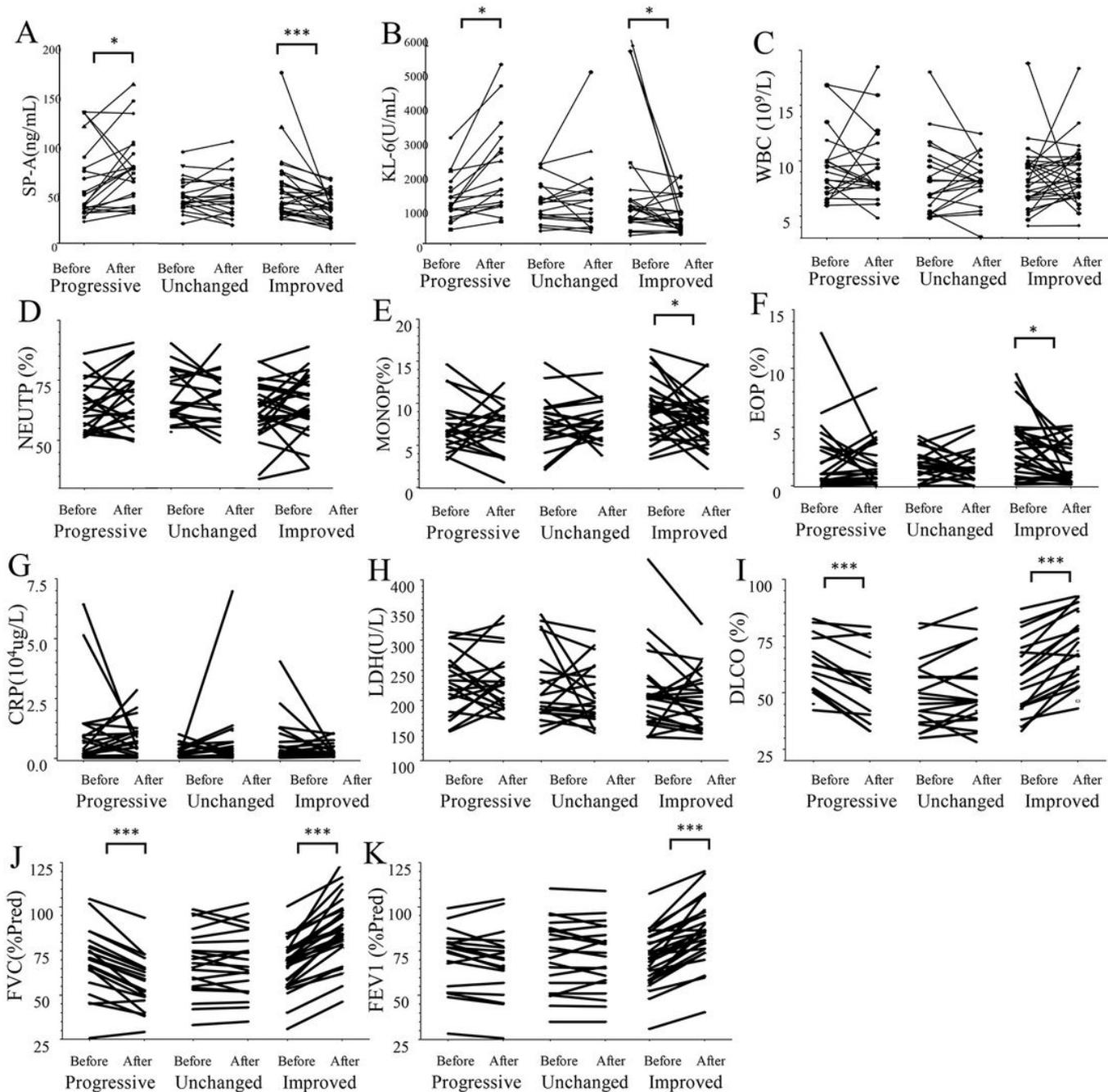


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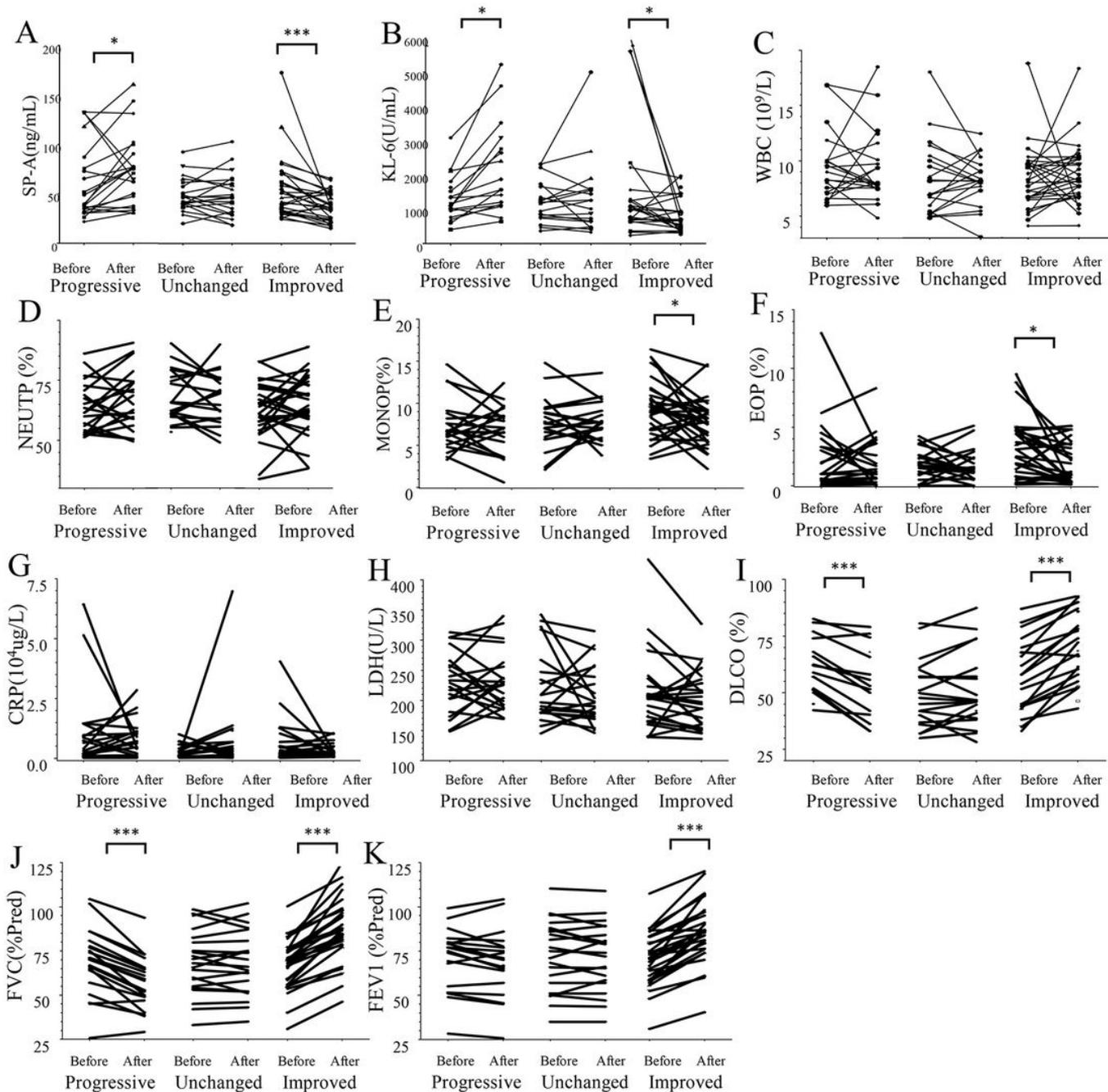


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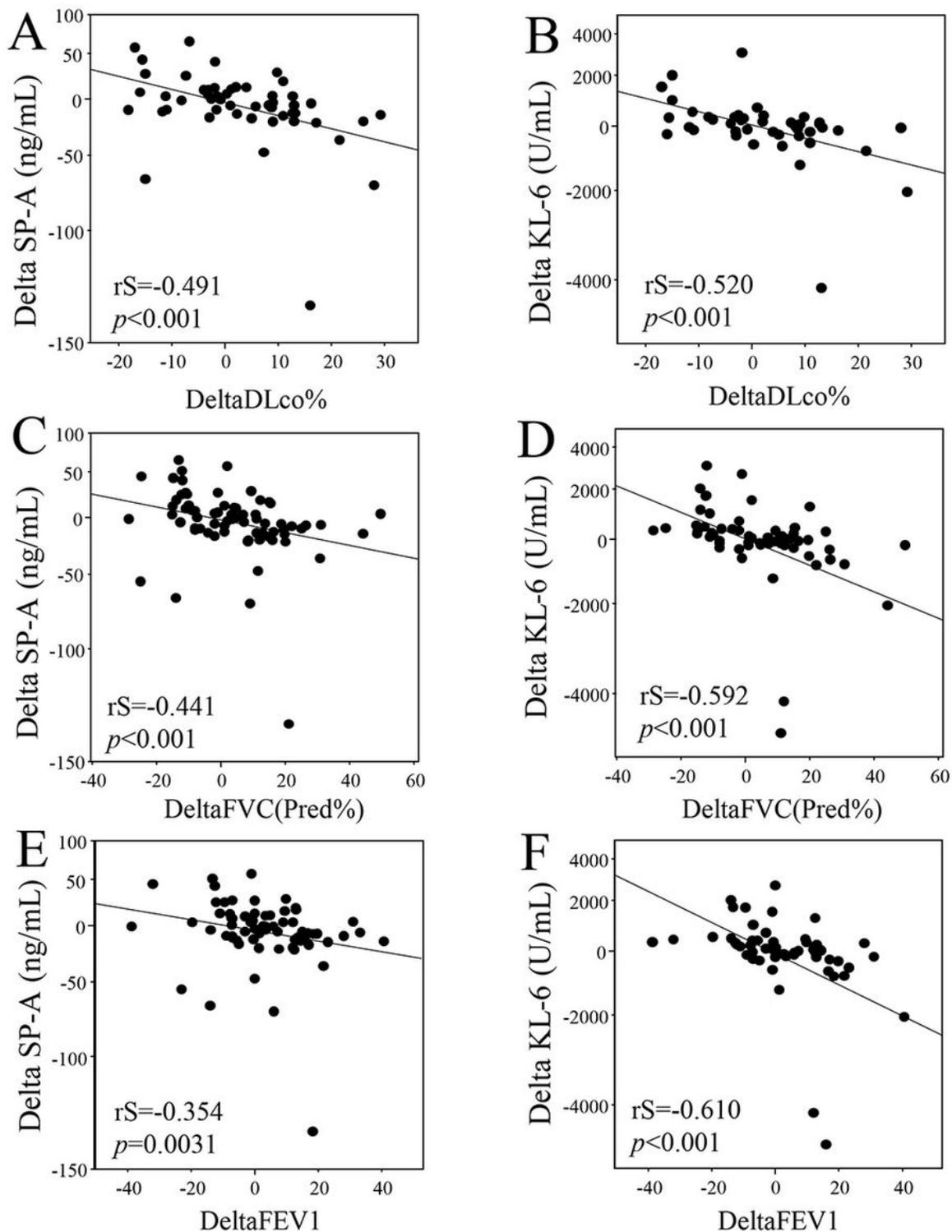


Figure 2

Correlations between Delta SP-A and Delta KL-6 and changes in pulmonary function test parameters. Surfactant protein-A (SP-A); Krebs von den Lungen-6 (KL-6); Diffusing capacity for carbon monoxide (DLCO); Forced vital capacity (FVC); Forced expiratory volume in 1s (FEV1).

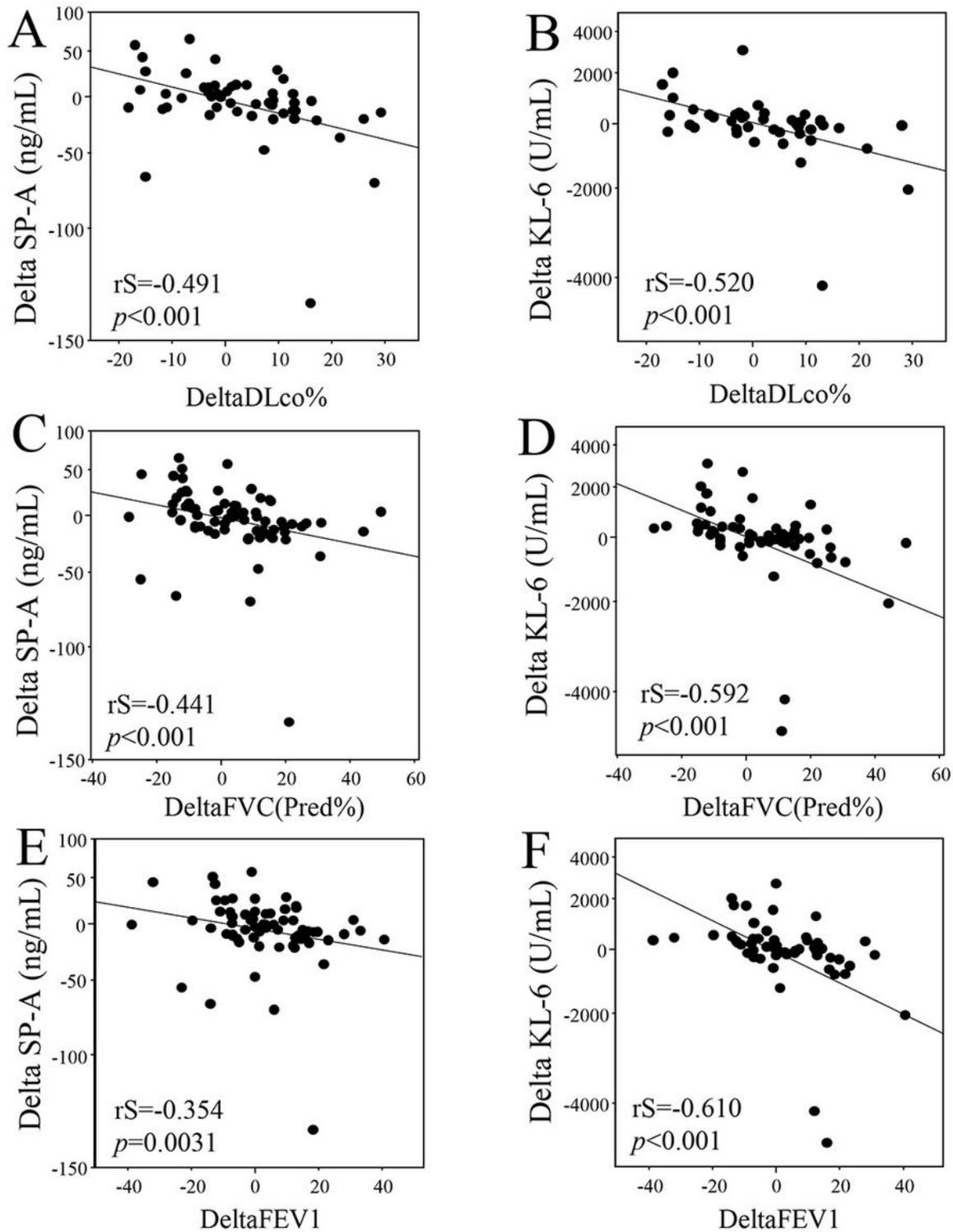


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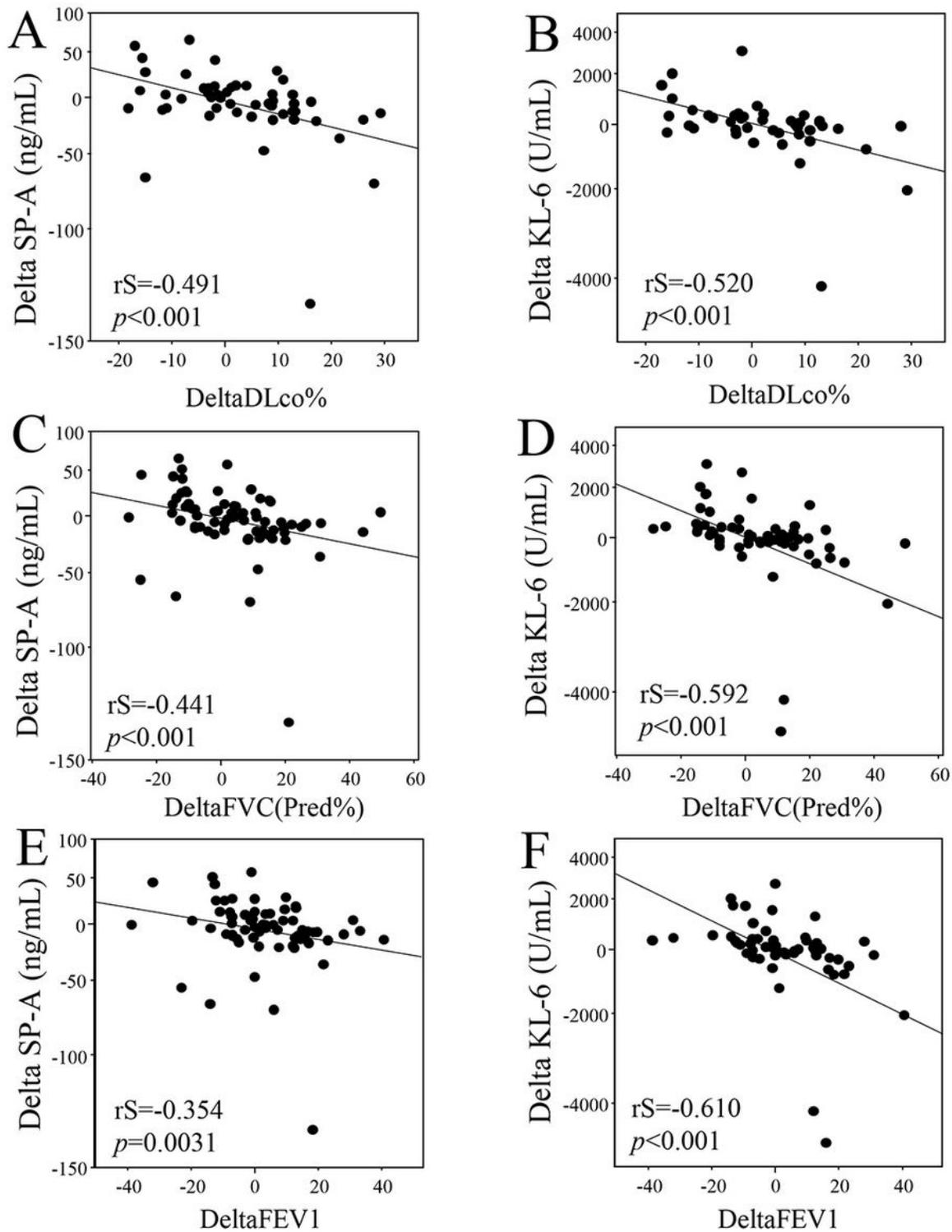


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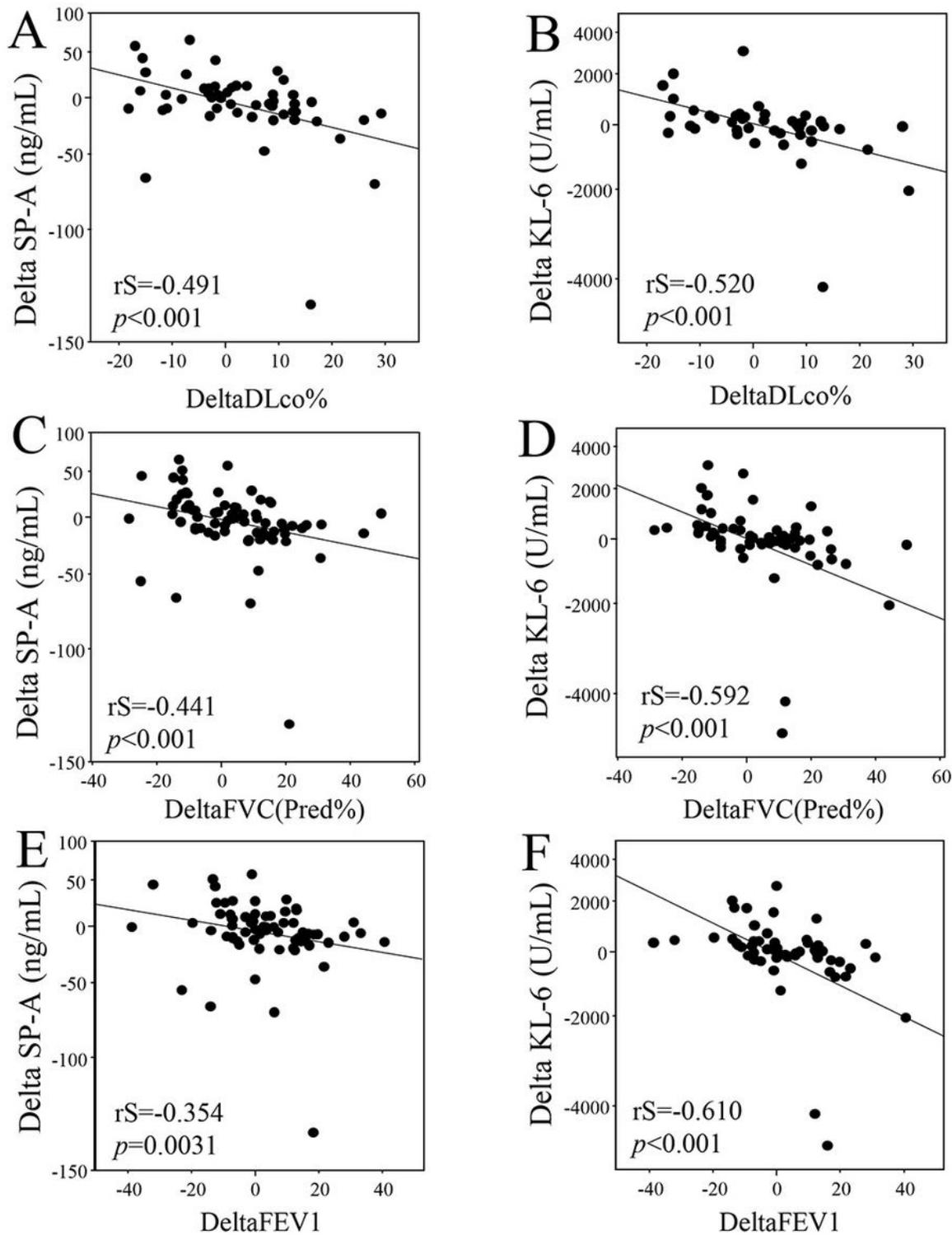


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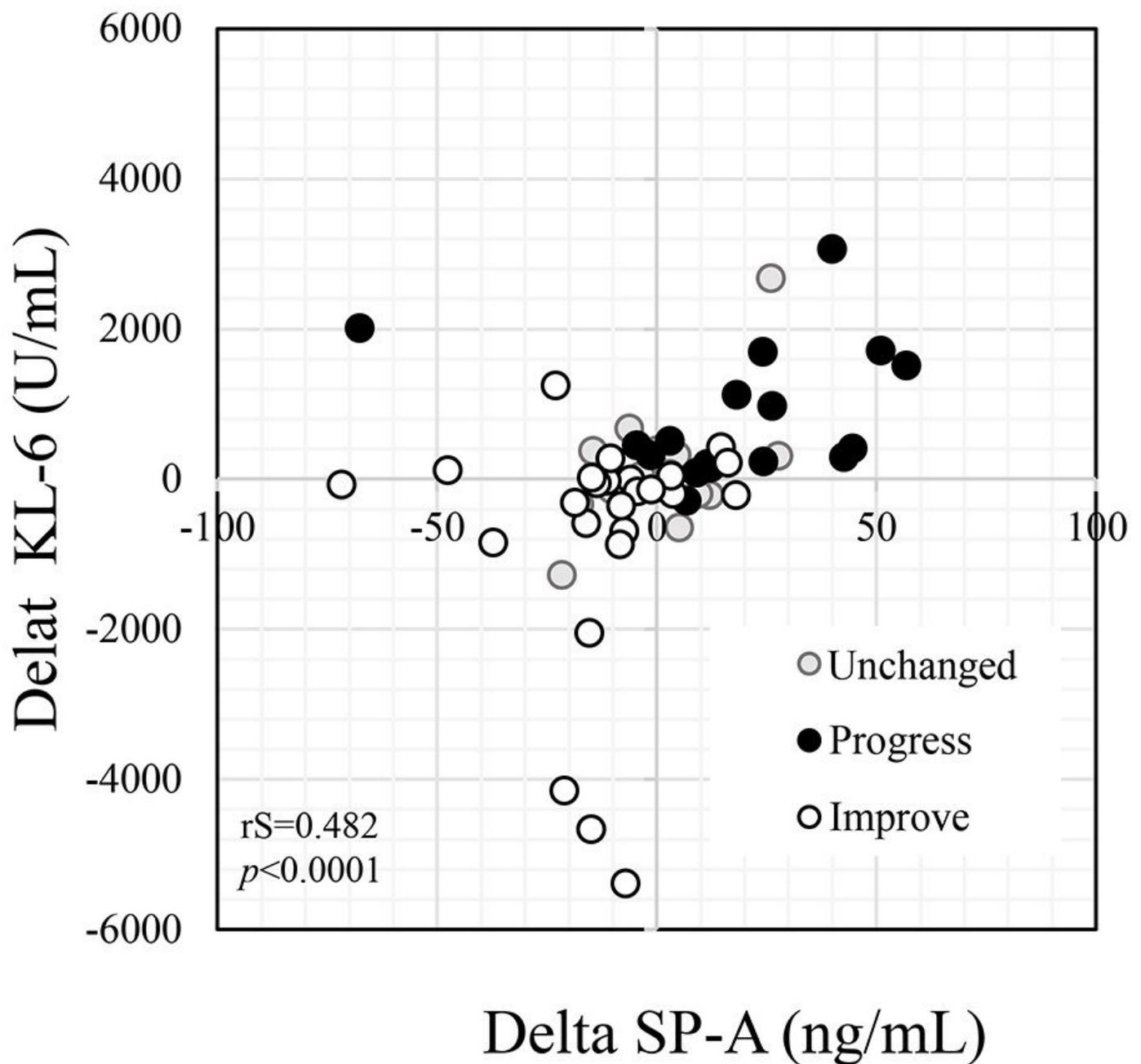


Figure 3

Correlation between serum Delta KL-6 and Delta SP-A values. The individual dots indicate patients in the Unchanged (gray), Progressive (black), and Improved (open) groups. SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6.

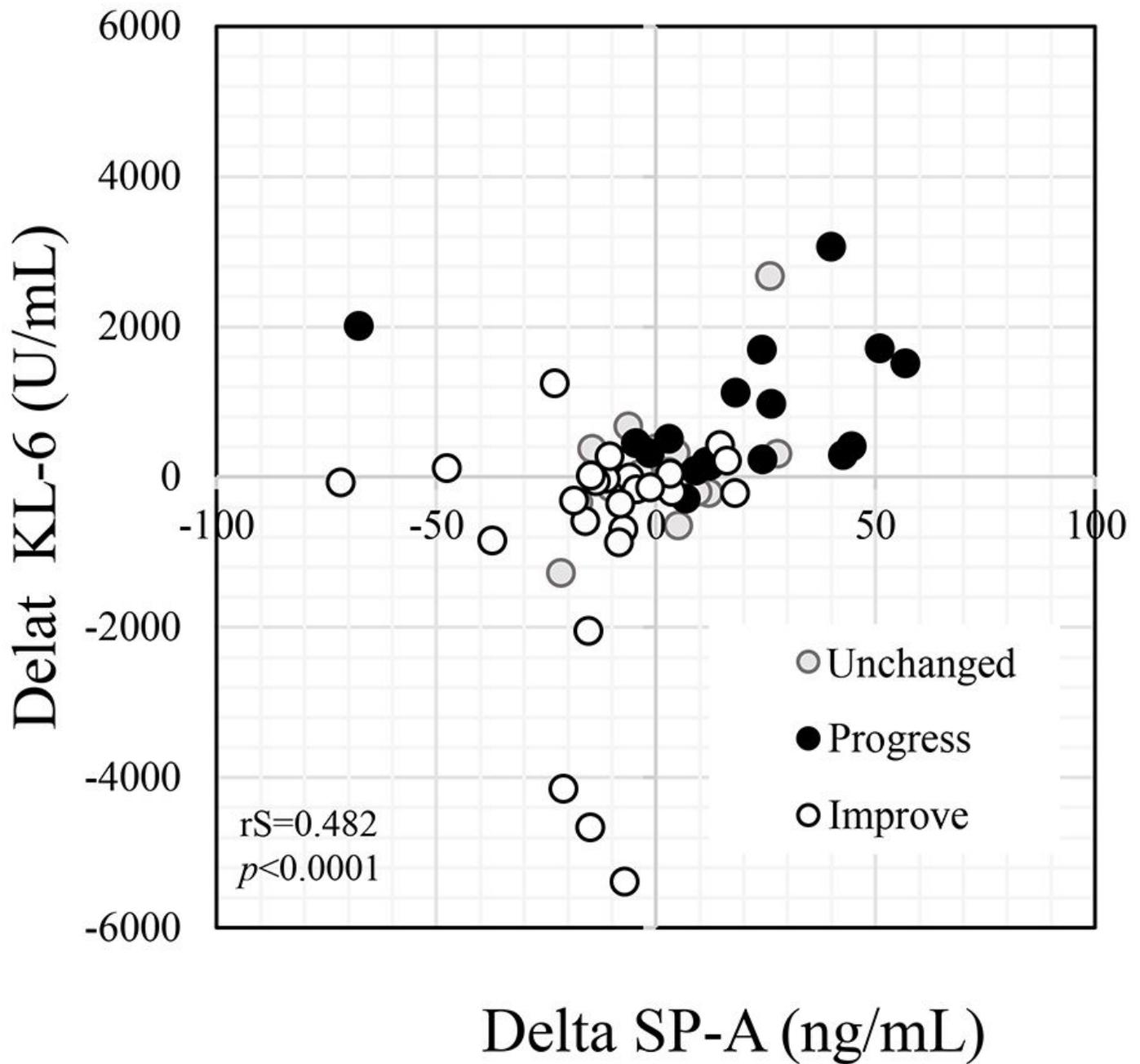


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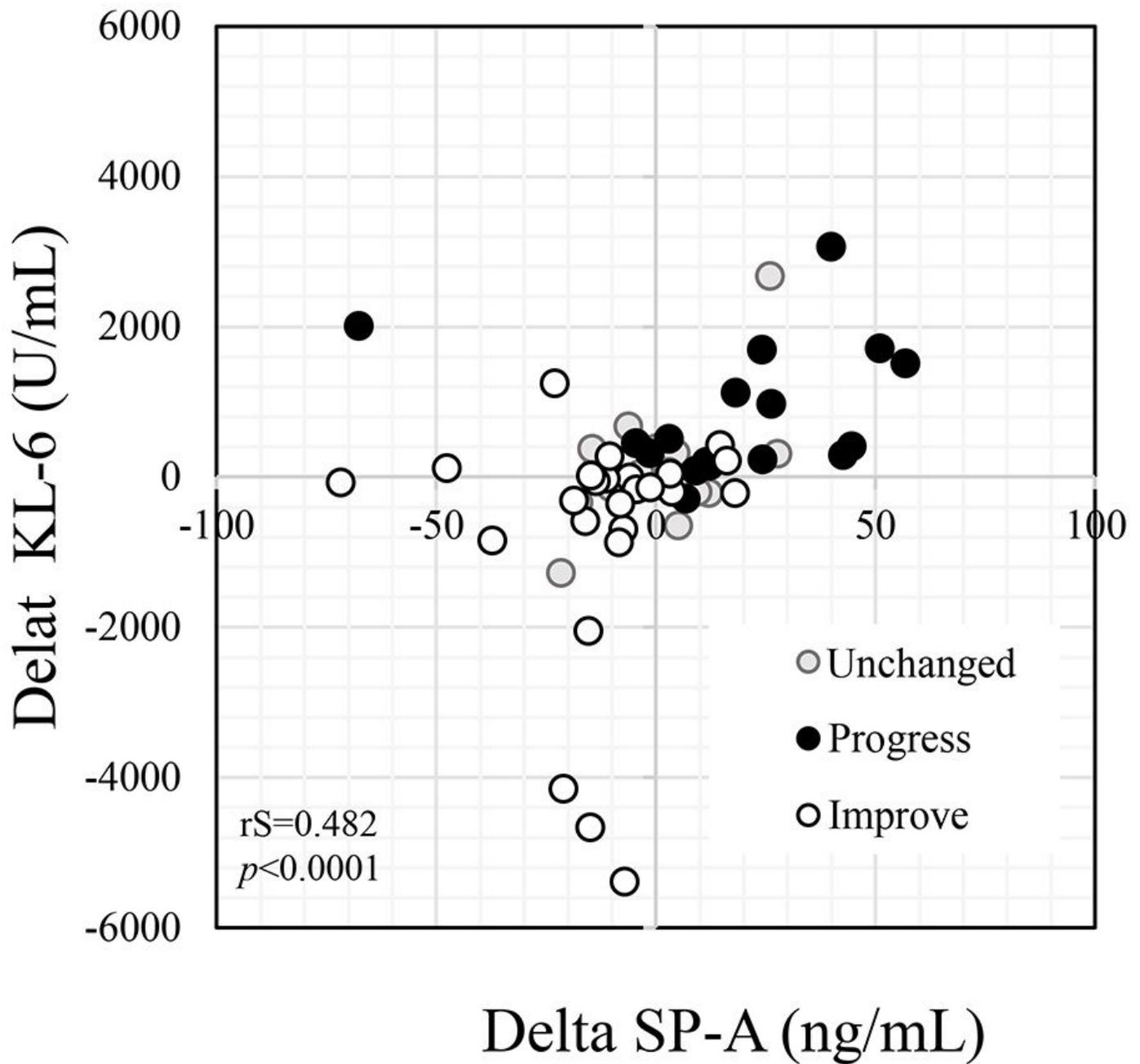


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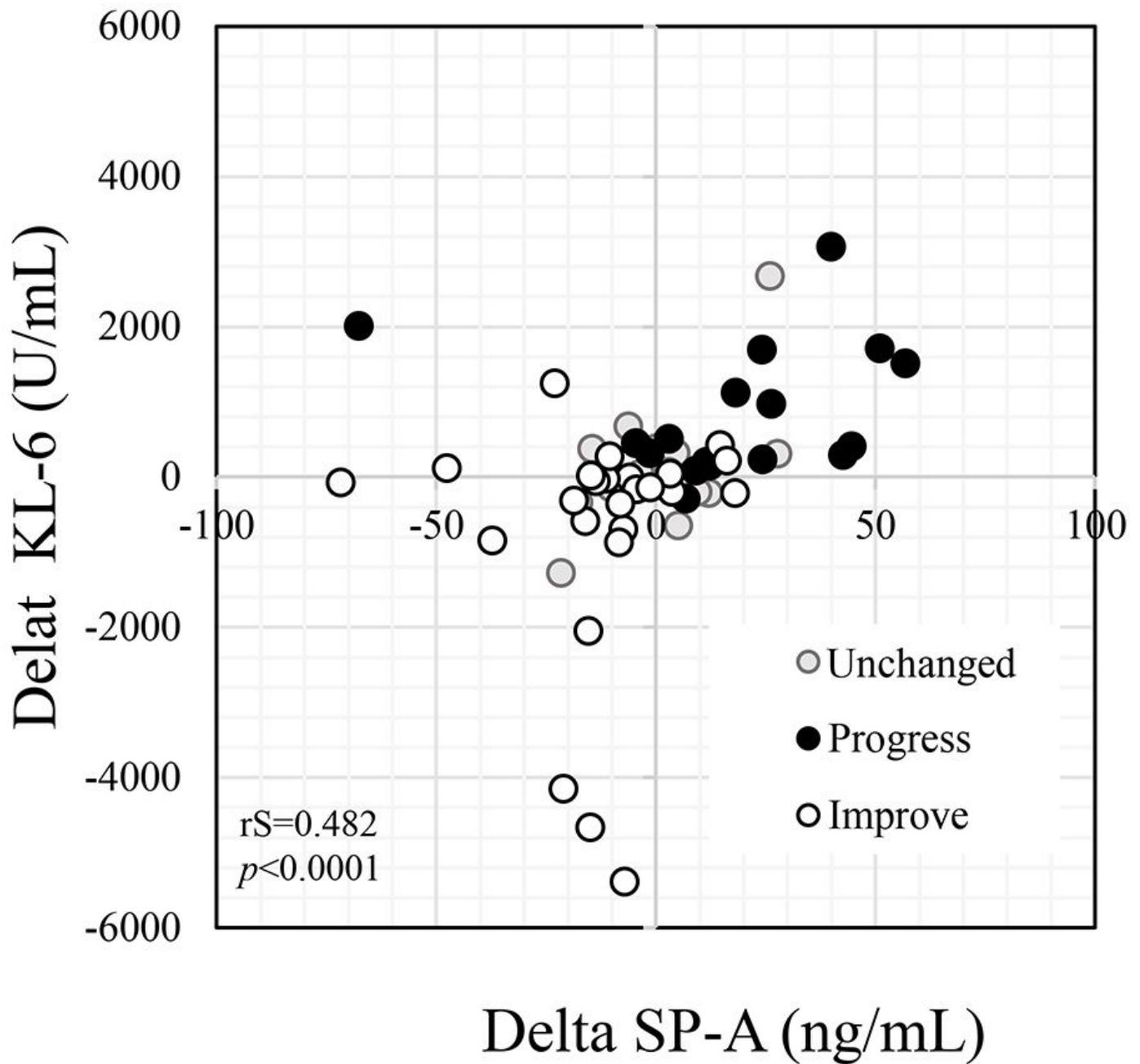


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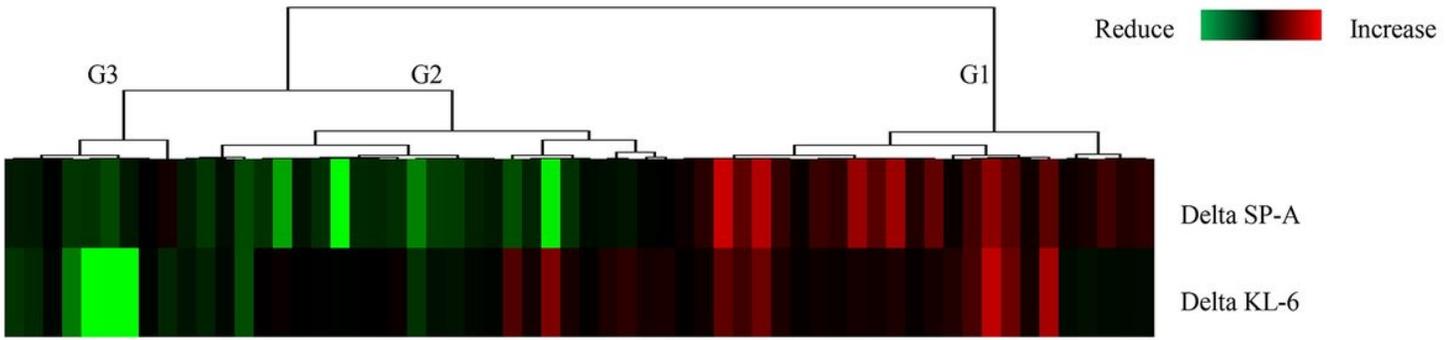


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Unsupervised, hierarchical clustering analysis of Delta SP-A and Delta KL-6 levels. The cluster analysis was performed by centroid linkage based on correlation distance. Red represents increased biomarker levels and green represents decreased biomarker levels. The clustering analysis was used to divide the patients into three groups (G1, G2, and G3). SP-A, surfactant protein-A; KL-6, Krebs von den Lungen-6

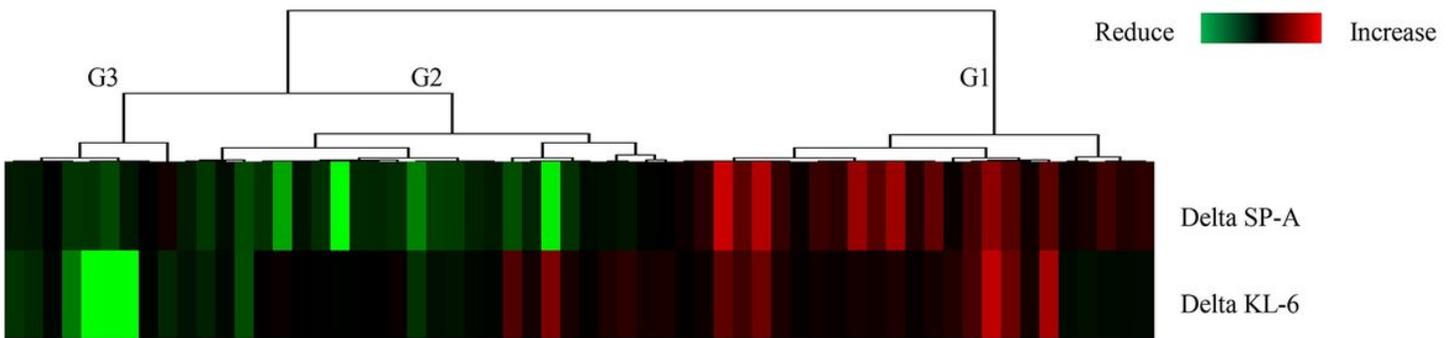


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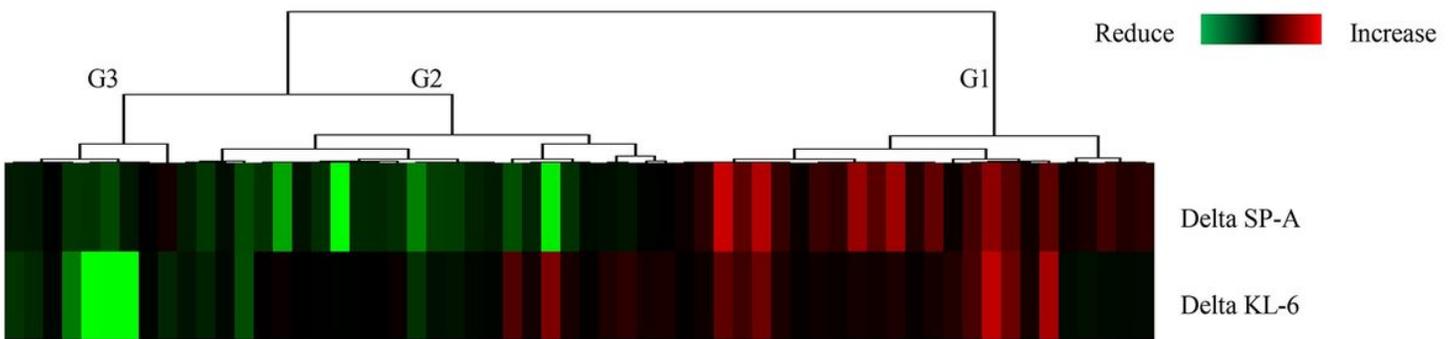


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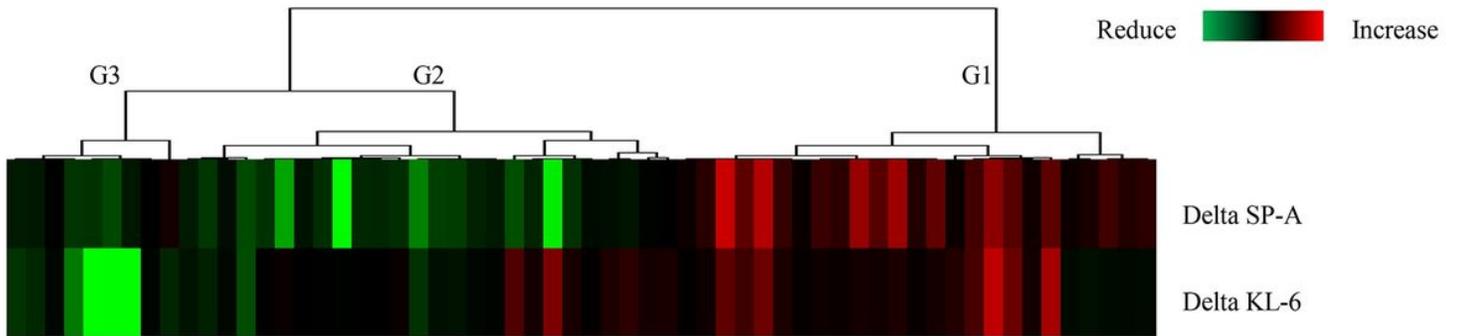


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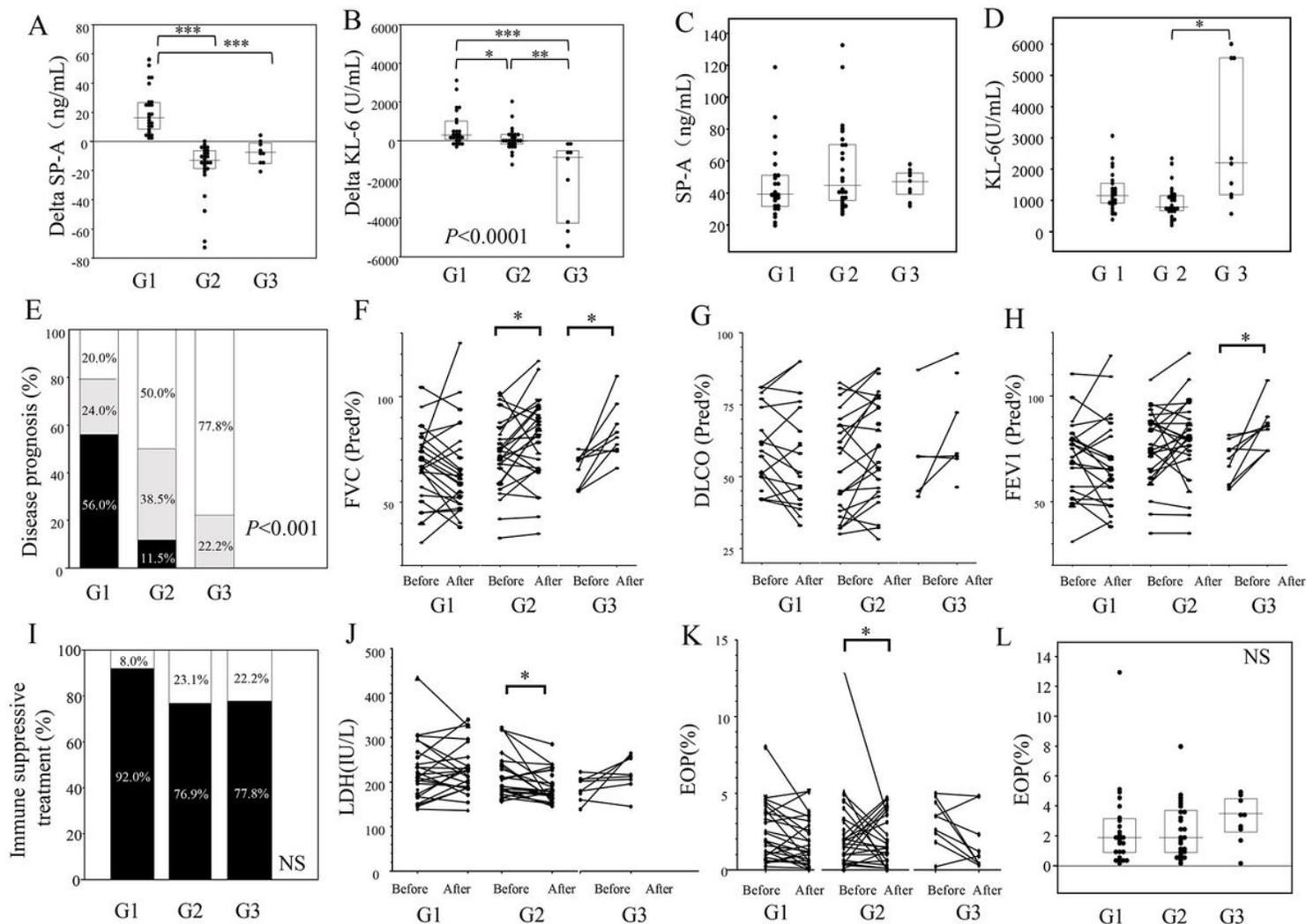


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Comparison of respiratory function and prognosis among the three groups identified in the cluster analysis. A: Delta surfactant protein-A (SP-A) levels. B: Delta Krebs von den Lungen-6 (KL-6) levels. C: Pretreatment serum SP-A levels. D: Pretreatment serum KL-6 levels. E: Differences in disease prognosis between the three groups (Black, Progressive group; Gray, Unchanged group; and White, Improved group). F: Change in percent-predicted forced vital capacity (FVC) pre and post treatment. G: Change in percent-predicted diffusing capacity for carbon monoxide (DLCO) pre and post treatment. H: Change in percent-predicted forced expiratory volume in 1s (FEV1) pre and post treatment. I: Differences in immune suppressive treatment between the three groups (Black, with immune suppressive treatment; White, without immune suppressive treatment). J: Change in serum lactate dehydrogenase (LDH) levels between pre and post treatment. K: Change in blood eosinophil ratio (EOP) between pre and post treatment. L: Pretreatment EOP. The results are presented as individual data points with medians (bars) and interquartile ranges (boxes). (A-G, L) P-values were calculated by steel-dwass test (A-G, L), Wilcoxon signed-rank test (F-H, J, K), and Fisher's exact test (E, I)

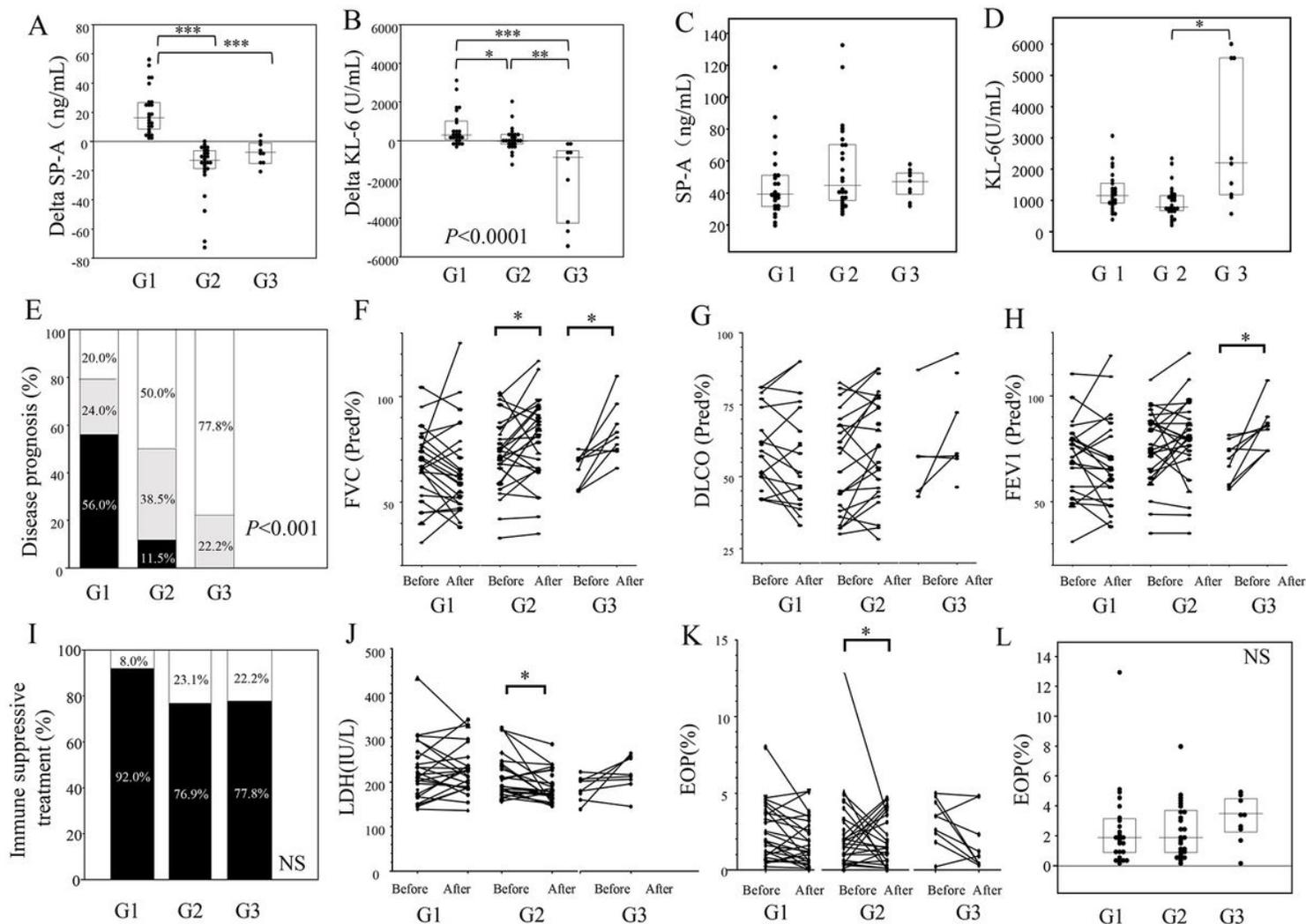


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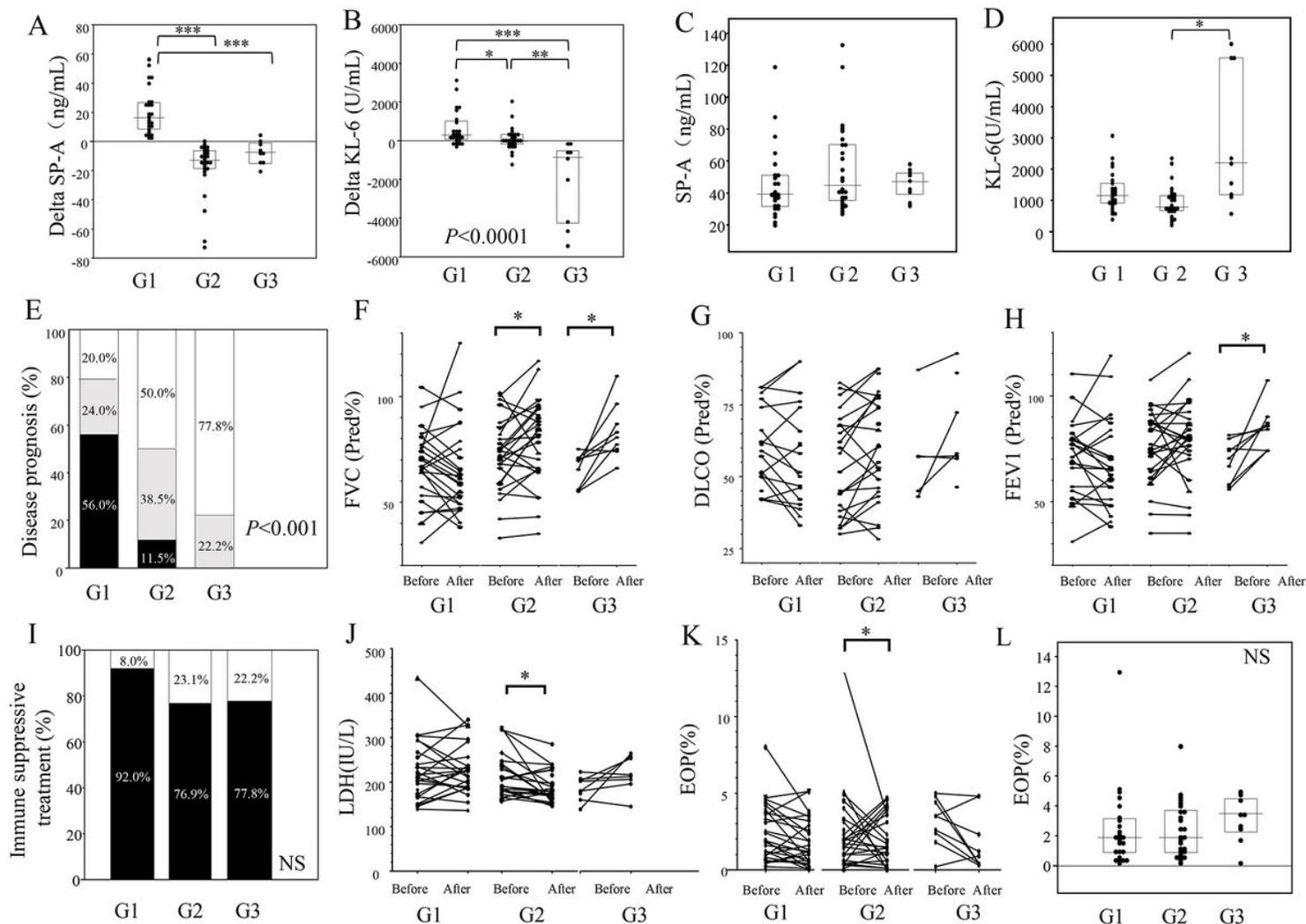


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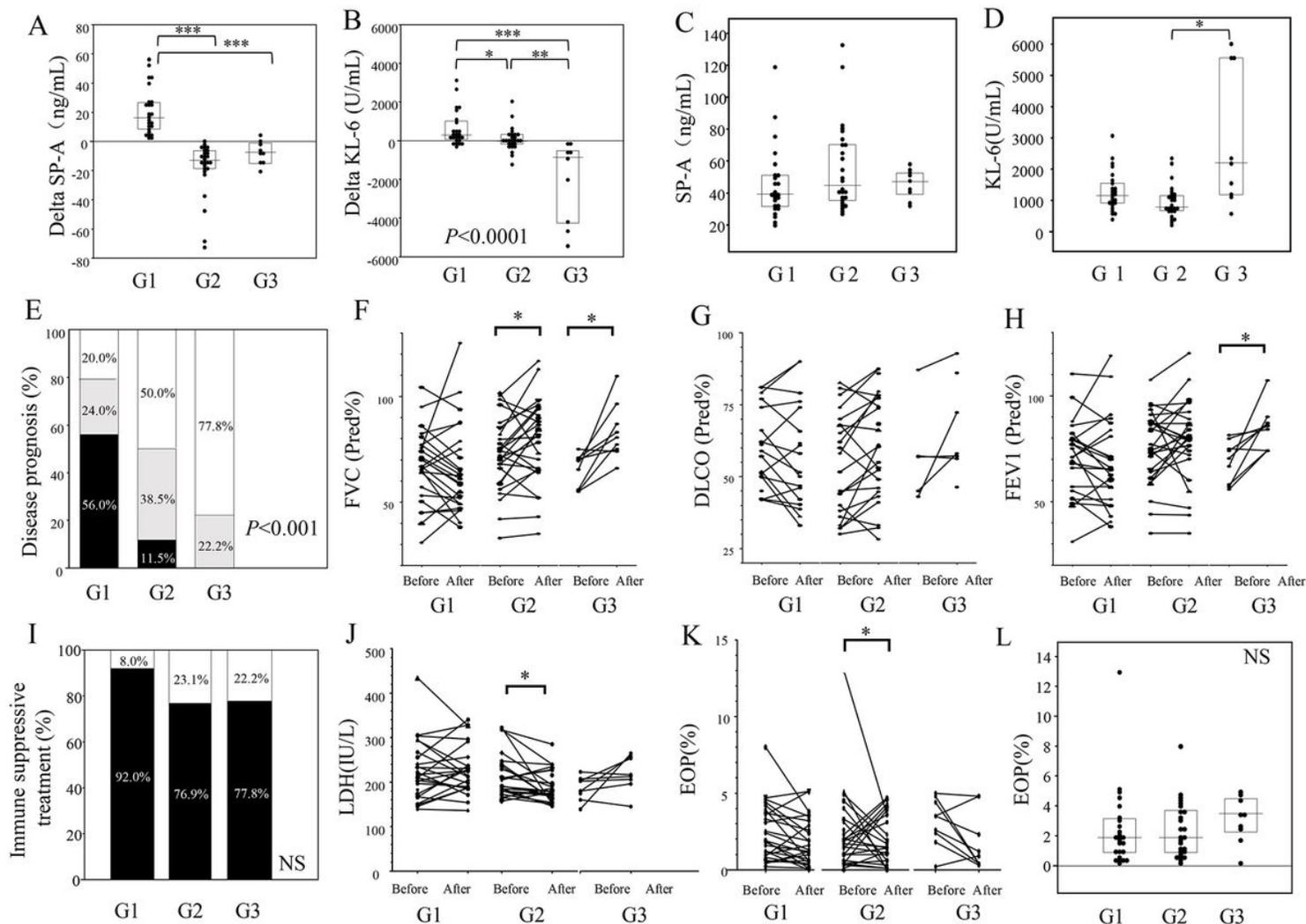


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