Serum Krebs von den Lungen-6 level predicts disease progression in interstitial lung disease

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Abstract

Disease progression (DP) in interstitial lung disease (ILD) is variable and difficult to predict. In previous reports, serum Krebs von den Lungen-6 (KL-6) was suggested to be useful in diagnosing and predicting survival in ILD. The aim of our study was to investigate the usefulness of serum KL-6 as a predictor of DP in ILD. Clinical data of 199 patients with ILD (idiopathic pulmonary fibrosis: 22.8%) were prospectively collected and serum KL-6 levels were measured. DP was defined as a relative decline in forced vital capacity (FVC) ≥ 10%, acute exacerbation, or death during follow-up. The median follow-up period was 11.1 months. The mean age of the subjects was 62.2 years, and 59.8% were male. DP occurred in 21.6% of patients. The progressed group showed lower FVC, lower diffusing capacity for carbon monoxide, lower the minimum oxygen saturation during the 6-minute walk test, higher fibrosis scores on high-resolution computed tomography, and higher KL-6 levels (826.3 vs. 629.0 U/mL; p < 0.001) than those of the non-progressed group. In receiver operating characteristic curve analysis, serum KL-6 levels were a significant predictor of DP in ILD (area under the curve = 0.629, p = 0.009, and the optimal cut-off level was 811 U/mL). In multivariable Cox analysis, high serum KL-6 levels (≥ 800 U/mL) were only independently associated with DP in ILD (HR 2.689, 95% CI 1.445–5.004, P = 0.002). Serum KL-6 levels might be useful to predict DP in patients with ILD.

Introduction

Interstitial lung disease (ILD) is defined as a group of lung diseases affecting the interstitium and includes more than 200 different types, including idiopathic pulmonary fibrosis (IPF) [1]. The clinical course of ILD is highly variable and unpredictable; some patients appear stable or show a slow decline, whereas others show rapid deterioration or periods of relative stability interposed with periods of acute respiratory decline [2]. Thus, predicting disease progression is difficult but important for the effective management of ILD. Previous studies reported that older age, male sex, lower lung function (forced vital capacity [FVC], diffusing capacity of the lung for carbon monoxide [DL CO]), a usual interstitial pneumonia pattern, and more extensive disease on high-resolution computed tomography (HRCT) are associated with poor prognosis in ILD [3–9].
However, their predictive capacity may be limited by insufficient respiratory effort, complications such as emphysema or pulmonary hypertension, or interobserver variability [10–12].

Blood biomarkers are relatively easy to test and independent of patient effort or reader ability [13]. A number of blood biomarkers were reported to be useful in predicting diagnosis or prognosis in ILD, including surfactant proteins A (SP-A) and D (SP-D), monocyte chemoattractant proteins 1 (MCP-1) and 7 (MCP-7), chemokine ligand 18 (CCL-18), interleukin-8 (IL-8), and Krebs von den Lungen-6 (KL-6) [14–18]. KL-6 is a high-molecular-weight glycoprotein located on the surface of alveolar epithelial cells. Surface expression of KL-6 is induced during the regeneration of type II pneumocytes, and destruction of the air-blood barrier of affected lungs increases the permeability of KL-6, leading to increased blood concentration of KL-6 [19]. Thus, blood KL-6 is considered an indicator for pulmonary damage, and has been reported to be a useful biomarker for diagnosis and for estimating disease severity, acute exacerbation, and prognosis in ILD [20–22]. However, biomarkers for predicting disease progression of ILD are not well-defined. Therefore, the aim of this study was to investigate the role of KL-6 as a predictor for disease progression in ILD.

**Materials and methods**

**Study population**

From June to December 2016, 230 patients with ILD visited the ILD clinic at Asan Medical Center (Seoul, Republic of Korea) and were screened for this study. Among them, patients who did not undergo PFT within 3 months of KL-6 measurement and those with lung cancer or pulmonary tuberculosis were excluded in this study. Therefore, a total of 199 patients were included in this study (Fig 1). All patients underwent pulmonary function tests (PFT) and 197 underwent HRCT. Diagnosis of IPF, idopathic non-specific interstitial pneumonia (iNSIP), cryptogenic organizing pneumonia (COP) and unclassifiable ILD was made according to the international guidelines [23, 24]. Diagnosis of hypersensitivity pneumonitis (HP) was based on on histopathological findings (all biopsy-proven cases) [25]. Diagnosis of ILD in connective tissue disease (CTD-ILD) was confirmed based on the HRCT findings.

The study protocol was approved by the Institutional Review Board of Asan Medical Center (approval number 2016–0377) and written informed consent for the use of blood samples for clinical research was obtained from all patients.

**Clinical information**

Clinical and survival data for all patients were prospectively collected, and all clinical parameters were obtained within 3 months of KL-6 measurement. Spirometry, total lung capacity (TLC) by plethysmography, and DLCO were measured according to the recommendations, and results were expressed as percentages of normal predicted values [26–28]. Pulmonary function tests were performed every 3 months. The 6-minute walk test (6MWT) was performed according to the American Thoracic Society guidelines [29]. Acute exacerbation was defined according to the recommendation by Collard et al. [30] and disease progression was defined as a 10% or greater relative decline in FVC, acute exacerbation, or death during follow-up [23].

**HRCT evaluation**

Two experienced chest radiologists (HJK and KHD), blinded to the clinical information, evaluated the HRCT images. HRCT findings were scored on a scale of 5% for all lobes, and classified based on a previous report by Ichikado et al., as follows: 1) normal attenuation, 2) ground-glass attenuation, 3) consolidation, 4) reticular abnormality, 5) traction bronchiectasis, and 6)
honeycombing [31]. The fibrosis score (%) was calculated as the sum of honeycombing and reticulation scores, and the ILD extent (%) was the sum of ground-glass attenuation, reticulation, traction bronchiectasis, and honeycombing scores [32]. Disagreement between the two readers was resolved via a consensus. Representative HRCT images of IPF, CTD-ILD, COP, and unclassifiable ILD were presented in S1 Fig.

Measurement of KL-6

Blood samples were obtained by venipuncture and were stored at -80°C until measurement. The serum levels of KL-6 were measured using an AU 5822 analyzer (Beckman Coulter, Brea, CA, USA) with the Nanopia KL-6 assay (Sekisui Medical, Tokyo, Japan). The KL-6 assay was performed using a latex-enhanced immunoturbidimetric assay method according to the manufacturer’s instructions.

Statistical analysis

Student’s t-tests or Mann-Whitney U tests were used for continuous data and chi-square tests or Fisher’s exact tests were used for categorical data. Survival was estimated by Kaplan-Meier
survival curves and compared by a log-rank test. Receiver operating characteristic (ROC) curve analysis was performed to confirm the optimal cut-off value of KL-6 for the prediction of disease progression. Risk factors were analyzed for disease progression with Cox proportional hazards models using backward elimination: variables with $P < 0.1$ in the univariate analysis were entered into the multivariable models. All $P$ values were two-tailed, with statistical significance set at $P < 0.05$. All statistical analyses were performed using SPSS 21.0 (IBM, Armonk, NY).

**Results**

**Baseline characteristics**

The median follow-up period was 11.1 months (interquartile range [IQR], 10.0–13.0 months). For the 199 patients with ILD, the mean age was 62.2 years, and 59.8% were male. Moreover, 52.3% were ever-smokers (Table 1). The mean value of KL-6 was 671.6 U/mL (median: 487.5 U/mL, IQR: 319.5–801.5 U/mL). Among ILD cases, unclassifiable ILD was the most common

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**Table 1. Comparison of baseline characteristics between the IPF and non-IPF groups.**

| Characteristics                        | Total     | IPF       | Non-IPF   | $P$ value |
|----------------------------------------|-----------|-----------|-----------|-----------|
| No. of patients                        | 199       | 43        | 156       |           |
| Age, years                             | 62.2 (11.7)| 66.0 (8.1)| 61.1 (12.3)| 0.002     |
| Male                                   | 118 (59.8)| 35 (81.3) | 83 (53.2) | 0.002     |
| BMI, kg/m²                              | 24.3 (3.2)| 25.1 (2.5)| 24.0 (3.3)| 0.021     |
| Ever-smoker                            | 104 (52.3)| 30 (69.8) | 74 (47.4) | 0.015     |
| Smoking amount, pack-years             | 15.9 (21.1)| 23.6 (21.2)| 13.8 (20.6)| 0.007     |
| Interval between diagnosis and enrollment (years) | 2.3 [1.2–5.0] | 4.1 [2.0–6.8] | 2.1 [1.1–4.6] | 0.004     |
| CRP, mg/L                              | 0.5 (1.6) | 0.8 (3.0) | 0.4 (0.9) | 0.400     |
| KL-6, U/mL                             | 671.6 (534.5)| 877.2 (494.9)| 614.9 (532.5)| 0.004     |
| FVC, % predicted                       | 75.2 (18.2)| 67.0 (15.5)| 79.1 (17.4)| 0.001     |
| DLCO, % predicted                      | 56.2 (18.4)| 47.7 (13.9)| 59.1 (18.4)| < 0.001   |
| TLC, % predicted                       | 76.3 (17.3)| 66.1 (12.9)| 77.4 (18.2)| < 0.001   |
| 6MWT distance, m                       | 438.4 (92.4)| 435.1 (89.2)| 439.4 (93.7)| 0.796     |
| 6MWT, lowest SpO₂, %                   | 91.8 (4.6) | 89.0 (5.0) | 92.7 (4.1) | < 0.001   |
| Bronchoalveolar lavage                 |           |           |           |           |
| Neutrophils, %                         | 9.9 (17.3)| 9.7 (13.7)| 9.9 (18.2)| 0.936     |
| Lymphocytes, %                         | 21.4 (19.3)| 13.2 (11.7)| 23.6 (20.3)| < 0.001   |
| HRCT, %                                |           |           |           |           |
| Honeycombing                           | 5.5 (8.8) | 12.1 (11.0)| 3.3 (6.6) | < 0.001   |
| Reticular abnormality                  | 10.3 (6.8)| 12.1 (5.3)| 9.7 (7.2) | 0.019     |
| Ground-glass attenuation               | 9.2 (10.6)| 7.9 (7.1) | 9.6 (11.6) | 0.252     |
| Traction bronchiectasis                | 6.9 (5.4) | 9.4 (4.4) | 6.0 (5.4) | < 0.001   |
| Fibrosis score                         | 15.8 (18.2)| 24.2 (13.9)| 13.0 (11.0)| < 0.001   |
| ILD extent                             | 34.0 (19.0)| 43.8 (15.2)| 30.7 (19.0)| < 0.001   |
| Treatments                             |           |           |           | < 0.001   |
| Steroid ± immunosuppressants           | 75 (37.7)| 4 (9.3)   | 71 (45.5) |           |
| Antifibrotics                          | 39 (19.6)| 32 (74.4)| 7 (4.5)   |           |
| None                                   | 85 (42.7)| 7 (16.3) | 78 (50.0) |           |

Data are presented as mean (standard deviation), median [interquartile range] or number (%), unless otherwise indicated.

IPF, idiopathic pulmonary fibrosis; BMI, body mass index; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; TLC, total lung capacity; 6MWT, 6-minute walk test; SpO₂, peripheral blood oxygen saturation; HRCT, high-resolution computed tomography; ILD, interstitial lung disease

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(28.6%), followed by IPF (21.6%), connective tissue disease-associated interstitial lung disease (CTD-ILD; 19.6%), and cryptogenic organizing pneumonia (8.5%).

The IPF group showed older age, more proportion of male subjects and ever-smokers, higher KL-6 levels, higher body mass index (BMI), lower lung function (FVC, DLCO, TLC), and the lowest oxygen saturation during the 6MWT than the non-IPF group (Table 1). The IPF group also had higher scores of honeycombing, reticulation, traction bronchiectasis, fibrosis, and ILD extent on HRCT compared with the non-IPF group.

Correlations between KL-6 levels and disease severity

Serum KL-6 levels were inversely correlated with FVC (r = -0.27, P < 0.001) and DLCO (r = -0.36, P < 0.001) (Fig 2A and 2B). Serum KL-6 levels were positively correlated with fibrosis scores (r = 0.33, P < 0.001) and ILD extent (r = 0.54, P < 0.001) on HRCT (Fig 2C and 2D).

Predicting factors for disease progression

During follow-up, 43 patients (21.6%) experienced disease progression (acute exacerbation in 3 patients, decline in FVC ≥ 10% in 40 patients, and no death; the mean interval of PFT: 3 months). The progressed group had higher KL-6 levels, lower FVC, lower TLC, lower
minimum oxygen saturation during the 6MWT, and a higher ILD extent on HRCT than the non-progressed group (Table 2).

In ROC curve analysis, serum KL-6 level was a significant predictor of disease progression in ILD, and the optimal cut-off value was 811 U/mL (area under the curve [AUC] = 0.629, \( P = 0.009 \), sensitivity of 46.5%, specificity of 81.4%) (Fig 3). In an unadjusted Cox proportional hazards model, high KL-6 levels \( \geq 800 \) U/mL, lower lung function (FVC), and lower the minimum oxygen saturation during the 6MWT, diagnosis of IPF and use of antifibrotics, were significantly related to disease progression (Table 3). In the multivariable analysis including age, KL-6 level \( \geq 800 \) U/mL, FVC, lowest oxygen saturation during 6MWT, and diagnosis of IPF, only KL-6 levels \( \geq 800 \) U/mL were the independent predictive factor for disease progression (HR 2.689, 95% CI 1.445–5.004, \( P = 0.002 \)).

Table 2. Comparison of baseline characteristics between progressed and non-progressed groups.

| Characteristics                          | DP         | Non-DP     | \( P \) value |
|-----------------------------------------|------------|------------|---------------|
| No. of patients                         | 43         | 156        |               |
| Age, years                              | 64.2 (10.9)| 61.6 (11.8)| 0.206         |
| Male                                    | 25 (58.1)  | 93 (59.6)  | 1.000         |
| BMI, kg/m\(^2\)                         | 25.0 (3.5) | 24.1 (3.1) | 0.091         |
| Ever-smoker                             | 22 (51.2)  | 82 (52.6)  | 1.000         |
| Smoking amount, pack-years              | 18.5 (24.2)| 15.2 (20.2)| 0.363         |
| Interval between diagnosis and enrollment (years) | 2.0 [1.1–5.2] | 2.6 [1.2–4.8] | 0.459 |
| CRP, mg/L                               | 0.8 (3.0)  | 0.4 (0.9)  | 0.369         |
| KL-6, U/mL                              | 826.3 (531.6)| 629.0 (529.0)| 0.032        |
| FVC, % predicted                        | 68.7 (17.5)| 77.0 (18.0)| 0.008         |
| DL\(_{CO}\), % predicted                | 54.5 (17.0)| 57.2 (18.4)| 0.382         |
| TLC, % predicted                        | 69.1 (17.2)| 78.2 (16.9)| 0.002         |
| 6MWT distance, m                        | 424.0 (95.4)| 442.8 (91.4)| 0.262        |
| 6MWT, lowest SpO\(_2\), %               | 89.9 (5.2) | 92.4 (4.2) | 0.002         |
| Bronchoalveolar lavage                  |            |            |               |
| Neutrophils, %                          | 6.5 (9.8)  | 10.9 (18.9)| 0.05          |
| Lymphocytes, %                          | 20.8 (17.1)| 21.6 (20.0)| 0.81          |
| HRCT, %                                 |            |            |               |
| Honeycombing                            | 6.4 (9.5)  | 5.2 (8.6)  | 0.459         |
| Reticular abnormality                   | 12.4 (8.3) | 9.6 (6.2)  | 0.056         |
| Ground-glass attenuation                | 11.8 (15.2)| 8.4 (8.9)  | 0.193         |
| Traction bronchiectasis                 | 7.4 (4.8)  | 6.7 (5.5)  | 0.440         |
| Fibrosis score                          | 18.8 (13.8)| 14.9 (12.3)| 0.085         |
| ILD extent                              | 41.0 (18.0)| 31.9 (18.8)| 0.008         |
| IPF                                     | 15 (34.9)  | 28 (17.9)  | 0.029         |
| Treatments                              |            |            | < 0.001       |
| Steroid ± immunosuppressants            | 17 (39.5)  | 58 (37.2)  |               |
| Antifibrotics                           | 17 (39.5)  | 22 (14.1)  |               |
| None                                    | 9 (20.9)   | 76 (48.7)  |               |

Data are presented as mean (standard deviation), median [interquartile range] or number (%).

DP, disease progression; BMI, body mass index; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity; DL\(_{CO}\), percentage predicted diffusing capacity of the lung for carbon monoxide; TLC, total lung capacity; 6MWT, 6-minute walk test; BAL, bronchoalveolar lavage; SpO\(_2\), peripheral blood oxygen saturation; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis

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Comparison of survival between low and high KL-6 groups
The high KL-6 group (≥ 800 U/mL) showed a higher BMI, lower lung function (FVC, DL_CO, TLC), and poorer exercise capacity (the lowest oxygen saturation and distance during 6MWT) than the low KL-6 group (< 800 U/mL) (Table 4). The high KL-6 group also showed higher scores of honeycombing, reticulation, ground-glass opacity, traction bronchiectasis, fibrosis, and ILD extent compared to the low KL-6 group. The high KL-6 group had a significantly lower progression-free survival rate than the low KL-6 group (median survival period: 419 days vs. not reached, \( P = 0.002 \), Fig 4).

Discussion
In our study, baseline serum KL-6 levels were significantly higher in patients who experienced disease progression during follow-up compared to those who remained stable. Moreover, a high baseline serum KL-6 level (≥800 U/mL) was an independent risk factor for disease progression in ILD. These findings indicate that KL-6 is a useful prognostic marker for disease progression in patients with ILD.

Our study showed that high serum KL-6 was an independent prognostic factor in ILD. In previous studies, serum KL-6 was also suggested as an indicator of disease activity or progression of ILD [22, 33]. A study of 77 patients with IPF by Ohshimo et al. demonstrated that patients who developed acute exacerbation (n = 13) had significantly higher baseline serum KL-6 levels (2528 ± 1645 U/mL vs. 1584 ± 1000 U/mL; \( P < 0.0001 \)) than those without acute exacerbation, suggesting that serum KL-6 could be a predictive marker for disease progression in IPF [22]. A study of 14 patients with rapidly progressive IPF by Yokoyama et al. also
demonstrated that KL-6 levels in patients who survived (n = 8) significantly decreased from 2661 ± 1178 U/mL to 2160 ± 910 U/mL (P < 0.05) at 1 week and to 1801 ± 899 U/mL (P < 0.05) at 3 weeks after weekly steroid pulse therapy [33].

Our study also showed that a serum KL-6 level of 811 U/mL was the most discriminatory cut-off value predicting disease progression in patients with ILD. Previous studies also reported similar findings [34, 35]. Among two studies performed in Japan, one study of 27 IPF patients identified a baseline serum KL-6 level of 1000 U/mL as the most discriminatory cut-off value in predicting mortality (sensitivity 90.0%, specificity 70.6%) [34]. The other study, with a larger group of 219 patients with idiopathic interstitial pneumonias (IIPs) and CTD-ILD, also identified a baseline serum KL-6 level of 1000 U/mL as the most discriminatory cut-off value in predicting mortality (sensitivity 67.2%, specificity 60.2%) [35]. Moreover, another study of 77 IPF patients showed that a serum KL-6 level of 1300 U/mL was useful to predict the acute exacerbation of IPF (AUC = 0.736, sensitivity 92%, specificity 61%, P = 0.008) [22].

In our study, serum KL-6 levels showed a positive correlation with lung function or ILD extent on HRCT. Previous studies also reported that serum KL-6 levels were positively correlated with the disease severity of ILDs [36–38]. In a study of 101 patients with sarcoidosis by

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Table 3. Risk factors for disease progression in patients with ILD assessed by Cox regression analysis.

| Variables                                | HR (95% CI)       | P value |
|------------------------------------------|------------------|---------|
| **Unadjusted analysis**                  |                  |         |
| Age, years                               | 1.028 (0.999–1.058) | 0.058   |
| Male                                     | 0.939 (0.512–1.722) | 0.839   |
| BMI, kg/m²                                | 1.076 (0.978–1.183) | 0.132   |
| Ever-smoker                              | 1.082 (0.595–1.969) | 0.796   |
| CRP, mg/L                                | 1.076 (0.963–1.203) | 0.197   |
| KL-6 (≥ 800 U/mL)                        | 2.533 (1.389–4.618) | 0.002   |
| Interval between diagnosis and enrollment (years) | 1.004 (0.905–1.114) | 0.937   |
| FVC, % predicted                         | 0.980 (0.964–0.997) | 0.019   |
| DLCO, % predicted                        | 0.995 (0.979–1.012) | 0.562   |
| Distance during 6MWT, m                  | 0.998 (0.995–1.001) | 0.238   |
| Lowest oxygen saturation during 6MWT, %  | 0.928 (0.877–0.982) | 0.010   |
| BAL, Neutrophils, %                      | 0.985 (0.956–1.015) | 0.316   |
| BAL, lymphocytes, %                      | 1.000 (0.982–1.018) | 0.965   |
| Fibrosis score on HRCT, %                | 1.016 (0.993–1.039) | 0.172   |
| **ILD subtype**                           |                  |         |
| None-IPF (Ref.)                          | 1                |         |
| IPF                                      | 2.115 (1.129–3.963) | 0.019   |
| Treatment†                                |                  |         |
| Antifibrotics                            | 4.086 (1.813–9.209) | 0.001   |
| Steroid ± immunosuppressants             | 1.920 (0.849–4.344) | 0.117   |
| **Multivariable analysis**               |                  |         |
| KL-6 (≥ 800 U/mL)                        | 2.689 (1.445–5.004) | 0.002   |

ILD, interstitial lung disease; BMI, body mass index; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity; DLCO, percentage predicted diffusing capacity of the lung for carbon monoxide; 6MWT, 6-minute walk test; TLC, total lung capacity; BAL, bronchoalveolar lavage; HRCT, high-resolution computed tomography; IPF, idiopathic pulmonary fibrosis; Ref, reference

* Because treatment is highly related to ILD subtype (antifibrotic agents have been used mostly in IPF patients), treatment was not included in the multivariable analysis.

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Honda et al., patients with elevated KL-6 levels (mean 802.4 U/mL) had significantly more frequent parenchymal involvement (ground-glass opacities, nodules, interlobular septal thickening, traction bronchiectasis, architectural distortion, and bronchial wall thickening) on chest CT than those with normal KL-6 levels (mean 305.7 U/mL) [37]. A study of 47 patients with rheumatoid arthritis-associated pulmonary disease by Kinoshita et al. also reported a positive correlation between serum KL-6 levels and total CT scores ($r = 0.83$, $P < 0.001$) [38]. In addition, a study of 98 patients with ILD by Qin et al. showed that serum KL-6 levels were significantly correlated with $DL_{CO}$ ($r = -0.513$, $P < 0.001$) and CT scores ($r = 0.539$, $P = 0.000$) [39]. These results support that serum KL-6 levels may correlate with the severity of ILDs.

There are some limitations in this study. First, this was a study conducted in a single tertiary referral center. Further prospective multicenter studies are needed to confirm our findings.

Table 4. Comparison of baseline characteristics between high and low KL-6 groups.

| Characteristics                          | High KL-6 $^a$ | Low KL-6 $^b$ | $P$ value |
|------------------------------------------|---------------|---------------|-----------|
| No. of patients                          | 51            | 148           | 0.315     |
| Age, years                               | 63.6 (10.0)   | 61.7 (12.2)   | 0.315     |
| Male                                     | 31 (60.8)     | 87 (58.8)     | 0.932     |
| BMI, kg/m$^2$                            | 25.6 (2.5)    | 23.8 (3.3)    | < 0.001   |
| Ever-smoker                              | 29 (56.9)     | 75 (50.7)     | 0.548     |
| Smoking amount, pack-years               | 17.5 (23.7)   | 15.3 (20.2)   | 0.522     |
| CRP, mg/L                                | 0.7 (2.8)     | 0.3 (1.0)     | 0.283     |
| KL-6, U/mL                               | 1388.7 (576.2)| 424.6 (179.0)| < 0.001   |
| Interval between diagnosis and enrollment (years) | 2.1 [1.2–4.5]| 2.4 [1.2–5.1]| 0.451     |
| FVC, % predicted                         | 62.3 (15.5)   | 79.6 (16.9)   | < 0.001   |
| TLC, % predicted                         | 61.8 (12.8)   | 81.1 (15.9)   | < 0.001   |
| $DL_{CO}$, % predicted                   | 47.4 (14.6)   | 59.8 (18.1)   | < 0.001   |
| 6MWT distance, m                         | 410.9 (88.8)  | 449.4 (91.8)  | 0.013     |
| 6MWT, lowest SpO$_2$, %                  | 88.6 (4.5)    | 93.1 (4.0)    | < 0.001   |
| Bronchoalveolar lavage                   |               |               |           |
| Neutrophils, %                           | 7.8 (10.6)    | 10.7 (19.6)   | 0.264     |
| Lymphocytes, %                           | 20.0 (18.9)   | 22.1 (19.6)   | 0.565     |
| HRCT, %                                  |               |               |           |
| Honeycombing                             | 9.0 (11.3)    | 4.1 (7.1)     | 0.007     |
| Reticular abnormality                    | 13.0 (8.3)    | 9.2 (5.8)     | 0.005     |
| Ground-glass attenuation                 | 13.6 (13.7)   | 7.5 (8.6)     | 0.005     |
| Traction bronchiectasis                  | 10.2 (5.1)    | 5.5 (4.9)     | < 0.001   |
| Fibrosis score                           | 21.9 (15.3)   | 13.3 (10.7)   | 0.001     |
| ILD extent                               | 47.7 (15.6)   | 28.5 (17.5)   | < 0.001   |
| IPF                                      | 21 (41.2)     | 22 (14.9)     | < 0.001   |
| Treatments                               |               |               |           |
| None                                     | 8 (15.7)      | 77 (52.0)     |           |
| Antifibrotics                            | 20 (39.2)     | 19 (12.8)     |           |
| Steroid ± immunosuppressants             | 23 (45.1)     | 52 (35.1)     |           |

Data are presented as mean (standard deviation), median [interquartile range] or number (%), unless otherwise indicated.

KL-6, Krebs von den Lungen-6; BMI, body mass index; CRP, C-reactive protein; FVC, forced vital capacity; TLC, total lung capacity; $DL_{CO}$, diffusing capacity of the lung for carbon monoxide; 6MWT, 6-minute walk test; SpO$_2$, peripheral blood oxygen saturation; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis

$a$: KL-6 $\geq$ 800 U/mL

$b$: KL-6 $<$ 800 U/mL

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Second, the median follow-up period was relatively short. However, disease progression occurred in one-fifth of the patients, which was sufficient to confirm the significance of predicting disease progression by KL-6 levels. Finally, we analyzed heterogenous ILD patients together due to small numbers in each subgroup. However, in our previous study, KL-6 levels were not different between ILD subtypes [40].

**Conclusion**

Baseline serum KL-6 levels are useful in predicting disease progression in patients with ILD. Larger-scale prospective studies are needed to confirm these findings.

**Supporting information**

S1 Fig. Representative HRCT images of ILD patients. (A) IPF (B) CTD-ILD (C) COP (D) unclassifiable ILD. HRCT, high-resolution computed tomography; ILD interstitial lung disease; IPF, idiopathic pulmonary fibrosis; CTD-ILD, connective tissue disease-associated interstitial lung disease; COP, cryptogenic organizing pneumonia. (PPTX)

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