# Elevated Serum Krebs von den Lungen-6 in Early Disease Predicts Subsequent Deterioration of Pulmonary Function in Patients with Systemic Sclerosis and Interstitial Lung Disease

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*ABSTRACT.* ***Objective.*** To identify predictors of poor prognosis in patients with systemic sclerosis (SSc) associated with interstitial lung disease (ILD).

***Methods.*** Fifty patients with early-stage SSc-ILD who had never received disease-modifying drugs and were either observed for ≥ 10 years or died from ILD-related causes were enrolled. The baseline variables of patients who developed endstage lung disease (ESLD) were compared with those of patients who remained ESLD-free, and the Cox proportional hazard model was used to identify initial factors that correlated with ESLD development.

***Results.*** Sixteen patients (32%) developed ESLD during 173.5 ± 64.7 months of followup. Elevated serum Krebs von den Lungen-6 (KL-6) at initial assessment was highly correlated with ESLD development ($p = 0.0002$). Receiver-operating characteristic curve analysis revealed that a KL-6 value of 1273 U/ml effectively discriminated patients who developed ESLD from those who did not. Patients with KL-6 > 1273 U/ml were less likely to remain ESLD-free compared with those with lower KL-6 levels ($p < 0.0001$). Multivariate analysis showed that KL-6 > 1273 U/ml was the most reliable predictor of ESLD development (OR 51.2, 95% CI 7.6–343, $p < 0.0001$). Finally, the initial KL-6 level correlated with the forced vital capacity (FVC) decline rate ($r = 0.58$, $p < 0.0001$).

***Conclusion.*** The natural course of SSc-ILD is highly variable. Baseline serum KL-6 is a biomarker potentially useful for predicting FVC decline. (J Rheumatol First Release August 1 2016; doi:10.3899/jrheum.160339)

*Key Indexing Terms:*
SYSTEMIC SCLEROSIS                           INTERSTITIAL LUNG DISEASE
OUTCOME ASSESSMENT                           SURVIVAL ANALYSIS

Interstitial lung disease (ILD) is the leading cause of disease-related morbidity and mortality in patients with systemic sclerosis (SSc)[1]. Further, the University of Pittsburgh Scleroderma database shows that the proportion of patients who died of ILD over the past 30 years increased from 6% to 33%[2]. SSc-associated ILD (SSc-ILD) is currently treated with immunosuppressive agents such as cyclophos-

phamide (CYC) and mycophenolate mofetil (MMF)[3]. However, these treatments have limited efficacy, and are primarily used to prevent disease progression rather than to induce remission. Therefore, it is critical to identify patients in the early stages of progressive disease, prior to the onset of functional impairment, and to initiate treatment as early as possible. However, there is substantial variability in the disease course of SSc-ILD; some patients show progressive decline in lung function, leading to endstage lung disease (ESLD) and subsequent death, whereas others exhibit pulmonary function that remains stable for many years[4,5]. The dilemma for the clinician is whether to treat patients in the early stages of SSc-ILD with immunosuppressive agents. Because immunosuppressive medications, such as CYC, have a significant risk for severe side effects, including infection, carcinogenesis, and impaired fertility[6], the treatment of patients with intrinsically stable ILD should be avoided. To select patients who may benefit from treatment, it is paramount to be able to accurately predict the future progression of ILD and mortality.

Factors associated with ILD progression and poor prognosis have been examined in patients with SSc. Older

age[7], African American race[8], male sex[7,8], reduced forced vital capacity (FVC)[7,8,9], reduced DLCO[7,9], and extensive disease by high-resolution computed tomography (HRCT)[7,10] have been shown to predict poor ILD outcomes. Goh, *et al* proposed a simple staging system based on a combined evaluation using HRCT and pulmonary function testing (PFT); the extensive stage defined by an HRCT disease extent > 20% and/or an FVC < 70% was found to be predictive of high mortality[7]. However, these features are typically observed in the advanced and irreversible stages of ILD, and are rarely detected in patients with early SSc-ILD. A systematic review suggested that observing a combination of variables may be useful in predicting ILD progression and mortality in patients with SSc; however, an accurate clinical prediction model has yet to be developed[11].

At our institution, SSc-ILD was not considered a treatment target before 2000. The longterm outcomes of untreated patients could provide extremely useful information on the natural course of SSc-ILD. Thus, our study examined a group of untreated patients with SSc-ILD to identify early predictors of ILD progression.

## MATERIALS AND METHODS

*Subjects*. The subjects enrolled in our study were 50 patients with early SSc-ILD who had never received potential disease-modifying drugs. They were chosen from a prospective cohort of the SSc database from the Keio University Hospital, Tokyo, Japan. Of the 221 patients registered in the SSc database between 1985 and 1995, 94 were first selected for the cohort because they (1) satisfied the American College of Rheumatology preliminary classification criteria for SSc[12], (2) exhibited disease symptoms except Raynaud phenomenon for ≤ 3 years prior to enrollment, (3) did not have overlap with definitive polymyositis/dermatomyositis, systemic lupus erythematosus, or rheumatoid arthritis, and (4) were diagnosed with ILD, detected as bibasilar reticulation and/or fibrosis on a chest radiograph, during the initial assessment. Fifty-two subjects were further selected based on the availability of records of ≥ 4 PFT examinations performed over the course of their disease, and an observation period of ≥ 10 years after entering the study (unless they had died earlier from ILD-related causes). The final 50 subjects analyzed in our longitudinal study had never received potential disease-modifying drugs, including corticosteroids (> 10 mg daily prednisolone or equivalent), D-penicillamine, or immunosuppressive agents, including CYC, MMF, azathioprine, cyclosporine, or tacrolimus. Two patients, including 1 with thrombotic microangiopathy and 1 with necrotizing vasculitis, were excluded because of their treatment with potential disease-modifying drugs. This study was approved by the Institutional Review Board of the Keio University School of Medicine.

*Clinical analysis*. The patients were registered in a database upon the diagnosis of SSc-ILD, and prospective records of clinical and laboratory findings were kept for all patients. A complete medical history, physical examination, and laboratory workup were performed for each patient at database entry, with more limited evaluations performed during followup visits (at least once every 3 mos). The SSc was classified as diffuse cutaneous SSc (dcSSc) or limited cutaneous SSc (lcSSc) according to the Medsger criteria[13]. The modified Rodnan total skin thickness score (mRSS; scale 0–51) was used to evaluate the extent of skin thickening. All patients underwent PFT at diagnosis and were repeatedly evaluated during followup. FVC was measured by spirometry, and DLCO was assessed by the single-breath method and was corrected for hemoglobin levels. The predictive values for each subject, based on sex, age, and height, were obtained from standard tables, and the final data were expressed as percentages of the predicted values[14,15]. ESLD was defined as SSc-ILD resulting in at least 1 of the following: an FVC < 50%, a requirement for continuous oxygen supplementation, or death[16,17]. The annual change in FVC in the entire disease course was calculated by the change of FVC between the first (baseline) and last examinations divided by the interval (year) between 2 examinations. The FVC change in the early disease phase was also calculated by the change of FVC between the first and last examinations carried out within 5 years after the enrollment divided by the interval between 2 examinations.

*Serum biomarkers*. Serum samples were obtained at the initial assessment and stored at –70°C until use. To detect anticentromere antibodies (ACA), the samples were analyzed by indirect immunofluorescence with commercially available HEp-2 cell slides (MBL International Corp.). The presence of ACA in sera showing a discrete speckled pattern was confirmed by a commercially available enzyme immunoassay (MBL). To test for the presence of anti-DNA topoisomerase I (topo I) antibodies, the sera were subjected to an enzyme immunoassay (MBL) and an immunoprecipitation assay[18]. Anti-RNA polymerase III antibodies were detected by an immuno-precipitation assay with reference sera[18]. Serum Krebs von den Lungen-6 (KL-6) levels were measured in duplicate by an assay system using a latex-fixed anti-KL-6 monoclonal antibody, according to the manufacturer's instructions (Nanopia KL-6; Eidia Co. Ltd.).

*Statistical analysis*. All continuous values are shown as the mean ± SD. Unpaired comparisons of continuous variables were performed using the Mann-Whitney U test. Categorical variables were compared using Fisher's exact test or a chi-square test when appropriate. The correlation coefficient (r) was calculated using Spearman regression model. The cutoff value that best discriminated 2 groups was determined by receiver-operating characteristic (ROC) curve analysis. The Cox proportional hazard model was used to identify independent variables that increased the risk for developing ESLD. The results are presented as an OR with a 95% CI. Survival analysis was performed using the Kaplan-Meier method, and the difference in survival between 2 groups was determined by the log-rank test. All of the statistical analyses were performed using SPSS 21.0 statistical software (SPSS).

## RESULTS

*Longterm outcomes in patients with SSc-ILD*. The enrolled cohort of patients with early SSc-ILD who had never received potential disease-modifying drugs exhibited an average disease duration of 14.2 ± 7.2 months and consisted of 26 patients with dcSSc and 24 with lcSSc. The patients underwent PFT evaluations 5.86 ± 2.01 times (range 4–13) during 173.5 ± 64.7 months (range 28–204) of followup. Of the 50 patients, 16 (32%) developed ESLD between 18 and 204 months after entering the study (Figure 1). Eight of these patients exhibited FVC < 50%, and 8 required continuous oxygen supplementation.

Thirteen (81%) of 16 patients who developed ESLD died during followup, and all of the deaths resulted from ILD-related causes, including cardiopulmonary insufficiency in the presence or absence of pulmonary hypertension, respiratory tract infection, and pneumothorax. Only 5 deaths (15%) were observed in patients who remained ESLD-free, and the causes of death included solid and hematologic malignancies in 4 and cerebrovascular hemorrhage in 1.

*Initial variables predicting future development of ESLD*. Clinical characteristics at the initial assessment were compared between the stratified patient groups (Table 1). The
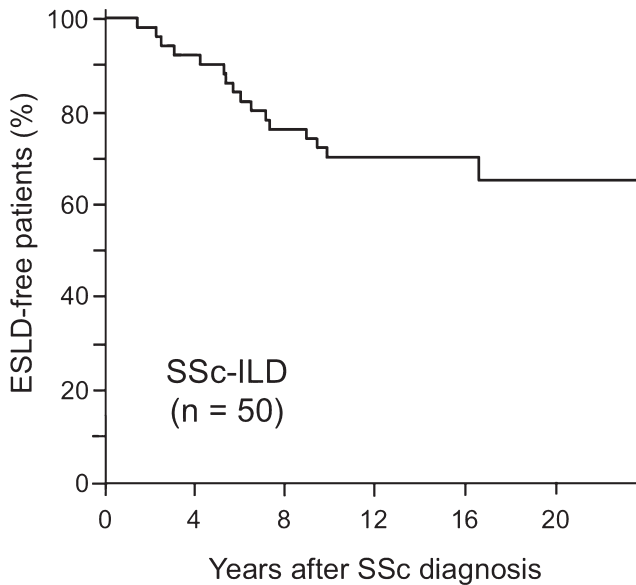
*Figure 1*. Percentage of patients remaining ESLD-free over the course of the study, in untreated patients with SSc-ILD. ESLD: endstage lung disease; SSc: systemic sclerosis; ILD: interstitial lung disease.

*Table 1*. Clinical variables at diagnosis in patients with early SSc-ILD stratified by the subsequent development of ESLD. Variables are mean ± SD unless otherwise specified.

| Variables at Diagnosis | ESLD, n = 16 | Non-ESLD, n = 34 | p |
|---|---|---|---|
| Female, % | 81 | 97 | 0.06 |
| Age at SSc diagnosis, yrs | 45.1 ± 11.0 | 42.9 ± 11.7 | 0.26 |
| Time between disease onset and study entry, mos | 13.7 ± 7.5 | 14.4 ± 7.2 | 0.37 |
| dcSSc, % | 81 | 38 | 0.006 |
| mRSS | 13.1 ± 5.8 | 9.6 ± 5.7 | 0.045 |
| mRSS in patients with dcSSc, n = 13 | 15.0 ± 4.5 | 15.5 ± 4.4 | 0.79 |
| Current and previous smoking, % | 25 | 21 | 0.72 |
| Exertional dyspnea, % | 69 | 32 | 0.03 |
| Anti-topo I antibody, % | 94 | 53 | 0.004 |
| ACA, % | 0 | 29 | 0.02 |
| Anti-RNA polymerase III, % | 0 | 6 | 0.83 |
| FVC, % predicted | 76.6 ± 12.8 | 87.1 ± 13.8 | 0.007 |
| DLCO, % predicted | 48.6 ± 13.4 | 59.0 ± 9.6 | 0.001 |
| Serum KL-6, U/mL | 2189 ± 904 | 694 ± 322 | < 0.0001 |

SSc: systemic sclerosis; ILD: interstitial lung disease; ESLD: endstage lung disease; dcSSc: diffuse cutaneous SSc; mRSS: modified Rodnan skin score; anti-topo I: antitopoisomerase I; ACA: anticentromere antibodies; FVC: forced vital capacity; KL-6: Krebs von den Lungen-6.

initial variables associated with future ESLD development included dcSSc, high mRSS, the presence of exertional dyspnea, anti-topo I antibody positivity, ACA negativity, reduced FVC, reduced DLCO, and elevated serum KL-6. The mRSS was higher in the ESLD group than in the ESLD-free group, but this difference was lost when the analysis was

conducted using only patients with dcSSc, indicating that dcSSc was the primary predictor for ESLD development.

We next conducted ROC analysis to determine the optimal KL-6 cutoff level that differentiated between patients who went on to develop ESLD and those who remained ESLD-free (Figure 2A). This analysis confirmed that the KL-6 measurement at diagnosis was effective for predicting the future development of ESLD (area under the curve 0.93, p < 0.0001) and that a KL-6 level > 1273 U/ml was the optimal cutoff for predicting the development of ESLD (sensitivity 87.5%, specificity 100%). Our results showed that patients exhibiting a KL-6 level > 1273 U/ml were less likely to remain ESLD-free than those exhibiting a KL-6 level ≤ 1273 U/ml (p < 0.0001; Figure 2B).
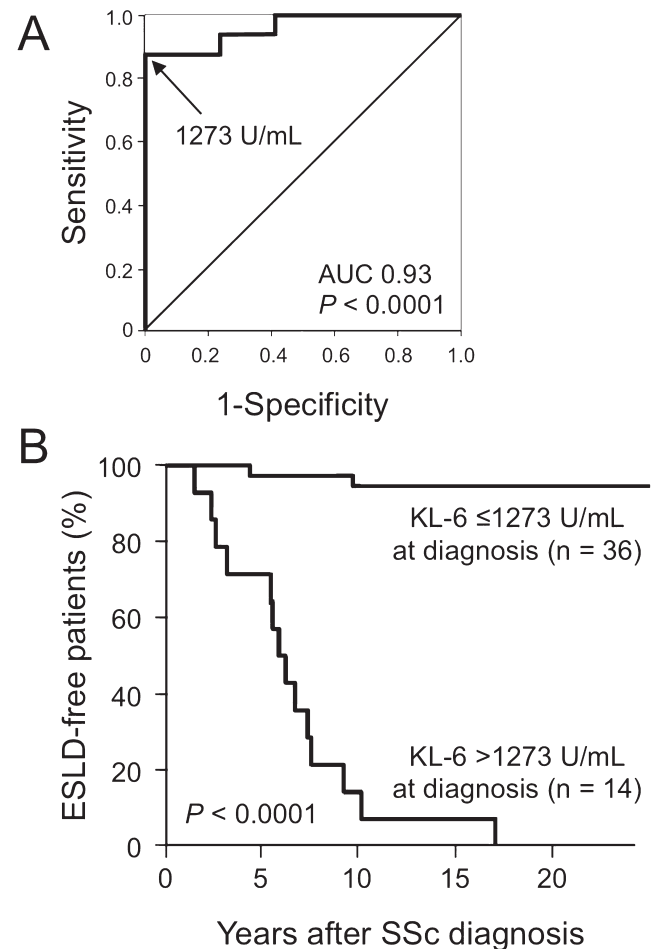


*Figure 2*. Correlation between elevated KL-6 levels in early SSc-ILD and subsequent development of ESLD. A. Receiver-operating characteristic curve analysis to determine the optimal KL-6 cutoff value that differentiated between patients who subsequently developed ESLD and those who remained ESLD-free. A serum KL-6 level > 1273 U/ml was shown to reliably predict subsequent ESLD development. B. Percentage of patients remaining ESLD-free of those with a KL-6 level > 1273 U/ml and those with a KL-6 level ≤ 1273 U/ml at diagnosis. KL-6: Krebs von den Lungen-6; SSc: systemic sclerosis; ILD: interstitial lung disease; ESLD: endstage lung disease; AUC: area under the curve.

To identify independent variables that predicted ESLD development, multivariate analysis was conducted using initial variables extracted by univariate analysis, including dcSSc, mRSS, exertional dyspnea, anti-topo I antibody, FVC, DLCO, and serum KL-6 > 1273 U/ml (Table 2). The results demonstrated that serum KL-6 > 1273 U/ml was the most reliable predictor of ESLD development (OR 51.2, 95% CI 7.6–343, p < 0.0001). After adjusting for age and smoking, serum KL-6 > 1273 remained the best predictor of ESLD development (OR 80.1, 95% CI 10.0–642, p < 0.0001).

*Serial changes in FVC*. Figure 3 shows the results of serial FVC measurements of the ESLD-stratified patient groups. In the 16 patients who developed ESLD, FVC declined gradually over the course of the disease, and the annual change over the entire disease course was –4.6 ± 2.3%. We assessed whether FVC declined more rapidly early in the disease course, but the annual FVC change within 5 years after diagnosis was –4.6 ± 2.9, which was comparable to that observed over the entire disease course. On the other hand, the FVC remained relatively stable in the patients who did not develop ESLD, indicating that FVC decline rate is highly variable among patients with SSc-ILD.

We further evaluated the relationship between the FVC, DLCO, or serum KL-6 at diagnosis and the subsequent change in FVC over the disease course (Figure 4). There was no correlation between the FVC at diagnosis and the subsequent decline in FVC. DLCO tended to be correlated with the subsequent change in FVC (p = 0.053), and the serum KL-6 level was significantly correlated with the subsequent change in FVC (p < 0.0001).

## DISCUSSION

In our study, we demonstrated that the natural course of SSc-ILD was highly variable, and that only 32% of patients with early stage SSc-ILD who remained untreated over the course of the study developed ESLD. In addition, elevated serum KL-6 was identified as an independent predictor of subsequent FVC decline and ESLD development. Thus, serum KL-6 measurement early in the disease process could aid clinicians in identifying patients with SSc at risk of progressive ILD, who would benefit from careful monitoring and early therapeutic intervention.

The prevalence of ESLD development in patients with SSc has been examined previously in patient populations with and without ILD. In the University of Pittsburgh Scleroderma databank, of 890 patients with SSc, 60% never developed an FVC ≥ 75%, 27% developed moderate restrictive disease with an FVC of 50%–75%, and only 13% developed a severe restrictive disease with an FVC ≤ 50%[8]. In a British cohort of 561 patients with SSc whose disease duration was < 5 years at entry, the cumulative probability of ESLD development over the subsequent 14 years was only 12%[19]. Recently, Man, *et al* retrospectively assessed changes in FVC over the disease course of 254 patients with SSc, and identified 7 distinct FVC trajectories over 12 years[20]. Only 15% of the patients underwent a slow or fast decline in pulmonary function. Considering that ~50% of patients with SSc develop ILD[1], these previous results are largely consistent with our finding that 32% of the patients enrolled upon the diagnosis of SSc-ILD subsequently developed ESLD, although our study is unique in that only patients who had not received any potential disease-modifying treatment were included in the analysis. Notably, ~70% of the patients with SSc-ILD maintained their pulmonary function over the course of the disease even though they remained untreated. For these patients, treatment with immunosuppressive medications that have severe toxicity risks should be avoided.

Clinical variables identified in patients with early-stage SSc-ILD with normal pulmonary function are highly desirable in clinical practice. Our analysis using a prospective cohort of untreated patients with early SSc-ILD identified serum KL-6 as a useful biomarker that predicted subsequent FVC decline. KL-6 is an antigen that corresponds to a high molecular-weight glycoprotein, MUC1, which is produced primarily by regenerating type II pneumocytes, and has been used as a biomarker for detecting and assessing the activity of various types of ILD[21]. Elevated KL-6 is associated with radiologic evidence of ILD in patients with SSc, and its level correlates inversely with vital capacity or DLCO[22,23,24,25,26]. In addition, elevated KL-6 is associated with alveolitis, as defined by either bronchoalveolar lavage or HRCT using the Scleroderma Lung Study criteria[27]. In a retrospective study to investigate predictive value of serum IL-6 level for outcomes of SSc-ILD, elevated KL-6 level at study entry was useful for prediction of death, but was not associated with subsequent deterioration or improvement of lung function[26]. Lack of the association with lung function outcomes in that particular study might be explained by a short followup

*Table 2*. Multivariate analysis to identify independent variables that predict ESLD development in patients with SSc-ILD.

| Variables at Diagnosis | OR (95% CI), p | OR (95% CI), p, Adjusted for Age and Smoking |
|---|---|---|
| dcSSc | 5.57 (0.86–36.1), p = 0.07 | 4.64 (0.62–34.7), p = 0.14 |
| mRSS | 0.96 (0.79–1.18), p = 0.71 | 0.91 (0.73–1.14), p = 0.41 |
| Exertional dyspnea | 1.91 (0.11–34.0), p = 0.66 | 1.93 (0.81–45.9), p = 0.68 |
| Anti-topo I antibody | 0.84 (0.04–17.5), p = 0.91 | 1.70 (0.09–33.7), p = 0.73 |
| FVC | 1.01 (0.89–1.15), p = 0.84 | 1.00 (0.87–1.14), p = 0.97 |
| DLCO | 0.94 (0.86–1.03), p = 0.20 | 0.97 (0.87–1.08), p = 0.57 |
| Serum KL-6 > 1273 U/ml | 51.2 (7.6–343), p < 0.0001 | 80.1 (10.0–642), p < 0.0001 |

ESLD: endstage lung disease; SSc: systemic sclerosis; dcSSc: diffuse cutaneous SSc; ILD: interstitial lung disease; mRSS: modified Rodnan skin score; anti-topo I: antitopoisomerase I; FVC: forced vital capacity; KL-6: Krebs von den Lungen-6.
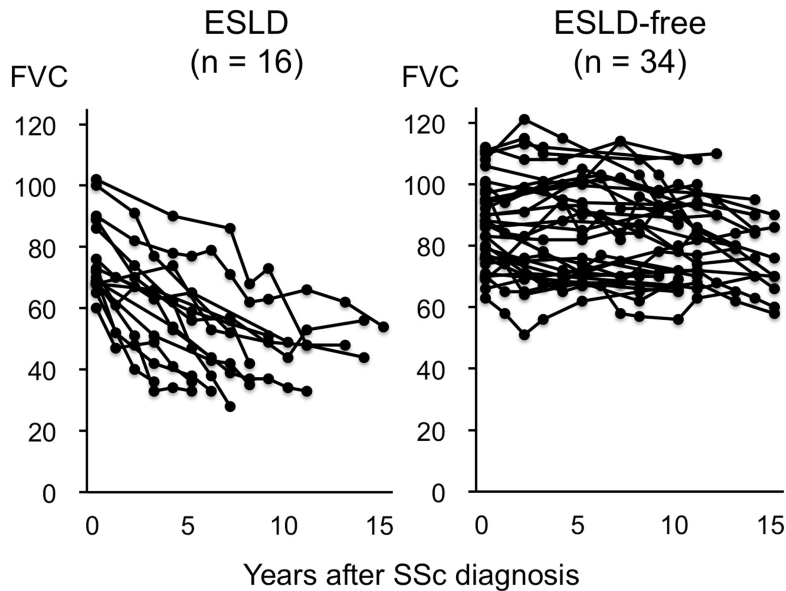
*Figure 3*. Serial measurements of FVC in patients with SSc-ILD, stratified by their subsequent development of ESLD. FVC: forced vital capacity; SSc: systemic sclerosis; ILD: interstitial lung disease; ESLD: endstage lung disease.
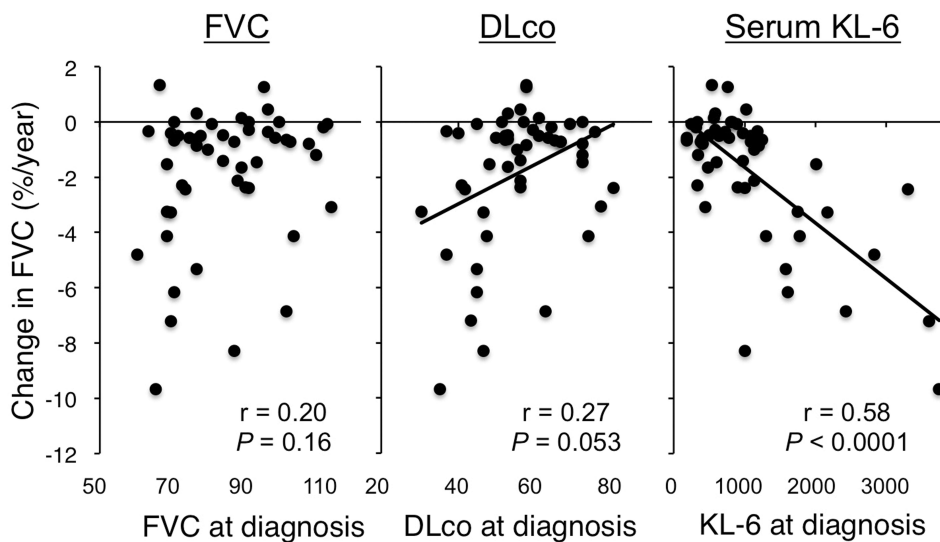


*Figure 4*. Correlations between the FVC, DLCO, or serum KL-6 level at diagnosis and the subsequent change in FVC over the study course in all 50 patients with SSc-ILD. FVC: forced vital capacity; KL-6: Krebs von den Lungen-6; SSc: systemic sclerosis; ILD: interstitial lung disease.

period (average of 29 mos) and the use of CYC, which was likely to be introduced to patients with progressive ILD. To our knowledge, our study is the first to demonstrate the utility of KL-6 measurements in predicting the progression of SSc-ILD using a longterm observational study cohort of untreated patients. Several other serum biomarkers have also been shown to predict FVC decline in patients with SSc. These include CC-chemokine ligand 18[28,29], COMP/throm-bospondin-5[30], C-reactive protein[31], carbohydrate antigen

15.3[32], and interleukin 6[33]. KL-6 has several advantages over these potential biomarkers because of its relatively specific association with ILD and the commercial availability of automated and validated detection systems[21]. However, future prospective studies comparing the utility of these candidate biomarkers in patients with early SSc-ILD are warranted.

In our cohort, FVC declined gradually in the patients who developed ESLD. The annual decline in the FVC of these

patients was 4.6 ± 2.3%, which was comparable to the 2%–3% reduction reported in a previous study[20]. These findings, in addition to the results of other investigations[20,34,35], suggest that SSc-ILD is a gradually developing disease. In contrast, the results of an earlier study showed that the FVC decline in patients with SSc-ILD occurs more rapidly within the first 4 years of diagnosis and then slows down[8], but in our study, there was no difference in the annual FVC decline between the entire disease course and early disease phase within 5 years after diagnosis.

The following limitations of our study deserve discussion. Because our study enrolled patients registered in 1985 to 1995 for analysis, the ILD diagnosis was based on chest radiograph results because HRCT was not available then. Thus, cases of mild ILD may have been missed, resulting in a biased selection of patients with more prominent ILD. In addition, information on the extent of disease, pattern, and scoring by HRCT, which are shown to be useful for prediction of outcomes of SSc-ILD[11,36], was missing. Another limitation that could have affected our results was the variable timing of PFT examinations among the patients. Finally, because we intended to focus on longterm outcomes of lung function, our study excluded patients who died of non-ILD complications (such as renal crisis and myocardial involvement) within 10 years after diagnosis. However, only 4 patients with SSc-ILD were excluded because of early deaths that occurred in association with non-ILD complications. Despite these limitations, we believe that the cohort of patients with SSc-ILD who were left untreated for long periods of time should be valuable.

Our study suggested that patients with SSc-ILD who exhibited an elevated serum KL-6 level early in the disease course are at increased risk for developing ESLD, and thus are good candidates for aggressive therapeutic intervention and inclusion in future clinical trials for the treatment of SSc-ILD, although further largescale prospective studies are necessary to confirm our finding.

## ACKNOWLEDGMENT

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