ORIGINAL ARTICLE

Serum markers of pulmonary epithelial damage in systemic sclerosis-associated interstitial lung disease and disease progression

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ABSTRACT

Background and objective: The course of systemic sclerosis-associated interstitial lung disease (SSc-ILD) is highly variable, and accurate prognostic markers are needed. KL-6 is a mucin-like glycoprotein (MUC1) expressed by type II pneumocytes, while CYFRA 21-1 is expressed by alveolar and bronchiolar epithelial cells. Both are released into the blood from cell injury. Methods: Serum KL-6 and CYFRA 21-1 levels were measured in a retrospective (n = 189) and a prospective (n = 118) cohort of SSc patients. Genotyping of MUC1 rs4072037 was performed. Linear mixed-effect models were used to evaluate the relationship with change in lung function parameters over time, while association with survival was evaluated with Cox proportional hazard analysis. Results: In both cohorts, KL-6 and CYFRA 21-1 were highest in patients with lung involvement, and in patients with extensive rather than limited ILD. KL-6 was higher in patients carrying the MUC1 rs4072037 G allele in both cohorts. In patients with SSc-ILD, serum KL-6, but not CYFRA 21-1, was significantly associated with DL_{CO} decline in both cohorts (P = 0.001 and P = 0.004, respectively), and with FVC decline in the retrospective cohort (P = 0.005), but not the prospective cohort. When combining the cohorts and subgrouping by severity (median CPI = 45.97), KL-6 remained predictive of decline in DL_{CO} in both milder (P = 0.007) and more severe disease (P = 0.02) on multivariable analysis correcting for age, gender, ethnicity, smoking history and MUC1 allele carriage. Conclusion: Our results suggest serum KL-6 predicts decline in lung function in SSc, suggesting its clinical utility in risk stratification for progressive SSc-ILD.

SUMMARY AT A GLANCE

The clinical course of systemic sclerosis-associated interstitial lung disease (SSc-ILD) is highly variable and easily measurable biomarkers are needed to predict disease progression. Serum epithelial biomarker KL-6 is predictive of disease progression measured by a decline in DL_{CO}, regardless of ILD severity, and could provide increased prognostic ability to inform risk stratification in SSc-ILD.

Key words: biomarker, CYFRA 21-1, disease progression, Krebs von den Lungen-6, MUC1 allele, systemic sclerosis-associated interstitial lung disease.

INTRODUCTION

Interstitial lung disease in scleroderma (systemic sclerosis-associated interstitial lung disease, SSc-ILD) is the leading cause of death in SSc.1 Although many patients have relatively mild and/or stable ILD, many others have progressive disease with reduced life expectancy. Patients at higher risk of ILD progression...
need to be identified in order to ensure optimal treatment and monitoring.

Krebs von den Lungen-6 (KL-6), a glycoprotein expressed mainly on type II pneumocytes, is highly expressed by proliferating and regenerating cells. Serum KL-6 levels are increased in SSc-ILD, with higher levels associated with more extensive SSc-ILD and more rapid short-term decline in forced vital capacity (FVC) and development of end-stage lung disease. However, the prognostic value of serum KL-6 levels has not been evaluated against changes in measures of gas transfer, known to be strongly linked to mortality in SSc-ILD, particularly a categorical worsening in diffusing capacity of the lung for carbon monoxide (DLCO) by ≥15% at 2 years, an independent predictor of survival in SSc-ILD. A single-nucleotide polymorphism (SNP) in the KL-6 gene MUC1, rs4072037, relates to KL-6 serum levels in SSc, with higher levels in individuals carrying the G allele.

Cytokeratin 19 fragment, CYFRA 21-1, is expressed on type I/II pneumocytes and respiratory bronchiolar epithelial cells. Cytokeratin proteolytic fragments are soluble and released into the blood from cell lysis or necrosis. Serum CYFRA 21-1 appears to distinguish idiopathic pulmonary fibrosis (IPF) patients from controls, with higher levels associated with increased mortality. In a study of patients with connective tissue diseases, CYFRA 21-1 was associated with ILD, although the number of patients was small (n = 23), with only seven SSc-ILD patients.

In this study, we evaluate serum KL-6 and CYFRA 21-1 as biomarkers of SSc-ILD and its worsening in SSc patients with long-term follow-up.

METHODS

Study populations
Consecutive SSc patients attending clinics at the Royal Brompton and Royal Free Hospitals, London (retrospective cohort: 1991–2013, prospective cohort: 2014–2016), were recruited. A diagnosis of SSc was made according to established criteria. Only patients with lung function within 6 months of serum collection were included. Patients with malignancies at the time of serum collection were excluded. All participants gave written informed consent, and the Ethics Committees of the Royal Brompton and the Royal Free Hospitals gave authorization for the study (REC 13/LO/0857).

Clinical assessment
Clinical data were recorded at the time of serum collection. ILD was defined as the presence of interstitial changes on chest imaging. Lung function tests were performed in a single lab, including FVC and (DLCO) levels, as previously reported. Further details are available in Appendix S1 (Supplementary Information).

Quantification of disease severity
Goh et al.’s severity staging system was used to subclassify disease severity at presentation as ‘limited’ or ‘extensive’ lung fibrosis. As a marker of ILD severity, the composite physiological index (CPI) was calculated as: CPI = 91.0 – (0.65 × FVC% predicted) – (0.53 × DLCO% predicted) + (0.34 × FEV1% predicted), where FEV1 is forced expiratory volume in 1 s.

Genotyping
DNA was extracted from blood using the Gentra PureGene DNA kit (Qiagen, Venlo, The Netherlands). Genotyping was carried out using a TaqMan assay (catalogue number: 4351379; Applied Biosystems, Waltham, MA, USA) on a Rotor-Gene 6000 real-time PCR machine (Qiagen).

Biomarker measurements
Sera were drawn and separated using a standardized protocol and aliquots were stored at −80°C until use. KL-6 was measured using the KL-6 nanopart kit (Sensus Diagnostics, Burlington, MA, USA) on the Beckman Au680 autoanalyser (Beckman Coulter, Brea, CA, USA). CYFRA 21-1 was measured using the Elecsys CYFRA 21-1 kit (Roche Diagnostics, Basel, Switzerland) on the Cobas E411 immunoassay analyser (Roche Diagnostics).

Statistical analysis
Analyses were performed using STATA15.1 software (StataCorp, College Station, Texas). Group comparisons were made using Wilcoxon’s rank sum, Mann-Whitney or chi-square tests, as appropriate. KL-6 and CYFRA 21-1 levels were log transformed to normalize the data. Generalized linear models were used to assess whether the association between serum KL-6 and ILD extent was modified by MUC1 genotype. We performed linear mixed-effects analysis, which takes into account variations in test intervals, using FVC (L) and DLCO (mmol/min), as outcome measures, with subject as a random effect and time from baseline, age, gender, ethnicity and smoking status as fixed effects. A P-value of <0.05 was considered significant. Further details are available in Appendix S1 (Supplementary Information).

RESULTS

Patient cohorts
A total of 189 patients were recruited for the retrospective cohort and 118 patients for the prospective cohort. Further details are available in Appendix S2 (Supplementary Information).

Patient characteristics are described in Table 1. Compared to the retrospective cohort, patients in the prospective cohort were significantly older and more likely to be of non-European ancestry. Patients in the prospective cohort were also more likely to have more severe lung disease, be on active treatment, have estimated pulmonary artery systolic pressure (PASP) ≥40 mm Hg on echocardiogram and less likely to have anti-centromere antibodies (ACA) (Table 1).
Serum KL-6 and CYFRA 21-1 are associated with the presence and extent of ILD and MUC1 rs4072037 allele carriage

Serum KL-6 and CYFRA 21-1 correlated with the presence and severity of ILD in both cohorts, with higher levels in SSc-ILD compared to SSc-no ILD, and in extensive compared to limited ILD (Fig. 1). In both cohorts, KL-6 levels were significantly higher in patients carrying the G allele of MUC1 rs4072037 (Fig. 2). Further details are available in Appendix S2 (Supplementary Information).

KL-6 and CYFRA 21-1 correlate with baseline levels of lung function

Serum levels of KL-6 and CYFRA-21-1 were inversely correlated with the baseline lung function measurements (Appendix S2, Figs S1,S2 in Supplementary Information).

KL-6 and CYFRA 21-1 levels and active treatment

In both cohorts, patients on active treatment (Table S1 in Supplementary Information) at the time of serum collection had higher levels of KL-6 compared to those not on active treatment ($P < 0.03$ and $P = 0.04$, respectively). This association was lost once the disease severity (CPI) was taken into account (Table S2 in Supplementary Information). There was no significant difference in CYFRA 21-1 levels in either cohort according to treatment status.

Association between KL-6 and CYFRA 21-1 and SSc-ILD progression

The association between serum KL-6 and CYFRA 21-1 and lung function worsening was evaluated in patients with SSc-ILD (retrospective $n = 146$, prospective $n = 114$). Only associations identified as significant in the retrospective cohort were tested in the prospective cohort for validation.

On linear mixed-effect model analysis, KL-6 was significantly associated with FVC ($P < 0.005$) and $\text{DL}_{\text{CO}}$ ($P < 0.001$) decline in the respective cohort. The association with $\text{DL}_{\text{CO}}$ decline was confirmed in the prospective cohort ($P = 0.004$) (Table 2). Serum CYFRA 21-1 was not significantly associated with decline in FVC or $\text{DL}_{\text{CO}}$ in the retrospective cohort (Table 3). Having confirmed an association between serum KL-6 and $\text{DL}_{\text{CO}}$ decline in the prospective cohort, we evaluated whether KL-6 was predictive of lung function decline independent of disease severity. As the two cohorts differed significantly in ILD severity and had markedly different follow-up time, to correct for ILD severity but have adequate numbers to allow statistical

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**Table 1** Baseline demographic and clinical characteristics of the retrospective and prospective cohorts

|                          | Retrospective ($n = 189$) | Prospective ($n = 118$) | $P$-value |
|--------------------------|---------------------------|--------------------------|-----------|
| Age at serum collection  | 49.1 (47.08–51.05)        | 56.4 (54.10–58.73)       | <0.001    |
| Gender (female (%))      | 146 (77.25)               | 90 (76.27)               | 0.84      |
| Ethnicity (Caucasian (%))| 156 (82.54)               | 65 (55.08)               | <0.001    |
| Smoking (never (%))      | 131 (69.31)               | 82 (69.49)               | 0.93      |
| Cutaneous involvement† (limited (%)) | 115 (63.54) | 61 (63.54)               | 0.99      |
| Mortality: deaths (%)    | 92 (48.68)                | 17 (14.41)               | <0.001    |
| Follow-up length (years) | 8.44 (0.62–25.24)         | 2.77 (0.62–4.56)         | <0.001    |
| Estimated PASP ≥ 40 mm Hg on echo‡ (%)| 6 (5)    | 15 (16.85)               | <0.001    |
| Active treatment (%)     | 79 (41.80)                | 100 (84.75)              | <0.001    |
| Presence of ILD (%)      | 146 (77.25)               | 114 (96.61)              | <0.001    |
| Extent of ILD (extensive (%)) | 43 (22.75) | 72 (61.02)               | <0.001    |
| CPI                      | 37.8 (26.9–47.7)          | 51.3 (44–60.1)           | <0.001    |
| Autoantibody (%)         |                           |                          |           |
| ATA                      | 85 (44.97)                | 53 (44.92)               | 0.98      |
| ACA                      | 20 (10.58)                | 2 (1.69)                 | 0.003     |
| RNP                      | 18 (9.52)                 | 8 (6.78)                 | 0.42      |
| Other autoantibodies     | 34 (17.99)                | 33 (27.97)               | 0.05      |
| BNP (pmol/L)             | 8 (3–152)                 | 44.5 (22–70)             | 0.06      |
| $\text{DL}_{\text{CO}}$% predicted | 55.5 (44.3–68.35) | 39.9 (29.2–48.8)         | <0.001    |
| $K_{\text{CO}}$% predicted | 79.6 (67.1–92.2) | 68.7 (56.6–78.1)        | <0.001    |
| FEV1% predicted          | 79.8 (68.5–89)            | 73 (55.9–83.6)           | 0.005     |
| FVC% predicted           | 80.1 (67.2–95.5)          | 73.8 (57.2–87)           | <0.001    |

Age is presented as mean (range), and all other data are presented as median (interquartile range).

†Data are available for 181 patients in the retrospective cohort and 96 patients in the prospective cohort.

‡Estimated echocardiographic assessment of PASP within 18 months of serum collection was available for 120 patients in the retrospective cohort and 89 patients in the prospective cohort.

AC, anti-centromere antibody; ATA, anti-topoisomerase antibody; BNP, brain natriuretic peptide; CPI, composite physiological index; $\text{DL}_{\text{CO}}$, diffusing capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; ILD, interstitial lung disease; $K_{\text{CO}}$, CO transfer coefficient; PASP, pulmonary artery systolic pressure; RNP, ribonucleoprotein antibody.
Figure 1  Serum Krebs von den Lungen-6 (KL-6) and CYFRA 21-1 levels according to interstitial lung disease (ILD) status. Serum KL-6 levels in no-ILD, limited ILD and extensive ILD in the (A) retrospective and (B) prospective cohorts. Serum CYFRA 21-1 levels in no-ILD, limited ILD and extensive ILD in the (C) retrospective and (D) prospective cohorts. Central lines indicate median values with boxes showing the 25th and 75th percentiles, whiskers indicate upper and lower quartile + 1.5× interquartile range (IQR).

Figure 2  Serum Krebs von den Lungen-6 (KL-6) according to allele carriage status. Serum KL-6 levels according to MUC1 rs4072037 allele carrier status in the (A) retrospective and (B) prospective cohorts. Central lines indicate median values with boxes showing the 25th and 75th percentiles, whiskers indicate upper and lower quartile + 1.5× interquartile range (IQR).
power in each subgroup, we combined the two cohorts and stratified according to median CPI (45.97). This definition of ILD severity was selected as it resulted in an even number of patients in each severity group \((n = 128/129)\), while subgrouping according to Goh \textit{et al.}'s staging system would have resulted in unequal cohort sizes \((n = 134 \text{ and } n = 123)\). In patients with less severe ILD \((\text{CPI} < 45.97)\), KL-6 was significantly associated with decline in DLCO \((P = 0.03)\). This association remained significant following correction for age, gender, ethnicity, smoking status and allele carriage \((P = 0.007)\). Although the trend towards an association with FVC decline did not reach significance on univariable analysis, KL-6 was also significantly associated with decline in FVC on multivariable analysis \((P = 0.01)\). In patients with more severe ILD \((\text{CPI} \geq 45.99)\), KL-6 was significantly associated with decline in DLCO on both univariable \((P = 0.007)\), and multivariable analyses \((P = 0.02)\) (Table 4). For clinical purposes, we wanted to test if knowledge of \textit{MUC1} rs4072037 allele carriage was necessary for the prognostic utility of KL-6. The associations with DLCO remained significant when allele carriage was omitted from the multivariable analysis (Table S3 in Supplementary Information). All associations remained significant when estimated PASP \(\geq 40\) mm Hg on echocardiogram was added as a covariate in the smaller group with echocardiographic data (Table S4 in Supplementary Information).

### Predictive cut-off value for serum KL-6 in predicting DLCO decline by \(\geq 15\%\)

We sought to establish the optimal serum KL-6 threshold in predicting decline in DLCO by \(\geq 15\%\) at 2 years, an established surrogate marker of mortality in SSc-ILD,\(^\text{16}\) by performing receiver operating characteristic (ROC) analysis in the retrospective cohort. The best cut-off level for serum KL-6 was 1472 U/mL, with a sensitivity of 41.94\%, specificity of 80.67\% and 74.03\% of patients correctly classified. This cut-off value successfully predicted time to decline in DLCO by \(\geq 15\%\) in the prospective cohort \((P = 0.003)\) (Fig. S3 in Supplementary Information).

### KL-6 and CYFRA 21-1 and mortality

Both KL-6 \((P = 0.015)\) and CYFRA 21-1 \((P = 0.001)\) were significantly associated with mortality in patients with SSc-ILD in the retrospective cohort on univariable analysis, although only bordered on statistical significance \((P = 0.06 \text{ for both})\) after adjustment for CPI, age, gender, ethnicity, smoking status and allele carriage when appropriate (Table S5 in Supplementary Information). As the findings were borderline significant, we also tested association with survival in the prospective

### Table 2  Association between serum KL-6 levels and lung function decline in patients with SSc-ILD

|                        | Coefficient (95% CI) | \(P\)-value |
|------------------------|----------------------|-------------|
| **Retrospective cohort \((n = 146)\)** |                      |             |
| FVC                    | -0.68 (−1.15, −2.00) | 0.005       |
| DLCO                   | -2.47 (−3.94, −1.01) | 0.001       |
| **Prospective cohort \((n = 114)\)** |                      |             |
| FVC                    | -0.29 (−0.82, 0.25)  | 0.29        |
| DLCO                   | -1.17 (−1.95, −0.38) | 0.004       |

Linear mixed-effect analysis.

**Note:** DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; SSc-ILD, systemic sclerosis-associated interstitial lung disease.

### Table 3  Association between serum CYFRA 21-1 levels and lung function decline in patients with SSc-ILD

|                        | Coefficient (95% CI) | \(P\)-value |
|------------------------|----------------------|-------------|
| **Retrospective cohort \((n = 146)\)** |                      |             |
| FVC                    | -0.60 (−1.33, 0.13)  | 0.11        |
| DLCO                   | -1.60 (−3.98, 0.77)  | 0.19        |

Linear mixed-effect model analysis.

**Note:** DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; SSc-ILD, systemic sclerosis-associated interstitial lung disease.

### Table 4  Association between serum KL-6 levels and lung function decline according to ILD severity

| CPI \(< 45.97 \((n = 128)\)\)   | Coefficient (95% CI)     | \(P\)-value |
|--------------------------------|-------------------------|-------------|
| FVC                           | -0.49 (−1.09, 0.12)     | 0.12        |
| DLCO                          | -1.34 (−2.58, −0.11)    | 0.03        |

| CPI \(\geq 45.97 \((n = 129)\)\) | Coefficient (95% CI)     | \(P\)-value |
|---------------------------------|-------------------------|-------------|
| FVC                             | 0.07 (−0.28, 0.42)      | 0.70        |
| DLCO                            | -3.80 (−6.53, −1.06)    | 0.007       |

**Notes:** Linear mixed-effect model analysis; Multivariable analysis correcting for age, gender, ethnicity, smoking status and allele carriage.

**Note:** CPI, composite physiological index; DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; ILD, interstitial lung disease; KL-6, Krebs von den Lungen-6.

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cohort in patients with SSc-ILD, and did not find an association with mortality.

**DISCUSSION**

In this study, we found that serum levels of KL-6 and of CYFRA 21-1 were highest in SSC patients with lung involvement, and in those with extensive rather than limited ILD. In patients with SSc-ILD, KL-6, but not CYFRA 21-1, was significantly associated with lung function decline, regardless of ILD severity.

Despite advances in the management of SSc-ILD, its impact on quality of life and mortality remains high. Accurate prognostication remains difficult. Evidence supports the need to treat patients with extensive and/or progressive SSc-ILD, while only a subset of patients with milder ILD may require treatment. The last decade has seen the publication of landmark clinical trials for SSc-ILD. While immunosuppression remains the mainstay of treatment, there is a subgroup of patients with progressive fibrotic disease despite treatment. Their early identification and prevention of progressive fibrosis remain a key objective. In addition to KL-6, a number of biomarkers have been reported to be associated with ILD presence and/or progression in SSc-ILD, including serum CCL18, although none are currently available for routine clinical use in Europe. Our results suggest that serum KL-6 is a more powerful biomarker than CYFRA 21-1 for predicting SSc-ILD progression across ILD severity. In particular, KL-6 is predictive of lung function decline in patients with less severe SSc-ILD, the group for which predictive markers are most needed, particularly now that the range of options to treat progressive fibrotic lung disease has increased to include anti-fibrotic agents, and further novel treatments are under investigation.

Interestingly, although carriage of the MUC1 allele was associated with ILD severity in both cohorts, the significance of the association between serum KL-6 and DLCO did not change even after omitting the allele carriage data from the multivariable analysis, suggesting that for clinical purposes, knowledge of the MUC1 allele carriage status is not indispensable for KL-6 to provide prognostically useful information.

Having observed an association between serum KL-6 and lung function worsening, in order to establish the best predictive cut-off value, we utilized DLCO decline at 2 years, identified as a stronger surrogate mortality marker than changes in FVC in SSc-ILD. We identified optimal thresholds predictive of decline in DLCO by ≥15% at 2 years from baseline in the retrospective cohort, and confirmed that KL-6 ≥ 1472 U/mL was also significantly associated with earlier decline in DLCO by ≥15% in the prospective group. Considering the majority of patients in the prospective cohort were on treatment for their SSc-ILD, serum KL-6 thresholds could aid in identifying patients more likely to require intensification of treatment to prevent progression of disease. In particular, whether serum KL-6 thresholds could help in identifying patients more likely to benefit from the addition of anti-fibrotic treatments will require further study.

Our study has limitations. The prospective cohort was not an ideal validation cohort, as ILD severity was greater and follow-up time was much shorter than in the retrospective cohort. The difference reflected unexpected changes in referral patterns during the study period, with a shift in recent times towards the selective referral of severe SSc-ILD patients. As a result, meaningful analysis of prognostic differences between severe and less severe SSc-ILD was not possible in the prospective cohort, with shorter follow-up time in this cohort as an additional constraint. In view of the importance of severity distinctions, we therefore conducted a post hoc analysis in which the two cohorts were combined and subdivided according to median CPI. This definition for ILD severity was selected as it resulted in an even number of patients in each severity group. Although the CPI has not specifically been tested in SSc-ILD, Wells et al. had observed that the relationship between spirometric lung volumes and DLCO, components of the CPI score and HRCT extent did not differ between SSc-ILD and IPF, suggesting that it is reasonable to use CPI as a measure of severity in SSc-ILD.

Another unavoidable limitation of our study is the inability to adjust for treatment differences. Although categorized broadly as active treatment within 3 months of serum collection, the later introduction of treatment could not be accounted for in the analyses. Baseline KL-6 levels were higher in patients on treatment in both cohorts, but this association was lost with adjustment for disease severity, with treatment status linked to disease severity, as expected. CYFRA 21-1 levels did not vary according to treatment status. Treatment regimens in SSc-ILD are too variable to allow categorical sub-analysis during longer term follow-up. There is a major variability in the choice, timing and duration of treatment with large modifications often made due to side effects or non-efficacy. Finally, although our main focus was the utility of serum KL-6 and CYFRA-21-1 as potential markers of SSc-ILD progression, we recognize that the relatively small number of patients without ILD is a limitation of the study.

Serum KL-6 and CYFRA 21-1 are markers of epithelial cell damage. Rapid clearance of radio-labelled DTPA, reflecting impaired alveolar epithelial integrity, is associated with progression of SSc-ILD, suggesting that epithelial cell damage plays an important role in SSc-ILD pathogenesis. Interestingly, DTPA clearance was associated with lung function worsening, but not with mortality in SSc-ILD, similar to our observations where we found only a weak association with mortality on multivariable analysis and only in the retrospective cohort. This would again suggest that KL-6, like DTPA clearance, is specifically a marker of epithelial events, and therefore linked with lung function worsening. It would be of interest to investigate whether KL-6 is purely a marker of progression in SSc-ILD or if it has a direct role in promoting fibrosis. There is evidence that KL-6 may promote a fibrotic phenotype in human lung fibroblasts, although further data on its potential role are needed.

In conclusion, despite advances in the knowledge of SSc-ILD staging and pathogenesis, management of the disease remains challenging, with the need for more
accurate predictors of disease progression. Serum biomarkers are easily obtainable, and could provide increased prognostic ability and potentially new insights into pathogenesis and potential therapeutic targets in SSc-ILD. Both serum KL-6 and CYFRA 21-1 are markers of pulmonary epithelium injury and abnormal repair. From our study, we conclude that serum KL-6 appears to be a better marker of progressive SSc-ILD than CYFRA 21-1. Ultimately, we need to develop an individualized risk index that incorporates clinical variables including ILD severity, integrated by easily obtainable biomarkers to inform selective early treatment and frequent monitoring of patients with SSc-ILD at high risk of progression.

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Abbreviations: ACA, anti-centromere antibody; CPI, composite physiological index; DLCO, diffusing capacity of the lung for carbon monoxide; DTPA, Diethylenetriamine pentaacetate; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; HRCT, high-resolution computed tomography; ILD, interstitial lung disease; IPPF, idiopathic pulmonary fibrosis; IQR, interquartile range; KL-6, Krebs von den Lungen-6; PASP, pulmonary artery systolic pressure; PCR, polymerase chain reaction; SSc-ILD, systemic sclerosis-associated ILD

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Supplementary Information

Additional supplementary information can be accessed via the html version of this article at the publisher’s website.

Appendix S1 Additional methods.
Appendix S2 Additional results.
Figure S1 KL-6 correlation with baseline lung function.
Figure S2 CYFRA 21-1 correlation with baseline lung function.
Figure S3 KL-6 cut-off with decline in DLCO ≥15%.
Table S1 Type of treatment at baseline.
Table S2 Serum levels according to treatment status.
Table S3 Multivariable analysis for association between KL-6 and lung function decline according to ILD severity, excluding allele carriage as a covariate in the model, but including age, gender, ethnicity and smoking history.
Table S4 Multivariable analysis for association between KL-6 and lung function decline according to ILD severity, including estimated PASP ≥40 mm Hg on echocardiogram as a covariate in the model, in addition to allele carriage, age, gender, ethnicity and smoking history.
Table S5 Mortality analysis in patients with SSc-ILD.