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# Progression of Interstitial Lung Disease in Systemic Sclerosis: The Importance of Pneumoproteins Krebs von den Lungen 6 and CCL18

Elizabeth R. Volkmann,[1] Donald P. Tashkin,[1] Masataka Kuwana,[2] Ning Li,[3] Michael D. Roth,[1] Julio Charles,[4] Faye N. Hant,[5] Galina S. Bogatkevich,[5] Tanjina Akter,[5] Grace Kim,[6] Jonathan Goldin,[7] Dinesh Khanna,[8] Philip J. Clements,[1] Daniel E. Furst,[9] Robert M. Elashoff,[3] Richard M. Silver,[5] and Shervin Assassi[4]

**Objective.** To investigate the relationship between Krebs von den Lungen 6 (KL-6) and CCL18 levels and the severity and progression of systemic sclerosis (SSc)–related interstitial lung disease (ILD).

**Methods.** Patients enrolled in the Scleroderma Lung Study II (cyclophosphamide [CYC] versus mycophenolate mofetil [MMF]) were included. Baseline and 12-month plasma samples were analyzed by enzyme-linked immunosorbent assay to assess CCL18 and KL-6 levels. The forced vital capacity (FVC) and the diffusing capacity for carbon monoxide (DLco) were measured every 3 months. Joint models were created to investigate the relationship between baseline CCL18 and KL-6 levels and the course of the FVC and DLco over 1 year according to treatment arm.

**Results.** Baseline KL-6 and CCL18 levels each correlated with the extent of radiographic fibrosis. Levels of both CCL18 and KL-6 declined significantly at 1 year. In both treatment arms (n = 71 for CYC, n = 62 for MMF), a higher baseline KL-6 level predicted progression of ILD based on the course of FVC ($P = 0.024$ for CYC; $P = 0.005$ for MMF) and DLco ($P < 0.001$ for CYC; $P = 0.004$ for MMF) over 1 year. A higher baseline CCL18 level predicted progression of ILD based on the course of the FVC ($P < 0.001$ for CYC; $P = 0.007$ for MMF) and DLco ($P = 0.001$ for CYC; $P < 0.001$ for MMF) over 1 year, as well as mortality ($P = 0.0008$ for CYC arm only).

**Conclusion.** In a rigorously conducted clinical trial for SSc-related ILD, KL-6 and CCL18 levels correlated with ILD severity and declined with immunosuppression. Patients with higher baseline KL-6 and CCL18 levels were more likely to experience disease progression despite treatment. KL-6 and CCL18 levels could be used to identify patients with a progressive ILD phenotype who may benefit from a more aggressive initial treatment approach.

## INTRODUCTION

Interstitial lung disease (ILD) occurs in the majority of patients with systemic sclerosis (SSc) (1). While ILD is the leading cause of disease-related mortality among patients with SSc (2,3), ILD progression rates vary considerably. Results of randomized controlled trials (RCTs) have demonstrated that some patients experience an improvement in lung function after treatment with immunosuppression, while other patients experience progression of ILD despite early and aggressive

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[1]Elizabeth R. Volkmann, MD, MS, Donald P. Tashkin, MD, Michael D. Roth, MD, Philip J. Clements, MD, MPH: David Geffen School of Medicine, University of California, Los Angeles; [2]Masataka Kuwana, MD, PhD: Nippon Medical School, Tokyo, Japan; [3]Ning Li, PhD, Robert M. Elashoff, PhD: University of California, Los Angeles; [4]Julio Charles, MS, Shervin Assassi, MD, MS: University of Texas McGovern Medical School, Houston; [5]Faye N. Hant, DO, MSCR, Galina S. Bogatkevich, MD, PhD, Tanjina Akter, PhD, Richard M. Silver, MD: Medical University of South Carolina, Charleston; [6]Grace Kim, PhD: David Geffen School of Medicine and Fielding School of Public Health, University of California, Los Angeles; [7]Jonathan Goldin, MD, PhD: Fielding School of Public Health, University of California, Los Angeles; [8]Dinesh Khanna, MD, MS: University of Michigan Medical School, Ann Arbor, and CiVi BioPharma, Inc., Chevy Chase, Maryland; [9]Daniel E. Furst, MD: David Geffen School of Medicine, University of California, Los Angeles, University of Washington, Seattle, and University of Florence, Florence, Italy.

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Address correspondence to Elizabeth R. Volkmann, MD, MS, 1000 Veteran Avenue, Suite 32-59, Los Angeles, CA 90095. E-mail: evolkmann@mednet.ucla.edu.

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treatment (4,5). Furthermore, not all patients with ILD will develop symptoms or will have progressive disease even in the absence of treatment (1,6–8).

Evidenced-based clinical tools to predict which patients with SSc-related ILD are more likely to experience ILD progression do not exist. Specific clinical and biological factors have been associated with progression of ILD in observational studies (e.g., low forced vital capacity [FVC] [9], greater extent of ILD on high-resolution computed tomography [HRCT] [10,11], low diffusing capacity for carbon monoxide [$DL_{CO}$] [9,12], and anti–topoisomerase I antibody positivity [9,12]). Moreover, several studies have identified serum/plasma protein candidate biomarkers that predict SSc-related ILD progression, including interleukin-6 (IL-6) (13), C-reactive protein (CRP) level (14), CCL2 (15), CCL18 (16,17), CXCL4 (18), and Krebs von den Lungen 6 (KL-6) (19,20).

Among these candidate biomarkers, KL-6 and CCL18 have been found to predict outcomes in several different SSc-related ILD populations (16,17,19,20). Because KL-6 and CCL18 are pneumoproteins associated with lung parenchymal injury (21,22), they may be more specific markers for monitoring and predicting the course of ILD in SSc. For example, in contrast to general inflammatory markers (e.g., IL-6 or CRP level), the levels of KL-6 and CCL18 may be less likely to be affected by extrapulmonary fibrotic processes such as cutaneous sclerosis or infections.

Furthermore, KL-6 correlates with disease severity in different SSc-related ILD populations (23–28). Two observational studies (19,20) have shown that high KL-6 levels predict worse outcomes in SSc-related ILD. A recent, small observational study demonstrated that a high serum KL-6 level was associated with poor response to immunosuppression with cyclophosphamide (CYC) in patients with SSc-related ILD (29).

CCL18 is a chemokine that was previously known as pulmonary and activation-regulated chemokine, and studies have demonstrated higher levels of this chemokine in both serum and bronchoalveolar lavage fluid samples of patients with ILD (30). Observational studies have demonstrated that CCL18 also predicts various ILD-related outcomes in SSc (16,17,31,32).

Given the accumulating evidence that KL-6 and CCL18 may be key markers of disease activity and progression in SSc-related ILD, the present study sought to evaluate the predictive role of CCL18 and KL-6 levels in the context of an RCT, in which all patients have equal access to care, uniform follow-up, and a standardized treatment approach. The present study aimed to determine whether KL-6 and CCL18 are associated with the severity of ILD in a clinical trial cohort comprising patients with well-characterized and active SSc-related ILD. A secondary aim was to determine whether baseline levels of these peripherally measured lung glycoproteins predict the progression of SSc-related ILD in patients receiving immunosuppressive treatment with either mycophenolate mofetil (MMF) or CYC.

## PATIENTS AND METHODS

**Study participants.** Data and plasma samples from participants enrolled in the Scleroderma Lung Study (SLS) II (5) were analyzed for this study. Eligibility criteria included the following key requirements: 1) adults ages 18–75 years, 2) limited or diffuse cutaneous SSc (33), 3) active ILD as demonstrated by restrictive to borderline restrictive ventilatory impairment (FVC <80–85% but ≥45% predicted) AND the presence of any ground-glass opacity (GGO; hazy opacity through which normal lung markings can be discerned) on HRCT, and 4) exertional dyspnea (grade ≥2 on the magnitude of task component of the Baseline Dyspnea Index) (34). Key exclusion criteria included pulmonary hypertension, clinically significant abnormalities on HRCT not attributable to SSc, smoking within the past 6 months, and evidence of significant airflow obstruction. Complete details of the SLS II design have been previously reported (5). See Appendix A for SLS II investigators and institutions.

Unaffected control participants were independently recruited at the University of Texas, Houston and age-, ethnicity-, and sex-matched to SLS II participants in an approximately 1 (control) to 3 (SLS II) ratio. The same unaffected controls were used for both the KL-6 and CCL18 analyses. The institutional review board of each site approved the studies, and only participants who provided informed consent were included in the present analyses.

**Patient and public involvement.** Patients and the public were not involved in the design or reporting of the results of this research study. Patients were involved in the conduct of the study because they served as participants.

**SLS II design.** In SLS II, enrolled patients were randomized in a similar manner to receive either oral CYC for 1 year followed by 1 year of placebo (supplied by Hoffmann-La Roche/Genentech) or MMF for 2 years. For complete details of the SLS II protocol, please see the supplementary online appendix accompanying the SLS II study by Tashkin et al (5). The FVC (primary SLS II end point) and $DL_{CO}$ (secondary SLS II end point) were measured every 3 months, and total lung capacity (TLC) was measured every 6 months during the trial. HRCT thoracic imaging was obtained at baseline in SLS II, and a computer-aided design scoring system was employed to provide quantitative measures of different patterns of ILD as previously described (35). The quantitative ILD (QILD) score was the sum of all scores classified as abnormal, including scores for quantitative lung fibrosis (QLF; linear reticular markings with architectural distortion), GGO, and honeycomb changes (clustered air-filled cysts with dense walls). Scores were calculated as the percentage of total counted voxels for both the whole lung (WL), including both lungs, and for the

zone of maximal involvement (ZM; area-equivalent upper, middle, or lower lung zone).

**KL-6 and CCL18 assays.** SLS II plasma samples were collected at the baseline and 12-month study visits in EDTA tubes and were immediately processed onsite on the day of collection, stored at –70°C, and shipped on dry ice to the central repository at the University of Texas. All SLS II patients with an available baseline plasma sample were included in the present study. Plasma samples from unaffected controls collected at the University of Texas were handled in the same manner except that no shipping was required. CCL18 was assayed by commercially available enzyme-linked immunosorbent assay kits (MIP-4/CCL18; Cell Sciences), while KL-6 was measured using latex-fixed anti–KL-6 monoclonal antibody with an automated analyzer (Nanopia KL-6; Sekisui Medical). All plasma assays were performed in duplicate, and the coefficient of variance was <20%. Technicians performing the assays were blinded to the clinical diagnosis and outcome data.

**Statistical analysis.** *Baseline characteristics*. Summary statistics were generated for baseline characteristics. A 2-sample *t*-test or a Wilcoxon rank sum test was used to compare continuous variables, and a chi-square test was used to compare categorical variables. Kendall's tau correlations were performed to examine the relationship of KL-6 and CCL18 levels with the baseline measures of the extent of ILD, as measured by the FVC, DLco, QILD score, and QLF score.

*Change in KL-6 and CCL18 level from baseline to 12 months.* Summary statistics of KL-6 and CCL18 levels were calculated for baseline and 12 months. A Wilcoxon signed rank test was used to compare the data collected at the 2 time points.

*Relationship between baseline KL-6 and CCL18 levels and the progression of SSc-related ILD.* A joint model analysis was used to determine whether baseline levels of KL-6 or CCL18 predicted progression of SSc-related ILD. The joint model (used also in the main SLS II analysis [5]) adjusts for nonignorable missing data due to treatment failure, death, and dropouts (36). The end point for the primary outcome model was the course of % predicted FVC measured in 3-month increments from 3 to 12 months. The longitudinal model of the joint analysis included the following covariates: baseline KL-6 or CCL18 level, baseline % predicted FVC, and a linear time trend. The end point for the secondary outcome model was the course of % predicted DLco measured in 3-month increments from 3 to 12 months. The longitudinal model of the joint analysis included the following covariates: baseline KL-6 or CCL18 level, baseline % predicted DLco, and a linear time trend. KL-6 and CCL18 were log transformed (with a base of 2) in these analyses to correct data skewness. We generated models for examining baseline KL-6 and CCL18 levels as a continuous variable and also as a dichotomous variable (using the median as the cut point). The median was selected because there are no valid thresholds for defining high versus low KL-6 and CCL18 levels. In an exploratory analysis, we generated receiver operating characteristic (ROC) curve and logistic regression analysis to determine whether we could identify a threshold for KL-6 and CCL18 levels that predicted disease progression. Since there is no universally accepted definition of disease progression in SSc-related ILD, we used the following 2 definitions: 1) FVC decline of –5% or more, and 2) FVC decline of –10% or more OR FVC decline between –5% and –9% accompanied by a DLco decline of –15% or more. The time course of 3–12 months was selected as this was the time period in which patients in both study arms (CYC and MMF) were receiving active treatment.

**Table 1.** Baseline demographic and clinical characteristics of SLS II participants by study group and unaffected controls*

| Measure | SLS II | | Controls (n = 39) |
|---|---|---|---|
| | CYC group (n = 71) | MMF group (n = 62) | |
| Age, years† | 52.3 ± 9.5 | 52.9 ± 10.0 | 52.2 ± 9.5 |
| Female, no. (%) | 55 (77.5) | 44 (71.0) | 28 (71.8) |
| Race, no. (%)‡ | | | |
| White | 47 (66.2) | 46 (74.2) | 27 (69.2) |
| African American | 18 (25.4) | 10 (16.1) | 9 (23.1) |
| Asian | 3 (4.2) | 6 (9.7) | 3 (7.7) |
| Other | 3 (4.2) | 0 (0) | 0 (0) |
| Diffuse cutaneous sclerosis | 39 (54.9) | 38 (61.3) | |
| Disease duration, years§ | 2.5 ± 1.8 | 2.7 ± 1.7 | |
| FVC, % predicted | 66.2 ± 9.9 | 66.5 ± 8.3 | |
| $FEV_1$/FVC, % | 83.5 ± 5.6 | 82.0 ± 5.7 | |
| TLC, % predicted | 65.4 ± 12.1 | 66.4 ± 10.2 | |
| DLco, % predicted‡ | 53.8 ± 14.2 | 54.9 ± 11.3 | |
| BDI focal score (range 0–12)‡ | 7.0 ± 2.3 | 7.3 ± 2.2 | |
| HAQ DI score (range 1–3) | 0.7 ± 0.7 | 0.7 ± 0.6 | |
| MRSS (range 0–51) | 14.1 ± 10.8 | 15.2 ± 10.3 | |
| QLF-WL score, %§ | 9.1 ± 7.0 | 8.4 ± 7.1 | |
| QLF-ZM score, worst zone, %§ | 23.2 ± 19.2 | 22.8 ± 20.4 | |
| QILD-WL score, %‡ | 32.1 ± 14.2 | 27.7 ± 13.8 | |
| QILD-ZM score, %‡ | 53.2 ± 19.3 | 49.7 ± 21.2 | |

* Except where indicated otherwise, values are the mean ± SD. SLS = Scleroderma Lung Study; CYC = cyclophosphamide; MMF = mycophenolate mofetil; FVC = forced vital capacity; $FEV_1$ = forced expiratory volume in 1 second; TLC = total lung capacity; DLco = diffusing capacity for carbon monoxide; BDI = Baseline Dyspnea Index (lower scores indicate worse dyspnea); HAQ DI = Health Assessment Questionnaire disability index (higher scores indicate greater disability); MRSS = modified Rodnan skin thickness score; QLF-WL = quantitative extent of lung fibrosis (reticulations on high-resolution computed tomography [HRCT]) for the whole lung; QLF-ZM = QLF (on HRCT) for zone of maximal involvement; QILD-WL = quantitative extent of interstitial lung disease (on HRCT; includes scores for fibrosis, ground-glass opacity, and honeycombing) for the whole lung; QILD-ZM = QILD (on HRCT) for zone of maximal involvement.
† $P < 0.01$, CYC group versus MMF group.
‡ $P < 0.001$, CYC group versus MMF group.
§ $P < 0.05$, CYC group versus MMF group.

*Relationship of baseline KL-6 and CCL18 levels with long-term survival in SSc-related ILD.* Cox regression was used to assess the association between baseline KL-6 and CCL18 levels and long-term survival in SLS II. The model included baseline KL-6 and CCL18 levels (log transformed) and baseline % predicted FVC as covariates. The methods for obtaining long-term survival data in SLS II are described in detail in our recent publication (37).

All tests were 2-sided. The joint analyses were performed using the R package JMbayes, and all other analyses were conducted using SAS version 9.4.

## RESULTS

**Participant characteristics.** Baseline characteristics of SLS II participants who underwent KL-6 and CCL18 analysis are shown in Table 1. Among the 142 SLS II participants, 133 and 99 participants had both KL-6 and CCL18 measurements at baseline and 12 months, respectively. Compared with the SLS II cohort, unaffected controls (n = 39) were similar in age (mean ± SD 52.2 ± 9.5 years), sex (71.8% female), race (69.2% white, 23.1% African American, and 7.7% Asian), and ethnicity (12.8% Hispanic/Latino).

**Association of KL-6 levels with disease severity.** Mean ± SD KL-6 levels were significantly higher in SSc patients (n = 133) compared with unaffected controls (n = 39) (1,752.05 ± 1,274.67 versus 330.70 ± 125.74 units/ml; $P < 0.0001$). KL-6 levels correlated with SSc disease severity at baseline (Table 2). Specifically, increased KL-6 levels were associated with decreased $DL_{CO}$, decreased TLC, and increased radiographic extent of lung fibrosis as measured by the QILD and QLF scores for WL and ZM.

**Association of CCL18 levels with disease severity.** Mean ± SD CCL18 levels were significantly higher in SSc patients (n = 133) compared with unaffected controls (n = 39) (191.29 ± 111.08 versus 87.71 ± 28.28 ng/ml; $P = 0.0009$). In addition, increased CCL18 levels were associated with increased radiographic extent of lung fibrosis as measured by the QILD score for WL and the QLF score for ZM (Table 2).

**Table 2.** Baseline correlations between KL-6 and CCL18 levels and SSc disease activity measures*

| Disease measure | KL-6 (n = 133) | CCL18 (n = 133) |
|---|---|---|
| FVC, % predicted | −0.01 | 0.11 |
| $DL_{CO}$, % predicted | −0.23† | −0.04 |
| TLC, % predicted | −0.21† | 0.01 |
| QILD-WL | 0.35† | 0.14‡ |
| QILD-ZM | 0.35† | 0.18§ |
| QLF-WL | 0.36† | 0.08 |
| QLF-ZM | 0.33† | 0.10 |

* KL-6 = Krebs von den Lungen 6; SSc = systemic sclerosis; SLS = Scleroderma Lung Study; FVC = forced vital capacity; $DL_{CO}$ = diffusing capacity for carbon monoxide; QILD-WL = quantitative extent of interstitial lung disease (on high-resolution computed tomography [HRCT]; includes scores for fibrosis, ground-glass opacity, and honeycombing) for the whole lung; QILD-ZM = QILD (on HRCT) for zone of maximal involvement. QLF-WL = quantitative extent of lung fibrosis (reticulations) (on HRCT) for the whole lung; QLF-ZM = QLF (on HRCT) for zone of maximal involvement.
† $P < 0.001$
‡ $P < 0.05$
§ $P < 0.01$

**Relationship between KL-6 and CCL18 levels.** CCL18 levels correlated with KL-6 levels at baseline (r = 0.18, $P = 0.036$) and at 12 months (r = 0.15, $P = 0.032$). The change in CCL18 levels from baseline to 12 months was not correlated with the change in KL-6 levels from baseline to 12 months in all participants (r = 0.063, $P = 0.34$), or in participants randomized to receive CYC (r = 0.094, $P = 0.34$) or those randomized to receive MMF (r = 0.0068, $P = 0.95$).

**Decrease in KL-6 and CCL18 levels after 1 year of immunosuppression.** Among SLS II participants with baseline and 12-month KL-6 and CCL18 measurements (n = 99), treatment with CYC or MMF for 1 year led to significant reductions in these peripheral pneumoprotein levels (Figure 1). The average decline in KL-6 levels was 100.60 units/ml (n = 99; $P = 0.045$), while the average decline in CCL18 levels was 61.24 ng/ml (n = 98; $P < 0.0001$). Among patients assigned to receive MMF, both KL-6 levels (n = 49; $P = 0.016$) and CCL18 levels (n = 51; $P < 0.0001$) decreased significantly over 1 year (Supplementary Tables 1 and 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.41020/abstract).

Among patients assigned to receive CYC (n = 49), CCL18 levels decreased significantly over 1 year ($P = 0.0008$), although KL-6 levels did not (Supplementary Tables 1 and 2). The mean ± SD decline in KL-6 levels among patients assigned to CYC and those assigned to MMF was 55.72 ± 819.44 and 146.40 ± 458.69 units/ml, respectively (Supplementary Table 2). The mean ± SD decline in CCL18 levels among patients assigned to CYC and those assigned to MMF was 46.94 ± 87.10 and 75.55 ± 105.75 ng/ml, respectively (Supplementary Table 1).

**Prediction of SSc-related ILD progression by baseline KL-6 levels.** The predictive significance of KL-6 and CCL18 levels was analyzed in each treatment arm separately. Among SLS II participants, higher baseline KL-6 levels predicted progression of ILD as measured by the course of the % predicted FVC (estimate −0.32 for CYC [$P = 0.024$] and −0.72 for MMF [$P = 0.005$]) and % predicted $DL_{CO}$ (estimate −1.30 for CYC [$P < 0.001$] and −1.28 for MMF [$P = 0.004$]) over 1 year in the MMF and CYC treatment arms, even after adjustment for baseline disease severity (Table 3).

After dichotomizing the KL-6 variable based on the median level in baseline SLS II samples (1,448.2 units/ml), a high baseline KL-6 level was associated with increased progression of ILD as measured by the course of the FVC in the MMF arm (estimate −1.19; $P = 0.018$), but not in the CYC arm (estimate −0.19; $P = 0.44$) (Supplementary Table 3, http://onlinelibrary.wiley.com/doi/10.1002/art.41020/abstract). A high baseline

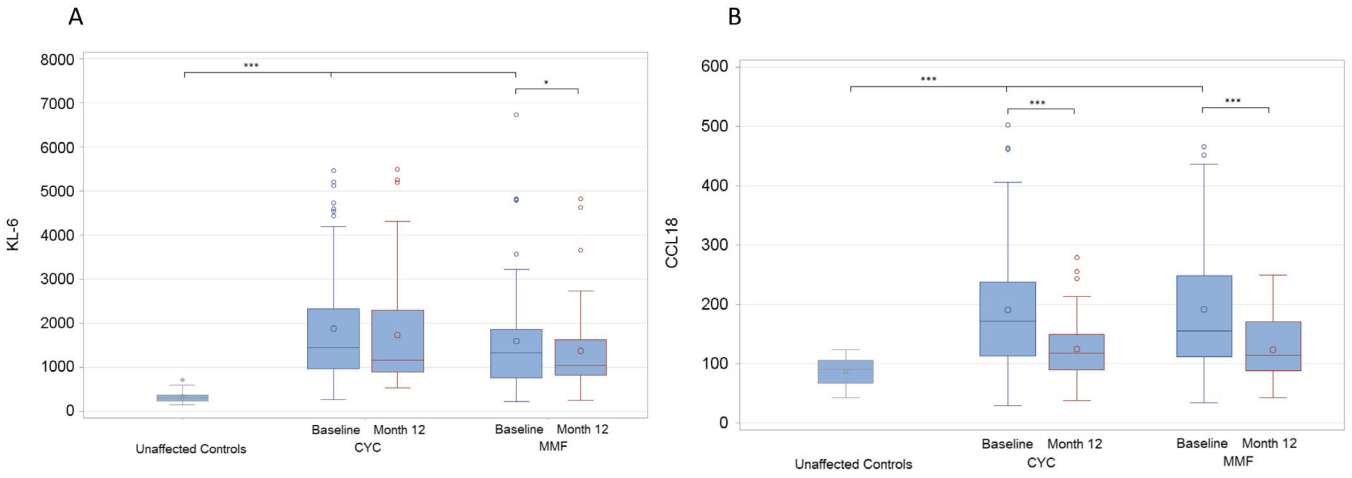


**Figure 1.** Comparison of Krebs von den Lungen 6 (KL-6) (**A**) and CCL18 (**B**) levels from baseline to 12 months during treatment with cyclophosphamide (CYC) or mycophenolate mofetil (MMF) for 1 year in the Scleroderma Lung Study II. Values for KL-6 are shown as units/ml. Values for CCL18 are shown as ng/ml. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines inside the boxes represent the median. Lines outside the boxes represent the maximum and minimum range. Circles indicate outliers. * = $P < 0.05$, *** = $P < 0.001$.

KL-6 level was associated with increased progression of ILD as measured by the course of the DLco in the MMF arm (estimate −0.46; $P = 0.030$), but not in the CYC arm (estimate −0.034; $P = 0.720$) (Supplementary Table 4, http://onlinelibrary.wiley.com/doi/10.1002/art.41020/abstract).

**Table 3.** Prediction of progression of ILD by high baseline KL-6 level, based on the course of the FVC and DLco over 1 year in patients randomized to receive CYC or MMF*

| Variable | Estimate | 95% CI | *P* |
|---|---|---|---|
| Outcome: course of FVC over 12 months in CYC arm | | | |
| Intercept | 9.99 | 7.02, 12.26 | 0.001 |
| KL-6 | −0.32 | −0.50, −0.11 | 0.024 |
| Baseline FVC | 0.88 | 0.87, 0.90 | <0.001 |
| Time | 0.10 | 0.028, 0.17 | 0.004 |
| Outcome: course of FVC over 12 months in MMF arm | | | |
| Intercept | 16.92 | 12.12, 21.99 | <0.001 |
| KL-6 | −0.72 | −1.03, −0.32, | 0.005 |
| Baseline FVC | 0.85 | 0.80, 0.89 | <0.001 |
| Time | 0.054 | −0.015, 0.12 | 0.128 |
| Outcome: course of DLco over 12 months in CYC arm | | | |
| Intercept | 18.11 | 14.63, 20.60 | <0.001 |
| KL-6 | −1.30 | −1.51, −1.00 | <0.001 |
| Baseline DLco | 0.85 | 0.84, 0.87 | <0.001 |
| Time | −0.024 | −0.12, 0.076 | 0.634 |
| Outcome: course of DLco over 12 months in MMF arm | | | |
| Intercept | 23.80 | 17.42, 26.21 | 0.001 |
| KL-6 | −1.28 | −1.46, −0.84 | 0.004 |
| Baseline DLco | 0.80 | 0.78, 0.84 | <0.001 |
| Time | 0.030 | −0.051, 0.11 | 0.428 |

* ILD = interstitial lung disease; KL-6 = Krebs von den Lungen 6; FVC = forced vital capacity; DLco = diffusing capacity for carbon monoxide; CYC = cyclophosphamide; MMF = mycophenolate mofetil; 95% CI = 95% confidence interval.

The results of the ROC analysis demonstrated that a KL-6 level of >1,549 units/ml in the MMF arm was associated with an increased risk of progression using both definitions of ILD worsening. The sensitivity and specificity were 100% and 74%, respectively, when we used the definition of FVC decline of −5% or more. The sensitivity and specificity were 100% and 71%, respectively, when we used the definition of FVC decline of −10% or more OR FVC decline between −5% and −9% accompanied by a DLco decline of −15% or more. We were unable to identify a threshold for KL-6 with an adequate sensitivity and specificity in the CYC arm (Supplementary Figure 1, http://onlinelibrary.wiley.com/doi/10.1002/art.41020/abstract).

**Prediction of SSc-related ILD progression by baseline CCL18 levels.** Higher baseline CCL18 levels predicted progression of ILD as measured by the course of the FVC (estimate −1.24 for CYC [$P < 0.001$] and −0.35 for MMF [$P = 0.007$]) and DLco (estimate −1.87 for CYC [$P = 0.001$] and −1.26 for MMF [$P < 0.001$]) over 1 year for both treatment arms, even after adjustment for baseline disease severity (Table 4). After dichotomizing the CCL18 variable based on the median level in baseline SLS II samples (163.1 ng/ml), a high baseline CCL18 level was associated with increased progression of ILD as measured by the course of the FVC both in the MMF arm (estimate −0.61; $P = 0.039$) and in the CYC arm (estimate −0.01; $P = 0.010$) (Supplementary Table 3). High baseline CCL18 level was associated with increased progression of ILD as measured by the course of the DLco in the MMF arm (estimate −0.94; $P < 0.001$) and in the CYC arm (estimate −2.13; $P < 0.001$) (Supplementary Table 4). The ROC analysis failed to reveal a significant CCL18 threshold for predicting ILD progression in either treatment arm with an adequate sensitivity and specificity (Supplementary

**Table 4.** Prediction of progression of ILD by high baseline CCL18 level, based on the course of the FVC and DLco over 1 year in patients randomized to receive CYC or MMF*

| Variable | Estimate | 95% CI | *P* |
|---|---|---|---|
| Outcome: course of FVC over 12 months in CYC arm | | | |
| Intercept | 13.80 | 11.92, 15.65 | <0.001 |
| CCL18 | −1.24 | −1.46, −1.03 | <0.001 |
| Baseline FVC | 0.91 | 0.90, 0.93 | <0.001 |
| Time | 0.10 | 0.025, 0.17 | 0.012 |
| Outcome: course of FVC over 12 months in MMF arm | | | |
| Intercept | 10.21 | 8.34, 11.89 | <0.001 |
| CCL18 | −0.35 | −0.52, −0.16 | 0.007 |
| Baseline FVC | 0.88 | 0.86, 0.90 | <0.001 |
| Time | 0.057 | −0.014, 0.13 | 0.114 |
| Outcome: course of DLco over 12 months in CYC arm | | | |
| Intercept | 16.91 | 12.16, 19.61 | <0.001 |
| CCL18 | −1.87 | −2.17, −1.19 | 0.001 |
| Baseline DLco | 0.87 | 0.84, 0.89 | <0.001 |
| Time | −0.020 | −0.11, 0.065 | 0.642 |
| Outcome: course of DLco over 12 months in MMF arm | | | |
| Intercept | 17.36 | 14.39, 19.45 | <0.001 |
| CCL18 | −1.26 | −1.49, −0.92 | <0.001 |
| Baseline DLco | 0.85 | 0.82, 0.87 | <0.001 |
| Time | 0.040 | −0.040, 0.12 | 0.327 |

* ILD = interstitial lung disease; FVC = forced vital capacity; DLco = diffusing capacity for carbon monoxide; CYC = cyclophosphamide; MMF = mycophenolate mofetil; 95% CI = 95% confidence interval.

Figure 2, http://onlinelibrary.wiley.com/doi/10.1002/art.41020/abstract).

**Prediction of long-term survival in SSc-related ILD by CCL18, but not KL-6.** Data from the SLS II long-term follow-up study (37) were used to explore whether baseline KL-6 or CCL18 predicted long-term survival in patients with SSc-related ILD. At the time of this analysis, 30 (16 CYC, 14 MMF) of 142 SLS II participants (21%) had died within 8 years after the first patient was randomized. The median follow-up time for all patients was 4 years. The majority of deaths in both cohorts were due to respiratory failure from underlying SSc (n = 16) (37).

The Cox proportional hazards model analysis demonstrated that SLS II participants with increased CCL18 at baseline had an increased risk of mortality due to respiratory failure even after controlling for baseline disease severity in the CYC arm (hazard ratio [HR] 3.09, $P = 0.018$) but not in the MMF arm. Baseline KL-6 level was not associated with mortality due to respiratory failure in either treatment arm.

Similarly, baseline CCL18 level was associated with mortality due to all causes (HR 3.31, $P = 0.0008$) in the CYC arm, but not the MMF arm. Patients with high CCL18 level based on the median had an increased risk of mortality in the CYC arm ($P = 0.006$ by log rank test) but not in the MMF arm. Baseline KL-6 level was not associated with all-cause mortality in either treatment arm. High KL-6 level based on the median was not associated with an increased risk of mortality in the CYC arm or in the MMF arm. For details on the above analyses, see Supplementary Tables 5–8 and Supplementary Figures 3–6, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.41020/abstract.

## DISCUSSION

To our knowledge, this is the first study to evaluate the relationship between plasma levels of KL-6 and CCL18 and progression of ILD in the context of a relatively large RCT for SSc-related ILD. Elevated levels of both KL-6 and CCL18 at baseline predicted poor response to immunosuppressive therapy with either CYC or MMF.

At baseline, both KL-6 and CCL18 levels each correlated with surrogate measures of ILD severity, including extent of radiographic fibrosis (KL-6 and CCL18), and % predicted TLC (KL-6) and DLco (KL-6). These findings are consistent with those from our previous study of patients who participated in SLS I (CYC versus placebo), in which baseline KL-6 levels correlated with the extent of radiographic fibrosis and with the DLco (23). In contrast, neither KL-6 (SLS I and II) nor CCL18 (SLS II) levels were associated with the baseline % predicted FVC. While the severity of SSc-related ILD is often defined by the degree of ventilatory restriction (i.e., % predicted FVC), all pulmonary function test parameters are indirect and variable measures of the extent of structural lung disease. This may explain why these peripheral pneumoproteins correlate more strongly with the extent of radiographic fibrosis as measured by quantitative computer-aided diagnostic techniques.

KL-6 and CCL18 levels decreased in response to treatment with CYC and MMF for 1 year, although the magnitude of the decline was greater for CCL18 than for KL-6. This may be due to the fact that CCL18 is secreted by type 2 macrophages, whereas KL-6 is excreted by type II pneumocytes. Macrophages as inflammatory cells would be more likely to decrease their activity in response to immunosuppressive treatment than type II pneumocytes, which are epithelial in origin. For both pneumoproteins, patients assigned to receive MMF experienced the greatest decline in CCL18 and KL-6 levels. This discrepancy could have been due to several factors. As reported previously (5), MMF was better tolerated than CYC in patients in SLS II; thus, patients may have had better adherence to therapy with MMF than CYC and were more likely to achieve and maintain the target treatment dose. Another possibility is that MMF targets pathways involving KL-6 and CCL18 with greater potency than CYC. In SLS II, no difference was noted in the course of the FVC over 2 years between patients assigned to receive MMF versus CYC; however, there was a difference in the course of the DLco, favoring MMF (5). More research is needed to further explore why KL-6 and CCL18 levels declined to a greater degree in response to MMF than to CYC treatment.

Even after adjustment for baseline disease severity, higher levels of KL-6 and CCL18 predicted progression (worsening) of ILD in each of the 2 SLS II treatment arms. We opted to examine treatment arms separately since MMF and CYC have markedly different mechanisms of action; however, even in the combined cohort, both baseline KL-6 and CCL18 levels predicted progression of ILD, as measured by the course of the DLco and FVC over 1 year (results available upon request from the corresponding author).

The finding that high baseline KL-6 and CCL18 levels predicted progression of ILD even after adjustment for baseline disease severity suggests that these 2 pneumoproteins could be used to identify patients with a more aggressive ILD phenotype. Despite treatment with MMF, patients with high baseline KL-6 and CCL18 levels experienced a decline in their FVC and DLco over 12 months. Among patients assigned to receive CYC, those who had high baseline CCL18 levels, but not KL-6 levels, also experienced a decline in their FVC and DLco over 12 months, as well as increased risk of long-term mortality. In addition to helping identify patients who may benefit from closer monitoring, KL-6 and CCL18 measurements could also be used to select patients for combination ILD therapy (2 immunosuppressants or an immunosuppressant plus an antifibrotic) or for cohort enrichment to identify patients who may be eligible for clinical trials investigating other novel therapies for progressive SSc-related ILD.

We attempted to identify a threshold for KL-6 and CCL18 for predicting worsening of ILD. We discovered that a KL-6 level of >1,549 units/ml in the MMF arm was associated with an increased risk of progression using both definitions of ILD worsening, with an excellent sensitivity and good specificity. However, we were unable to identify a threshold with adequate sensitivity and specificity in the CYC arm for KL-6, or for either treatment arm for CCL18. This may have been due to loss of power due to dichotomization of the outcome. Moreover, while we used 2 different definitions of ILD progression, there is currently no consensus on a universally accepted definition of ILD progression in SSc.

This study has some limitations. We did not include an external validation cohort. We had planned to use the SLS I cohort as an external validation cohort, but the sample size of participants who underwent KL-6 measurement in SLS I and had complete follow-up data was too small (n = 40) to perform the joint model analysis. However, the baseline correlations between KL-6 and surrogate measures of ILD severity were similar between both SLS cohorts, suggesting that our findings are likely reproducible. Moreover, we demonstrated predictive potential of both KL-6 and CCL18 in both treatment arms of SLS II, with each arm analyzed separately and 1 arm being CYC, as a means of semi-internal validation.

This study has important strengths. We evaluated ILD progression by using a joint model that included repeated measures of the FVC and DLco. Trends in the FVC and DLco determined from measurements at several time points may more accurately reflect true progression of ILD compared with changes in the FVC and DLco using measurements at only 2 time points. Indeed, our recent analysis of the long-term follow-up data from SLS I and II revealed that the course of the FVC and DLco were better predictors of long-term mortality than the baseline FVC or DLco (37).

Using data from a rigorously conducted clinical trial to study candidate biomarkers also limits potential confounding from variables, such as access to care and therapy as well as missing outcome data, that often occurs in the setting of observational studies in which patients receive varying medication regimens at baseline and subsequent visits (type, dose, and duration) and varying follow-up. Furthermore, in an exploratory analysis, we also found that high CCL18 levels at baseline were associated with an increased risk of long-term mortality due to respiratory failure. These findings substantiate previously published work linking CCL18 with progressive ILD and poor outcomes (16,17,31).

In conclusion, the present findings strongly suggest that KL-6 and CCL18 are important peripheral markers of both disease severity and disease progression in patients with SSc-related ILD. Measurement of these 2 pneumoproteins early in the course of SSc-related ILD may help to identify those patients with a more aggressive SSc-related ILD phenotype in both clinical practice and in research. Additional mechanistic studies are needed to determine precisely how KL-6 and CCL18 contribute to the pathobiology of SSc-related ILD. These additional studies may also reveal new therapeutic targets for intervention in SSc-related ILD since currently available treatment options for this often fatal condition are still limited.

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## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Volkmann had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Volkmann, Tashkin, Assassi.

**Acquisition of data.** Volkmann, Tashkin, Kuwana, Roth, Charles, Hant, Bogatkevich, Akter, Kim, Goldin, Khanna, Clements, Furst, Silver, Assassi.

**Analysis and interpretation of data.** Volkmann, Tashkin, Li, Kim, Elashoff, Assassi.

## ADDITIONAL DISCLOSURE

Author Khanna is employed by CiVi BioPharma, Inc.

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## APPENDIX A: INVESTIGATORS AND INSTITUTIONS IN THE SCLERODERMA LUNG STUDY II

Investigators and institutions who participated in the Scleroderma Lung Study II are as follows: A. C. Theodore, R. W. Simms, E. Kissin, F. Y. Cheong (University of Boston); V. D. Steen, C. A. Read Jr., C. Fridley, M. Zulmatashvili (Georgetown University, Washington, DC); R. A. Wise, F. M. Wigley, L. Hummers, G. Leatherman (Johns Hopkins University, Baltimore); R. M. Silver, C. Strange, F. N. Hant, J. Ham, K. Gibson, D. Rosson (Medical University of South Carolina, Charleston); D. P. Tashkin, R. M. Elashoff, M. D. Roth, P. J. Clements, D. Furst, E. R. Volkmann, S. Kafaja, E. Kleerup, D. Elashoff, J. Goldin, E. Ariola, G. Marlis, J. Mason-Berry, P. Saffold, M. Rodriguez, L. Guzman, J. Brook (University of California, Los Angeles); J. Golden, M. K. Connolly, A. Eller, D. Leong, M. Lalosh, J. Obata (University of California, San Francisco); S. Volkov, D. Schraufnagel, S. Arami, D. Franklin (University of Illinois, Chicago); J. Varga, J. Dematte, M. Hinchcliff, C. DeLuca, H. Donnelly, C. Marlin (Northwestern University, Chicago); D. J. Riley, V. M. Hsu, D. A. McCloskey; (University of Medicine and Dentistry of New Jersey, New Brunswick); K. Phillips, D. Khanna, F. J. Martinez, E. Schiopu, J. Konkle (University of Michigan, Ann Arbor); M. Mayes, B. Patel, S. Assassi, F. Tan (University of Texas, Houston); A. Fischer, J. Swigris, R. Meehan, K. Brown, T. Warren, M. Morrison (National Jewish Health, Denver); M. B. Scholand, T. Frecht, P. Carey, M. Villegas (University of Utah, Salt Lake City); J. Molitor, P. Carlson (University of Minnesota, Minneapolis).