Received: 27 November 2017 | Revised: 20 November 2018 | Accepted: 8 November 2018

DOI: 10.1111/1756-185X.13452

ORIGINAL ARTICLE

WILEY International Journal of Rheumatic Diseases

Serum levels of Krebs von den Lungen-6 as a promising marker for predicting occurrence and deterioration of systemic sclerosis-associated interstitial lung disease from a Chinese cohort

Xiao-yu Cao | Sha-sha Hu | Dong Xu | Meng-tao Li | Qian Wang | Yong Hou | Xiao-feng Zeng

Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College, Beijing, China

Correspondence
Yong Hou, Xiao-feng Zeng, Department of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Graduate School of Peking Union Medical College, Beijing, China.
Emails: houyongjia@hotmail.com; xiaofeng.zeng@cstar.org.cn

Funding information
The National Natural Science Foundation of China, Grant/Award Number: 81671614; Chinese Academy of Medical Sciences Initiative for Innovative Medicine, Grant/Award Number: 2017-12M-3-001; Peking Union Medical College Hospital, Grant/Award Number: 2015zlgc0110 and 2015zlgc0709

Abstract

Aim: A prospective and longitudinal study to investigate the correlations between Krebs von den Lungen-6 (KL-6) serum levels and systemic sclerosis associated with interstitial lung disease (SSc-ILD).

Method: Blood samples of baseline and the time point at 2 years follow-up intervals were collected for the measurement of serum KL-6 levels. The baseline clinical, laboratory characteristics, and incidence density of newly diagnosed ILD during the follow up were compared between SSc patients with elevated serum KL-6 levels (KL-6 > 500 U/mL) and those with normal KL-6 levels (KL-6 ≤ 500 U/mL) at baseline. Further, we explored the association between serum KL-6 and deterioration of ILD measured by lung function parameters during follow-up of 2 years.

Results: Patients with elevated baseline serum KL-6 had a significant tendency to have disappearance of the finger pad. The incidence density of new-onset ILD in SSc patients with elevated baseline serum KL-6 and those with normal baseline serum KL-6 was 1.33% and 0.51%, respectively. Among the mild lung injury group, the incidence density of ILD deterioration in SSc patients with elevated baseline serum KL-6 and those with normal serum KL-6 was 1.2% and 0.74%, respectively.

Conclusion: Serum KL-6 level correlates with the clinical manifestations of microvascular injury. Baseline elevated serum KL-6 may predict deterioration of lung function of SSc-ILD patients with mild lung injury.

KEYWORDS
biomarker, Chinese, interstitial lung disease, serum KL-6, systemic sclerosis

1 | INTRODUCTION

Systemic sclerosis (SSc) is a kind of autoimmune diseases characterized by skin thickening and fibrosis. The pathogenesis of SSc is still unknown. Many organs including the heart, lung, kidney and gastrointestinal tract can be involved. Interstitial lung disease (ILD) is a significant contributor to disease-related deaths in SSc patients.[1] Conventional methods of ILD assessment comprise pulmonary

Xiao-yu Cao and Sha-sha Hu equally contributed to this work.

function test (PFT) and chest high-resolution computerized tomography (HRCT). However, PFT needs the cooperation of patients, and the substantial radiation exposure of HRCT makes it inconvenient to be frequently pursued. These necessitate a convenient and non-invasive tool to determine existence and progression of ILD earlier in clinical practice.

In fact, as early as 2000, Kenichi Yamane et al found serum levels of Krebs von den Lungen-6 (KL-6), a kind of salivary liquefied glycoprotein mainly expressed on the surface of type II alveolar epithelial cells,[2] correlated with SSc-associated ILD (SSc-ILD).[3] The associations between serum KL-6 and the activity and severity of SSc-ILD had been explored in some previous studies.[4-6] In recent years, the value of serum KL-6 for predicting onset and prognosis of SSc-ILD has drawn more and more attention. A study identifying the role of KL-6 in predicting the deterioration in SSc-ILD was performed,[7] whereas another research found that serum KL-6 in late phase of disease (disease duration >3 years) cannot predict subsequent progression of ILD in a large cohort of SSc patients including overlap syndrome.[5]

As we know, ethnicity differences existed in the clinical manifestations and laboratory results of SSc.[8] To our best knowledge, there was only one study from a Chinese cohort retrieved in Pubmed that probed into the correlation between serum KL-6 and patients with ILD, and they confirmed the diagnosis value of KL-6 for ILD patients.[9] Our present cohort was intended to investigate the predictive and prognostic value of serum KL-6 for Chinese SSc-ILD patients.

2 | MATERIALS AND METHODS

2.1 | Patients

This study was approved by the Ethics Committee of Peking Union Medical College Hospital (PUMCH). We received approval from all patients by obtaining written informed consent. Patients who were diagnosed as SSc at Department of Rheumatology and Clinical Immunology in PUMCH between February 2009 and December 2015 were recruited. They met either the 1980 or 2013 classification criteria for SSc initiated by the American College of Rheumatology/European League Against Rheumatism,[10,11] and were older than 14 years. Patients who were lost to follow up and with overlap syndrome were excluded. There were 157 patients with SSc. Among them, 16 patients with overlap syndrome that overlapped systemic lupus erythematosus, rheumatoid arthritis, polymyositis/dermatomyositis were excluded. The remaining 141 patients made up the study cohort. Baseline serum KL-6 levels of a total of 141 patients were determined.

2.2 | Clinical data

There were 127 women (90%). Sixty patients were with diffuse cutaneous SSc (42.6%) based on the SSc classification system.[12] The diagnosis of SSc-ILD was made by the approach of combining respiratory symptoms, PFT and chest HRCT.[13] During the follow-up of SSc patients without ILD at baseline, the chest HRCT was the mainstay for diagnosis of new-onset ILD.

We defined lung deterioration of SSc-ILD as >10% decrease in percentage of the predicted forced vital capacity value (FVC%), or >15% decrease in percentage diffusion capacity for carbon monoxide value (DLCO%) at follow-up interval of 2 years.[14] Both serum levels of KL-6 and PFT were measured twice. According to FVC% and DLCO% performed at baseline, the extent of baseline lung injury was defined as grade 0 (FVC% or DLCO% >80%), grade 1 (FVC% or DLCO% 60%-80%), grade 2 (FVC% 40%-60% or DLCO% 45%-60%), grade 3 (FVC% <40% or DLCO% <45%). If the grades of FVC% and DLCO% of one patient were different, the higher grade was selected. Patients with grade 0 and grade 1 were integrated into the group of mild lung injury, and patients with grade 2 and grade 3 were incorporated into the group of moderate-severe lung injury.

2.3 | KL-6 measurement

Two hundred microliters of patients' serum samples were subpacked in a frozen storage tube and stored in the refrigerator at −80°C. Serum KL-6 concentrations were measured using a chemiluminescent enzyme immunoassay kit (FU JIREBIO INC, Tokyo, Japan), according to the manufacturer's instructions. The detection range of the assay was 50 U/mL ~10 000 U/mL.

2.4 | Statistical analysis

All statistical analyses were applied using SPSS 19.0 statistical software (SPSS Inc, Chicago, IL, USA). Number or percentage was used for categorical variables, and χ^2 test was used for comparison between groups. The continuous variables were tested by Kolmogorov-Smirnov normal distribution. Mean ± standard deviation was used for measurement data following the normal distribution, and independent sample *t* test was used to compare the difference between groups. Median was used for non-normal distribution measurement data. The 2-tailed test was applied for all tests. A value of $P < 0.05$ suggested the difference was statistically significant. Pearson correlation analysis was used for bivariate standard distribution data. Fisher exact test was used to compare the incidence density of different groups.

3 | RESULTS

3.1 | Comparison of baseline characteristics between SSc patients with elevated serum KL-6 levels and those with normal levels

According to the kit instructions and many research findings,[15-18] elevated serum KL-6 was defined if KL-6 > 500 U/mL, and normal serum KL-6 was defined if KL-6 ≤ 500 U/mL. The baseline

TABLE 1 Baseline demographic and clinical characteristics of SSc patients with elevated KL-6 levels and normal KL-6 levels

	KL-6 > 500 N = 77 N (%)/mean ± SD	KL-6 ≤ 500 N = 64 N (%)/mean ± SD	*P*
Sex, women	67 (87.0)	60 (93.8)	0.183
Type of disease, diffuse cutaneous SSc	38 (49.4)	22 (34.4)	0.073
Duration of disease course, y	7 (1-34)	5 (0.04-40)	0.740
Age at onset	39.0 ± 11.3	37.6 ± 11.8	0.479
Age at diagnosis	43.0 ± 10.9	41.0 ± 11.1	0.291
Death	3 (3.9)	4 (6.3)	0.802
Initial treatment	25 (32.5)	17 (26.6)	0.445
Smoking present/past	7 (9.1)	2 (3.1)	0.273
Skin score	7 (0-30)	5 (0-40)	0.125
Vascular involvement			
Reynaud's phenomenon	73 (94.8)	61 (95.3)	1.000
Digital ulcers	26 (33.8)	14 (21.9)	0.119
Disappearance of finger pad	27 (35.1)	12 (18.8)	0.031
Arthritis	16 (20.8)	12 (18.8)	0.764
Muscle involvement	9 (11.7)	6 (9.4)	0.657
Gastroesophageal reflux	36 (46.8)	23 (35.9)	0.195
Esophageal involvement	45 (8.4)	30 (46.9)	0.171
Gastric involvement	23 (29.9)	15 (23.4)	0.391
Intestinal involvement	11 (14.3)	13 (20.3)	0.343
Respiratory symptoms			
Shortness of breath	53 (68.8)	25 (39.1)	0.000
Cough	30 (39.0)	7 (10.9)	0.000
Pulmonary artery hypertension	5 (8.2)	3 (5.5)	0.830
Renal crisis	0	3 (4.7)	0.182
Cardiac involvement			
Arrhythmia	3 (4.5)	0	0.283
Myocardial lesions	0	1	-
Diastolic dysfunction of left ventricle	12 (17.9)	4 (6.7)	0.057
Pericardial effusion	6 (9.0)	10 (16.7)	0.191
Valve disease	17 (25.4)	9 (15.0)	0.148
Lung function			
TLC%	82.1 ± 18.4	95.1 ± 17.3	0.000
DLCO%	59.1 ± 16.4	73.5 ± 18.6	0.000
FEV1%	81.5 ± 15.2	90.0 ± 13.6	0.001

(Continues)

TABLE 1 (Continued)

	KL-6 > 500 N = 77 N (%)/mean ± SD	KL-6 ≤ 500 N = 64 N (%)/mean ± SD	*P*
FVC%	76.1 ± 18.2	88.7 ± 14.2	0.000
Increase in erythocyte sedimentation rate	24 (35.3)	12 (22.2)	0.116
Antinuclear antibody (+)	61 (98.4)	53 (96.4)	0.916
Anti-Scl-70 (+)	38 (61.3)	24 (44.4)	0.070
Anticentromere antibody (+)	6 (7.7)	10 (23.3)	0.033

DLCO%, percentage of the predicted diffusion capacity for carbon monoxide value; FEV_1%, percentage of the predicted forced expiratory volume value in 1 s; FVC%, percentage of the predicted forced vital capacity value; KL-6, Krebs von den Lungen-6; SSc, systemic sclerosis; TLC%, percentage of the predicted total lung capacity value.

characteristics of SSc patients with different serum KL-6 levels were compared in Table 1. As shown in Table 1, SSc patients with elevated serum KL-6 levels were more likely to have respiratory symptoms (such as shortness of breath after activity, cough) than those with normal serum KL-6 levels (68.8% vs 39.1%, *P* = 0.000; 39.0% vs 10.9%, *P* = 0.000, respectively). Incidence rates of disappearance of the finger pad were higher in patients with elevated serum KL-6 levels than in those with normal serum KL-6 levels (35.1% vs 18.8%, *P* = 0.031). Anticentromere antibodies (ACA) were found in a smaller proportion of SSc patients with elevated

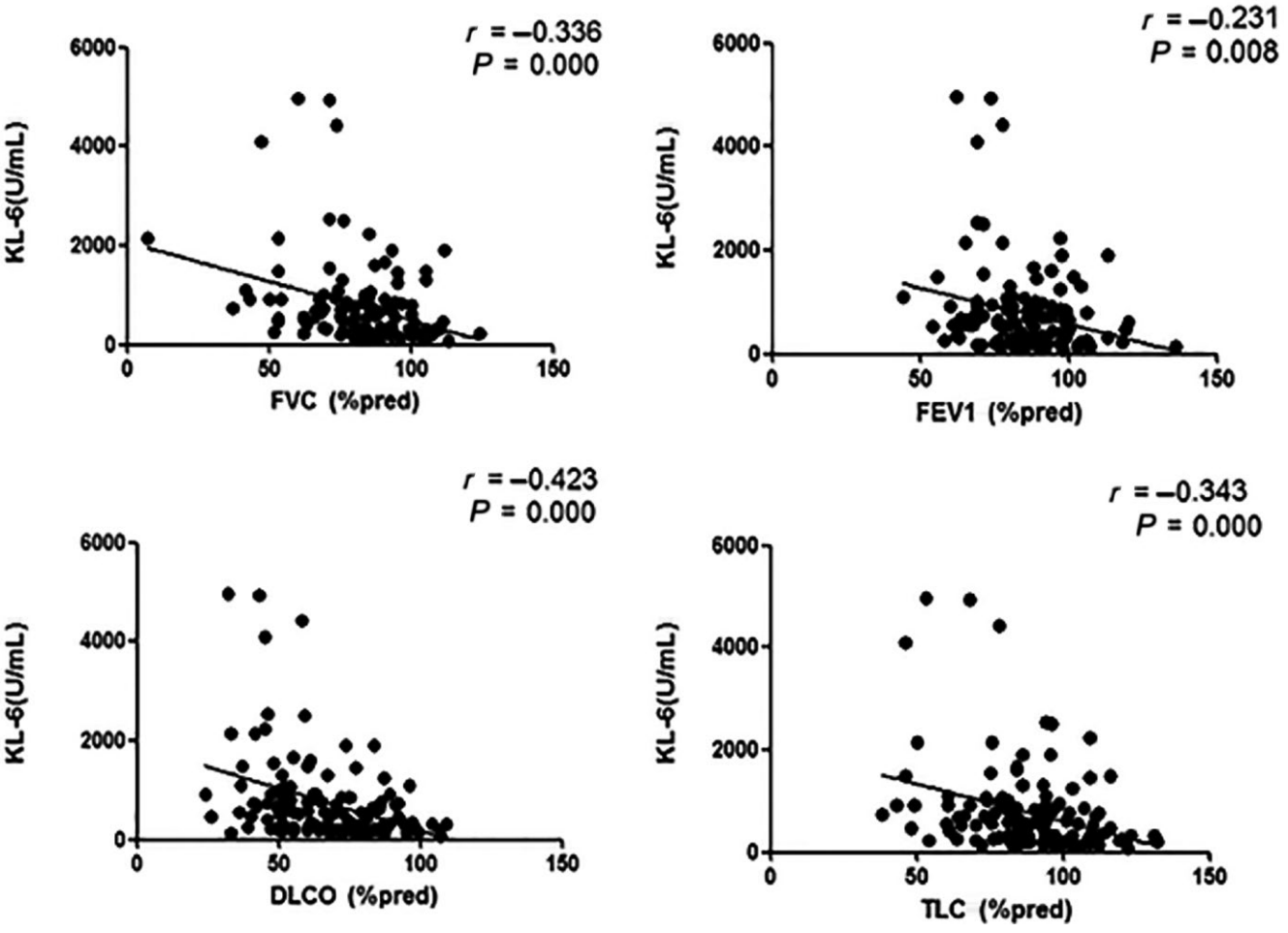

FIGURE 1 Analysis of correlation between serum Krebs von den Lungen-6 (KL-6) levels and pulmonary function test (PFT) parameters

KL-6 levels (7.7%) than those with normal KL-6 levels (23.3%), and the difference was statistically significant ($P = 0.033$). There was no statistically significant difference in skin score between patients with elevated KL-6 levels and patients with normal serum KL-6 levels (7 vs 5, $P = 0.125$).

Pulmonary function test parameters were significantly decreased in patients with elevated KL-6 levels than patients with normal serum KL-6 levels (percentage of the predicted total lung capacity value [TLC%] 82.1 ± 18.4 vs 95.1 ± 17.3, $P = 0.000$; DLCO% 59.1 ± 16.4 vs 73.5 ± 18.6, $P = 0.000$; percentage of the predicted forced expiratory volume value in 1 second [FEV_1%] 81.5 ± 15.2 vs 90.0 ± 13.6, $P = 0.001$; FVC% 76.1 ± 18.2 vs 88.7 ± 14.2, $P = 0.000$), especially the value of DLCO% related to lung diffusing capacity decreased obviously compared with the normal range. In detail, the correlations between PFT parameters and KL-6 levels of 141 patients were displayed intuitively in the form of a scatter plot (Figure 1). We can see that KL-6 levels were inversely associated with PFT parameters to a degree, and the association was statistically significant by Spearman correlation test, and KL-6 levels were inversely correlated with DLCO%, FVC% and TLC% to a low degree.

3.2 | Exploration of the suitable KL-6 cut-off level for Chinese based on ROC analysis

The cut-off value of baseline serum KL-6 levels to identify SSc-ILD and SSc non-ILD was 352 U/mL, which was determined by receiver operating characteristic (ROC) curve. Its sensitivity was 74.8%, and specificity was 90%. The area under the ROC curve was 0.858 (Figure 2, $P = 0.000$, 95% CI, 0.791-0.924).

3.3 | Validation of the value of KL-6 in the diagnosis of SSc-ILD

Among the 141 patients, 42 patients (29.8%) did not receive any previous treatment; 111 patients had SSc-ILD, and 30 patients had SSc non-ILD. Baseline serum levels of KL-6 of 141 patients were determined. Logarithm values of serum KL-6 levels of SSc-ILD patients were significantly higher than that of SSc non-ILD patients by *t* test (Figure 3, 2.81 ± 0.35 vs 2.35 ± 0.23, $P = 0.000$).

3.4 | Preliminary exploration of the predictive value of baseline serum KL-6 levels for newly diagnosed ILD

Thirty out of 141 patients were SSc non-ILD at baseline, then the 30 patients were followed up. Four patients met the exclusive criteria. Newly diagnosed ILD occurred in eight patients during the follow-up period. There was no significant difference in the logarithm value of baseline KL-6 levels between patients with recently diagnosed ILD and those without newly diagnosed ILD (2.43 ± 0.30 vs 2.32 ± 0.20, $P = 0.252$), although baseline KL-6 levels of newly diagnosed ILD patients were higher.

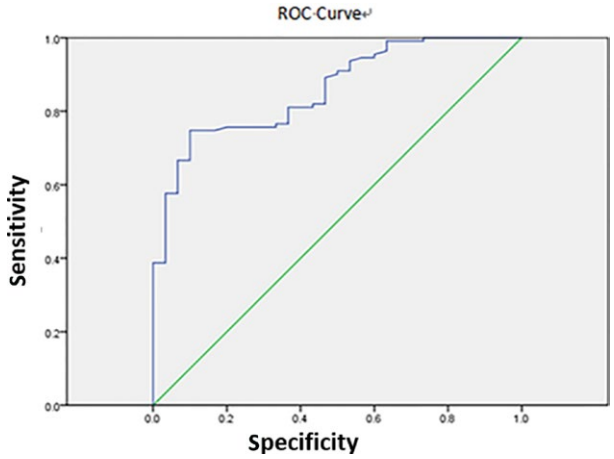

FIGURE 2 Receiver-operating characteristic (ROC) curve was used to determine the optimal Krebs von den Lungen-6 (KL-6) cut-off value to diagnose system sclerosis with interstitial lung disease (SSc-ILD) and SSc non-ILD

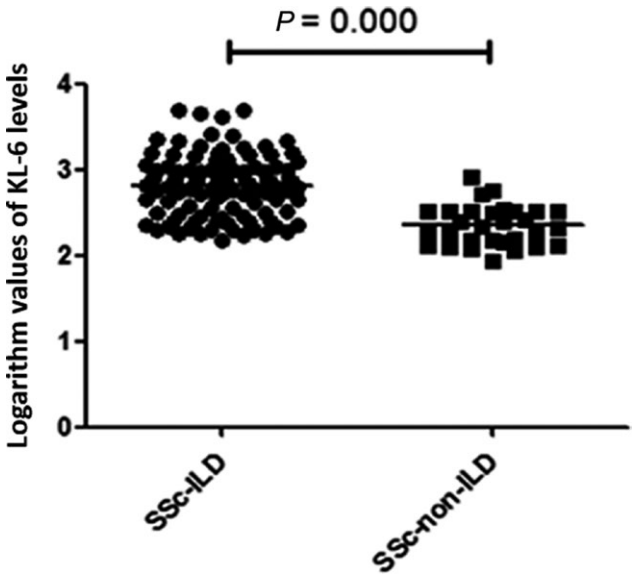

FIGURE 3 The comparison between logarithm values of serum Krebs von den Lungen-6 (KL-6) levels in system sclerosis with interstitial lung disease (SSc-ILD) and SSc non-ILD patients by *t* test

During the follow-up period of 150 persons per month, two of three patients with baseline elevated serum levels of KL-6 had newly diagnosed ILD. Then the incidence density of ILD in patients with baseline raised serum levels of KL-6 was 2/150, that is 1.33%. In contrast, during the follow-up period of 1166 persons per month, six of 23 patients with baseline normal serum levels of KL-6 had newly diagnosed ILD, and the incidence density of ILD in patients with baseline normal serum levels of KL-6 was 0.51%. The incidence difference of ILD development between patients with normal KL-6 levels and those with elevated KL-6 levels at baseline was not statistically significant (1.33% vs 0.51%, $P = 0.229$).

TABLE 2 Grades of lung injury at baseline and mean value ± standard deviations of serum Krebs von den Lungen-6 levels of every grade

	Extent of lung injury	FVC%	DLCO%	Case-s	Mean value ± SD	ANOVA test
Grade 0		>80%	>80%	7	2.42 ± 0.38	$P_{3\text{-}0}$ = 0.002
Grade 1	Mild	60%-80%	60%-80%	14	2.62 ± 0.36	$P_{3\text{-}1}$ = 0.008
Grade 2		40%-60%	45%-60%	10	2.74 ± 0.43	$P_{3\text{-}2}$ = 0.046
Grade 3	Moderate-severe	<40%	<45%	5	3.18 ± 0.34	

ANOVA, analysis of variance test; DLCO%, percentage of the predicted diffusion capacity for carbon monoxide value; FVC%, percentage of the predicted forced vital capacity value.

3.5 | Correlation between serum KL-6 levels and deterioration of pulmonary function during the follow-up interval of 2 years

Thirty-six patients with SSc-ILD from the cohort were included based on complete follow-up data and two serum samples, which were preserved for measurement of serum KL-6 levels before and after the follow-up period of 2 years. During the follow up, six out of 36 patients had lung deterioration, that is, DLCO% was decreased by more than 15% in five patients, and FVC% was reduced by more than 10% in one patient. Mean logarithm value of baseline serum KL-6 levels were not statistically different if we compared these data in patients with lung deterioration and those without lung deterioration (2.49 ± 0.46 vs 2.74 ± 0.42, P = 0.207).

The extent of lung injury of 36 patients with SSc-ILD at baseline was graded, as shown in Table 2. It can be seen that with the aggravation of degree of lung injury, the levels of KL-6 were accordingly increased. Only the difference in the KL-6 levels between patients with grade 3 and those with other classes was statistically significant.

According to FVC% and DLCO% levels of PFT patients performed at first measurement, 36 patients were divided into mild lung injury group and moderate-severe lung injury group. Then we calculated occurrence density of lung deterioration of intragroup patients with different KL-6 levels at baseline. Twenty-one patients were in the mild lung injury group. Among these, three of eight patients with elevated serum levels of KL-6 at baseline had lung deterioration, that is, DLCO% was decreased by more than 10% after follow up for 250 person-months. Then the occurrence density of lung deterioration was 1.2%. In contrast, in the mild lung injury group, three of the remaining 13 patients with normal serum levels of KL-6 at baseline had lung deterioration after follow up for 404 person-months, and the occurrence density of lung deterioration was 0.74%, whereas the occurrence density of ILD deterioration between patients with elevated KL-6 levels and those with normal KL-6 levels at baseline was not statistically significant (1.2% vs 0.74%, P = 0.68). Fifteen patients were in the moderate-severe lung injury group. Among them, 11 patients had elevated serum levels of KL-6, and four patients had normal serum levels of KL-6 at baseline. After follow-up for 399 person-months, no one developed lung deterioration.

We found that among SSc-ILD patients with mild lung injury, patients with elevated serum levels of KL-6 at baseline had a greater possibility to develop lung deterioration than those with normal serum levels of KL-6 after follow up for 2 years. However, among the moderate-severe lung injury group, it was still not clear whether serum levels of KL-6 could predict lung deterioration after monitoring for 2 years.

We also used the Pearson correlation test to investigate the correlation between △KL-6 (ie the difference between the last serum level of KL-6 and the first serum level of KL-6) and changes of PFT parameters during the follow-up of 2 years (Figure 4). There were no apparent correlations between △KL-6 and △FVC%, △DLCO%. △KL-6 was inversely correlated with △TLC to a low degree, and △KL-6 was positively associated with △FEV_1% to a low degree.

4 | DISCUSSION

We found elevated KL-6 levels had high correlations with the disappearance of finger pad, which was the clinical manifestation of microvascular injury. As far as we know, our study was the first to propose the association between elevated serum KL-6 levels and microvascular injury of extrapulmonary tissue in SSc patients. Different from the serum KL-6 correlated with the modified Rodnan skin scores reported by Bonella et al,[19] the correlation between skin scores and serum KL-6 levels was not statistically significant in our cohort. Suliman et al[20] found that ACA less frequently occurred in SSc patients with the manifestations of fibrosis on chest HRCT than those with normal HRCT, which can explain that patients with elevated KL-6 levels were more likely to have lower ACA positive rates in our cohort.

Our research found that KL-6 levels were negatively correlated with TLC%, FVC%, and DLCO%. Previous studies only indicated that KL-6 levels were inversely related with FVC% or DLCO%.[3,15,16,19] Many researchers had demonstrated that serum KL-6 levels of patients with SSc-ILD were significantly higher than those of SSc non-ILD patients.[3,16] Our study was in agreement with that, and further confirmed the diagnostic value of serum KL-6 for SSc-ILD.

We speculated baseline serum KL-6 levels had some extent of predictive value for newly diagnosed ILD. Thirty SSc patients did not have ILD at baseline, and eight patients had new-onset ILD during follow up. Although there were no significant differences, a clinical trend was observed in our study which deserved deeper investigation.

Yanaba et al continuously monitored serum KL-6 levels and pulmonary fibrosis (PF) lesions of 39 patients with SSc in a retrospective

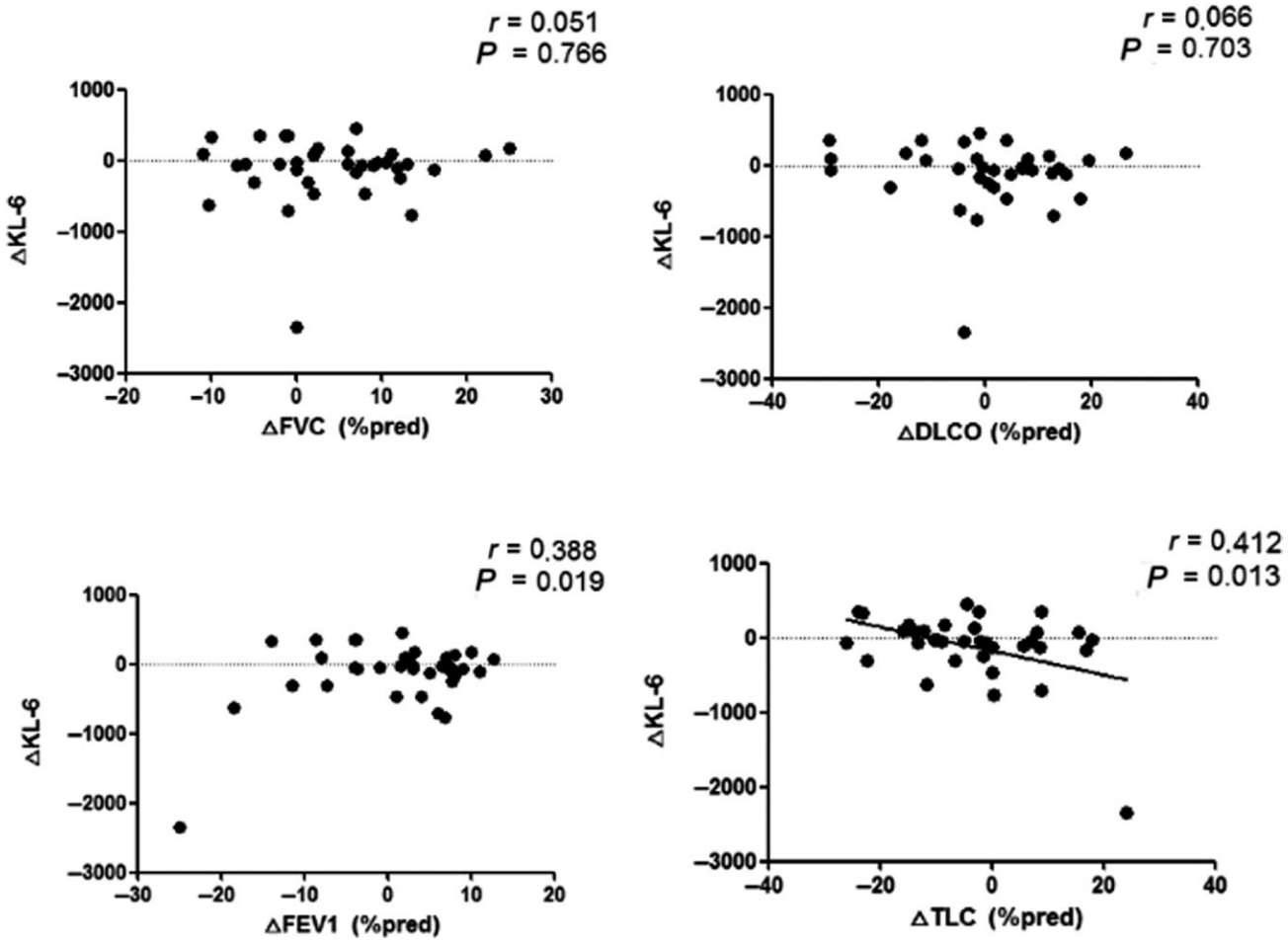

FIGURE 4 Analysis of correlation between △KL-6 (ie the difference between the last serum level of Krebs von den Lungen-6 [KL-6] and the first serum level of KL-6) and changes of pulmonary function test (PFT) parameters

longitudinal study. They found that KL-6 levels of patients with positive anti-Scl-70 antibodies elevated rapidly, and the increased trend was consistent with the trend of progression of PF. However, serum KL-6 levels of PF patients with stable lung lesions always steadily elevated. Thirty-one patients with normal serum KL-6 levels throughout did not have deterioration of pulmonary lesions or new-onset ILD.[21] So we continuously monitored serum KL-6 levels and speculated elevated KL-6 levels might be correlated with deterioration of ILD. In our study, serum KL-6 levels before and after the follow-up intervals of 2 years were determined twice, and parameters of PFT performed near the time of collecting serum were used to reflect the condition of lung involvement at that time. Patient stratification was based on the different degrees of lung involvement at the time of first follow up, and we found, among SSc-ILD patients with mild lung injury, patients with elevated serum KL-6 levels were more likely to develop lung deterioration after monitoring for 2 years. However, among the moderate-severe lung injury group, we did not find the prediction effect of KL-6 for lung deterioration after follow-up for 2 years.

Median course from disease onset to the first determination of serum KL-6 levels was 57 months in the moderate-severe lung injury group, compared to 40 months in the mild lung injury group. The past study showed that the process of interstitial lung injury was more active in the early stage of the disease.[22] Mean disease course of patients in the moderate-severe lung injury group was longer, and lung lesions were already somewhat stable. Therefore , the risk of ILD deterioration was reduced. In the group of mild lung injury patients, who were with shorter disease course, the predictive effect of KL-6 on the progression of ILD was more pronounced. Yamakawa et al[6] demonstrated that there was a significant correlation between the changes in serum levels of KL-6 and the changes in FVC at a median follow-up interval of 1.9 years in a small size of patients with SSc/mixed connective tissue disease-associated ILD. Unfortunately, although we found there was a lower degree of correlation between the changes of KL-6 levels and the changes of TLC% during the follow-up of 2 years, we did not confirm the relationship between changes in KL-6 levels and alterations in FVC% and DLCO%.

There are some limitations to our study. First, our sample cohort was small. Larger groups investigating the role of KL-6 levels in assessment of SSc-ILD patients are warranted. Second, in the study that investigated the predictive value of serum KL-6 levels for ILD deterioration, the follow-up time was not long enough.

In summary, we found elevated KL-6 levels were significantly correlated with reduction of PFT parameters, lower ACA positive rate, and the clinical manifestation of microvascular injury (disappearance

of the finger pad). Our study validated the diagnostic value of serum KL-6 levels for SSc-ILD and confirmed that patients with elevated baseline serum KL-6 levels were more likely to have newly diagnosed ILD during the follow-up period. Among SSc-ILD patients with mild lung injury, elevated serum KL-6 levels at baseline were to some extent predictive of ILD deterioration.

ACKNOWLEDGEMENTS

Dr. Yong Hou is funded by grants from the National Natural Science Foundation of China (81671614, the website: www.nsfc.gov.cn/), Chinese Academy of Medical Sciences Initiative for Innovative Medicine 2017-12M-3-001, and Peking Union Medical College Hospital 2015zlgc0110, 2015zlgc0709.

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Study design: CXY, HSS, XD, LMT, WQ, HY, ZXF. Acquisition of data: CXY, HSS. Analysis of data: XD, LMT, WQ, HY, ZXF. Draft the manuscript: CXY, HSS. Final approval of the paper and responsibility for all aspects of the article: all authors.

ORCID

Xiao-yu Cao https://orcid.org/0000-0002-3918-7146

REFERENCES

1. Tyndall AJ, Bannert B, Vonk M, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis*. 2010;69(10):1809-1815.
2. Kohno N, Akiyama M, Kyoizumi S, et al. Detection of soluble tumor-associated antigens in sera and effusions using novel monoclonal antibodies, KL-3 and KL-6, against lung adenocarcinoma. *Jpn J Clin Oncol*. 1988;18(3):203-216.
3. Yamane K, Ihn H, Kubo M, et al. Serum levels of KL-6 as a useful marker for evaluating pulmonary fibrosis in patients with systemic sclerosis. *J Rheumatol*. 2000;27(4):930-934.
4. Kumanovics G, Minier T, Radics J, et al. Comprehensive investigation of novel serum markers of pulmonary fibrosis associated with systemic sclerosis and dermato/polymyositis. *Clin Exp Rheumatol*. 2008;26(3):414-420.
5. Kumanovics G, Gorbe E, Minier T, et al. Follow-up of serum KL-6 lung fibrosis biomarker levels in 173 patients with systemic sclerosis. *Clin Exp Rheumatol*. 2014;32(6 Suppl 86):S-138-44.
6. Yamakawa H, Hagiwara E, Kitamura H, et al. Serum KL-6 and surfactant protein-D as monitoring and predictive markers of interstitial lung disease in patients with systemic sclerosis and mixed connective tissue disease. *J Thorac Dis*. 2017;9(2):362-371.
7. Kuwana M, Shirai Y, Takeuchi T. 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. *J Rheumatol*. 2016;43(10):1825-1831.
8. Nashid M, Khanna PP, Furst DE, et al. Gender and ethnicity differences in patients with diffuse systemic sclerosis–analysis from three large randomized clinical trials. *Rheumatology*. 2011;50(2):335-342.
9. Hu Y, Wang LS, Jin YP, et al. Serum Krebs von den Lungen-6 level as a diagnostic biomarker for interstitial lung disease in Chinese patients. *Clin Respir J*. 2017;11(3):337-345.
10. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Subcommittee for scleroderma criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. *Arthritis Rheum*. 1980;23(5):581-590.
11. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. *Ann Rheum Dis*. 2013;72(11):1747-1755.
12. LeRoy EC, Black C, Fleischmajer R, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol*. 1988;15(2):202-205.
13. Antin-Ozerkis D, Rubinowitz A, Evans J, et al. Interstitial lung disease in the connective tissue diseases. *Clin Chest Med*. 2012;33(1):123-149.
14. Goh NS, Hoyles RK, Denton CP, et al. Short-term pulmonary function trends are predictive of mortality in interstitial lung disease associated with systemic sclerosis. *Arthritis Rheumatol*. 2017;69(8):1670-1678.
15. Sato S, Nagaoka T, Hasegawa M, et al. Elevated serum KL-6 levels in patients with systemic sclerosis: association with the severity of pulmonary fibrosis. *Dermatology*. 2000;200(3):196-201.
16. Hant FN, Ludwicka-Bradley A, Wang HJ, et al. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. *J Rheumatol*. 2009;36(4):773-780.
17. Ishikawa N, Hattori N, Yokoyama A, et al. Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases. *Respir Investig*. 2012;50(1):3-13.
18. Bonella F, Costabel U. Biomarkers in connective tissue disease-associated interstitial lung disease. *Semin Respir Crit Care Med*. 2014;35(2):181-200.
19. Bonella F, Volpe A, Caramaschi P, et al. Surfactant protein D and KL-6 serum levels in systemic sclerosis: correlation with lung and systemic involvement. *Sarcoidosis Vasc Diffuse Lung Dis*. 2011;28(1):27-33.
20. Suliman YA, Dobrota R, Huscher D, et al. Brief report: pulmonary function tests: high rate of false-negative results in the early detection and screening of scleroderma-related interstitial lung disease. *Arthritis Rheumatol*. 2015;67(12):3256-3261.
21. Yanaba K, Hasegawa M, Hamaguchi Y, et al. Longitudinal analysis of serum KL-6 levels in patients with systemic sclerosis: association with the activity of pulmonary fibrosis. *Clin Exp Rheumatol*. 2003;21(4):429-436.
22. Steen VD, Conte C, Owens GR, Medsger TA Jr. Severe restrictive lung disease in systemic sclerosis. *Arthritis Rheum*. 1994;37(9):1283-1289.

How to cite this article: Cao X-Y, Hu S-S, Xu D, et al. Serum levels of Krebs von den Lungen-6 as a promising marker for predicting occurrence and deterioration of systemic sclerosis-associated interstitial lung disease from a Chinese cohort. *Int J Rheum Dis*. 2019;22:108–115. https://doi.org/10.1111/1756-185X.13452