ORIGINAL ARTICLE

Circulating Biomarkers and Disease Activity in Systemic Sclerosis-Associated Interstitial Lung Disease

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Background: Systemic sclerosis-associated interstitial lung disease (SSc-ILD) is a major cause of death in SSc patients. Several studies have reported that serum anti-Scl-70, Krebs von Lungren-6 (KL-6), and surfactant protein D (SP-D) levels are associated with the presence and progression of ILD in SSc patients.

Objective: To examine the correlation between levels of these serum biomarkers and disease severity determined by baseline dyspnea index (BDI), high-resolution computed tomography (HRCT) score, and pulmonary function tests.

Materials and Methods: The present study was a single-center, cross-sectional study. Serum anti-Scl-70, KL-6, and SP-D from 20 SSc-ILD patients and five non-ILD subjects were measured. The BDI, HRCT score, and pulmonary function tests were used to assess the severity of ILD in SSc-ILD patients. HRCT abnormalities, including ground-glass opacity (GGO), fibrosis, and honeycombing, were scored by using the semi-quantitative scoring system.

Results: Serum anti-Scl-70, KL-6, and SP-D in SSc-ILD patients were significantly higher than those in non-ILD subjects. There was a moderate correlation between diffusing capacity for carbon monoxide (DLCO) and serum KL-6 levels (r=−0.551, p=0.022), while the pulmonary fibrosis (PF) score exhibited a strong correlation with serum KL-6 levels (r=0.630, p=0.003). The PF score had a moderate negative correlation with forced vital capacity (FVC) (r=−0.515, p=0.034) and a strong negative correlation with total lung capacity (TLC) and DLCO (r=−0.625, p=0.007, and r=−0.762, p<0.001, respectively).

Conclusion: The levels of serum KL-6, and SP-D are elevated in SSc-ILD patients. Serum KL-6 may be a useful non-invasive biomarker for the disease severity, as determined by DLCO and the extent of fibrosis on HRCT, in patients with SSc-ILD.

Trial registration: Thai Clinical Trials Registry, TCTR20200314001, registered 13 March 2020, retrospectively registered at http://www.thaiclinicaltrials.org/show/TCTR20200314001

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Interstitial lung disease (ILD) is a major cause of death in systemic sclerosis (SSc), which occurred in 90% of SSc patients detected by high-resolution computed tomography (HRCT)[1]. The main challenge is to identify parameters for the measurement of disease activity and response to treatment. The most widely used activity score in SSc studies included forced vital capacity (FVC), diffusing capacity for carbon monoxide (DLCO), modified Rodnan skin score (mRSS), and erythrocyte sedimentation rate (ESR)[2-4].

Currently, there are no biomarkers that predict the presence and progression of ILD in SSc-ILD patients. Various studies demonstrated that anti-topoisomerase (anti-Scl-70) antibodies and several markers are candidate biomarkers that need further validation. Serum biomarkers aim to detect the disease at a subclinical stage to early diagnosis, monitoring, and treatment decision. Several studies have reported the use of serum biomarkers, including anti-topoisomerase (anti-Scl-70) antibody, Krebs von Lungren-6 (KL-6), and surfactant protein D (SP-D),

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to diagnoses and determine the activity of the disease.

Anti-Scl-70 antibody frequently presents in patients with diffuse SSc and is associated with the presence of ILD. Serum anti-Scl-70 is associated with the degree of pulmonary fibrosis (PF) on HRCT, pulmonary function, and mortality[5,6]. However, serum anti-Scl-70 can be increased from other organ involvement, including skin, musculoskeletal, or cardiovascular systems[7]. KL-6 and SP-D are predominantly expressed in the lungs and are associated with lung damage, fibrosis, and inflammation[8,9].

KL-6 is a high-molecular-weight mucin-like glycoprotein produced by type II alveolar epithelial cells (AEC). KL-6 has been shown to be elevated in SSc-ILD patients with active disease. SP-D is lipoprotein complexes secreted by type II AEC and Clara cells to decrease the surface tension of alveoli. Various studies have demonstrated that serum KL-6 and SP-D levels are higher in the serum of patients with SSc-ILD, compared to SSc patients without ILD and healthy subjects[10-13]. The levels of KL-6 and SP-D were potentially used to determine the prognosis and progression of SSc-ILD[13-16]. The present study aims to determine the correlation between the level of serum anti-Scl-70, KL-6, and SP-D and disease severity in patients with SSc-ILD.

Materials and Methods

The present study was a single-center, cross-sectional study. Serum samples were obtained from patients with SSc-ILD and non-ILD subjects. All SSc patients fulfilled the criteria for diagnosis of SSc proposed by the American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR). Non-ILD subjects included healthy individuals or patients without connective tissue disease, parenchymal lung disease, airway disease, and no evidence of ILD on chest computed tomography (CT). The exclusion criteria included patients younger than 18 years old, pregnant, and patients who had the following conditions, adenocarcinoma of any organs and bacterial or viral pneumonia, and patients who would not perform pulmonary function tests. The present study was approved by the Institutional Review Board, Faculty of Medicine Siriraj Hospital (Si188/2017). Informed consent was obtained from all patients before the procedure. The present study was supported by the Siriraj Research Development Fund, Faculty of Medicine, Siriraj Hospital.

Outcomes

The primary outcome included the correlation between the level of serum anti-Scl-70, KL-6, and SP-D and disease severity in patients with SSc-ILD. The secondary outcomes were to compare the levels of these serum biomarkers in SSc-ILD patients and non-ILD subjects and to determine the interobserver agreement on HRCT interpretation and grading of abnormalities between radiologists.

Clinical assessment

Clinical data and baseline dyspnea index (BDI) were collected at the time of enrollment. Pulmonary function tests, including FVC, total lung capacity (TLC), DLCO, and 6-minute walk test, were conducted within three months before enrollment.

High-resolution computed tomography

All subjects performed HRCT within three months before enrollment. All HRCT were reviewed and scored by two independent pulmonary radiologists blinded to clinical data and serum biomarker results. Disease extent was determined using the scoring system described by Goldin et al., which higher scores indicated more extensive disease[17]. HRCT abnormalities were divided into three patterns including any ground-glass opacities (GGO), PF that included thickened reticular markings, traction bronchiolectasis, and bronchiectasis, and honeycombing (HC) pattern. Each lung was divided into three zones including upper for lung apex to aortic arch, middle for aortic arch to inferior pulmonary veins, and lower for inferior pulmonary veins to lung bases. The abnormalities in each six zones were scored, using a scale from 0 to 4, with 0 for absent, 1 for 1% to 25%, 2 for 26% to 50%, 3 for 51% to 75%, and 4 for 76% to 100%. The sum of these scores in all six zones, thus between 0 and 24, made up the global score for each abnormality.

Measurement of serum biomarkers

The serum samples collected from the patients were stored at –80°C until use and subsequently analyzed in a blinded fashion about the patient's clinical status. Serum anti-Scl-70, KL-6, and SP-D were measured with specific ELISA kits (MyBioSource, USA and R&D Systems, USA) according to the manufacturer`s protocols. Ninety-six-well plates were coated with a specific monoclonal antibody. The absorbance of the color change was measured spectrophotometrically at 450 nm. All

Table 1. Baseline characteristics

	SSc-ILD	Non-ILD
Total; n	20	5
Age (years); mean±SD (range)	53±12 (26 to 73)	64±7 (52 to 71)
Female; n (%)	19 (95)	4 (80)
Non-smoker; n (%)	19 (95)	4 (80)
Diffuse disease; n (%)	20 (100)	0 (0)
BDI; mean±SD (range)	10±2 (2 to 12)	11±1 (9 to 12)
FVC (% predicted); mean±SD (range)	67.5±16.2 (35.8 to 102)	83±8.6 (83 to 102.5)
TLC (% predicted); mean±SD (range)	75.1±15.7 (45.5 to 97.7)	85±13.6 (85 to 115.9)
DLCO (% predicted); mean±SD (range)	41.6±12.1 (21 to 62)	82±4.3 (74 to 84)
6MWD (meters); mean±SD (range)	356±73 (240 to 466)	395±34 (383 to 460)

6MWD=6-minute walk distance; BDI=baseline dyspnea index; DLCO=diffusing capacity for carbon monoxide; FVC=forced vital capacity; TLC=total lung capacity; SD=standard deviation; SSc-ILD=systemic sclerosis-associated interstitial lung disease

Table 2. The levels of serum anti-Scl-70, KL-6, and SP-D in SSc-ILD patients and non-ILD subjects

Biomarkers	SSc-ILD; mean±SD	Non-ILD subjects; mean±SD	p-value	95% CI
Anti-Scl-70 (RU/mL)	106.9±64.6	<2	0.003	42.9 to 171.0
KL-6 (U/mL)	906.1±598.0	336.3±173.3	0.049	2.6 to 1187.4
SP-D (ng/mL)	806.3±629.8	211.3±49.0	0.049	2.6 to 1136.9

Anti-Scl-70=anti-topoisomerase I; CI=confidence interval; KL-6=Krebs von Lungren-6; SP-D=surfactant protein D; SD=standard deviation; SSc-ILD=systemic sclerosis-associated interstitial lung disease

assays were performed in duplicate, and results were given as the mean value.

Statistical analyses

Sample size calculation was designed to detect a correlation between levels of these serum biomarkers and lung function using a correlation coefficient of 0.6 to provide an 80% power of detecting a correlation at a 5% level of significance[9]. The number of patients needed would be 20. Continuous variables were presented as the mean or median and standard deviation (SD) and were compared between groups by using an unpaired t-test or Mann-Whitney U test. Categorical variables were presented as numbers and percentages and were compared between groups by using Fisher's exact test. The correlation between the level of serum biomarkers and disease severity was determined by using Spearman's non-parametric correlation. A coefficient between 0.40 and 0.59 was considered to indicate a moderate correlation, while a coefficient between 0.60 and 0.79 was considered to indicate a strong correlation[18]. A p-value of less than 0.05 was considered to indicate statistical significance. Interobserver agreement among the two radiologists on each HRCT abnormality was determined by intraclass correlation coefficients. All data analyses were performed using PASW Statistics for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Twenty patients with SSc-ILD and five non-ILD subjects were enrolled between May and December 2017. Most of the SSc-ILD patients were female (95%) with a mean age of 53 years as shown in Table 1. Mean FVC and DLCO in SSc-ILD patients were 67.5% and 41.6% of the predicted value, respectively. The levels of serum anti-Scl-70, KL-6, and SP-D in SSc-ILD patients were significantly higher than those in non-ILD subjects as shown in Table 2. All non-ILD patients had negative anti-Scl-70 antibody. Serum KL-6 levels were 906.1 U/mL in SSc-ILD patients compared to 336.3 U/mL in non-ILD subjects (p=0.049). Serum SP-D levels were 806.3 ng/mL in SSc-ILD patients compared to 211 ng/mL in non-ILD subjects (p=0.049).

All SSc-ILD patients had GGO and PF on HRCT with global scores of 6.7 and 7.0, respectively, as shown in Table 3. HC was found in 15% with a global score of 1.0. The correlations between serum biomarkers and disease severity determined by BDI, lung functions, and HRCT scores are shown in Table 4. There was a moderate correlation between DLCO and serum KL-6 levels (r=–0.551, p=0.022),

Table 3. HRCT scores of SSc-ILD patients

Abnormalities	n (%)	Global score mean±SD (range)
Ground-glass opacities (GGO)	20 (100)	6.7±4.7 (2 to 19)
Pulmonary fibrosis (PF)	20 (100)	7.0±2.8 (1 to 11.5)
Honeycombing (HC)	3 (15)	1.0±2.6 (0 to 9)

HRCT=high-resolution computed tomography; SD=standard deviation; SSc-ILD=systemic sclerosis-associated interstitial lung disease

Table 4. The correlation coefficients of anti-Scl-70 and serum biomarkers with the severity of SSc-ILD

Variables	Anti-Scl-70		KL-6		SP-D	
	r	p-value	r	p-value	r	p-value
BDI	0.393	0.295	0.105	0.659	–0.175	0.460
FVC	–0.238	0.570	–0.211	0.417	–0.426	0.088
TLC	–0.262	0.531	–0.372	0.142	–0.374	0.139
DLCO	0.084	0.844	–0.551	0.022	–0.291	0.258
6MWD	0.048	0.911	–0.001	0.996	–0.285	0.268
GGO	–0.235	0.542	0.289	0.217	0.378	0.100
PF	–0.254	0.509	0.630	0.003	0.330	0.155
HC	0.000	1.000	0.201	0.396	–0.098	0.681

6MWD=6-minute walk distance; BDI=baseline dyspnea index; DLCO=diffusing capacity for carbon monoxide; FVC=forced vital capacity; GGO=ground-glass opacities; HC=honeycombing; PF=pulmonary fibrosis; SSc-ILD=systemic sclerosis-associated interstitial lung disease; TLC=total lung capacity

Table 5. The correlation coefficients of HRCT score and the severity of SSc-ILD determined by BDI and pulmonary function tests

Variables	GGO score		PF score		HC score	
	r	p-value	r	p-value	r	p-value
BDI	0.315	0.176	0.076	0.751	0.219	0.354
FVC	–0.028	0.914	–0.515	0.034	–0.225	0.386
TLC	–0.109	0.678	–0.625	0.007	–0.332	0.193
DLCO	–0.362	0.153	–0.762	<0.001	–0.361	0.154
6MWD	0.099	0.706	–0.383	0.130	0.257	0.319

BDI=baseline dyspnea index; FVC=forced vital capacity; GGO=ground-glass opacities; HC=honeycombing; PF=pulmonary fibrosis; SSc-ILD=systemic sclerosis-associated interstitial lung disease

while the PF score on HRCT exhibited a strong correlation with serum KL-6 levels (r=0.630, p=0.003). The PF score had a moderate negative correlation with FVC (r=–0.515, p=0.034) and a strong negative correlation with TLC and DLCO as shown in Table 5 (r=–0.625, p=0.007, and r=–0.762, p<0.001, respectively).

Interobserver agreement for HRCT scoring was good as shown in Table 6 (intraclass correlation coefficients 0.770, 95% CI 0.51 to 0.90 and 0.981, 95% CI 0.95 to 0.99 for GGO and HC scoring, respectively), whereas the interobserver agreement for PF scoring was moderate (intraclass correlation coefficients 0.550, 95% CI 0.15 to 0.79).

Table 6. Interobserver agreement on HRCT scoring

HRCT abnormality	ICC	95% CI	p-value
GGO	0.770	0.51 to 0.90	<0.001
PF	0.550	0.15 to 0.79	0.005
HC	0.981	0.95 to 0.99	<0.001

CI=confidence interval; GGO=ground-glass opacities; HC=honeycombing; HRCT=high-resolution computed tomography; ICC=intraclass correlation; PF=pulmonary fibrosis

Discussion

Circulating biomarkers are molecules that can be measured in the blood and reflect the presence and severity of the disease. Several serum biomarkers have been studied in patients with ILD associated with various conditions, including SSc to assess disease activity and progression with variable results. Serum KL-6 and SP-D are biomarkers that have been extensively studied in ILD, including SSc-ILD. Anti-Scl-70 antibody is also associated with the presence of ILD, the degree of PF on HRCT, pulmonary function, and mortality but it can be increased from other organ involvement[5-7]. The present study results were consistent with the previous studies that showed significantly higher levels of anti-Scl-70, serum KL-6 and SP-D in SSc-ILD compared to non-ILD subjects[13,19,20]. The elevation of serum KL-6 and SP-D in SSc-ILD patients reflects alveolar damage and inflammation. Several studies demonstrated that serum KL-6 and SP-D in SSc-ILD patients were significantly higher than in subjects without ILD[12,13,21]. These findings suggest that these serum biomarkers can be a clinically useful tools for detecting ILD.

KL-6 has shown to be the strongest sensitivity and accuracy for ILD diagnosis, and SP-D also appears to be a sensitive biomarker. Serum KL-6 had high specificity, higher than 85%, and variable sensitivity, between 70% and 94%, in the diagnosis of ILD. The cut-off levels of 465 U/mL for the diagnosis of ILD had sensitivity and specificity of 93.9% and 96.3%, respectively[15]. The present study showed the association between the level of serum KL-6 and disease activity determined by DLCO and PF score, which were consistent with the previous studies[9,11,21]. These findings support the usefulness of serum KL-6 to determine the presence of ILD and

disease severity in SSc-ILD patients. However, the authors cannot demonstrate the association between the serum KL-6 and FVC, which is an important parameter to determine the severity and progression of ILD. Other than fibrosis, the previous studies also found that increased serum KL-6 was associated with the presence of alveolitis, defined by bronchoalveolar lavage (BAL) fluid cellular analysis with 3% or more neutrophils, 2% or more eosinophils, or any GGO on HRCT, but the authors cannot demonstrate these findings[13].

Serum SP-D is likely to be specific to the lung. Several studies demonstrated that serum SP-D increased in SSc-ILD patients[12,13,16,20,22]. Takahashi et al. found that serum SP-D levels had a higher sensitivity for the presence of ILD in SSc patients than SP-A levels at 77% and 33%, respectively[12]. Several studies have reported the cut-off levels of SP-D of 116 to 200 ng/mL for the presence of ILD in SSc patients with variable sensitivity and specificity[15]. The present study found that the serum SP-D level in non-ILD subjects was 211.3 ng/mL, which was higher than the cut-off levels from the previous studies, however, it was significantly lower than in SSc-ILD patients. This finding reflects that serum SP-D levels may also be elevated in other diseases or conditions. Therefore, the elevation of serum SP-D should be carefully interpreted in conjunction with clinical data and radiographic findings. In terms of disease severity and quality of life, the authors found no significant correlation between serum SP-D levels and HRCT score, pulmonary function tests, and BDI, which was consistent with the study of Honda et al.[23]. However, these findings were different from various studies that have reported that serum SP-D was correlated with the presence of GGO, PF, and HC on HRCT and decreased DLCO[12,20,24,25]. In further analysis of HRCT extent, the present study demonstrated moderate to good correlations between the PF score and FVC, TLC, and DLCO in accordance with several studies[26-28].

The usefulness of serum biomarkers besides the detection of ILD and assessment of disease severity at baseline is to determine the progression and prognosis of the disease. The authors could not conclude the utility of these serum biomarkers in this advantage because the present study did not have longitudinal data on these patients. Several studies have demonstrated that higher KL-6 and SP-D levels were associated with the progression of the disease and worse ILD progression[4,20,29,30].

Limitations exist in the present study. It is a single-center, cross-sectional study that included a small number of patients. It lacks the longitudinal data to assess the progression of the disease. A larger population is required to demonstrate the significant correlation between the levels of these serum biomarkers and lung function parameters and quality of life.

Conclusion

The levels of serum KL-6, and SP-D are elevated in SSc-ILD patients. Serum KL-6 may be a useful non-invasive biomarker for the disease severity, as determined by DLCO and the extent of fibrosis on HRCT, in patients with SSc-ILD.

What is already known on this topic?

Serum KL-6 and SP-D are elevated in SSc-ILD patients. Several studies have shown that these serum biomarkers are associated with disease activity and disease progression with variable results.

What this study adds?

The present study confirmed that serum KL-6 appears to be the useful biomarker associated with the disease severity, determining by DLCO and the extent of fibrosis on HRCT, in patients with SSc-ILD.

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Conflicts of interest

All authors declare no conflict of interest regarding the publication of the research.

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