ORIGINAL PAPER

Serum KL-6 as predictive and prognostic marker of interstitial lung disease in childhood connective tissue diseases: a pilot study

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SUMMARY

This study was aimed to evaluate serum KL-6 levels to determine if this marker can be used for diagnosing and assessing severity of interstitial lung disease (ILD) in children with connective tissue disorders.

In total, 40 patients [18 patients with juvenile systemic lupus erythematosus (JSLE), 10 patients with juvenile idiopathic arthritis (JIA), 8 patients with juvenile mixed connective tissue disease (JMCTD), 3 patients with juvenile systemic sclerosis (JSSc), and 1 patient with juvenile dermatomyositis (JDM)] and 20 healthy controls were included in this study. Age, sex, and duration of CTD and ILD (if any) were recorded. Blood samples from all the patients and controls were examined by ELISA.

20 of the 40 patients with CTD (50%) had ILD, 12 were mild and 8 were severe as assessed by spirometry. The median serum KL-6 level was 102.7 U/mL (76.1-180.8) in the CTD with severe ILD group, 72.2 U/mL (58.4-100.5) in the CTD with mild ILD group, 56.7 U/mL (35.8-68.5) in the CTD without ILD group, and 52.3 U/mL (32.8-62.4) in the control group. KL-6 levels were significantly higher in the CTD with ILD ($p<0.05$), at a cut-off of 63.4 U/ml identified by ROC curve, serum KL-6 showed a sensitivity of 95.2% and specificity of 89.7%. KL-6 is a valuable biomarker for diagnostic purposes and to detect severity in ILD in childhood CTD.

Key words: *Interstitial lung diseases, connective tissue diseases, KL-6, pulmonary function test.*

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INTRODUCTION

Juvenile rheumatoid arthritis (JRA), juvenile systemic lupus erythematosus (JSLE), juvenile systemic sclerosis (JSSc), juvenile dermatomyositis (JDM), and juvenile mixed connective tissue disease (JMCTD) are a dissimilar group of systemic inflammatory diseases characterized by the presence of circulating autoantibodies and autoimmune-mediated multiorgan system involvement (1-4). Pediatric onset of these disorders causes diagnostic stress for the clinician due to the probable years of disease burden and complications to be expected (5). The lungs are a common target in CTD, and all components of the respiratory system are at risk. Diffuse lung disease (DLD) in children with rheumatologic disease represents a pattern of pulmonary diseases as pleuritis, interstitial lung disease (ILD), thromboembolic disease, pulmonary hypertension, and pulmonary hemorrhage secondary to vasculitis. In this cases, complex care and monitoring of disease activity and progression are compulsory for life (6).

Interstitial lung disease (ILD) represents a broad group of diffuse parenchymal lung injury patterns characterized by variable degrees of inflammation and fibrosis, which is a common manifestation of CTD and is a leading cause of significant morbidity and mortality. ILD may arise at any time over the course of CTD or may be the first manifestation of disease. ILD may be subclinical (radiographic or physiologic abnormalities without symptoms), chronically progressive, or may present in a fulminant, life-

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threatening manner (7). The diagnosis of ILD necessitates meticulous evaluation of clinical, radiological, physiological, and pathological changes. Pulmonary function tests (PFTs) and radiological imaging may be used to detect ILD (8, 9). Consequently, novel noninvasive procedures for early detection are essential.

KL-6 is a high molecular weight mucin-like protein produced by alveolar type-2 pneumocytes. KL-6 is elevated in regenerated normal lung tissue, and when the air-blood barrier is disturbed in the lungs or expression of alveolar type-2 pneumocytes is increased (10-12). KL-6 is suggested as a sensitive and specific marker of the development of ILD in adults and is also increased in cases of CTD with comorbid ILD (13-16). There are few studies to determine the relationship between KL-6 and CTD-ILD in children.

Therefore, our study aims to evaluate the correlation between serum KL-6 levels with both the presence of interstitial lung disease (ILD) and its severity in childhood connective tissue diseases for early detection and better management of interstitial lung disease in CTD children.

PATIENTS AND METHODS

Study population

CTD children were selected from the medical record database between October 2019 and April 2020 from Rheumatology and Rehabilitation Department, and Pediatric department; Pulmonology, Immunology and Allergy Unit, faculty of medicine, Zagazig University. Written informed consent was obtained from all participants. The study was done according to the Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans, and approved accordance with the guidelines of the Institutional Review Board (IRB) of the Faculty of Medicine, Zagazig University, Egypt (Approval No.: ZU-IRB #544459/14-7-2019). Out of 40 CTD children, 16 were already diagnosed with interstitial lung disease, and 4 patients were newly diagnosed.

The study included 20 patients with CTD without ILD, 20 patients with CTD-ILD and 20 healthy control children attending the outpatient clinic. They all had a similar age and sex distribution. In total, the study included children with connective tissue diseases aged 5-15 years at diagnosis selected according to the classification criteria of the European League Against Rheumatism (EULAR) for rheumatoid arthritis (RA), 2010 (17), systemic lupus erythematosus (SLE), 2019 (18), dermatomyositis (DM), 2017 (19), and juvenile systemic sclerosis (SSc), 2007 (20). The selection was also based on the diagnostic criteria for mixed connective tissue disease (MCTD) from the Japan research committee of the ministry of health, labor, and welfare for systemic autoimmune diseases, 2019 (21). We excluded patients with an active malignancy, and active acute infections. History taking, general and local examinations were performed for all the children included.

Diagnostic approach to ILD in CTD children

In patients with respiratory symptoms (dry cough, rapid and/or difficult breathing, or exercise intolerance) and/or respiratory signs (tachypnea at rest, adventitious sounds, retractions, digital clubbing, failure to thrive, or respiratory failure) or even in asymptomatic patients with restrictive lung pattern on spirometry, the diagnosis of ILD required the following (22, 23):

1) exclusion of other known causes of diffuse lung disease, infection (by CRB, ESR, sputum culture), cystic fibrosis (proven by sweat chloride test), primary *vs* secondary immunodeficiency (based on complete blood count and differential WBCs count, antibodies to HIV, vaccine response), gastroesophageal reflux (barium swallow study), congenital heart disease (shown by echocardiography) (23).
2) The presence of the pattern of usual interstitial pneumonia (UIP) at High-Resolution Computed Tomography (HRCT).
3) Specific combinations of HRCT and histopathology patterns in patients subjected to lung tissue biopsy (22).

Lung HRCT

Patients were evaluated by lung HRCT. All patients were scanned from the apex to the

basis of the lung by routine end-inspiratory spiral CT with a slice thickness of 5.0 mm. High-resolution re-establishment was used to scan the patients twice for confirmation. Lung X-rays and HRCT were interpreted by both the radiologist and the pediatric pulmonologist to check whether any specific criteria of definite UIP were met, such as subpleural and basal predominant component, heterogeneous distribution, honeycombing with or without peripheral traction, or bronchiectasis).

Surgical lung biopsy

SLB was performed only for four patients who had a non-specific HRCT in spite of their clear clinical manifestations and restricted pulmonary function. In these four cases, lung biopsy was completed with a mini thoracotomy. Two large lung samples were harvested from two separate portions using a chest tube inserted prior to stitching. Then these biopsy samples were assessed by histopathological examination. The lung histology patterns observed revealed a Non-Specific Interstitial Pneumonia (NSIP) in 3 patients, and a Desquamative Interstitial Pneumonia (DIP) in one patient. NSIP is characterized by mild to moderate interstitial chronic inflammation and type 2 alveolar epithelial cells hyperplasia in inflammation areas. DIP is characterized by airspaces filled with alveolar macrophages, thickened alveolar septa, scattered mixed inflammatory cells and minimal fibrosis.

■ ASSESSMENT OF ILD SEVERITY

Spirometry

Patients underwent the PFT using a Jeager Vyntus spirometry. The following parameters were recorded: forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, expressed as a percentage of measured value/predicted value. The interpretation of PFT according to Jiang et al. (24) was adopted, whereby FVC, FEV1 >80% of predicted values was considered as normal; FVC, FEV1 <80% and >60% of predicted values was considered as mild ILD Patients, and FVC, FEV1 <60% of predicted values was considered as severe ILD Patients.

6-minute walk test

6MWT was performed in all participants according to Lancaster (25). Participants held pulse oximeters. Heart rate and oxygen saturation (SpO2) were recorded before and immediately after the 6-minute walk. To assess the grade of dyspnea, participants were asked to rate their dyspnea using the modified Borg scale by choosing a score from 0 to 10, with 0 being no significant dyspnea and 10 being highest grade of dyspnea.

Laboratory tests

Blood samples (10 mL) were drawn from all the patients and controls, and stored at –80°C after centrifugation at 1000 rpm for 15 min. KL-6 levels were calculated using ELISA (Bioneovan Co., Ltd., Beijing, China) with an assay range between 3.4 U/mL-200 U/mL.

Statistical analysis

The Kruskal-Wallis and Mann-Whitney U tests were used for the analysis of numerical independent data. Receiver operating characteristic (ROC) curves were set up to identify the cut off value for KL6 to differentiate patients with and without ILD, matching them with the Youden method (13, 26), which detects sensitivity and specificity. The following parameters were also calculated: estimation of the area under the ROC curve and its 95% confidence interval, sensitivity, specificity, positive and negative predictive values related to the cut off value. A multiple regression analysis was used to analyze dichotomous dependent variables. All statistical analyses were applied using SPSS for Windows 15 statistical software. A value of $p<0.05$ was statistically significant, a value of $p<0.001$ was highly statistically significant, and a value of $p>0.05$ was non-statistically significant.

■ RESULTS

Patient demographic and clinical characteristics

The study population consisted of 40 patients with CTD, 20 of whom had ILD (12 mild, 8

severe). Their data were compared to those of 20 healthy control subjects. In the control group the mean age was 12±2.4 years, *versus* 13±2.2 years in CTD only group and 11.9±3.5 years in CTD+ILD group. CTD duration was 3.2±2.5 in CTD only group and 2.6±1.2 in CTD+ILD group. The ILD duration was 1 year (min 0, max 3 years). A female predominance was reported in all groups (Table I). The common symptoms/signs at presentation were dyspnea (80%), cough (60%, dry with no sleep interruption), exercise limitation (60%), frequent respiratory infections (50%), wheezing (33.3%), tachypnea and chest wall retraction (10%) and clubbing (10%). Two of children with mild ILD were asymptomatic and diagnosed by HRCT (10%). The symptoms/signs severity correlated positively with severity ILD, as assessed by clinical examination.

Serum KL-6 estimation among different groups

The median serum level of KL-6 was 52.3 U/mL (min 32.8; max 62.4 U/mL) in the control group, 56.7 U/mL (min 35.8; max 68.5 U/mL) in the CTD only group, 72.2 U/mL (min 58.4; max 100.5 U/mL) in mild ILD group, and 102.7 U/mL (min 76.1; max 180.8 U/mL) in the severe ILD group. The KL-6 levels were higher in the severe ILD, intermediate in mild ILD group and lower in the control groups (Table II).

Comparison of PFT, 6MWT among the different groups

The pulmonary function test reflected a restrictive lung problem with decreased lung compliance and lung volumes. CTD only group showed a forced expiratory volume in 1 s (FEV1) (% predicted) of 101±7, forced vital capacity (FVC) (% predicted) of 95±7, the mild ILD group showed a FEV1% of 77±10, FVC% of 72±7, and the severe ILD group showed a FEV1% of 55±5, FVC% of 49±8, with significant differences between groups. In 6MWT, the mean end-test SpO2 was 96%±3 of CTD only group *vs* 92%±2, 67%±5 in mild ILD and severe ILD groups, respectively. The mean end-test dyspnea

Table I - Demographic and clinical characteristics of the study groups.

	Control group (n 20)	CTD groups		p value
		CTD only group (n 20)	CTD+ILD group (n 20)	
Age (years)	12±2.4	13±2.2	11.9±3.5	0.3847
Number of female patients	10 (50%)	12 (60%)	13 (65%)	0.09072
Duration of CTD (years)	–	3.2±2.5	2.6±1.2	0.3394
Duration of ILD (years)	–	–	1 year (0 months-3 years)	–
CTD subgroup				
JSLE	–	8 (40%)	10 (50%)	0.97036
JRA	–	5 (25%)	5 (25%)	0.97036
JDM	–	1 (5%)	0 (0%)	0.97036
JSSc	–	2 (10%)	1 (5%)	0.97036
JMCTD	–	4 (20%)	4 (20%)	0.97036
Immunosuppressive agents at the baseline visit				
prednisone > 5 mg	–	14 (70%)	16 (80%)	0.1024
methotrexate	–	7 (35%)	9 (45%)	0.1489
azathioprine	–	2 (10%)	1 (5%)	0.1794
cyclophosphamide	–	3 (15%)	3 (15%)	1

JRA, juvenile rheumatoid arthritis; JSLE, juvenile systemic lupus erythematosus; JSSc, juvenile systemic sclerosis; JDM, juvenile dermatomyositis; JMCTD, juvenile mixed connective tissue disease. One-way ANOVA, Chi-Square, and Paired sample T tests, $p>0.05$: no significant differences, $p<0.05$: significant differences, $p<0.001$: highly significant differences.

Table II - Comparison of KL-6 levels among groups.

	KL-6 levels (U/mL)		
	Min-max	Median	p value
Control group	32.8-62.4	52.3	<0.05[@,#]
CTD only group	35.8-68.5	56.7	<0.05[@,#]
Mild ILD subgroup	58.4-100.5	72.2	<0.05[*,$,#]
Severe ILD subgroup	76.1-180.8	102.7	<0.05[*,$,@]

Kruskal-Wallis and Mann-Whitney U tests. *Significant differences vs control group, $significant differences vs CTD group, @significant differences vs mild ILD group), #significant differences vs severe ILD group).

level graded with the modified Borg scale was 2±1 in the CTD only group *vs* 3±2, 4±3 in mild ILD and severe ILD groups, respectively (Table III).

Associations between serum KL-6 level and spirometry results

Figure 1 shows a significant inverse correlation between serum KL-6 levels and predicted FEV1 (%Pred) (r=–0.7056, p=0.000), and a significant inverse correlation between serum KL-6 levels and FVC (%Pred) (r=–0.7745, p=0.000).

Prediction of ILD by multiple regression analysis and ROC curve analysis

Table IV showed that the most sensitive variable to detect ILD in CTD children was the serum KL-6 level and FVC% of predicted value. The other variables were not significant in terms of ILD detection. According to the Receiver Operating Characteristic (ROC) curve analysis, the area under the curve was of 0.977 and the cut-off value of serum KL-6 to distinguish ILD was 63.4 U/mL, with sensitivity of 95.2%, specificity of 89.7%, positive predictive value of 83.26% and negative predictive value of 97.2% (Figure 2). Moreover, FVC% Receiver Operating Characteristic (ROC) curve analysis showed that the area under the curve was of 0.979 and the cut-off value of FVC% to distinguish ILD was 79, with sensitivity of 95.2%, specificity of 100%, positive predictive value of 100% and negative predictive value of 95.4% (Figure 3). The comparison between KL6 and FVC% ROC curves showed that there is no statistical difference (p=0.7385) between KL6 and FVC% ROC curves.

DISCUSSION AND CONCLUSIONS

Juvenile CTD is marked by high morbidity and mortality and can affect any organs. ILD is one of the most significant complication of CTD (27, 28). The serum KL-6 level is se-

Table III - Comparison of PFT, 6MWT among the different groups.

Variable	CTD only group (20 n.)	CTD+ILD group		p value
		Mild ILD (12 n.)	Severe ILD (8 n.)	
FVC, % predicted	95±7	72±7*	49±8*,**	0.0000
FEV1, % predicted	101±7	77±10*	55±5*,**	0.0000
[1]SpO2 at start of the test, %	98±2	95±3	88±4*,**	0.0000
[2]SpO2 at end of the test, %	97±3	92±2*	67±5*,**	0.0000
p value [(1 vs 2)]	0.2225	0.0086	0,0001	
[3]Dyspnea at start of the test	0	1±0.5*	3±1*,**	0.0000
[4]Dyspnea at end of the test	2±1	3±2	4±3*	0.0101
p value [(3 vs 4)]	0.0001	0.0028	0.3862	

One-way ANOVA, least significant difference (LSD), paired T test (before and after the test): p>0.05: no significant differences, p<0.05: significant differences, p<0.001: highly significant differences, (* *Vs* CTD only group, ** *Vs* mild ILD group).

creted normally by type II alveolar epithelium and abnormally increased in case of lung tissue regeneration (10-12). KL-6 can be considered as a sensitive and specific marker for the development of ILD (13-16). As a result of this review, our study aimed to evaluate the correlation between serum KL-6 levels with both the presence of ILD and its severity in children with connective tissue diseases to ensure early detection and better management of ILD in children with CTD.
As to the associations between serum KL-6 levels and other spirometry pulmonary function parameters, this study determined

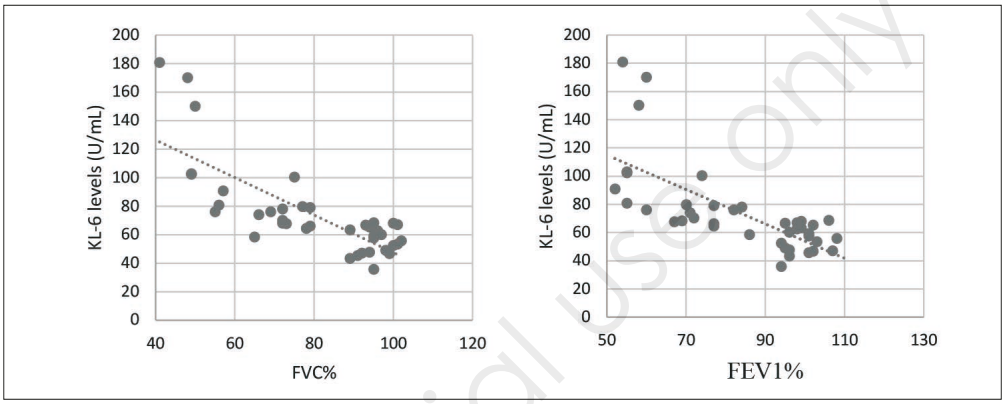

Figure 1 - Correlations between serum KL-6 levels and other PFT parameters.

Table IV - Prediction of ILD by multiple regression analysis.

Variables	Coefficient	Std. Error	t	p
Serum KL-6	0.1608	0.03694	4.354	0.0001*
FEV1% of predicted	–0.02472	0.1107	–0.223	0.8247
FVC% of predicted	0.3104	0.1313	2.363	0.0246*
Dyspnea at end of the test	0.4410	0.4961	0.889	0.3808
SpO2 at end of the test	0.1582	0.1271	1.244	0.2228

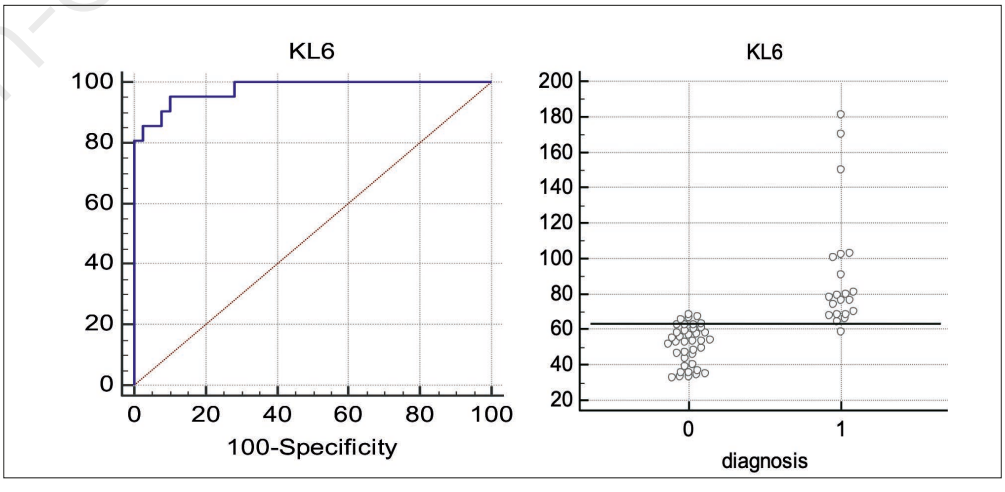

Figure 2 - KL-6 Receiver-Operating Characteristic (ROC) curve to diagnose CTD with interstitial lung disease, and the optimal cut off value (diagnosis: 0: controls and patients without ILD; 1: patients with ILD).

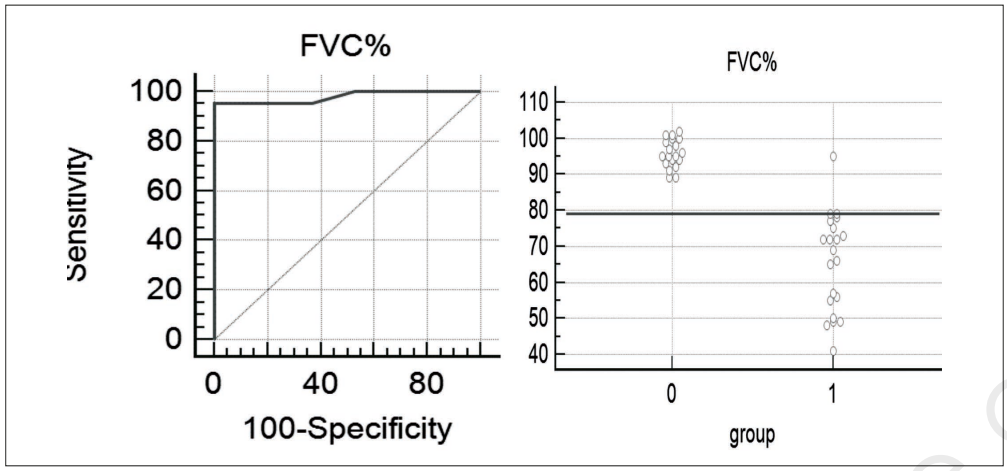

Figure 3 - FVC% Receiver-Operating Characteristic (ROC) curve to diagnose CTD with interstitial lung disease, and the optimal cut off value (diagnosis: 0: controls and patients without ILD; 1: patients with ILD).

that there is a significant inverse correlation between serum KL-6 levels and all spirometry results: predicted FEV1 (%Pred), and FVC (%Pred). This was in agreement with Fathi et al., who reported a negative correlation between serum KL-6 level and spirometry results in patients with ILD (29). In another study, Hu et al. reported that the KL-6 levels were significantly inversely correlated with PFT (12). The same result was achieved by Xue et al. in a study on ILD associated with asbestosis and silicosis; these authors stated that serum KL-6 concentration was negatively correlated with predicted FVC% (30).

Our multiple regression analysis showed that both KL-6 and FVC% can be considered as indicators of ILD. According to the Receiver Operating Characteristic Curve (ROC) analysis for KL-6, the area under the curve was of 0.977 and the optimal cut-off value of serum KL-6 to discriminate the presence of ILD was 63.4 U/mL, with sensitivity of 95.2%, specificity of 89.7%, positive predictive value of 83.26% and negative predictive value of 97.2%, while the FVC ROC curve analysis showed that the area under the curve was of 0.979 and the optimal cut-off value of serum FVC% to discriminate the presence of ILD was 79, with sensitivity of 95.2%, specificity of 100%, positive predictive value of 100% and negative predictive value of 95.4%. This matched with results achieved by Kobayashi et al. who mentioned that KL-6 could discriminate between ILD and other common lung diseases. A cut-off of 500 U/ml was established to distinguish adult patients with ILD from healthy controls and those with other lung disorders (31). Also, Zheng et al. mentioned that the area under the curve for serum KL-6 was 0.911, indicating a good diagnostic performance for idiopathic interstitial pneumonia, with sensitivity of 85.33%, specificity of 90.00%, positive predictive value of 95.52%, negative predictive value of 71.05%, and Kappa value of 0.70 (32). Furthermore, KL-6-ROC curve analysis by Lee et al. showed that 275.1 U/mL was the serum KL-6 concentration for detecting the presence of ILD in CTD patients (sensitivity 79.4%, specificity 79.9%) (33).

Pairwise comparison of ROC curves showed that there is no statistical difference between KL6 and FVC% ROC curves, but KL-6 is more valuable in early detection of mild patients, easy to measure with routine blood investigations, and does not require any cooperation from the patient, particularly in pediatric cases.

Furthermore, Ishikawa et al. mentioned that patients with ILD had abnormal KL-6 levels, as opposed to only 10% of patients with pneumonia, asthma, or chronic obstructive pulmonary disorder and 28% of patients with active pulmonary tuberculosis (34).

This means that KL-6 is more specific for ILD than FVC. Our conclusion agrees with the results reported by Salazar et al., who stated that KL-6 was predictive of early SSc-ILD progression and higher KL-6 levels were predictive of faster FVC% decline at the 1-year follow up (11). This is also in agreement with Fukaya et al. who reported that the KL-6 level should be sequentially measured in CTD patients, when interstitial lung diseases develop (35). Moreover, Hu et al. (12) showed that post-treatment serum KL-6 levels were reduced in patients with improved ILDs and were increased in patients with advanced ILDs, thus showing the importance of KL-6 level as a prognostic tool and for treatment monitoring purposes. As to the comparison of KL-6 and HRCT for the early detection of ILD, we agree with Lee at al., who reported that regular chest HRCT in patients with these CTDs is not currently recommended. Considering cost-effectiveness and radiation hazard, KL-6 measurement using a simple blood test would be a good alternative to chest HRCT for evaluating the current status of ILD, regardless of the CTD type. Furthermore, chest HRCT can provide objective evidence of exacerbation with overt clinical symptoms, but the optimal time interval between CT scans in asymptomatic ILD patients is difficult to determine. Regular chest HRCT, for instance yearly, combined with more frequent KL-6 measurements could be an ideal protocol for detecting worsening CTD-ILD (33).

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Conflict of interest
The authors declare no potential conflicts of interest.

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