Raynaud`s Phenomenon and MMP-7 are Clinicoserologic Biomarkers in Patients with Connective Tissue Disease Associated Interstitial Lung Disease.

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Abstract

Background: The aim of this study was to investigate clinicoserologic biomarkers associated with the development, progression, and prognosis of connective tissue diseases associated interstitial lung disease (CTD-ILD).

Methods: We conducted a single center, a retrospective study including 70 incident patients diagnosed with CTD-ILD and 70 age-, sex-, and type of CTD-matched patients without ILD. Clinical informations, pulmonary function test, and chest CT findings were reviewed using medical records. To identify serologic biomarkers, serum interferon-r-induced protein 10 (IP-10), interleukin (IL)-6, IL-8, IL-10, and matrix metalloproteinase 7 (MMP-7) in patients with CTD-ILD and CTD without ILD were measured.

Results: A total of 140 patients were enrolled. The mean was 63.3 ± 11.2 years, and 102 (72.9%) patients were female. Raynaud`s phenomenon (OR 5.96, 95% CI 2.11–16.86) was proved to be a risk factor for developing ILD in CTD by multivariable logistic regression analysis. To analyze distinctive features according to the onset of ILD, CTD-ILD was stratified into three groups: ILD-preceding, simultaneous, and CTD-preceding. The majority of the ILD-preceding group (75%) had worse baseline pulmonary function requiring treatment (DLCO <65%). Serum levels of MMP-7 were associated with the development of ILD in patients with CTD, and also had a significant correlation with CT extent score in the present study.

Conclusion: In this study, Raynaud`s phenomenon, and serum levels of MMP-7 were clinicoserologic biomarkers CTD-ILD. Therefore, clinicoserologic biomarkers associated with ILD should be assessed in patients with CTD to provide proper management.

Introduction

Connective tissue diseases (CTD) comprise a group of systemic rheumatologic disorders characterized by autoimmune-mediated organ damage that cause symptomatic presentation. CTD consists of rheumatoid arthritis (RA), idiopathic inflammatory myositis (IIM), systemic sclerosis (SSc), and systemic lupus erythematosus (SLE) [1]. Diverse organs can be involved in CTD, such as the kidneys, gastrointestinal tract, and heart. The lung is a common target of autoimmune-mediated organ injury in patients with CTD. Pulmonary involvement is a leading cause of morbidity and mortality in patients with CTD. Pulmonary damage caused by CTD is characterized by considerable heterogeneity in prevalence, severity, and multi-compartment involvement including lung parenchyma, pleura, airways, and vessels [2]. Among various forms of lung involvement in rheumatic disease, the most common and serious pulmonary complication is interstitial lung disease (ILD).

ILD is a group of heterogeneous disorders characterized by diffuse parenchymal lung involvement with shared clinical, imaging, and pathologic characteristics [3]. CTD-associated ILD (CTD-ILD) has a more benign prognosis compared to idiopathic pulmonary fibrosis (IPF), and it can be treated differently from IPF. CTD-ILD can be managed with immune-suppressants, while IPF cannot [4, 5]. It is important to identify patients with CTD at risk for developing ILD to ensure proper decision-making with established and emerging therapeutic options. Pulmonary involvement can be the first manifestation of CTD or it can also occur later in the course of disease [6]. However, there is a lack of research on the differences in the clinical features and prognosis of ILD according to the time of development in relation to CTD.

Biomarkers have been actively investigated as predictors of therapeutic response and progression of ILD. Several cytokines and chemokines have been studied sporadically in relation to CTD-ILD. Interleukin (IL)-6, a pleiotropic cytokine, was described as a prognostic marker in a large cohort of SSc-ILD [7]. The contributions of IL-6, IL-8, and IL-10 to rapidly progressive ILD in IIM were reported in 2014 [8]. Chen and colleagues reported that serum levels of serum interferon-r-induced protein 10 (IP-10) and matrix metalloproteinase 7 (MMP-7) are elevated in RA-ILD [9]. Our understanding of the natural history of pulmonary complications in CTD has been progressed, but epidemiologic data and study of clinicoserologic biomarkers in Korean patients remain sparse. Therefore, this study was conducted to identify the clinical features and serologic biomarkers associated with the development and the progression of ILD in Korean patients with CTD.

Methods

Study population

Seventy patients with CTD diagnosed with ILD were enrolled. Diagnosis of ILD was established based on diffuse parenchymal abnormalities seen on images obtained by high-resolution computed tomography (HRCT) or computed tomography (CT). To evaluate risk factors for developing ILD, we selected 70 age-, sex-, and type of CTD-matched patients without ILD were selected as a control group. Therefore, the study included 140 patients in total from Kyung Hee University Medical Center. Data collected from medical records was retrospectively reviewed. Clinical variables included smoking history, comorbidities, and accompanying autoimmune features; mechanic hand, digital edema, skin rash, Raynaud`s phenomenon, digital ulcer, and inflammatory arthritis which were defined using clinical classification criteria for interstitial pneumonia with autoimmune features (IPAF) [10]. In some patients with CTD-ILD, the onset of ILD preceded that of CTD. Patients with CTD-ILD were stratified into three groups: ILD-preceding (12/70, 17.1%), simultaneous (25/70, 35.7%), and CTD-preceding (33/70, 47.1%). Simultaneous onset was defined as when ILD was developed within 2 months before or after CTD diagnosis. ILD-preceding was defined ILD as being diagnosed more than 2 months earlier than CTD. Finally, CTD-preceding was defined as when ILD followed CTD diagnosis by more than 2 months after CTD diagnosis. The Institutional Review Board of Kyung Hee University Medical Center approved this study (IRB No. 2018-07-003-011), and all patients provided written informed consent.

Imaging Evaluation For ILD

CT scoring

One trained radiologist (SSY) who was blinded to the clinical data analyzed CT. To assess both extent and distribution, each lung was divided into three zones. The upper zone was defined as scans from the apex of the lungs to the mid-aortic arch. The middle lung zone was defined as scans from the level of the aortic arch to the pulmonary venous confluence. Scans below the venous confluence down to the right dome of the diaphragm represented as the lower lung zone. The ILD extent of CT scans was graded semi-quantitatively: grade 0, 0–25%; grade 1, 26–50%; grade 2, 51–75% and grade 3, 76–100% of lung zone affected. This resulted in potential CT scores ranging from 0 to 9 points for each patient. To evaluate progression, changes in CT scores were compared between baseline and annual follow-up examinations. To investigate whether the candidate serum biomarkers are correlated with the CT scores, we used CT scans taken within 6 months of serum sampling.

Definition of CT pattern for ILD

The CT pattern for ILD was determined according to the American Thoracic Society/European Respiratory Society (ATS/ERS) statement [11]. Usual interstitial pneumonia (UIP) is defined as peripheral basilar predominant reticular abnormalities, honeycombing, and minimal to no ground glass opacities (GGOs). Conversely, non-specific interstitial pneumonia (NSIP) shows reticulation and GGOs with little architectural distortion and honeycombing. Cryptogenic organizing pneumonia (COP) is defined as patchy and often migratory consolidation in a subpleural or peribronchial pattern that is commonly associated with GGO.

Pulmonary Function Test (PFT) For ILD

PFT at baseline ILD diagnosis and at annual follow-up were analyzed. The following parameters were included for analysis: forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and diffusion capacity for carbon monoxide (DLCO). To determine if candidate biomarkers are correlated with lung physiology, PFT results within 6 months of each sampling were used.

Evaluation Of Clinical Course And Prognosis For ILD

To identify the predictors of ILD progression, annual laboratory results, image findings, and PFTs were analyzed. Progression of ILD was defined as an increase in CT grade or significant lung function decline [FVC $\geq$ 10% and/or DLCO $\geq$ 10%].

Measurement Of Cytokines

Serum samples were stored at -80 ^{o}C. Levels of serum cytokines, including IL-6, IL-8, IL-10, IP-10, and MMP-7 were measured by multiplex assay using the Luminex bead technology (Luminex, Austin, TX, USA)

Statistical analysis

The differences between patients with and without ILD were evaluated using the Student's t test or chi-square test. Fisher's exact test was used when appropriate and the Mann-Whitney U test was used to compare median values. To evaluate the differences between three or more groups, the Kruskal-Wallis test was used. The Pearson correlation coefficient was used to analyze the association between serum cytokine levels and PFT parameters. Analyses were performed using SPSS (version 22; SPSS Inc., Chicago, IL, USA). *P*-values less than 0.05 were considered statistically significant.

Results

Clinical risk factors for ILD

Baseline clinical characteristics of CTD patients with or without ILD are presented in Table 1. The mean age of the study group was 63.3 ± 11.2 years. Most of the patients were female (103, 73.6%) and non-smokers (122, 87.1%). Significantly more patients in the CTD-ILD group had more history of diabetes mellitus (DM).

Table 1
Demographic and clinical findings of study patients (n = 140)

	CTD-ILD (n = 70)	CTD without ILD (n = 70)	*p*-value
Male : Female	22 : 48	15 : 55	0.250
Mean age	64.05 ± 9.96	62.50 ± 12.25	0.411
Smoker, n (%)	13 (18.6)	5 (8.8)	0.132
Comorbidity, n (%)	49 (70)	41 (59.4)	0.217
DM	22 (31.4)	11 (15.9)	0.045
HTN	24 (34.3)	29 (42.0)	0.385
GERD	4 (5.7)	1 (1.4)	0.366
Accompanying autoimmune features at diagnosis of CTD, n (%)			
Raynaud`s phenomenon*	22 (31.4)	5 (7.1)	< 0.001
Arthritis	46 (65.7)	49 (72.1)	0.465
Mechanic hand	2 (2.9)	1 (1.4)	1.000
Digital edema	2 (2.9)	2 (2.9)	1.000
Skin rash	7 (10.0)	8 (11.6)	0.791
Digital ulcer	1 (1.4)	1 (1.4)	1.000
Type of CTD, n (%)			
RA	33 (47.1)	36 (51.4)	0.735
SSc	8 (11.4)	6 (8.6)	0.779
IIM	11 (15.7)	11 (15.7)	1.000
SLE	9 (12.9)	9 (12.9)	1.000
others	9 (12.9)	8 (11.4)	0.786
Inflammatory markers at diagnosis of CTD			
ESR (mm/hr)	52.21 ± 30.49	51.12 ± 32.15	0.837
CRP (mg/dL)	2.18 ± 3.44	2.39 ± 3.62	0.578
Inflammatory markers at diagnosis of ILD			
ESR (mm/hr)	43.4 ± 27.4		
CRP (mg/dL)	2.74 ± 3.02		
ANA positivity, n (%)	37 (52.9)	29 (41.4)	0.173

*P-value < 0.05; DM, diabetes mellitus; HTN, hypertension; GERD, gastroesophageal reflux disease; RA, rheumatoid arthritis; SSc, systemic sclerosis; IIM, idiopathic inflammatory myopathy; SLE, systemic lupus erythematosus; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ANA, anti-nuclear antibody

CTD patients with ILD had a statistically more frequent Raynaud`s phenomenon than those without ILD (31.4% vs. 7.1%, $p < 0.001$; Table 1). Initial ESR and CRP were similar between the two groups. The presence of antinuclear antibody (ANA) was more common in the CTD-ILD group, but the difference was not statistically significant. Multivariable logistic regression analysis indicated that Raynaud`s phenomenon was a clinical risk factors for developing ILD in CTD (OR 5.96, 95% CI 2.11–16.86).

Clinical and radiologic features of CTD-ILD according to the onset pattern

Differences in clinical features and radiologic features between three groups were stratified according to the onset time of ILD in relation to CTD. In the ILD-preceding group, patients had more Raynaud`s phenomenon, but the difference was not statistically significant. The ILD-preceding group showed NSIP and UIP patterns on CT in almost equal proportions as the CTD-preceding group. The simultaneous group had a larger proportion of the NSIP pattern than the two other groups (72% *vs* 50%, 51.5%). Total CT score at diagnosis of ILD was significantly higher in the ILD preceding group (1.67 ± 1.83 *vs*. 1.36 ± 1.32, 0.48 ± 0.94, $p = 0.006$). There were no significant differences in respiratory function. However, predicted DLCO at diagnosis of ILD was lowest in the ILD-preceding group (53.2 ± 17.1; Table 2). Annual changes in PFT were obtained from 31 patients (15 CTD-preceding, 10 simultaneous, and 6 ILD-preceding). ILD progression was observed in 15 of 31 patients (48.4%) and in 3 of 31 patients (9.7%) when it was defined by decreased pulmonary function and increased CT

score, respectively. FVC decreased significantly more in the ILD-preceding group, than in CTD-preceding or simultaneous groups (-4.67 ± 10.4, -2.40 ± 9.4, 10.1 ± 18.6, $p = 0.047$, Table 2).

Table 2. Stratification of 70 CTD-ILD patients according to onset pattern of ILD

	ILD preceding (n=12)	Simultaneous (n=25)	CTD preceding (n=33)	P-value
Raynaud`s phenomenon	5 (41.7)	8 (32.0)	9 (27.3)	0.656
CT patterns, n (%)				
NSIP	6 (50)	18 (72)	17 (51.5)	0.389
UIP	6 (50)	6 (24)	14 (42.4)	0.631
COP	0	1 (4)	3 (9.1)	0.508
CT score at diagnosis of ILD				
Upper	0.08±0.29	0	0	0.153
Mid	0.42±0.67	0.29±0.46	0.14±0.36	0.178
Lower	1.17±1.83	1.13±1.03	0.62±0.80	0.369
Total*	1.67±1.83	1.36±1.32	0.48±0.94	0.006
PFT at diagnosis of ILD				
FEV1,% predicted	86.4±25.7	84.8±19.5	87.5±18.7	0.919
FVC,% predicted	79.6±22.3	76.5±17.8	82.7±17.9	0.579
FEV1/FVC,%	81.1±6.07	82.3±7.23	79.9±10.3	0.704
DLCO,% predicted	53.2±17.1	62.1±19.7	72.6±17.5	0.063
DLCO<65%, n (%)	3 (25)	10 (40)	5 (15.2)	0.557
Change of PFT at 12 months from baseline (n=31)				
FEV1,% predicted	-4.33±13.8	7.40±17.7	-5.27±11.1	0.088
FVC,% predicted*	-4.67±10.4	10.1±18.6	-2.40±9.40	0.047
FEV1/FVC,%	0.33±3.08	-2.20±6.09	-0.20±1.42	0.342
DLCO,% predicted	-2.75±4.57	4.10±22.9	-6.33±15.5	0.366
ILD improvement at 12 months	0	5 (50)	2 (13.3)	0.031
ILD progression at 12 months	2 (33.3)	4 (26.7)	9 (60)	0.453

*P-value <0.05; Values are presented as mean ± SD or n (%); CTD, connective tissue disease; ILD, interstitial lung disease; UIP, Usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia; COP, cryptogenic organizing pneumonia; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide.

When stratified according to specific CTD, CTD-preceding was more frequent in RA- and SSc-ILD (Table 3), while simultaneous onset was more frequent in IIM- and SLE-ILD. Significantly more RA patients (63.6%, $p = 0.033$) developed ILD after diagnosis of CTD. RA patients tended to develop ILD a long time after diagnosis of RA (median 75 months, range: 3–199 months). However, IIM patients developed ILD early in the course of disease (median 6 months, range: 5–10 months).

Table 3
Radiologic evaluation and pulmonary function test of 70 CTD-ILD patients according to CTD subgroup

	CTD-ILD (n = 70)					
	RA-ILD (n = 33)	SSc-ILD (n = 8)	IIM-ILD (n = 11)	SLE-ILD (n = 9)	Other-ILD (n = 9)	Total (n = 70)
Gender (M:F)	12 : 21	2 : 6	3 : 8	0 : 9	5 : 4	22 : 48
Age	67.2 ± 8.6	58.0 ± 8.3	57.0 ± 9.2	62.5 ± 12.2	68.1 ± 8.9	64.05 ± 9.96
Time of ILD onset						
ILD-preceding	4 (12.1)	2 (25)	3 (27.2)	1 (11.1)	2 (22.2)	12 (17.1)
Simultaneous	8 (24.2)	2 (25)	6 (54.4)	4 (44.4)	5 (55.6)	25 (35.7)
CTD-preceding	21 (63.6) *	4 (50)	2 (18.2)	4 (44.4)	2 (22.2)	33 (47.1)
Time interval between CTD and ILD onset, months (median)						
ILD-preceding	36–118 (46)	3–36 (19)	5–10 (6)	17	5–41 (23)	2-118 (27)
CTD-preceding	3–199 (75)	6-182 (29)	12–27 (20)	13–182 (111)	3–71 (37)	2-199 (70)
CT pattern, n (%)						
UIP	17 (51.5)	1 (12.5)	1 (9.1)	2 (22.2)	4 (44.4)	25 (35.7)
NSIP	14 (42.4)	7 (87.5)	9 (81.8)	7 (77.8)	4 (44.4)	41 (58.6)
COP	2 (6.1)	0	1 (9.1)	0	1 (11.1)	4 (5.7)
CT score at diagnosis of ILD						
Upper	0	0	0	0.11 ± 0.31	0	0.02 ± 0.13
Mid	0.37 ± 0.58	0.29 ± 0.45	0.60 ± 0.66	033 ± 0.47	0	0.26 ± 0.48
Lower	0.95 ± 0.83	1.00 ± 1.07	1.6 ± 1.02	1.00 ± 1.05	0.86 ± 0.99	0.95 ± 0.98
Total*	1.32 ± 1.30	1.13 ± 1.45	2.00 ± 1.48	1.44 ± 1.71	0.67 ± 0.94	1.00 ± 1.33
PFT at diagnosis of ILD						
FEV1,% predicted*	87.8 ± 17.9	97.3 ± 21.6	63.3 ± 13.7	93.3 ± 11.6	94.0 ± 16.3	86.3 ± 19.7
FVC,% predicted*	85.8 ± 16.2	83.0 ± 20.8	56.9 ± 12.7	82.4 ± 11.1	86.3 ± 15.9	79.9 ± 18.7
FEV1/FVC,%*	75.8 ± 8.6	89.3 ± 5.5	86.5 ± 6.0	84.9 ± 5.8	79.7 ± 5.2	81.0 ± 9.4
DLCO,% predicted	71.9 ± 18.5	63.0 ± 13.1	51.1 ± 17.7	66.0 ± 12.6	70.3 ± 22.3	66.1 ± 18.6
DLCO < 65%	3 (9.1)	2 (25)	6 (54.5)	4 (44.4)	4 (44.4)	19 (27.1)
FVC < 75%	5 (15.2)	1 (12.5)	7 (63.6)	2 (22.2)	0	15 (21.4)

*P-value < 0.05; Values are presented as the mean ± SD or n (%); CTD, connective tissue disease; ILD, interstitial lung disease; RA, rheumatoid arthritis; SSc, systemic sclerosis; IIM, idiopathic inflammatory myopathy; SLE, systemic lupus erythematosus; UIP, Usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia; COP, cryptogenic organizing pneumonia; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxid

Table 4
Cytokine levels in all patients with CTD

	CTD-ILD (n = 61)	CTD without ILD (n = 61)	*P*- value
IP-10, pg/ml	24.4 ± 12.6	58.4 ± 56.8	< 0.001
IL-10, pg/ml	0.29 ± 0.40	2.63 ± 2.71	< 0.001
IL-6, pg/ml	3.21 ± 2.09	15.6 ± 19.5	< 0.001
IL-8, pg/ml	31.4 ± 31.2	48.4 ± 145.3	0.082
MMP-7, ng/ml	2.35 ± 1.52	1.64 ± 1.38	< 0.001
CTD, connective tissue disease; ILD, interstitial lung disease; IP-10, interferon-r-induced protein 10; IL, interleukin; MMP, matrix metalloproteinase			

Clinical and radiologic features of ILD according to the individual CTDs.

RA was the most common among various CTDs associated with ILD. NSIP was the most frequent radiographic pattern (41/70, 58.6%) in CTD-ILD. While UIP was the most common (17/33, 51.5%) pattern in RA-ILD. Total CT score representing involved extent was 1.0 ± 1.3 (mean ± SD), which was significantly higher (2.0 ± 1.48, $p = 0.027$) in IIM-ILD. Lower lung fields were predominantly affected in most of the patients. Most patients with RA-ILD, were seropositive with the high titer of anti-cyclic citrullinated peptide (CCP) than those without ILD. There were only two patients who were seronegative RA.

Baseline PFT parameters including, FEV1, FVC, and FEV1/FVC values at ILD diagnosis were significantly lower in the IIM-ILD group ($p < 0.002$, 0.002, 0.005) than in other CTD subgroups. In the IIM-ILD group, more than half (54.5%) of patients had low DLCO (DLCO < 65%) requiring active treatment, and 63.6% of patients showed moderately restrictive lung physiology (FVC < 75%) from the time of ILD diagnosis (Table 3).

Assessment Of Serum Biomarkers In Relation To Clinical Findings

Comparison of cytokine levels in CTD-ILD and CTD without ILD

122 serum samples were available to investigate the characteristics of cytokine profiles in patients with CTD associated ILD, the levels of each cytokine were compared in patients with CTD-ILD and in CTD patients without ILD (Table 5). IP-10, IL-6, IL-8, IL-10, and MMP-7 were analyzed. MMP-7 was significantly higher in CTD-ILD patients than in CTD without ILD ($p < 0.001$). Pro-inflammatory cytokines (IL-6, IL-8, IL-10, and IP-10) were significantly higher in CTD patients without ILD.

Table 5
Multivariate logistic regression analysis of cytokines adjusted with ESR in all CTD

	B	SE	*P*- value	OR	95% CI
All CTD-ILD (n = 61)					
ESR	0.009	0.009	0.285	1.009	0.99–1.01
IP-10	-0.017	0.013	0.183	0.983	0.96–1.01
IL-10	-0.373	0.215	0.083	0.689	0.45–1.05
IL-6	-0.025	0.018	0.155	0.975	0.94–1.01
IL-8	-0.001	0.001	0.447	0.999	0.99-1.00
MMP-7*	0.423	0.160	0.008	1.527	1.12–2.09
*P-value < 0.05; IP-10, interferon-r-induced protein 10; IL, interleukin; MMP, matrix metalloproteinase					

Analysis of biomarkers contributing to the development of ILD in CTD

To reveal the cytokines that contribute to ILD, multiple linear regression analysis was performed. ESR and CRP were analyzed because the concentrations of inflammatory cytokines can be affected by the disease activity of underlying CTD. MMP-7 was the only cytokine significantly associated with ILD in CTD ($p = 0.008$, OR 1.53, 95% CI 1.12–2.09; Table 5). Differences in cytokine levels between ILD-preceding, simultaneous, and CTD-preceding groups were not statistically significant. In RA-ILD, even after adjusting for disease activity score-28 with ESR (DAS28-ESR) in multivariable logistic regression analysis, MMP-7 was significantly associated with ILD in RA ($p = 0.026$, OR 1.77, 96% CI 1.07–2.94; data was not shown).

Biomarker reflecting ILD severity

Semiquantitative CT grades of the total score were significantly proportional to MMP-7 level in CTD-ILD ($p = 0.036$, Fig. 1A). Other cytokines (IP-10, IL-10, IL-6, IL-8) were not differentiated by CT grades. Serum MMP-7 levels also had a negative correlation with DLCO% in CTD-ILD (Pearson coefficient = − 0.302, $p =$

0.023) (Fig. 1B). After analyzing only in RA-ILD separately, similar results were obtained (Pearson coefficient = − 0.421, p = 0.029). There was no significant difference of cytokine levels between the progressive and the stable ILD.

Discussion

In this study, the CTD-ILD group had more Raynaud $sphenomenonwhichwasariskfac \rightarrow rf$ or $develo\pi ngILD \in \mu < ivariab \leq \log isticregressionanalysis.\ Previousstudiesmentio \neq dd$ s between idiopathic ILD and CTD-ILD [12]. However, it has been sporadically reported Raynaud`s phenomenon as a risk factor for developing ILD in CTD. Xie et al. reported that Raynaud's phenomenon was a risk factor for developing ILD in IIM [13]. Narula et al. reported that Raynaud`s phenomenon had a positive association with the development of ILD in mixed connective tissue diseas (MCTD) [14].

On radiologic HRCT evaluation in this study, 58.6% of CTD-ILD patients had NSIP pattern and 35.7% had UIP pattern, consistent with previous studies. The two most common histologic patterns are UIP and NSIP. UIP is the predominant pattern in IPF, and NSIP is seen more in CTD-ILD [15–17]. In our study, RA-ILD had a larger proportion of UIP. Up to 60% of RA cases had the UIP pattern [18]. In SSc, IIM, and SLE, the predominant pathologic pattern is NSIP, and overall survival in this cohort is more favorable than for UIP associated with a CTD [19]. When stratified according to CTD subgroup, the proportion of UIP pattern is consistent with previous studies.

There were no significant differences in clinical findings according to CTD subtype except for pulmonary function test in IIM. In our study, IIM-ILD had poorer pulmonary function than other CTD types. There was no well-designed study comparing pulmonary function between each type of CTD-ILD. However, this difference in function could be explained by differences in the clinical courses of IIM-ILD and other CTD-ILD. The clinical course of IIM-ILD can be categorized into three clinical patterns: rapidly progressive, chronic and asymptomatic form [20]. In contrast, most patients with other CTD-ILD, have chronic progression. This difference reflects that inflammatory change is more prominent in myositis-associated ILD and is generally proportional to the rate of symptom deterioration, with greatest severity in the rapidly progressive form [21].

Most cases of CTD-ILD are diagnosed in patients with a rheumatologic diagnosis of a well-established CTD. A substantial minority of patients, present with ILD first, and CTD is diagnosed at a later date [22–27]. In our study, 17.1% of patients were diagnosed with ILD first, consistent with previous research. In a previous study, up to 15% of patients initially diagnosed with idiopathic NSIP had underlying CTD on further investigation [12]. We tried to identify clinical risk factors present by the time ILD occurs. However there were no significant differences in clinical features. For future research, further well-designed prospective studies are warranted to uncover detailed clinical factors affecting the development of ILD in patients who have been diagnosed with CTD first.

CTD-ILD is known to have a better prognosis than idiopathic interstitial pneumonia [28–31]. In SSc, the predominant pathologic pattern is NSIP, and overall survival in this cohort is more favorable than in UIP [21]. However, recent data have shown that CTD-ILD has varying prognoses depending on the type of underlying CTD or ILD pattern. For example, the presence of UIP in association with any CTD portends a prognosis that rivals the mortality associated with IPF [28, 29]. Unlike idiopathic ILD, in which NSIP was associated with better survival than UIP, CTD-NSIP and CTD-UIP have shown similar prognoses [30, 31], except for RA-ILD. Therefore, the exact evaluation of CT pattern is important to predict prognosis and provide proper management. In our study, the ILD-preceding group had poorer lung function at initial diagnosis and more decreased pulmonary function at follow up. As far as we know, there is no previous study on the prognosis of CTD-ILD stratified by ILD onset time. This study has implications for us that it is necessary to looking for clues indicating CTD in patients with already diagnosed ILD.

IPF is characterized by progressive lung remodeling associated with excessive deposition of extracellular matrix (ECM) [32]. The contribution of MMPs to the ECM remodeling is very complex because MMPs have multiple effects. They are not only responsible for ECM degradation, but also cleave diverse bioactive mediators such as growth factors, cytokines, and chemokines to release them from the ECM [33]. MMP-7, the smallest MMP (247 amino acids), plays a crucial role in pulmonary inflammation and fibrosis. MMP-7 is expressed in alveolar macrophages and bronchiolar epithelial cells. MMP-7 levels were shown to be elevated in serum and bronchoalveolar lavage (BAL) fluid from patients with IPF compared with healthy controls [34, 35]. Rosas et al. reported that MMP-7 was significantly higher in IPF populations than in healthy controls. They documented an increased in MMP-7 in lung tissue and BAL fluid obtained from patients with IPF. In addition, they reported that the elevated MMP-7 levels were negatively correlated with percent FVC% predicted and DLCO% predicted [36]. In IPF, plasma MMP-7 levels could predict mortality [37].

The present study demonstrated that MMP-7 was the most significant cytokine in ILD with CTD. MMP-7 levels were associated with the development of ILD in CTD patients in the present study. Also, MMP-7 had a significant association with CT score and a negative correlation with DLCO% in CTD-ILD. The role of MMP-7 in ILD associated with CTD has been reported in some studies. Chen et al. reported similar findings in RA-ILD that serum levels of MMP-7 are significantly elevated in RA-ILD [9]. In a recent study, Nakatsuka et al. reported that MMP-7 levels were higher in anti-amimoacyl-tRNA synthetase (ARS) antibody positive IIM-ILD; they also reported higher serum MMP-7 levels among anti-melanoma differentiation-associated protein 5 (MDA5) antibody positive IIM-ILD patients were associated with a worse prognosis [38]. To our knowledge, this is the first study evaluating the correlation of MMP-7 in the entire CTD-ILD. Although there is no significant association with ILD progression in this study, further prospective studies with larger cohorts are needed on this point.

The results for IL-6, IL-8, IL-10, and IP-10 were different from those of previous studies. In our study, IL-6, IL-10, and IP-10 were significantly higher in the CTD without ILD group. This is thought probably because the underlying disease activity was not reflected. Differences in the levels of these cytokines and chemokine were not significant after adjustment for inflammatory markers, including ESR and CRP, and disease activity score (DAS28-ESR) in RA-ILD. IL-6, a representative pro-inflammatory cytokine, contributes to the pathogenesis of rheumatologic diseases. In RA, it has been demonstrated that IL-6 is involved in local inflammation that causes joint destruction and various systemic inflammatory signs and symptoms [39]. IL-6 is found in the serum of patients with RA and its level correlates with the disease activity [40]. IL-10, a potent cytokine synthesis inhibitory factor and anti-inflammatory cytokine, plays a central role in the pathogenesis of autoimmune diseases [41]. High levels of serum IL-10 detected in RA patients is reported in previous studies [42]. A more significant

increase in the level of IL-10 in patients having high disease activity (DAS > 3.2) shows that the level of IL-10 rises in response to higher inflammatory state of these patients [43]. The chemokine IP-10 regulates immune responses by activation and recruitment of leukocytes through its receptor, CXCR3 [44]. Increased serum levels of IP-10 were associated with inflammatory responses in previous studies. Serum IP-10 levels are elevated prior to the development of RA [45]. IP-10 levels also have significant correlation with disease activity scores (DAS) in RA [46].

This is the first study investigating the radiologic features and respiratory function of CTD-ILD in Korea regarding to onset time of ILD. Our findings provide insight that ILD preceding group has more deteriorated lung function, and patients with IIM have more chance to develop ILD earlier than CTD. In this study, serum MMP-7 was significantly higher in the CTD-ILD group significantly and had significant correlation with CT grade and DLCO.

Despite some significance, this study has a few limitations. It is difficult to establish a cause and effect relationship, because the study is retrospective and includes a small number of patients. It is also possible that clinical aspects related to autoimmune features including Raynaud`s phenomenon were underreported in the non-ILD patients, leading to a retrospective bias. The prognostic results did not match CT score and PFT. This is because the PFT was not performed at exactly the same time in patients with CT. In CTD-ILD patients, sophisticated analysis of PFT is needed, because CTD can involve the bronchi, pulmonary vessels, and muscular structures in addition to lung parenchyma [47]. As mentioned previously, CTD is a heterogenous group of inflammatory diseases, and disease activity at the time of enrollment could not be analyzed. The last limitation is that not all non-ILD groups were identified by CT.

Conclusion

Our study findings suggest that patients with CTD who have Raynaud's phenomenon are more likely to develop ILD. Patients with ILD preceding to CTD, especially in case of IIM, had poorer lung function at baseline, which was associated with the progression of ILD. Therefore, patients with ILD should be carefully assessed for the development of CTD to ensure proper management of patients with CTD-ILD. As a serologic biomarker, serum MMP-7 was increased in CTD-ILD patients and was correlated with the CT extent score. Thus, serum levels of MMP-7 could be a useful biomarker for the evaluation of CTD-ILD. Further prospective, longitudinal study with a larger number of patients will help to confirm the practicality of using serum MMP-7.

Abbreviations

CTD: Connective tissue diseases; RA: Rheumatoid arthritis; IIM: idiopathic inflammatory myositis; SSc: systemic sclerosis; SLE: systemic lupus erythematosus; ILD: interstitial lung disease; CTD-ILD: CTD-associated ILD; IPF: idiopathic pulmonary fibrosis; IL: interleukin; IP-10: interferon-r-induced protein 10; MMP-7: matrix metalloproteinase 7; HRCT: high-resolution computed tomography; CT: computed tomography; IPAF: interstitial pneumonia with autoimmune features; ATS/ERS: American Thoracic Society/European Respiratory Society; UIP: Usual interstitial pneumonia; GGO: ground glass opacities; NSIP: non-specific interstitial pneumonia; COP: Cryptogenic organizing pneumonia; PFT: Pulmonary function test; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 second; DLCO: diffusion capacity for carbon monoxide; ANA: antinuclear antibody; CCP : cyclic citrullinated peptide; DAS28-ESR: disease activity score-28 with ESR; ECM: extracellular matrix; BAL: bronchoalveolar lavage; ARS: amimoacyl-tRNA synthetase

Declarations

Authors' contributions

SWC and YAL contributed to the study design and the revision of the manuscript. SYS, SHL, HSC, MJP and SJH collected and coded the data. SWC analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data that support the findings of this study are available from corresponding author.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board (IRB) of Kyung Hee University Medical Center in Seoul, Korea (approval numbers: 2018-07-003), and all patients were informed about the study and consented to participate by signing the form recommended by the IRBs.

Competing interests

The authors declare that they have no competing interests

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Figures

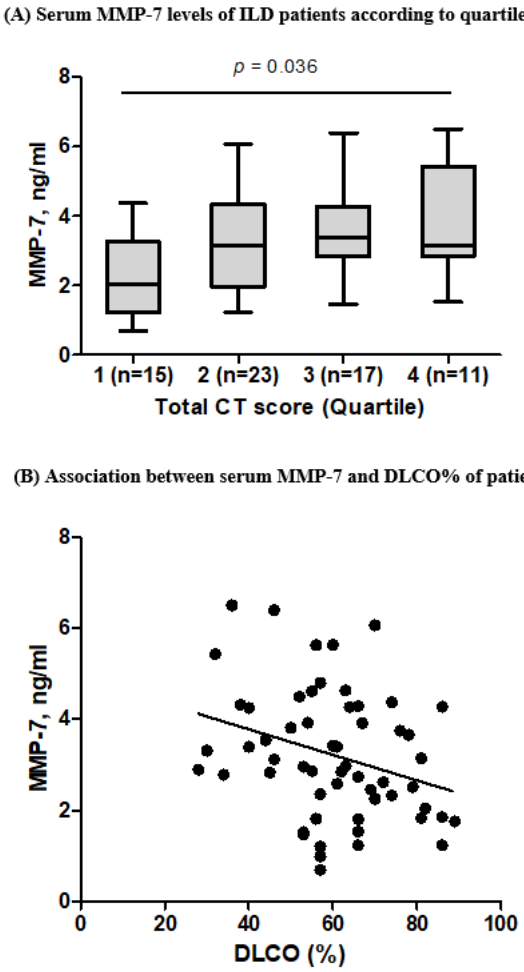

Figure 1

(A) Serum MMP-7 levels of ILD patients according to quartile of total CT score in CTD-ILD. (B) Association between serum MMP-7 and DLCO% of patients with CTD-ILD.