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Original Article

Clinical characteristics and serum levels of tumor markers of connective tissue disease-associated interstitial lung disease

Guifen Shen[1], Sheng Yang[3], Kejun Yao[2], Wenjie Shi[3], Qiuju Wang[1,4], Emanuela Haxhiu[5], Lingli Dong[1]

Departments of [1]Rheumatology, [2]Ophthalmology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China; [3]Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China; [4]Department of Rheumatology, Jingzhou Central Hospital, The Second Clinical Medical College, Yangtze University, Jingzhou 434020, Hubei, China; [5]Medical Center of Specialities No. 1, Tirana, Albania

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Abstract: Many connective tissue diseases present with various extraarticular manifestations. One of these is interstitial lung disease, which should be a focus of rheumatologists and physical clinicians because of its severe complications. The current study aimed to analyze clinical manifestations of various connective tissue disease-associated interstitial lung disease (CTD-ILDs) patients, evaluating differences in the inflammatory indexes, immunological serologies, and alterations of tumor-associated biomarkers in CTD-ILDs patients. Retrospective analysis of 332 CTD-ILDs patients, enrolled from the ward of the Rheumatology Department in Tongji Hospital, from January 2005 through December 2013, was carried out. The study included idiopathic pulmonary fibrosis (IPF) patients as the positive control group. Gender composition in different CTD-ILDs was statistically different with female predominance, while IPF patients consisted of slightly more males than females. Smoking, exertional dyspnea, and coughing were more common in patients with IPF, while velcro rales were more common in patients with RA-ILD. The percentage of patients with serum AFP, CEA, and NSE levels above upper normal limits was statistically different (more proportion in PM/DM-ILD patients). A positive correlation was found between blood levels of CA125, CA199, CA724, and CYFRA21-1 and patient age in CTD-ILD patients. Differences in gender, age, ILD symptoms, and tumor-associated markers existed not only between CTD-ILDs and IPF patients, but also between different subtypes of CTD-ILD. For early diagnosis of ILD in CTD patients, precise physical examinations are required in patients with subclinical disease. Tumor biomarkers can be screened in ILD patients due to their prognostic potential.

Keywords: Interstitial lung disease, connective tissue disease, clinical symptom, tumor marker

Introduction

Connective tissue disease-associated interstitial lung diseases (CTD-ILDs) are sometimes viewed as a homogeneous entity. However, CTD-ILDs are heterogeneous diseases, comprising different connective tissue diseases (CTDs) with various interstitial pneumonia patterns [1]. Interstitial lung disease (ILD) significantly increases morbidity and mortality in CTD patients and can be detected sub-clinically [2, 3]. Therefore, precisely phenotyped cohorts could be developed to better understand this disease. Moreover, respiratory symptoms are not consistent in diagnosed CTD-ILD patients.

Tumor biomarkers, including CEA, CA125, SCC, NSE, and CYFRA21-1, have been widely used to screen and diagnose lung cancer in high-risk populations. They may even be useful in the histological differentiation of non-small-cell lung cancer and small-cell lung cancer [4]. However, tumor biomarkers have also detected in other lung diseases besides lung cancer [5-8], some having been associated with interstitial lung disease. Indeed, serum CEA amounts are elevated in more than half of patients suffering from IPF and are correlated with disease severity [9]. Additionally, CEA and CA125 are elevated in ILD patients, increasing the risk of cancer [10]. In addition, CA153 was proposed for identification and monitoring IPF and other fibrotic ILDs, including extrinsic allergic alveolitis (EAA), nonspecific interstitial pneumonia (NSIP), nonclassifiable interstitial pneumonia (NCIP), inter-

stitial pneumonia (IP) and usual interstitial pneumonia (UIP) [11]. Besides CEA, CA125, and CA153, other tumor biomarkers, such as CYFRA21-1, CA199, and SCC [12], are elevated in patients with interstitial lung disease.

Recent evidence supports the notion that IPF represents a risk factor for lung cancer development [13-16]. Accumulating evidence has revealed the association of malignancy [17] or lung cancer [15] with CTD-ILD. However, differences in clinical manifestations and tumor biomarkers among various CTD-ILDs have been rarely reported. In this study, the common CTD-ILD types, including polymyositis/dermatomyositis (PM/DM), systemic sclerosis (SSc), undifferentiated connective tissue disease (UCTD), mixed connective tissue disease (MCTD), rheumatoid arthritis (RA), Sjögren's syndrome (SS), and systemic erythematosus lupus (SLE), were selected alongside idiopathic pulmonary fibrosis (IPF). The aim was to assess the main symptoms of interstitial lung disease and tumor biomarkers in various CTDs, further characterizing ILD with different underlying CTDs.

Material and methods

Study subjects

This retrospective study began by reviewing the hospital's medical records, providing access to all diagnoses of inpatients in Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. First, patients diagnosed with PM/DM, SSc, UCTD, MCTD, RA, SS, and SLE, from January 2005 to December 2013, with specific diagnosis criteria, were separately identified. Medical records of the patients were then reviewed, consecutively, generating a longitudinal database with ILD diagnosis. Data of IPF patients were collected for the same time period. Medical documents of IPF patients were reviewed, individually, to ensure that they did not have evidence of CTD. The present study was approved by the HUST Committee on Human Research of Tongji Medical College and all subjects provided informed consent.

Patients with a defined CTD according to current respective criteria (e.g. PM [18]/DM [19], SSc [20], MCTD [21], RA [22], SS [23], and SLE [24]) were diagnosed with specific CTD-related ILDs. Patients were considered to have UCTD with signs or symptoms and laboratory findings meeting UCTD criteria [25]. Patients included as IPF, according to published guidelines [26], were assessed as positive controls. ILD was diagnosed according to BTS Interstitial Lung Disease Guidelines [27]. Patients suffering from ILD with specific underlying causes (e.g., hypersensitivity pneumonitis, drug-induced lung disease, and environmental exposures) were excluded. Patients with tumors or any other severe pulmonary diseases, including bronchial asthma, chronic obstructive pulmonary disease, arterial pulmonary hypertension, pleural effusion/pneumothorax, and sleep apnea hypopnea syndrome, were also excluded.

Study plan

All medical records were collected based on the diagnosis code in the Inpatient Department of the hospital, identifying 472 PM/DM, 133 SSc, 486 UCTD, 77 MCTD, 811 RA, 230 SS, and 2,331 SLE patients. There were, respectively, 49, 35, 32, 61, 52, 53, and 50 patients with accompanying interstitial lung disease (**Figure 1**). Detailed medical information of both IPF and CTD-ILD patients was carefully reviewed. Lung biopsies were not performed and, therefore, histopathologic analysis was not possible.

Clinical and radiologic characteristics

Manually collected clinical baseline data included age at the time of enrollment, gender, smoking status, ILD symptoms or signs, physical exam findings, and serological test results. Acute-phase reactants, such as erythrocyte sedimentation rate (ESR) and C reactive protein (CRP), were recorded. Levels of serum tumor-related biomarkers were obtained routinely. All laboratory tests were collected as part of a clinical evaluation and not for the purposes of this study.

High resolution computed tomography (HRCT) imaging of the chest was performed in the hospital. Images were reviewed, independently, by 2 expert thoracic radiologists blinded to patient diagnoses. Specific interstitial lung abnormalities, such as septal lines, reticulation, traction bronchiectasis, cyst formation, and/or ground glass attenuation, were particularly targeted. Images were re-evaluated until a consensus was reached, in cases of disagreement.

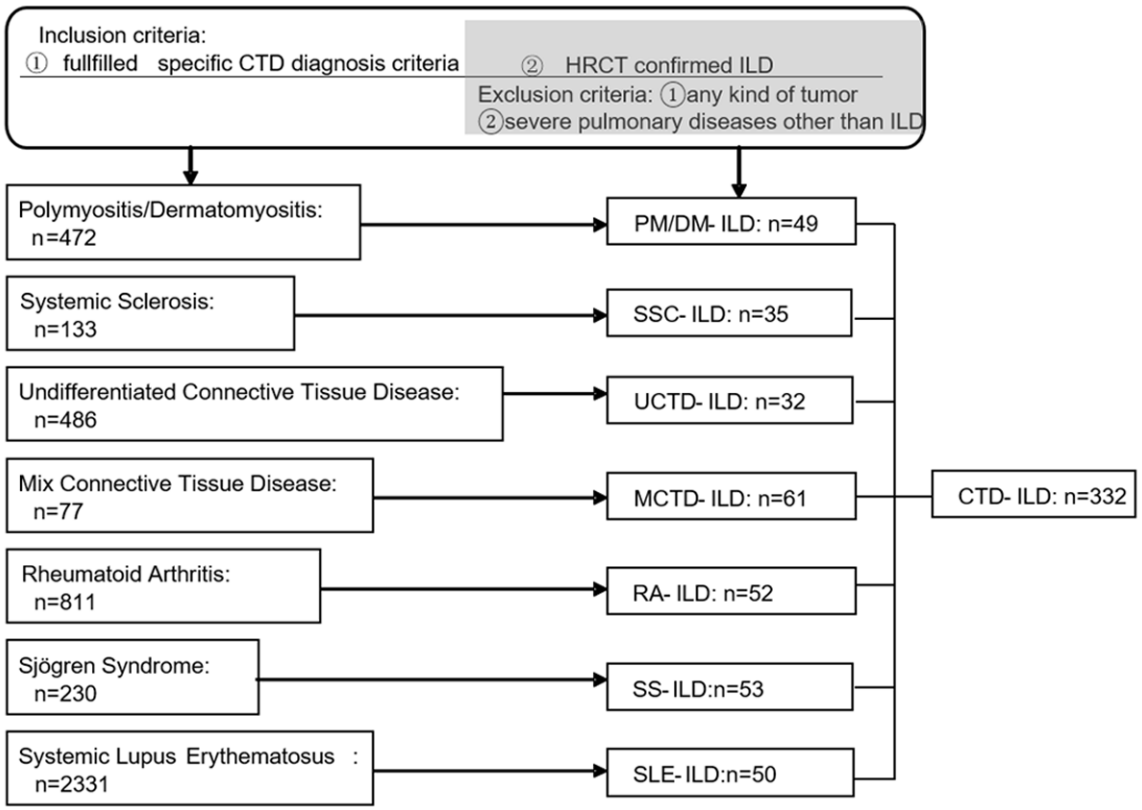

Figure 1. Flow chart of the data collection of CTD-ILD. CTD = connective tissue disease, CTD-ILD = connective tissue disease associated interstitial lung disease, HRCT = high-resolution computed tomography, ILD = interstitial lung disease, MCTD-ILD = mixed connective tissue disease associated interstitial lung disease, PM/DM-ILD = polymyositis/dermatomyositis associated interstitial lung disease, RA-ILD = rheumatoid arthritis associated interstitial lung disease, SLE-ILD = systemic erythematosus lupus associated interstitial lung disease, SSC-ILD = systemic sclerosis associated interstitial lung disease, SS-ILD = Sjögren syndrome associated interstitial lung disease, UCTD-ILD = undifferentiated connective tissue disease associated interstitial lung disease.

Statistical analysis

SPSS 22.0 (SPSS Inc., Chicago, IL, USA (www.spss.com)) and GraphPad Prism version 6.02 (GraphPad Software, Inc., San Diego, CA, USA (www.graphpad.com)) were used for statistical analysis. Descriptive data for continuous variables are reported as mean ± SEM. Two-tailed parametric ANOVA, nonparametric Mann-Whitney U-tests, and Kruskal Wallis tests were performed to assess levels of different biomarkers in various patient groups divided based on various CTD types. This was followed by the LSD test for differences in group pairs. Chi-squared test was performed for categorical data. Pearson's correlation test was utilized to analyze correlation between age and levels of tumor markers. $P < 0.05$ indicates statistical significance.

Results

Patient characteristics

Incidence rates of ILD in various CTD types were different (**Figure 1**). Gender composition was significantly different in various ILD types (**Table 1**, $P < 0.05$). SLE-ILD and SS-ILD patients were predominantly female. In other CTD-ILDs, such as PM/DM, SSc, UCTD, MCTD and RA, there were also more females than males. Additionally, IPF patients had slightly more males than females. Age of patients with various CTD-ILDs was used as a statistical variable. Age at ILD onset in various CTDs or IPF showed statistical differences ($P < 0.05$). After pairwise comparisons (Kruskal-Wallis test), RA-ILD patients (60.6 ± 10.9 years) were older than individuals with other ILD types, while SLE-

Table 1. Baseline characteristics at the time of diagnosis as interstitial lung disease

Type of ILD	Case (n)	Gender n (M/F)	Age (year) mean (min, max)	Ever Smoker (n)	Course of CTD to ILD (month) Mean (min, max)
IPF	50	28/22	51.0 (19, 86)	17	
PM/DM	49	17/31	46.0 (22, 65)	11	5.5 (-12.0, 36.0)
SSC	35	7/28	48.9 (26, 67)	0	37.3 (0, 192.0)
UCTD	32	7/25	54.8 (31, 79)	0	41.8 (-36.0, 778.0)
MCTD	61	7/54	50.5 (23, 75)	0	31.1 (0, 360.0)
RA	52	13/39	60.6 (40, 80)	11	17.5 (0, 228.0)
SS	53	1/52	50.5 (29, 77)	0	14.5 (-110, 108.0)
SLE	50	2/48	41.1 (20, 65)	1	36.9 (0, 275.0)
P		< 0.05	< 0.05	< 0.05	0.069

ILD = interstitial lung disease, IPF = idiopathic iopathic pulmonary fibrosis, MCTD = mixed connective tissue disease, PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis, SLE = systemic erythematosus lupus, SS = Sjögren syndrome, SSc = systemic sclerosis, UCTD = undifferentiated connective tissue disease. Chi-squared test was performed for categorical data.

Table 2. Comparison of ILD symptoms between IPF and different CTD-ILDs

ILD symptoms (n) (%)	IPF	PM/DM	SSC	UCTD	MCTD	RA	SS	SLE	*P*
Exertional dyspnea (%)	41 (82.0)	20 (40.8)	14 (40.0)	14 (43.8)	14 (23.0)	14 (26.9)	17 (32.1)	12 (24.0)	< 0.05
Cough (%)	44 (88.0)	8 (16.3)	8 (22.9)	9 (28.1)	11 (18.0)	17 (32.7)	30 (56.6)	22 (44.0)	< 0.05
Chest congestion (%)	18 (36.0)	10 (20.4)	14 (40.0)	10 (31.3)	10 (16.4)	0 (0.0)	13 (24.5)	18 (36.0)	< 0.05
Velcro rale (%)	18 (36.0)	17 (34.7)	16 (45.7)	11 (34.4)	6 (9.8)	45 (86.5)	23 (43.4)	18 (36.0)	< 0.05
Finger/Toe swelling (%)	4 (8.0)	12 (24.5)	11 (31.4)	16 (50.0)	20 (32.8)	43 (82.7)	13 (24.5)	30 (60.0)	< 0.05
Cyanosis (%)	5 (10.0)	11 (22.4)	3 (8.6)	4 (12.5)	5 (8.2)	6 (11.5)	4 (7.5)	4 (8.0)	0.290
Chest pain (%)	5 (10.0)	0 (0.0)	0 (0.0)	2 (6.3)	2 (3.3)	7 (13.5)	4 (7.5)	5 (10.0)	< 0.05
Weight loss (%)	10 (20.0)	10 (20.4)	5 (14.3)	8 (25.0)	9 (14.8)	16 (30.8)	9 (17.0)	8 (16.0)	0.439

ILD = interstitial lung disease, IPF = idiopathic pulmonary fibrosis, MCTD = mixed connective tissue disease, PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis, SLE = systemic erythematosus lupus, SS = Sjögren syndrome, SSc = systemic sclerosis, UCTD = undifferentiated connective tissue disease. Chi-squared test was performed for categorical data.

ILD occurred in younger patients (41.18 ± 11.8 years old). Similar differences appeared in age at CTD diagnosis (Supplementary Table 1). Ever smokers were predominately in IPF, PM/DM, and RA patients ($P < 0.05$). Interval between diagnosis of CTD and onset of ILD in various CTDs showed no statistical differences ($P < 0.069$). However, after pairwise comparison (Kruskal-Wallis test), ILD occurred in PM-DM patients earlier than in SSC, CTD, MCTD, and SLE patients ($P < 0.05$).

ILD symptoms

Main ILD symptoms, including exertional dyspnea, cough, chest congestion, Velcro rale, finger/toe swelling, cyanosis, chest pain, and weight loss, were evaluated in different ILDs (**Table 2**). Significant differences were found in occurrence rates of exertional dyspnea, cough, chest congestion, Velcro rale, finger/toe swelling, and chest pain in various CTD types ($P < 0.05$). Incidence rates of exertional dyspnea and cough were 82% and 88% in IPF, respectively, and 40.8% and 16.3% in PM/DM patients, respectively. The Velcro rale was auscultated in 86.5% of RA-ILD patients, but only in 36% of IPF patients.

Acute-phase reactants and immunological parameters

CRP and ESR were assessed in ILD patients (**Table 3**). In all CTD-ILD types, CRP and ESR were elevated, but only ESR showed statistically significant differences. CRP levels were 11.69 ± 13.08 mg/dl and 43.67 ± 79.16 mg/dl in SSc-ILD and SS-ILD patients, respectively ($P > 0.05$). Multiple comparisons indicated that ESR levels in IPF patients were lower than those of other CTD-ILD types, except DM/PM and SSc. ESR was lower in SSc-ILD than in other CTD-ILD types and lower in PM/DM-ILD patients than in individuals with SS-ILD and SLE-ILD.

Table 3. Analysis of acute-phase inflammatory reactants and immunologic panel in CTD-ILDs

Mean (SD)	IPF	PM/DM	SSC	UCTD	MCTD	RA	SS	SLE	*P*
CRP (mg/dl)	23.62 (25.13)	18.25 (19.16)	11.69 (13.08)	33.91 (40.86)	33.85 (55.97)	31.64 (30.79)	43.67 (79.16)	36.16 (52.19)	0.086
ESR (mm/1 h)	27.39 (23.42)	37.61 (27.49)	19.48 (19.15)	46.52 (29.23)	50.32 (34.58)	47.94 (32.66)	54.98 (39.69)	60.61 (41.84)	< 0.05
IgG (g/L)	12.88 (3.18)	15.30 (5.76)	12.82 (6.22)	16.75 (8.87)	21.06 (8.50)	12.95 (9.98)	20.37 (7.57)	19.04 (8.70)	< 0.05
IgA (g/L)	2.61 (1.05)	3.02 (1.66)	3.43 (4.74)	3.07 (2.53)	3.21 (1.54)	2.42 (1.00)	3.95 (3.80)	2.97 (2.92)	< 0.05
IgM (g/L)	1.48 (0.95)	1.90 (1.29)	1.49 (0.91)	1.70 (0.99)	1.77 (1.06)	1.72 (1.35)	1.65 (1.06)	1.56 (0.93)	0.786
C3 (g/L)	1.19 (0.38)	0.97 (0.24)	1.03 (0.23)	1.09 (0.24)	0.99 (0.31)	1.00 (0.23)	0.93 (0.28)	0.73 (0.38)	< 0.05
C4 (g/L)	0.24 (0.10)	0.29 (0.09)	0.28 (0.17)	0.31 (0.19)	0.23 (0.09)	0.23 (0.10)	0.22 (0.11)	0.16 (0.09)	< 0.05

Both ESR and CRP were detected in different CTD-ILDs, but only ESR values were found to be statistically significant. Besides, IgG, C3, and C4 values alterations were also significant. C3 = complement 3, C4 = complement 4, CRP = C reactive protein, ESR = erythrocyte sedimentation rate, IgA = immunoglobulin A, IgG = immunoglobulin G, IgM = immunoglobulin M, ILD = interstitial lung disease, IPF = idiopathic pulmonary fibrosis, MCTD = mixed connective tissue disease, PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis, SLE = systemic erythematosus lupus, SS = Sjögren syndrome, SSc = systemic sclerosis, UCTD = undifferentiated connective tissue disease. Two-tailed parametric ANOVA was performed to assess levels of different biomarkers in various patient groups divided based on various CTD types. This was followed by the LSD test for differences in group pairs.

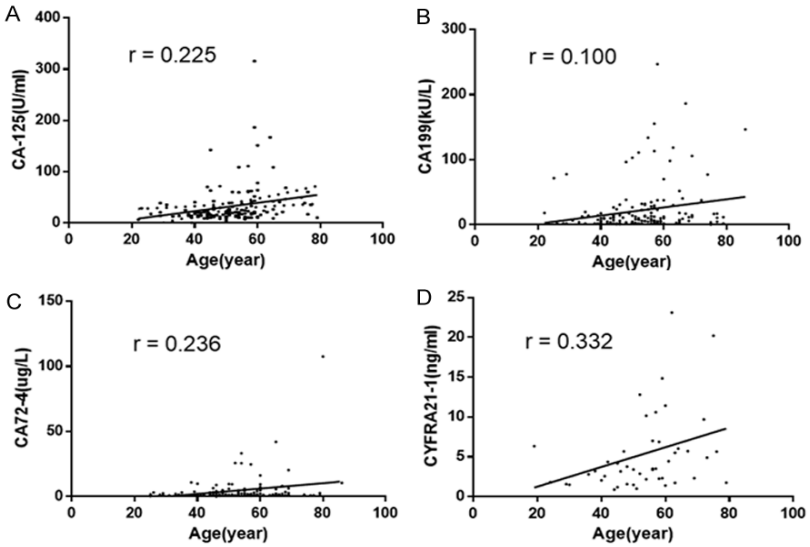

Figure 2. Correlation between age and CA125, CA199, CA724, or CYFRA21-1 in all patients. The relationship between age and values of tumor biomarkers of individual ILD patients was analyzed by Pearson's correlation test. R values for the correlation between age and CA-125, CA-199, CA724, or CYFRA21-1 are 0.225, 0.100, 0.236, and 0.332, respectively. Linear correlation between patient age and level of different tumor biomarkers was tested by linear regression analysis. Data shows the ages of individual patients against levels of CA125, CA199, CA724, or CYFRA21-1. Pearson's correlation test was utilized to analyze the correlation between age and levels of tumor markers.

IgG, C3, and C4 levels were significantly different in various CTD-ILD types. For example, IgG levels were significantly higher in MCTD-ILD patients than in individuals with SSc-ILD (21.06 ± 8.87 g/L vs. 12.82 ± 6.22 g/L, $P < 0.05$, according to multiple comparison test). C3 and C4 levels were lower in patients with SLE-CTD than those with other ILDs ($P < 0.05$, according to multiple comparison test).

Tumor biomarkers related to age

ILD data, including CTD-ILDs and IPF, assessed together, demonstrated that individual blood levels of CA125, CA199, CA724, and CYFRA21-1 were correlated with age, respectively. As shown in **Figure 2**, blood CA125, CA199, CA724, and CYFRA21-1 levels increased with age.

Discrepant tumor biomarkers

Patients with tumor biomarkers above the respective upper normal limits were analyzed in various CTD-ILD groups (**Table 4**). The numbers of ILD patients whose serum tumor biomarkers were assessed 241 are shown in Supplementary Table 2. The percentages of patients with serum AFP, CEA, and NSE levels above respective upper normal limits were significantly different in various CTD-ILDs (especially more in PM/DM-ILD patients). For example, blood AFP levels were higher than normal in 37.1% of DM/PM-ILD and 12.5% of UCTD-ILD patients, while remaining normal in other CTD-ILDs. Elevated CYFRA21-1 and NSE were found in various CTD-ILD patients. NSE showed statistically significant differences, while CYFRA21-1 did not. Elevated NSE and CYFRA21-1 levels were prominent in DM/PM and IPF patients, respectively. Similar to NSE, no CYFRA21-1 was detected in SLE-ILD patients, requiring further studies of CYFRA21-1 and NSE detection in SLE-ILD.

Table 4. Proportion of CTD-ILD patients with blood tumor markers above upper normal limits

%	IPF	DM/PM	SSC	UCTD	MCTD	RA	SS	SLE	*p* value
AFP	0.0	37.1	0.0	12.5	0.0	0.0	0.0	0.0	< 0.001
CA-125	46.2	8.7	27.3	20.0	33.3	41.7	29.0	44.4	0.235
CA-199	30.8	12.5	16.7	11.1	21.4	23.8	10.7	33.3	0.474
CA72-4	8.0	28.0	20.0	6.7	14.3	16.7	16.0	0.0	0.549
CA-153	42.9	25.0	31.3	30.0	25.0	18.	12.5	0.0	0.402
CEA	32.5	37.1	15.2	8.7	20.0	24.0	3.0	11.1	0.009
SCC	12.9	3.2	3.2	5.3	11.1	4.3	6.9	0.0	0.827
CYFRA21-1	68.0	50.0	57.1	25.0	40.0	40.0	25.0		0.537
NSE	20.0	80.0	40.0	37.5	57.1	40.0	16.7		0.03

The proportion of positive patients is presented as percentage. The difference of positive ratio between different CTD-ILD was analyzed by Pearson's Chi-squared test, with *P* value less than 0.05 indicating statistical differences. AFP = alpha fetal protein, CA (125, 199, 72-4, 193) = cancer antigen (125, 199, 72-4, 193), CEA = carcinoembryonic antigen, CYFRA21-1 = Cytokeratin fragment antigen21-1, ILD = interstitial lung disease, IPF = idiopathic pulmonary fibrosis, MCTD = mixed connective tissue disease, NSE = neuron-specific enolase, PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis; SS = Sjögren syndrome, SCC Squamous cell carcinoma associated antigen, SLE = systemic erythematosus lupus, SSC = systemic sclerosis, UCTD = undifferentiated connective tissue disease. Chi-squared test was performed for categorical data.

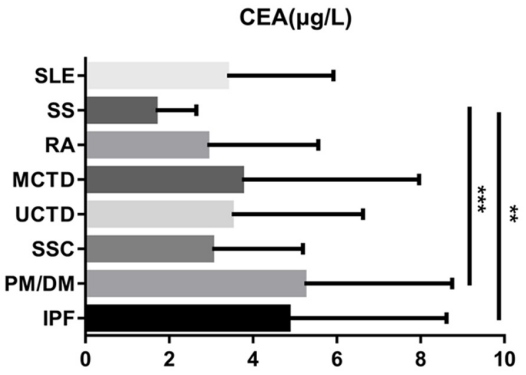

Figure 3. CEA levels were detected in ILD patients with different underlying CTDs, showing the mean value and standard deviation of tumor biomarkers. **: P < 0.01; ***: P < 0.001. CEA = carcinoembryonic antigen, ILD = interstitial lung disease, IPF = idiopathic pulmonary fibrosis, MCTD = mixed connective tissue disease, PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis, SLE = systemic erythematosus lupus, SS = Sjögren syndrome, SSC = systemic sclerosis, UCTD = undifferentiated connective tissue disease. Two-tailed parametric ANOVA was performed to assess levels of CEA in various patient groups divided based on various CTD types. This was followed by the LSD test for differences in group pairs.

Age correlated with levels of tumor biomarkers, as shown above, constituting a significant confounding factor in analyzing level differences of tumor biomarkers in various connective tissue diseases. To eliminate the confounding effects of age, patients were divided into three groups based on age, 20~40, 40~60, and 60~80, respectively. Since most included patients were between 40 and 60, this study deleted the data of patients younger than 20 or older than 60, largely controlling the confounding effects of age. In this way, the confounding effects of age can be largely controlled. Blood CEA levels were significantly lower in SS-ILD patients than in individuals with PM/DM-ILD and IPF ($P < 0.01$ and $P < 0.001$ respectively, **Figure 3**). Blood levels of the remaining tumor biomarkers showed no statistically significant differences after age-adjustment (Supplementary Figure 1).

Discussion

CTD-ILD is one of the major components of interstitial lung disease. While CTD-ILD is different from idiopathic interstitial lung disease, such as IPF, this study revealed that gender, age, respiratory symptoms, and tumor biomarkers showed differences not only between CTD-ILD and IPF, but also among CTD-ILD subtypes. Moreover, some tumor biomarkers found not to significantly differ in various CTD-ILDs were positively correlated with age.

Since CTD incidence is higher in female patients than in males [28, 29], it is not surprising that CTD-ILD patients were mostly female, especially SLE-ILD and SS-ILD patients. IPF patientshad similar proportions between the two genders. Age at onset in different CTD-ILD subtypes is certainly influenced by age at primary disease onset. However, this study did not compare ages of CTD patients without ILD with those of individuals with CTD-ILD. This deserves further attention.

Common symptoms, such as exertional dyspnea and coughing, frequently occurred in IPF, but not so often in CTD-ILD. In some CTD-ILD

subtypes, such as RA-ILD, incidence rates of exertional dyspnea and coughing were low, while Velcro rale was auscultated frequently. This finding indicates that, in CTD diseases, ILD cannot be excluded by the nonexistence of exertional dyspnea and coughing and thorough physical examinations (including auscultation) could provide clues for ILD. Although pulmonary function tests, HRCT, and lung biopsies are not routinely used, they can be helpful in ILD diagnosis. Some common respiratory symptoms did not occur as frequently in CTD-ILD as in IPF. Therefore, such examinations are recommended for early detection and diagnosis of CTD-ILD.

CRP and ESR are disease activity indicators, but elevated CRP and ESR may also indicate infections, tumors, and inflammatory conditions. Th-erefore, CRP and ESR are not specific for CTD-ILD diagnosis or activity detection. CRP and ESR levels were elevated mostly in the IPF and CTD-ILD subtypes. No significant differences in CRP levels were obtained between IPF and CTD-ILD patients, but ESR was lower in SSc-ILD patients than in individuals with other CTD-ILD types. Blood IgG, IgA, IgM, and complement C3 and C4 levels were also detected. Except for IgA and IgM, the remaining immunological parameters were significantly different between IPF and CTD-ILD subtypes. Different blood IgG, C3, and C4 levels might be due to the underlying CTD type. For instance, SLE-ILD patients showed higher IgG levels and lower C3 and C4 amounts, compared with other ILDs.

Lung cancer patients with CTD have a significantly higher incidence of ILD, as a complication, compared with those without CTD [30], indicating a potential relationship between lung cancer and CTD-ILD. In this study, besides lung cancer-associated tumor biomarkers (CEA, SCC, NSE, and CYFRA21-1), other tumor biomarkers (CA125 and CA153) were analyzed. Pathophysiological mechanisms of high levels of tumor biomarkers in patients with interstitial lung disease, with no evidence of malignancy, remain unclear. In this work, CEA levels were increased in 32.5% of IPF patients, far below previously reported values, with serum CEA amounts elevated in more than half of IPF patients [9]. Blood CEA levels were elevated not only in IPF patients, but also in individuals with PM/DM-ILD, as shown above. It is well-established that PM/DM is related to malignancy. Cancer risk was unchanged in patients with CTD without dermatomyositis or polymyositis, but increased after inclusion of individuals with dermatomyositis or polymyositis [31]. Whether blood CEA levels are increased in IPF or PM/DM-ILD patients for disease exacerbation or if they lead to cancer remains unclear, but such changes may result in poor prognosis. Other tumor biomarkers are related to the severity of IPF, including CA153 [32]. Additionally, serum CYFRA21-1 levels reflect the severity of lung injury in nonmalignant respiratory diseases and might be associated with prognosis in patients with IPF and collagen disease associated pulmonary fibrosis [33]. CYFRA21-1 has been considered a useful parameter in evaluating lung epithelial cell damage and repair [34], since cytokeratins are cytoskeletal structures expressed only in epithelial cells. Evidence indicates that cytokeratin fragment 19 levels constitute a useful variable for lung injury evaluation in interstitial pneumonia associated with PM/DM [35]. Other findings support this conclusion as well, demonstrating that the survival of IPF patients with high CYFRA 21.1 levels in BAL is worse, compared with that of the low CYFRA 21.1 group [36]. The usefulness of serum CA199 has been well-recognized for pancreatic cancer diagnosis. However, increased CA199 has also been found in other pathologies, such as bronchiectasis, bronchiolitis, emphysema, and interstitial fibrosis [37]. Interestingly, survival rates are significantly lower in patients with interstitial lung diseases (idiopathic interstitial lung disease and CDPF) and elevated serum CA199 than in counterparts with CA199 levels in the normal range [7]. NSE, a bioactive peptide, is produced and secreted by neuroendocrine cells of the lungs. To the best of our knowledge, the relationship between NSE and interstitial lung disease has not been reported yet.

Associations of ILD with tumor biomarkers were demonstrated above, with levels of serum tumor biomarkers related to severity of interstitial lung disease. The above findings of higher CEA levels in IPF and PM/DM-ILD patients, compared with those of SS patients, provide additional insight into the implication of tumor biomarkers in ILD. The current study suggests that serum tumor biomarkers should be tested not only in IPF patients but also in individuals with CTD-ILD, improving early screening of lung cancer and prognosis.

Several limitations of this study should be mentioned. First, biopsies or histopathologic examinations were not performed. Therefore, a comparative assessment of ILD pathology with various underlying CTDs is needed. Second, normal healthy individuals and CTD patients without ILD are needed as negative controls in such studies, excluding the effects of baseline differences. Third, long-term follow-ups are required to assess the prognostic values of clinical manifestations and tumor biomarkers for CTD-ILD patient survival, as well as the possibility of cancer occurrence.

Conclusion

This research revealed that gender, age, respiratory symptoms, and tumor biomarkers differ between CTD-ILD and IPF. Tumor biomarkers CA125, CA199, CA724, and CYFRA21-1 increased in blood levels with age in the whole ILD patient population. Assessment of tumor biomarkers elevated, to varying degrees, in various CTD-ILDs demonstrated that only CEA concentrations were significantly different after age-adjusted comparison. They were much lower in SS-ILD patients than in individuals with PM/DM-ILD or IPF. For early diagnosis of ILD in CTD patients, precise physical examinations are required in patients with subclinical disease. Tumor biomarkers can be screened in ILD patients due to their prognostic potential.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Lingli Dong, Department of Rheumatology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan 430030, Hubei, China. Tel: +86-27-83663614; Fax: +86-27-83663614; E-mail: dongll@tjh.tjmu.edu.cn

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Supplementary Table 1. Age of CTD onset

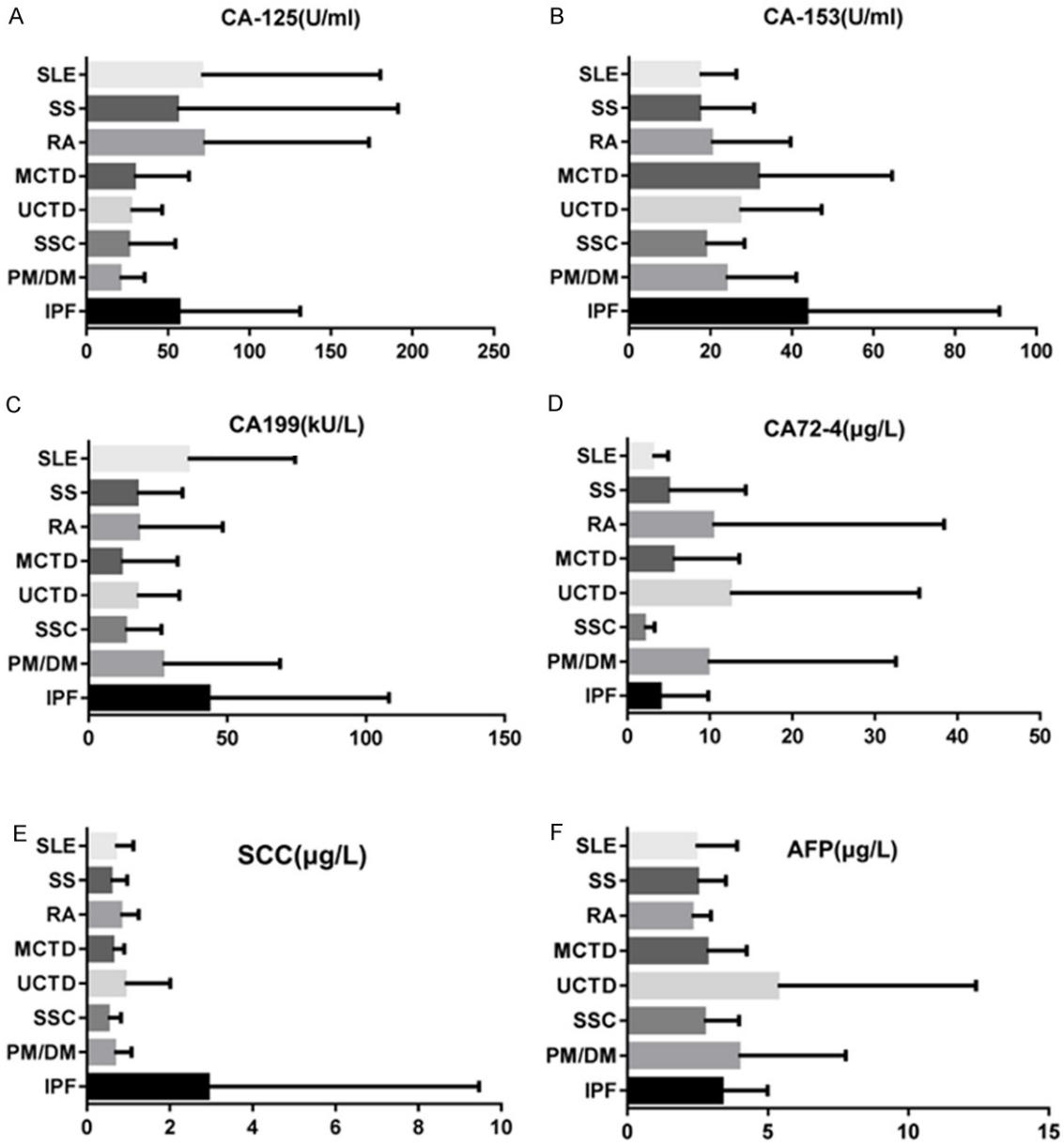
Type of disease	Age (year) of onsetmean (sd)
PM/DM	45.2 (9.2)
SSC	44.6 (12.7)
UCTD	51.1 (14.9)
MCTD	47.8 (11.1)
RA	54.1 (15.7)
SS	48.4 (10.9)
SLE	38.4 (12.9)
P	< 0.05

MCTD = mixed connective tissue disease, PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis, SLE = systemic erythematosus lupus, SS = Sjögren syndrome, SSC = systemic sclerosis, UCTD = undifferentiated connective tissue disease.

Supplementary Table 2. The number of ILD patients whose serum tumor markers were tested

ILD Patient (n)	AFP	CA-125	CA-199	CA72-4	CA-153	CEA	SSC	CFRA 21-1	NSE
IPF	15	13	26	25	14	40	31	19	30
PM	33	23	30	28	21	39	33	6	14
SSC	20	15	18	15	10	23	19	4	8
CTD	16	12	14	14	12	20	18	5	7
MCTD	28	22	24	15	16	33	17	7	10
RA	19	12	21	18	11	25	23	5	10
SS	28	31	28	25	24	33	29	4	6
SLE	9	10	6	4	6	10	5		
Total	168	138	167	144	114	223	175	50	85

AFP = alpha fetal protein, CA (125, 199, 72-4, 193) = cancer antigen (125, 199, 72-4, 193), CEA = carcinoembryonic antigen, CYFRA21-1 = Cytokeratin fragment antigen 21-1, ILD = interstitial lung disease, IPF = idiopathic pulmonary fibrosis, MCTD = mixed connective tissue disease, NSE = neuron-specific enolase PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis, SCC = Squamous cell carcinoma associated antigen, SLE = systemic erythematosus lupus, SS = Sjögren syndrome, SSc = systemic sclerosis, UCTD = undifferentiated connective tissue disease.



Supplementary Figure 1. CA-125, CA-153, CA199, CA72-4, SCC, and AFP levels were detected in ILD patients with different underlying CTDs, showing the mean value and standard deviation of tumor biomarkers. AFP = alpha fetal protein, CA (125, 199, 72-4, 193) = cancer antigen (125, 199, 72-4, 193), CEA = carcinoembryonic antigen, CYFRA21-1 = Cytokeratin fragment antigen21-1, ILD = interstitial lung disease, IPF = idiopathic pulmonary fibrosis, MCTD = mixed connective tissue disease, NSE = neuron-specific enolase, PM/DM = polymyositis/dermatomyositis, RA = rheumatoid arthritis, SCC Squamous cell carcinoma associated antigen, SLE = systemic erythematosus lupus, SS = Sjögren syndrome, SSC = systemic sclerosis, UCTD = undifferentiated connective tissue disease.