Advances in Rheumatology

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Serum MMP-9, SP-D, and VEGF levels reflect the severity of connective tissue disease-associated interstitial lung diseases

Chengna Lv, Qipan Zhang, Pan Tang, Lun Guo and Qunli Ding*

Abstract

Background: Interstitial lung disease (ILD) is a common pulmonary complication of connective tissue disease (CTD). This study aims to evaluate the clinical diagnostic value of matrix metalloproteinase-9 (MMP-9), surfactant protein-D (SP-D), and vascular endothelial growth factor (VEGF) as potential biomarkers for CTD-ILD.

Methods: This research included 33 CTD-ILD patients, 31 CTD patients without ILD, and 24 healthy control subjects. Then, the value of biomarkers for the diagnosis and evaluation of CTD-ILD was assessed through high-resolution computed tomography (HRCT) findings and pulmonary function test (PFT) parameters.

Results: The serum MMP-9, SP-D, and VEGF levels in the CTD-ILD group were higher than those in the CTD-NILD group and healthy group. The ROC curve indicates that VEGF has good to excellent diagnostic performance in diagnosing CTD-ILD, the cut-off that best optimizes sensitivity and specificity in diagnosing CTD-ILD is 277.60 pg/ml (sensitivity, 87.9%; specificity, 83.6%), with an area under the curve (AUC) of 0.905 (95% confidence interval (CI) 0.842–0.968); The ROC curve for MMP-9 suggests this biomarker is fair for diagnosis of CTD-ILD(sensitivity, 81.8%; specificity, 81.8%), with an AUC of 0.867 (95% CI 0.784–0.950), but SP-D only provided lower specificity with higher sensitivity in diagnosing CTD-ILD(sensitivity, 90.9%; specificity, 40.0%). The different serum biomarkers are more specific and sensitive when combined to diagnose ILD. The semiquantitative score for the degree of ILD severity on HRCT was positively correlated with SP-D and VEGF levels ($r=0.461$, $P=0.007$; $r=0.362$, $P=0.039$), and serum MMP-9 levels were elevated in the UIP subgroup compared to the non-UIP subgroup. The percentage of diffusing capacity of the lung for carbon monoxide (DLco) (% predicted) had a negative correlation with the SP-D level ($r=-0.407$, $P=0.044$) and a statistically negative correlation between MMP-9 and the forced vital capacity (FVC) ($r=-0.451$, $P=0.024$).

Conclusions: Serum MMP-9, SP-D, and VEGF levels may have clinical value in screening and evaluating the severity of CTD-ILD.

Key points

- Serum MMP-9, SP-D, and VEGF levels were increased in patients with CTD-ILD and they may have clinical value in screening and evaluating the severity of CTD-ILD.

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- Serum SP-D and VEGF levels had a positive correlation with ILD severity as measured using semiquantitative HRCT scores.
- Serum MMP-9 levels were elevated in the UIP subgroup compared to the non-UIP subgroup. Therefore, further research is required to determine the role of serum MMP-9 levels in the preliminary determination of the ILD subtype.
- Serum MMP-9 levels had a negative correlation with DLco, and serum SP-D levels had a negative correlation with FVC.

Keywords: Connective tissue disease-associated interstitial lung disease, Matrix metalloproteinase-9, Surfactant protein-D, Vascular endothelial growth factor

Introduction

Connective tissue disease (CTD) is a group of chronic inflammatory and autoimmune diseases that invade blood vessels and involve multiple systems and organs. Interstitial lung disease (ILD) is one of the most common and severe pulmonary complications in patients with CTD, who likely suffer from ILD at some point. The onset of CTD-ILD is insidious, and the clinical symptoms are atypical and not obvious, which may explain why ILD is often diagnosed in the late stage when there is widespread fibrosis. More importantly, the overall prognosis of CTD-ILD patients is poor, and their prognosis is related to pathology. NSIP is the most common pathology and HRCT subtype of CTD-ILD, and the prognosis is better than that of idiopathic pulmonary fibrosis (IPF), but the usual interstitial pneumonia (UIP) type has a worse prognosis and higher mortality than NSIP [1, 2]. The prognosis and treatment of CTD-ILD are very different from those of other ILDs [3], and correct diagnosis and treatment are critical to delaying the progression of fibrosis. However, how to predict and diagnose this group of patients remains difficult and unclear.

HRCT has a high value in the diagnosis of pulmonary fibrosis and can preliminarily judge the pathological classification, but it has a certain lag and is associated with harmful radiation in humans. Pulmonary function tests (PFTs) are useful in the diagnosis of ILD, but pulmonary function declines slightly and is not easy to detect, especially in patients with severe ILD. Currently, as a simple and less invasive diagnostic method, biomarkers have been actively studied to supplement the deficiencies of HRCT and PFTs to assess the severity, treatment response, and prognosis of CTD-ILD [4]. Surfactant protein-D (SP-D) is a hydrophobic, collagen-containing, calcium-dependent lectin that initiates an immune response in the lungs [5]. Matrix metalloproteinase-9 (MMP-9) belongs to the zinc ion-dependent protease family and is an indicator of chronic inflammatory and autoimmune diseases [6]. Vascular endothelial growth factor (VEGF) plays an important role in alveolar formation, rapid proliferation, and vascular bed development during lung development [7]. Recent studies suggest that SP-D, MMP-9, and VEGF have a certain correlation with CTD-ILD [8–11].

The main purpose of this study is to assess whether the serum biomarker level can be used as a reliable diagnostic method for patients with CTD-ILD and to evaluate the severity of disease in patients by exploring the relationship between serum biomarkers and laboratory indicators, HRCT findings, and PFT parameters.

Methods

Patients and healthy controls

A total of 64 patients with newly diagnosed CTD from December 2019 to December 2021 in our hospital were recruited and divided into two groups according to the recent diagnostic criteria of CTD-ILD [12]. All patients met the diagnostic criteria for the corresponding connective tissue disease. Patients were diagnosed with CTD-ILD according to the imaging characteristics on HRCT of the recent diagnostic criteria of CTD-ILD [12]. The CTD group with ILD was the experimental group (CTD-ILD group), and the CTD group without ILD was the control group (CTD-NILD group). Twenty-four healthy volunteers recruited in our hospital in the same period were selected and matched to patients on the basis of age and sex.

We excluded patients with chronic obstructive pulmonary disease, tuberculosis, pneumonia, or other lung conditions; patients with severe liver and kidney function impairment, heart failure, tumors, or AIDS; patients who are pregnant women or children, and patients who used methotrexate, steroids, mycophenolate mofetil, leflunomide, tripterygium glycosides, or other biological agents.

Clinical evaluation for ILD

The PFTs of the CTD-ILD group were performed by 2 pulmonary function physicians. Eight patients did not cooperate and were eliminated. HRCT was performed on 64 CTD patients by one of two radiologists. Each

expert independently scored each chest CT twice at random and took the average value. The HRCT subgroups: Two radiologists classified patients according to the diagnostic guidelines for IPF in 2018 [13]. Patients with UIP, possible UIP, and uncertain UIP were classified into the UIP group, and patients who did not meet the guidelines for the UIP group were classified into the non-UIP group. The HRCT score: Two trained radiologists assigned a semiquantitative score for the degree of ILD severity on the HRCT scan (at the level of 1 cm above the arch of the aorta, the protrusion, and the diaphragm, the percentages of lesions on the three levels in the lung field area were calculated), and the sum of the scores on the three levels was the HRCT score [14] (Fig. 1) (Unilateral lung area without lesion, score 0; Unilateral lesion area of 1% to 25%, score 1; The lesion area was 26–50%, and the score was 2; The lesion area was 51–75% and the score was 3; The lesion area was more than 75% and the score was 4).

Serum SP-D, MMP-9, and VEGF measurements

We collected venous blood after 8 h of fasting, centrifuged at 1200 × g for 10 min and the supernatant was aspirated and stored in a −80 °C refrigerator. The serum levels of MMP-9, SP-D, and VEGF were determined by enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Statistical analyses were performed using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA). To evaluate the differences among the three groups, baseline statistics (mean, standard deviation, or frequency) for each variable were generated, and their differences were evaluated using the Mann–Whitney U test or χ^2 test. Receiver-operating characteristic curves (ROC) were used to analyze the cut-off values of serum MMP-9, SP-D, and VEGF to diagnose ILD. The area under the curve with a 95% confidence interval (CI) and the sensitivity and specificity of the cut-off MMP-9, SP-D, and VEGF values were calculated. The *Pearson* or *Spearman* correlation coefficient was used to analyze the correlation between serum biomarker levels and autoantibodies, HRCT scores, subgroups, and PFT parameters. P values < 0.05 were considered significant.

Results

Clinical characteristics

The 64 enrolled CTD patients included 47 females (73.4%) and 17 males (26.5%), with an average age of 60.63 ± 11.60 years. In the general condition, there was no significant difference in age, sex, smoking history, disease duration, or disease composition among the three groups ($P > 0.05$) (Table 1). Regarding the clinical characteristics, there was no significant difference between the CTD-ILD and CTD-NILD groups ($P > 0.05$) (Table 2).

We measured the serum MMP-9, SP-D, and VEGF levels in the three groups. Patients with CTD-ILD

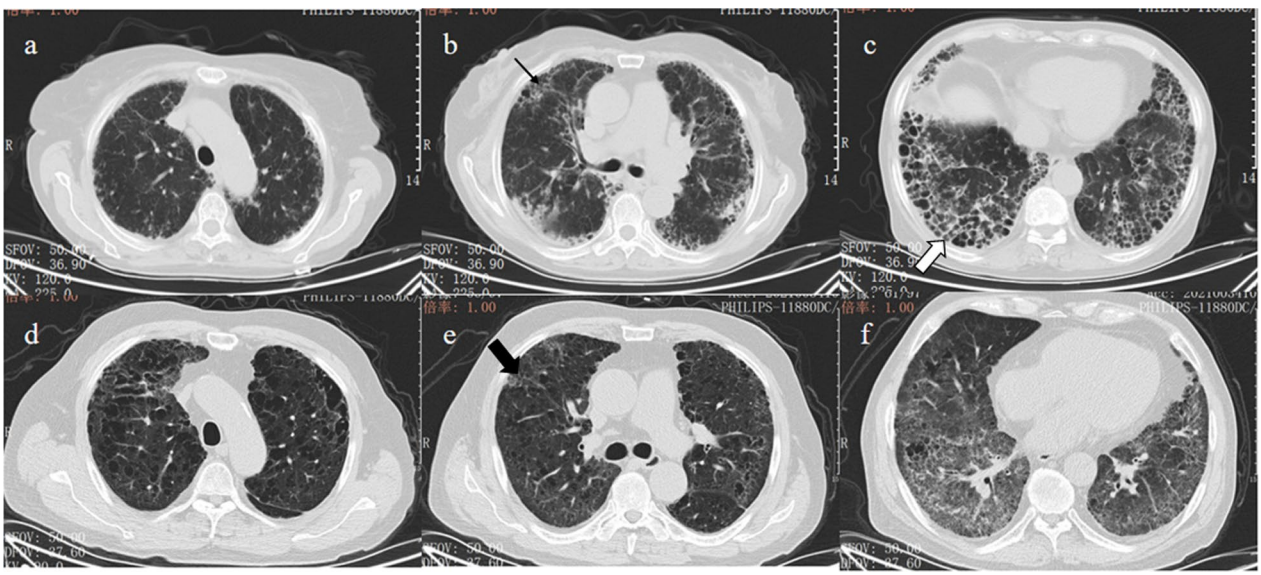

Fig. 1 **a**–**c** Three levels of chest CT scores in a middle-aged female with RA-ILD. Her CT findings showed interstitial pneumonia dominated by fibrosis, reticulation (**b**) and honeycombing (**c**) are visible at the arrow marker. We calculated her chest CT score at 15; **d**–**f** this is a chest CT of an elderly male patient with AAV-ILD. His CT findings showed interstitial pneumonia dominated by ground glass opacity (**e**), and we calculated his chest CT score at 10

Table 1 General conditions and serum biomarkers levels in the three groups

Group	CTD-ILD (n = 33)	CTD-NILD (n = 31)	HC (n = 24)
Male	9	8	6
Female	24	23	18
Age (years)	61.85 ± 10.35	59.32 ± 12.85	55.83 ± 12.70
Duration (years)	5.54 ± 2.19	6.28 ± 2.41	–
Smokers	8	7	5
MMP-9 (ng/ml)	466.63 ± 191.09	287.35 ± 110.55*	153.92(115.21–196.88)*[&]
SP-D(ng/ml)	90.44 ± 22.20	75.19 ± 17.15*	45.14 ± 13.03*[&]
VEGF (pg/ml)	445.86 ± 145.71	229.35(184.10–306.30) *	206.80 (159.83–226.95)*[&]

The CTD-NILD group and HC group were compared with the CTD-ILD group

*($P < 0.05$); HC group was compared with the CTD-NILD group, [&]($P < 0.05$)

Table 2 Clinical characteristics of patients between the CTD-ILD and CTD-NILD groups

Characteristics	CTD-ILD (n = 33)	CTD-NILD (n = 31)	Z/χ^2	*P*
RA	8 (24.2%)	9 (29.0%)	0.054	0.665
pSS	6 (18.2%)	7 (22.6%)	0.055	0.662
SSc	5 (15.2%)	3 (9.7%)	0.082	0.508
DM/PM	4 (12.1%)	3 (9.7%)	0.039	0.754
AAV	4 (12.1%)	3 (9.7%)	0.039	0.754
UCTD	6 (18.2%)	6 (19.4%)	0.015	0.904
Positive anti-CCP antibody	4 (12.1%)	4 (12.9%)	0.000	1.000
Positive ANA antibody	24 (72.7%)	18 (58.1%)	1.523	0.217
Positive SSA antibody	8 (24.2%)	12 (38.7%)	1.557	0.212
Positive SSB antibody	4 (12.1%)	8 (25.9%)	1.169	0.280
Positive p-ANAC antibody	8 (24.2%)	11 (35.5%)	0.968	0.325
Positive c-ANAC antibody	1 (3.0%)	1 (3.2%)	0.000	1.000
ESR (mm/h)	22.00 (9.50–38.50)	29.00 (12.00–51.00)	−0.974	0.330
CRP (mg/L)	3.90 (1.90–9.65)	5.70 (1.80–21.20)	−0.363	0.717
RF (IU/mL)	20.10 (20.00–167.00)	21.80 (20.00–42.30)	−0.233	0.816
IgM (g/L)	1.20 (0.95–1.73)	1.17 (0.87–1.97)	−0.013	0.989
IgA (g/L)	3.30 ± 1.89	2.89 ± 1.12	−0.598	0.550
IgG (g/L)	13.40 (11.60–16.60)	13.50 (12.00–15.80)	−0.114	0.909
C3 (g/L)	0.91 ± 0.30	0.91 ± 0.28	−0.430	0.667
C4 (g/L)	0.22 (0.18–0.29)	0.22 (0.14–0.28)	−0.282	0.778
Mean FEV_1 (n = 25)	91.30 ± 15.16	–	–	–
Mean FVC (n = 25)	84.26 ± 14.26	–	–	–
Mean DL_{CO}% (n = 25)	61.74 ± 13.90	–	–	–
Mean HRCT scores	8.24 ± 4.21	–	–	–

ESR erythrocyte sedimentation rate, *CRP* C-reactive protein, *RF* rheumatoid factors, *NEU* neutrophilic granulocyte, *HGB* hemoglobin, *RA* rheumatoid arthritis, *SSc* systemic sclerosis, *PM* polymyositis, *DM* dermatomyositis, *pSS* primary Sjogren syndrome, *UCTD* undifferentiated connective tissue disease, *AAV* anti- neutrophil cytoplasmic antibody associated vasculitis, *ANA* antinuclear antibody, *ANCA* anti- neutrophil cytoplasmic antibody, *C3* complement C3, *IgM* immunoglobulin M, *SSA* anti-Sjogren syndrome A antibody

(n = 33) had elevated serum MMP-9, SP-D, and VEGF levels compared to CTD patients without ILD (n = 31) and healthy volunteers (n = 24) ($p < 0.05$) (Table 1) (Fig. 2). We found that autoantibodies were not associated with ILD (Table 2), and there was no correlation between autoantibodies and serum biomarker levels in the ILD group (Table 3).

The sensitivity and specificity of VEGF, MMP-9, and SP-D in the diagnosis of CTD-ILD are summarized in Table 4. The ROC curve indicates that VEGF has good

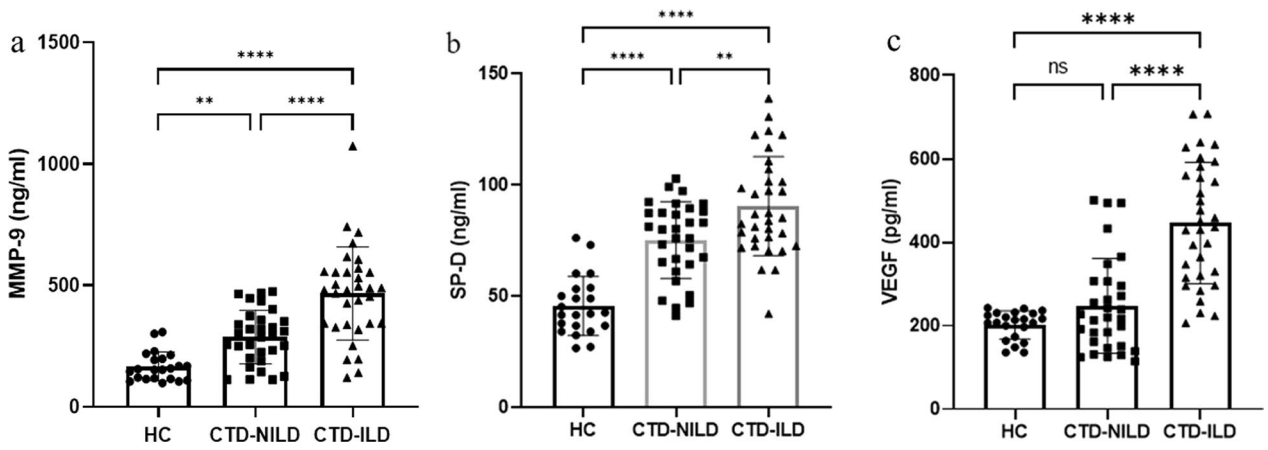

Fig. 2 **a**–**c** Serum MMP-9, SP-D, and VEGF levels in CTD-ILD, CTD-NILD, and HC groups

Table 3 Correlation analysis of serum marker level and traditional laboratory indicators in CTD-ILD

Indicators	MMP-9 (ng/ml)		SP-D (ng/ml)		VEGF (pg/ml)	
	r	*P*	*r*	*P*	*r*	*P*
ESR	−0.181	0.313	0.094	0.603	−0.129	0.474
CRP	−0.011	0.952	0.351	0.045*	0.012	0.946
RF	0.039	0.758	0.177	0.325	0.081	0.653
IgG	0.093	0.608	0.180	0.317	0.195	0.276
IgA	−0.074	0.684	−0.032	0.859	−0.268	0.132
IgM	0.007	0.968	0.080	0.659	0.038	0.833
C3	−0.034	0.849	0.260	0.144	−0.050	0.784
C4	−0.122	0.498	−0.179	0.318	−0.036	0.840
WBC	0.040	0.824	0.057	0.752	0.305	0.084
NEU	0.237	0.185	0.023	0.899	0.064	0.723
Positive anti−CCP antibody	−0.063	0.726	0.249	0.163	0.098	0.589
Positive ANA antibody	0.025	0.890	−0.029	0.875	−0.021	0.906
Positive SSA antibody	−0.089	0.622	−0.071	0.696	0.015	0.935
Positive SSB antibody	−0.199	0.266	−0.299	0.091	−0.205	0.253
Positive p-ANAC antibody	0.275	0.122	0.134	0.458	0.193	0.282
Positive c-ANAC antibody	−0.093	0.607	−0.279	0.093	−0.056	0.758

* mean P < 0.05 (correlation was considered significant)

ESR erythrocyte sedimentation rate, *CRP* c-reactive protein, *RF* rheumatoid factors, *IgG* immunoglobulin G, *C3* complement C3, *WBC* whitebloodcell, *NEU* neutrophilic granulocyte, *CCP* cyclic cirullinated peptide, *ANA* antinuclear antibody, *SSA* anti-Sjogren syndrome A antibody, *ANCA* anti-neutrophil cytoplasmic antibody

Table 4 ROC curves of the three markers for the independent and combined diagnosis of ILD

Biomarker	AUC of ROC	95% CI		Sensitivity %	Specificity %	Cut-off valve
MMP-9	0.867	0.784	0.950	81.8	81.8	326.80 ng/ml
SP-D	0.801	0.710	0.892	90.9	40.0	68.88 ng/ml
VEGF	0.905	0.842	0.968	87.9	83.6	277.60 pg/ml
Combine	0.952	0.912	0.992	96.0	78.7	–

AUC area under the curve, *ROC* receiver operating characteristic curve, *CI* confidence interval

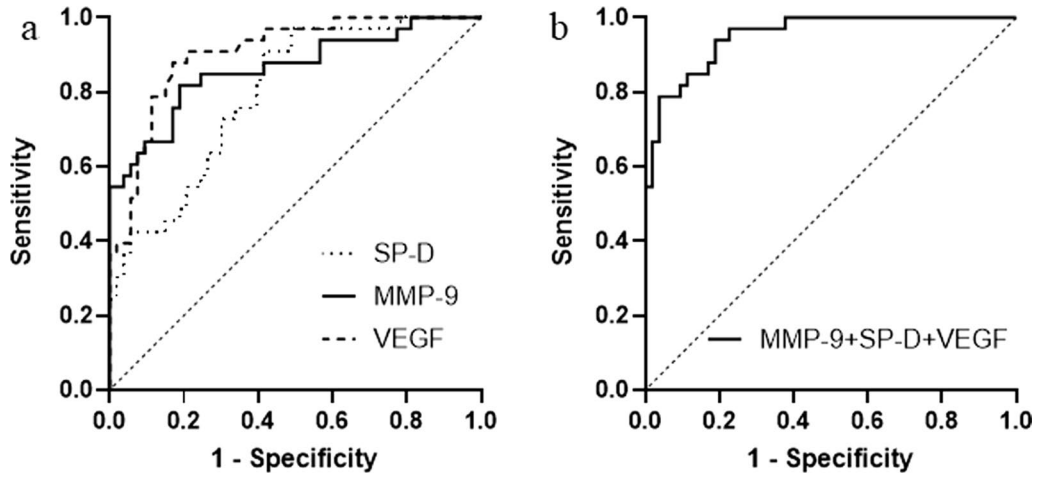

Fig. 3 ROC curves of the three biomarkers for the independent (**a**) and combined (**b**) diagnosis of ILD

to excellent diagnostic performance in diagnosing CTD-ILD, with an area under the curve (AUC) of 0.905 (95% CI 0.842–0.968). Based on this curve, the cut-off that best optimizes sensitivity and specificity in diagnosing CTD-ILD is 277.60 pg/ml (sensitivity, 87.9%; specificity, 83.6%). The ROC curve for MMP-9 suggests this biomarker is fair for diagnosis of CTD-ILD, with an AUC of 0.867 (95% CI 0.784–0.950), and an optimal cut-off is 326.80 ng/ml (sensitivity, 81.8%; specificity, 81.8%). However, SP-D only provided lower specificity with higher sensitivity in diagnosing CTD-ILD (sensitivity, 90.9%; specificity, 40.0%). (Table 4) (Fig. 3).

Correlation of the serum biomarkers with HRCT and PFT parameters

The imaging manifestations of the CTD-ILD group were varied. In our study, we found that ground glass opacity was the most common feature of CTD-ILD and was more common in pSS, SSc, UCTD, and PM/DM-related ILD. In addition, honeycomb lung and reticular opacity are more likely to be found in RA-ILD.

We used "SPSS 22.0 software" to calculate the intraclass correlation coefficient (ICC) of 0.928, which suggested that the two different senior radiologists' scoring results were in good agreement. We found that the semiquantitative scores for the degree of ILD on the HRCT scan were significantly proportional to the SP-D and VEGF levels ($r=0.461$, $P=0.007$; $r=0.362$, $P=0.039$) (Fig. 4). Among patients with CTD-ILD, serum MMP-9 levels were elevated in the UIP subgroup compared to the non-UIP subgroup ($P<0.05$) (Table 5), and there was no significant difference in serum SP-D and VEGF levels between the two groups ($P>0.05$) (Table 5). The serum MMP-9 level was negatively correlated with FVC ($r=-0.451$, $P=0.024$), and SP-D was negatively correlated with DLco (% predicted) ($r=-0.407$, $P=0.044$) (Fig. 4).

Discussion

In recent years, it has been found that serum biomarkers have clinical value for the diagnosis of CTD-ILD. Herein, we retrospectively investigated whether MMP-9, SP-D, and VEGF could be used as biomarkers that reflect the clinical status of ILD in CTD patients. We mainly focus on the clinical practice of using MMP-9, SP-D, and VEGF to diagnose and assess the severity of ILD in CTD patients and provide the relevant basis for clinicians.

Autoantibodies are involved in the process of pulmonary fibrosis in CTD. Studies have found that high titers of RF, positive anti-CCP antibodies and positive anti-ANA antibodies are risk factors for CTD-ILD [15]. Patients with positive anti-Jo-1 and anti-SSA antibodies have more severe ILD and are more resistant to immunosuppressive therapy than those who are negative for these antibodies [16]. Nonetheless, it has been reported that anti-SSA and anti-SSB antibody positivity is not associated with pSS-related ILD [17]. According to our research, autoantibodies were not associated with ILD, and there was no correlation between autoantibodies and serum biomarker levels in the ILD group. It is uncertain whether these antibodies contribute to the development of pulmonary fibrosis in patients with CTD, and their interaction should be investigated. It is noteworthy that traditional laboratory indicators could not reliably distinguish whether CTD was complicated with ILD.

An investigation of the association between novel serum biomarkers and CTD-ILD was conducted. As zinc

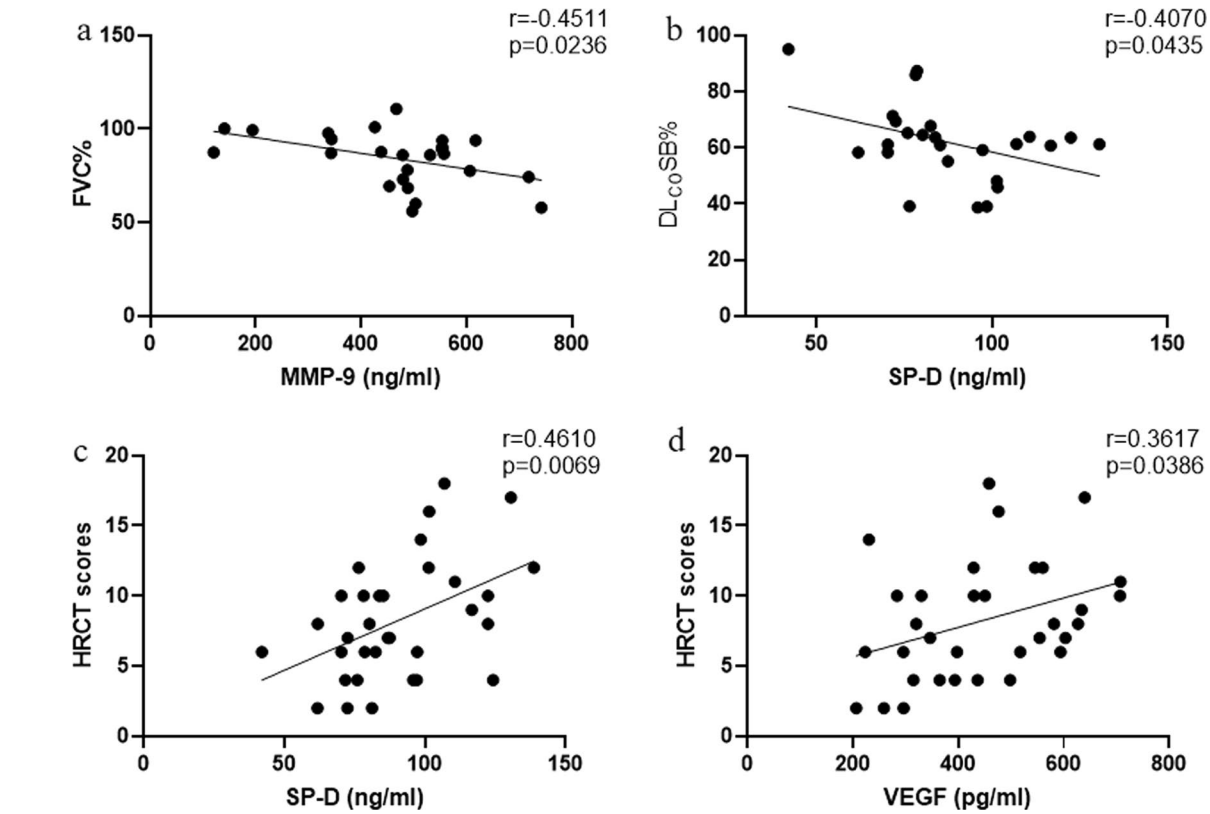

Fig. 4 **a** The correlation between serum MMP-9 level and FVC; **b** the correlation between serum SP-D level and DLco; **c** the correlation between serum SP-D level and semiquantitative HRCT scores; **d** the correlation between serum VEGF level and semiquantitative HRCT scores

Table 5 Comparison of serum biomarker level and HRCT subgroups in the CTD-ILD patients

HRCT subgroups	UIP(n = 9)	no-UIP (n = 24)	t	*p*
MMP-9 (ng/ml)	568.94 ± 215.88	415.47 ± 158.82	2.319	0.027
SP-D (ng/ml)	89.96 ± 22.09	90.67 ± 22.77	− 0.085	0.933
VEGF (pg/ml)	479.04 ± 116.07	429.27 ± 158.35	0.923	0.363

HRCT high-resolution computed tomography, *UIP* usual interstitial pneumonia

ion-dependent proteases, MMPs regulate inflammatory mediators and antifibrotic growth factors. A study has shown that PM patients have elevated serum levels of MMP-9 (a member of this family) [6]. Thirty-one patients with SSc-associated ILD had elevated serum MMP-9 levels and MMP-9 levels were negatively correlated with FVC [9]. We have found similar results in our research. There is evidence that PFT parameters may partially reflect the severity of ILD, and MMP-9 is also likely to indirectly reflect the severity of the disease. The SP-D lipoprotein plays a key role in the lung immune response and correlates with the activities of ILD and alveolar proteinosis. It is secreted by type II alveolar epithelial cells [18]. DLco (percent predicted) is negatively correlated with SP-D levels in early-stage pulmonary fibrosis patients with SSc [10]. As with the preceding study, our experimental results are comparable. As a diagnostic indicator for CTD-ILD, the SP-D level in the serum can be useful. In the progression of ILD, VEGF plays a critical role. According to recent research, VEGF plays a dual role in promoting and inhibiting pulmonary fibrosis, and an imbalance in VEGF subtype expression can contribute to the development of this disease, and the proliferation of endothelial cells and fibroblasts can be induced by VEGF [19]. Studies have shown elevated serum VEGF levels in microscopic polyvasculitis involving the lungs [20], and VEGF levels in patients with rheumatoid arthritis have been found to be higher than in healthy controls, which may indicate the progression of the disease [19]. A significant increase in VEGF levels was observed in the RA group with systemic involvement (including lung interstitial involvement) compared to the RA group without systemic involvement [11]. A high level of VEGF expression is not necessarily associated with ILD but may indicate increased disease activity. Our research has been

validated by these findings. It may be beneficial to measure VEGF in patients with CTD.

HRCT is the most common diagnostic method for CTD-ILD. KL-6 is significantly higher in RA-associated ILD than in RA without ILD, and its expression level is higher in the UIP subtype, which can reflect the subtype of ILD and predict the progression of the disease [21]. We measured the severity of ILD using a semiquantitative HRCT score and found that serum SP-D and VEGF levels were positively correlated with ILD severity. In addition, Japanese researchers discovered that UIP in the ILD subgroup can be differentiated by serum SP-A levels, whereas SP-D levels were insufficient [22]. However, the serum SP-D and VEGF levels failed to distinguish between UIP and non-UIP in our experiment. As we all know, IPF is a form of ILD with UIP-like histopathological and thoracic HRCT characteristics. Serum levels of MMP-9 and other matrix metalloproteinases are elevated in IPF patients [23], indicating an indirect association between serum MMP levels and UIP. We found that serum MMP-9 levels were significantly elevated in the UIP subgroup compared to the non-UIP subgroup. Therefore, further research is required to determine the role of serum MMP-9 levels in the preliminary determination of the ILD subtype. More clinical trials are necessary to confirm the role of these serum biomarkers in the diagnosis of ILD subtypes.

There was indeed some flaw in our research. Firstly, an analysis of the correlation between serum biomarker levels and the dynamic progression of ILD is lacking. Therefore, we are unable to assess the disease progression and prognosis of the patient. Secondly, A meta-analysis suggests serum SP-D level can also provide a convenient and non-invasive approach for screening and management of ILD among CTD patients, but serum SP-D is inferior to KL-6 in differentiating ILD from non-ILD [24]. Unfortunately, the concentration of KL-6 was not measured as a control in our study. Thirdly, we lacked data on lung function in the control group; The patients in the department of non-respiratory medicine did not routinely have pulmonary function tests performed, and some patients refused to undergo pulmonary function tests. Therefore, we could not dynamically compare the correlation between lung function parameters and biomarker levels, especially for patients with pure CTD who may potentially develop ILD in the later stage. Finally, this is a single cross-sectional investigation. With a small sample size and a cross-sectional design, the study's credibility and robustness are limited, and additional research with a larger sample size is required.

Conclusions

Serum MMP-9, SP-D, and VEGF levels were increased in patients with CTD-ILD; Serum SP-D and VEGF levels had a positive correlation with ILD severity as measured using semiquantitative HRCT scores, and serum MMP-9 levels were elevated in the UIP subgroup compared to the non-UIP subgroup. Serum MMP-9 levels had a negative correlation with DLco, and serum SP-D levels had a negative correlation with FVC. Serum MMP-9, SP-D, and VEGF levels may have clinical value in screening and evaluating the severity of CTD-ILD.

Acknowledgements
Not applicable.

Author contributions
CL: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing—original draft. QZ: Investigation. PT: Investigation. LG: Investigation. QD: Resources, Supervision, Conceptualization, Writing—review and editing. All authors read and approved the final manuscript.

Funding
This work was supported by the Zhejiang Provincial Natural Science Foundation of China (No. LBY22H180004); and the Ningbo Natural Science Foundation (No. 2019A610228).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate
This study was approved by The Affiliated Hospital of Medical School of Ningbo University Ethics Committee (Ethics number: KS20221010) and all subjects consented to participate.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests and have no financial interests to declare.

Received: 15 April 2022 Accepted: 12 October 2022
Published online: 27 October 2022

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