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Krebs von den Lungen-6 and surfactant protein-A in interstitial pneumonia with autoimmune features

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Abstract

Interstitial pneumonia with autoimmune features (IPAF) is a special subtype of interstitial lung disease that has received worldwide attention. Krebs von den Lungen-6 (KL-6) and surfactant protein-A (SP-A) can be used as an important biomarker of interstitial lung disease, but its exact relationship with IPAF is poorly understood.

A total of 65 IPAF patients were included in the study and were followed up for 52 weeks. The KL-6 and SP-A were evaluated by chemiluminescence enzyme immunoassay. The above indicators were tested at 2 time points, baseline (the first admission of patients) and 52 weeks. We also collected the indicators of antinuclear antibodies and rheumatoid factor. Based on high-resolution computed tomography evaluations, patients were divided into: aggravation, stable, and improvement group. At same time, 30 age-matched normal people as normal control were recruited, the same information was collected. Correlations among the groups were compared and analyzed.

The KL-6 and SP-A level in IPAF patients were significantly higher than normal controls (fold increase = 11.35 and 1.39, both $P < .001$) and differed significantly at baseline and 52 weeks in IPAF (difference ratio = 37.7% and 21.3%, $P < .05$, both). There were significant differences at baseline and 52 weeks (r values of aggravation, improvement, and stable groups for KL-6 were 0.705, 0.770, and 0.344, $P = .001$, .001, and .163, and for SP-A the r value were 0.672, 0.375, and 0.316, $P = .001$, .126, and .152). In aggravation group, KL-6 and SP-A were correlated with CT scores (both $P < .05$). Diffusing capacity of the lung for carbon monoxide (DLCO) and forced vital capacity (FVC), % predicted showed a progressive downward trend, with a significant difference at baseline and 52 weeks in IPAF patients (difference ratio = 23.8% and 20.6%, both $P < .05$). There was a significant correlation between KL-6 and FVC % predicted and DLCO (both $P < .05$), SP-A showed negatively correlated with DLCO, but not significantly correlated with FVC % predicted ($P < .05$ and .47).

This study demonstrated that KL-6 and SP-A can reflect disease progression, and both 2 play a key role at reflection of lung epithelial cell injury and fibrosis degree in IPAF.

Abbreviations: ANA = antinuclear antibodies, CTD-ILD = connective tissue disease-interstitial lung disease, DLCO = diffusing capacity of the lung for carbon monoxide, FEV1 = forced expiratory volume in 1 second, FVC = forced vital capacity, HRCT = high-

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MX and CC contributed equally to the study.

The study was approved by the ethics committee of the First Hospital of Guangzhou Medical University: NO. 201882.

Written informed consent for publication was obtained from all participants.

On the basis of not violating the participant confidentiality agreement. We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes.

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resolution computed tomography, ILD = interstitial lung disease, IPAF = interstitial pneumonia with autoimmune features, KL-6 = Krebs von den Lungen-6, RF = rheumatoid factor, SP-A = surfactant protein-A.

Keywords: interstitial pneumonia with autoimmune features, Krebs von den Lungen-6 (KL-6), surfactant protein-A (SP-A)

1. Introduction

Interstitial pneumonia with autoimmune features (IPAF) is a special subtype of interstitial lung disease (ILD) that was established by the American Thoracic Society and European Respiratory Society ATS/ERS in 2015 and is closely related to connective tissue disease-interstitial lung disease (CTD-ILD).[1–3] Both IPAF and CTD-ILD are immune-related ILDs with partially overlapping manifestations in high-resolution computed tomography (HRCT), histopathology, lung dysfunction, etc.[4] Therefore, the diagnostic and classification criteria for IPAF are also serological and clinical characteristics shared with CTD-ILD.[5–7] IPAF is diagnosed based on the exclusion of other known immune-related ILDs, and requires at least 2 of 3 clinical, serological, and imaging standards.[5] The underlying mechanisms and clinical manifestations of IPAF are complex.[6] The serology of IPAF is also poorly understood, and there is still a lack of consensus in substantive diagnosis.[4] In addition, the criteria for classification are currently being refined.[6,8]

HRCT and/or histopathological findings provide direct evidence for the identification of IPAF, and more than 90% of cases are serologically positive for antinuclear antibodies (ANAs) and/or rheumatic factor (RF).[9] Reduced diffuse function is a manifestation, rather than a feature, of the disease; however, it can still be used as a marker of progression.[4] Patients with IPAF may have a higher risk of disease progression than patients with CTD-ILD, and follow-up research may shed more light on the disease mechanism.[9,10]

Krebs von den Lungen-6 (KL-6) is a high molecular weight myxoid glycoprotein that is mainly found on type II alveolar cells and is expressed and secreted by thin bronchial epithelial cells. KL-6 is involved in fibroblast chemotaxis and antiapoptotic effects, can reflect the damage and regeneration of alveolar cells, and is an important biomarker of pulmonary fibrosis.[11,12] KL-6 is regarded as a core biomarker for the diagnosis of ILD in Japan, as it is closely related to disease activity and can provide prognostic information.[13,14] Alveolar surfactant proteins (SPs), including A, B, and D subtypes, are the most abundant. SP-A is a representative hydrophilic SP that is involved in the innate immune response, regulates inflammation, and plays a key role in limiting alveolar cavity inflammation as well as for II alveolar cells expressed.[15,16] KL-6 and SP-A have been extensively studied in CTD-ILD, including dermatomyositis-related ILD, systemic sclerosis-related ILD, and arthritis-related ILD.[14,17–19] Although IPAF is currently considered to be an independent diagnosis, many of its associated phenotypes overlap with CTD-ILD, and studies on KL-6 and SP-A in IPAF are lacking.[20]

In previous studies, we explored the levels of KL-6 and SP-A in IPAF, and found that although they could not be used as specific markers to distinguish between IPAF and CTD-ILD, their levels were higher in patients with these diseases than in normal people and differed significantly between patients with the 2 diseases.[21] The clinical manifestations of IPAF and other autoimmune diseases are similar and their classifications overlap. As the disease progresses, inconsistencies in lung function, imaging findings, clinical manifestations, and serology may occur; therefore, the application of a single indicator is not rigorous enough for comparative analysis.[22–25] In this study, we conducted a correlation analysis with multiple clinical indicators to comprehensively explore the value of KL-6 and SP-A in the progression of IPAF.

2. Methods

2.1. Characteristics of study participants

This study included 65 patients with IPAF evaluated at the First Affiliated Hospital of Guangzhou Medical University from October 2016 to October 2018. Diagnosis and classification were based on the IPAF expert consensus established by ERS/ATS in 2015 and were confirmed by imaging and/or pathological and serological evaluations. Patients had to meet the following criteria for inclusion:

1. males and females between 40 and 60 years of age;
2. no surgical history;
3. no metabolic diseases, such as diabetes or hyperthyroidism,
4. no cardiovascular diseases, such as coronary heart disease;
5. no history of cancer;
6. excluded the patients with allergic history of pollen, pet feeding, and fungal or pneumoconiosis patients; and
7. no history of other lung diseases.

General clinical information was collected, lung function was evaluated, and venous blood was collected in the morning on an empty stomach. Seven patients who were ultimately diagnosed with other types of ILD or developed tumors during follow-up were excluded. As a control group, 30 age-matched normal people were recruited, and serum samples were collected from them.

2.2. Blood sample preservation

Five milliliters of fasting venous blood was collected into a procoagulant tube. Then, the sample was centrifuged at 3000 rpm for 30 minutes at room temperature. The supernatant was collected and stored at −80°C until use.

2.3. Serological indicators

KL-6 and SP-A levels in serum were determined by chemiluminescence enzyme immunoassay (Sysmex, Japan). ANAs were detected by indirect immunofluorescence assay with Hep-2 cells as substrate, and the titer of 1:320 as positive defined (INOVA, America). RF was tested by detection Kit (Immunoturbidimetry) (MSKbio, China).

2.4. Pulmonary function tests

Lung function was evaluated using a lung quantifier (Jaeger, Germany), which was operated according to ATS/ERS standards.

Measured indicators included forced expiratory volume in one second (FEV1), forced vital capacity (FVC), FEV1/FVC, total lung capacity (TLC), vital capacity (VC), and dispersion function [(Diffusing capacity of the lung for carbon monoxide (DLCO)].

2.5. High resolution CT

All patients with IPAF underwent chest HRCT, with thin-layer (1-mm) scanning, at 2 time points, at baseline and 1 year later. Since there is no current unified scoring standard for IPAF, the IPAF classification standard published by ATS/ERS in 2015 was used, with reference to the image evaluation methods for ILD in Ying et al,[26] Man et al,[27] Zou et al,[28] and Lee et al.[29] Some of the morphological classification criteria for IPAF are the same as those for CTD-ILD; therefore, we tentatively used part of the CTD-ILD criteria (ground glass shadow, bronchial pull, nodules, and honeycombing in 2 CT sections of the diaphragm and aortic arch). For a more comprehensive assessment, we also combined this with the changes in lesion area and position (affected). Based on these assessments, the IPAF patients were divided into three groups, aggravation, stable, and improvement. All relevant evaluations and scores were conducted by 3 radiologists, 2 respiratory physicians, and 1 rheumatologist at our hospital to provide the most accurate diagnosis and to distinguish ILD from other identifiable diseases.

2.6. Statistical analysis

The data are presented as the mean ± standard deviation, and the variance, *t*-test, and rank-sum test were used to determine the statistical significance of differences at $P < .05$. IBM SPSS (Statistics for Windows, Version 22.0; IBM Corp., Chicago, IL, USA), GraphPad Prism 5.0 (GraphPad Software, San Diego, CA), and R statistical package (University of Auckland, New Zealand) were used for data analysis and graphic production.

3. Results

3.1. Characteristics of patients with interstitial pneumonia with autoimmune features (IPAF)

The levels of KL-6 and SP-A in IPAF patients were significantly higher than those in the normal controls (Fold increase = 7.06 and 2.84, Both $P < .001$). And the lung function index (FEV1% predicted, FVC %predicted, and DLCO) in patient of IPAF were lower than the normal control with significant difference (Difference ratio = 17.4%,16.7% and 32.0%, P all $< .01$). In this study, a higher proportion of IPAF patients were female, but the difference was not statistically significant due to the small sample size (Table 1).

Table 1

Participants characters.

	Normal Control	IPAF	*P*-value
N	30	65	——
Age, years	54.00 ± 12.61	51.00 ± 14.06	.227
Male/Female, n [%]	18/12 [150]	29/36 [81]	.190
BMI, kg/m^2	23.19 ± 3.84	24.11 ± 3.65	.435
WBC, 10^{12}/L	7.30 ± 2.31	7.04 ± 2.31	.399
Neutrophil ratio, %	68.12 ± 14.08	61.70 ± 10.12	.014
Lymphocyte ratio, %	23.00 ± 5.20	25.50 ± 7.32	.056
Mononuclear ratio, %	7.31 ± 2.44	8.40 ± 3.63	.068
Eosinophil ratio, %	1.32 ± 1.56	1.50 ± 1.62	.101
Basophil ratio, %	0.51 ± 0.21	0.40 ± 0.23	.278
RBC, 10^{12}/L	4.63 ± 0.29	4.42 ± 0.56	.187
Hemoglobin, g/L	134.00 ± 9.65	136.00 ± 14.04	.543
Thrombocyte, 10^9/L	168.00 ± 45.92	184.00 ± 60.96	.245
FEV1, %predicted	86.70 ± 7.41	68.00 ± 13.12	.001
FVC, %predicted	82.43 ± 5.39	68.40 ± 13.82	.003
DLCO, %	91.15 ± 10.44	59.90 ± 12.43	.001
KL-6	180.5 ± 71.51	1275.00 ± 1538.00	.001
SP-A	25.80 ± 18.31	37.15 ± 39.09	.001
CRP, mg/L	0.18 ± 0.53	0.26 ± 0.32	.137
LDH	177.06 ± 67.90	206.00 ± 48.45	.289

BMI = body mass index, CRP = C-reactive protein, DLCO = diffusing capacity of the lung for carbon monoxide, FEV1 = forced expiratory volume In 1 second, FVC = force vital capacity, KL-6 = krebs von den Lungen-6, LDH = lactate dehydrogenase, SP-A = surfactant protein-A, WBC = white blood cell.

3.2. Trends and correlation analysis of KL-6 and SP-A levels in the aggravation, improvement, and stable groups of IPAF patients

The total KL-6 and SP-A levels in patients with IPAF differed significantly at baseline and 52 weeks (Difference ratio = 37.7% and 21.3%, $r = 0.565$ and 0.556; $P < .05$, both). The trends in KL-6 and SP-A levels during the 52-week follow-up are shown in Figure 1. KL-6 and SP-A levels in the aggravation group differed significantly from those in the improvement and stable groups at both baseline and 52 weeks (the *r* values for KL-6 and SP-A in the aggravation, improvement, and stable groups were 0.705, 0.770, and 0.344, respectively, and 0.672, 0.375, and 0.316, respectively. The *P* values were .001, .001, and .163, respectively, and .001, .126, and .152, respectively.

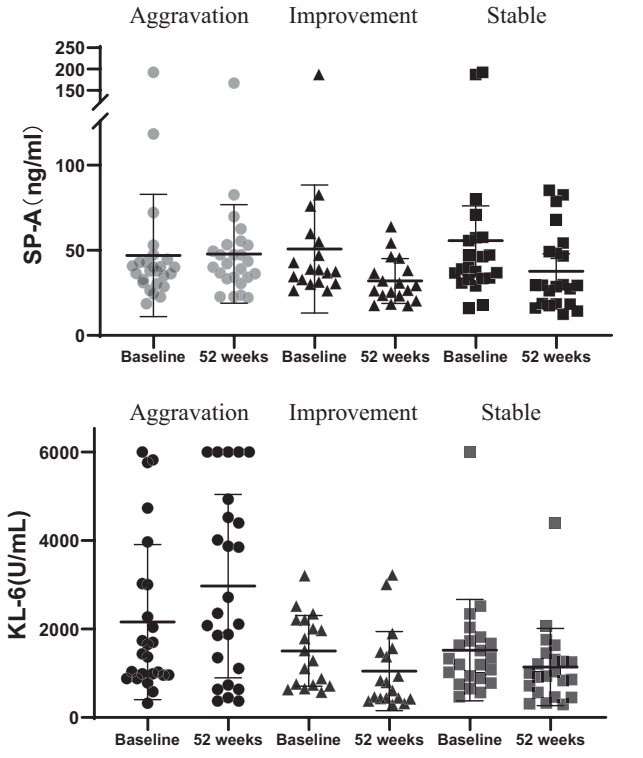

Figure 1. Trends of KL-6 and SP-A levels in IPAF patients during 52-week follow-up. IPAF = interstitial pneumonia with autoimmune features, KL-6 = Krebs von den Lungen-6, SP-A = surfactant protein-A. ***: $P < .001$.

Table 2

Imaging results.

	IPAF patients, N [%]
Characteristic pulmonary manifestations	
Reticular pattern	23 [35.4]
Honeycombing	25 [38.5]
Groud glass opacities	36 [55.4]
Nodular lesions	41 [63.1]
UIP	0
OP	3 [4.6]
NSIP and OP	1 [1.5]
LIP	0
Other manifestations	
Traction bronchiectasis or bronchiolectasis	11 [16.9]
Reduced lung volume	1 [1.5]
Hydropericardium	3 [4.6]
Hydrothorax	7 [10.8]
Pleural thickening	5 [7.7]
Mediastinal lymph node enlargement	48 [73.8]

LIP = lymphocytic interstitial pneumonia, NSIP = nonspecific interstitial pneumonia, OP = organizing pneumonia, UIP = usual interstitial pneumonia.

3.3. Correlation analysis of HRCT score with KL-6 and SP-A levels in patients with IPAF

The imaging results of patients with IPAF are shown in Table 2. To further analyze the correlation of KL-6 and SP-A levels with HRCT findings in patients with IPAF, we quantified KL-6 and SP-A levels and applied HRCT scores. The results of the analysis showed that KL-6 was significantly correlated with HRCT score ($r = 0.276$, $P < .05$), and SP-A was significantly correlated with HRCT score ($r = 0.246$, $P < .05$; Fig. 2). In the aggravation group of IPAF patients, both SP-A and KL-6 were significantly correlated with HRCT score ($r = -0.276$ and -0.479, respectively, $P < .05$ for both). In contrast, no significant correlations were found in the improvement and stable groups.

3.4. Correlation analysis of KL-6 and SP-A levels with lung function

IPAF is a special subtype of ILD that is characterized by interstitial lung damage, and pulmonary ventilation dysfunction often occurs.[30] In this study, DLCO and FVC, % predicted showed a progressive downward trend, with a significant difference between the 2 time points, baseline and 52 weeks (Difference ratio = 23.8% and 20.6%, $r = 0.438$ and 0.513, respectively; $P < .05$ for both). However, after grouping the IPAF patients, we found significant differences between DLCO and FVC, % predicted at baseline and 52 weeks ($r = 0.726$ and 0.641, respectively; $P < .05$), while no significant differences were found in the improvement and stable groups.

Comparison of DLCO and FVC, % predicted with KL-6 and SP-A levels showed that KL-6 was negatively correlated with DLCO and FVC, % predicted ($r = -0.378$ and –0.345, respectively; $P < .05$), and SP-A was negatively correlated with DLCO but not with FVC, % predicted ($r = -0.263$ and –0.091, respectively; $P < .05$ and 0.47, respectively; Fig. 3). In addition, the correlation of DLCO and FVC, % predicted with age and sex was also analyzed, but no significant correlations or trends were observed.

3.5. Clinical manifestations of patients with IPAF

In this study, Raynaud phenomenon was the most common symptom, which was present in 18.5% of IPAF patients, followed by joint pain/swelling (13.8%), weight loss (12.3%), and morning stiffness (10.8%). Distal digital fissuring and tip ulceration were observed in only 1 case (Table 3).

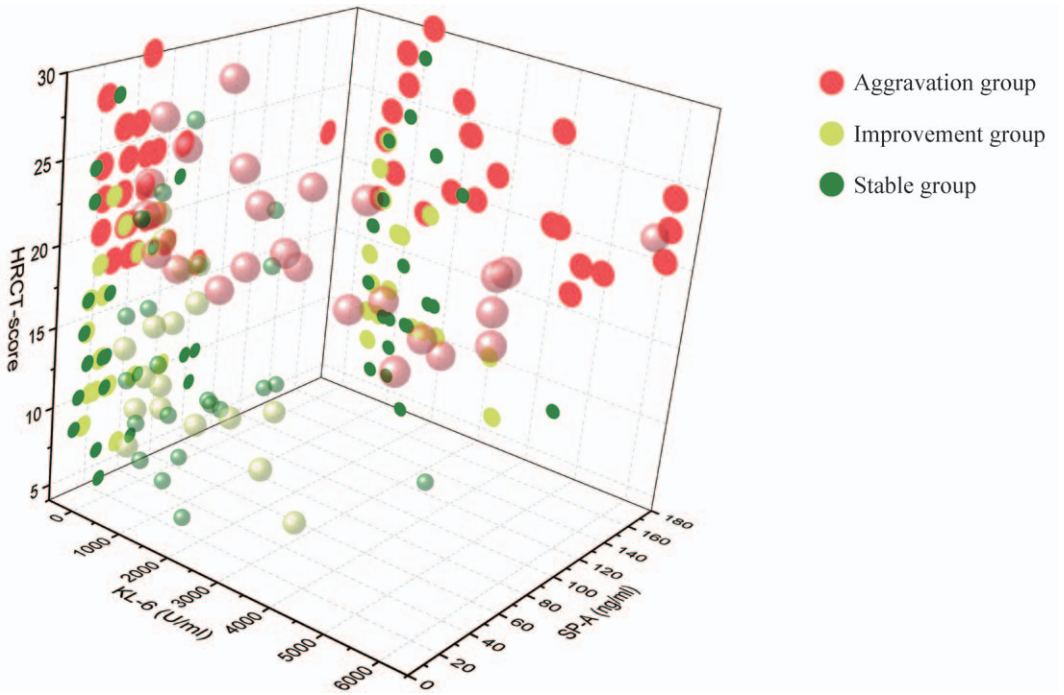

Figure 2. Scatter plot of HRCT scores and KL-6 and SP-A levels.

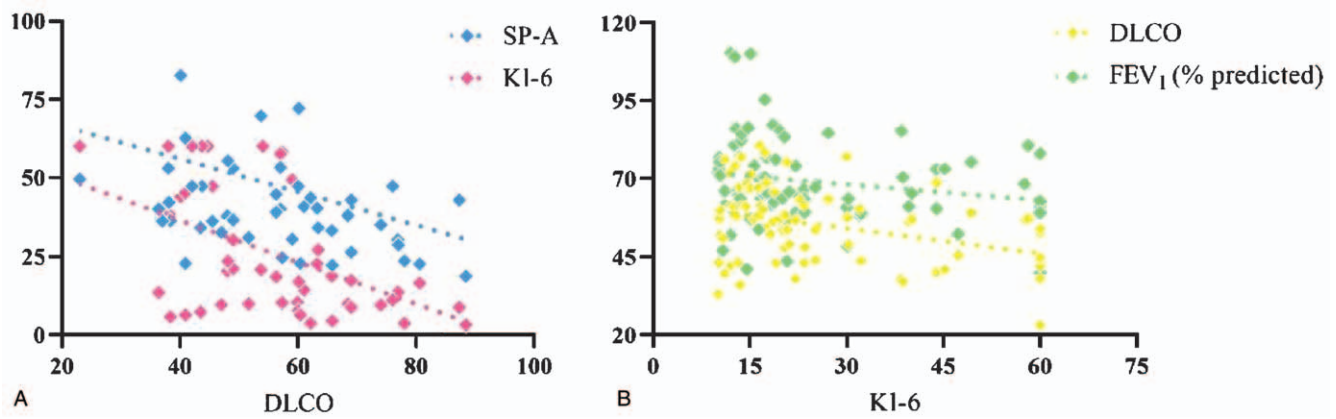

Figure 3. The correlation of KL-6 and SP-A levels with DLCO and FEV1. DLCO = carbon monoxide diffusing capacity, FEV1 = forced expiratory volume in 1 second, KL-6 = Krebs von den Lungen-6, SP-A = surfactant protein-A.

In addition, we referred to the research results of Doishita et al[31] and Zheng et al[32] as well as our previous research results.[21] There was a significant difference in KL-6 levels at baseline and 52 weeks ($r = 0.325$, $P < .05$), whereas SP-A levels did not differ significantly between these 2 time points ($r = 0.203$, $P > .05$). The differences in KL-6 and SP-A levels at baseline and 52 weeks in the aggravation, improvement, and stable groups were ($r = 0.703$, 0.473, and 0.638, respectively; $P < .05$, for all; and $r = 0.553$, −0.194, and 0.402, respectively; $P < .05$, =.44, and =.06, respectively). In addition, correlation analyses were performed for KL-6 and SP-A levels and the rates of various signs and symptoms, and for most, no significant correlation was found. However, KL-6 was significantly correlated with Raynaud phenomenon ($r = 0.326$, $P < .05$).

Table 3

The symptoms of IPAF patients.

Clinical manifestations	IPAF patients, N [%]
Main classification criteria	
Raynauds phenomenon	12 [18.5]
Palmar telangiectasia	3 [4.6]
Distal digital fissuring	1 [1.5]
Distal digital tip ulceration	1 [1.5]
Unexplained digital edema	0
Dry eyes or mouth	1 [1.5]
Gastroesophageal reflux	6 [9.2]
Weight loss	8 [12.3]
Leg/foot swelling	4 [6.2]
Joint pain/swelling	9 [13.8]
Rash photosensitivity	0
Dysphagia	0
Hand ulcers	1 [1.5]
Proximal muscle weakness	2 [3.1]
Oral ulceration	3 [4.6]
Morning stiffness	7 [10.8]
Other clinical manifestations	
Cough	60 [92.3]
Wheeze	46 [70.8]
Dyspnea	27 [41.5]
Chest congestion	39 [60.0]
Chest pain	13 [20.0]
Weakness	24 [36.9]

3.6. Correlation of KL-6 and SP-A with autoimmune factors

The positive rates of autoimmune factor detection in patients with IPAF are summarized in Table 4. No significant positive distribution of specific autoimmune factors was found in the aggravation, improvement, and stable groups. Correlation analysis of KL-6 and SP-A levels with various autoimmune factors yielded no significant correlation.

4. Discussion

IPAF is an independent diagnostic classification for ILD with immune characteristics that do not meet the criteria for CTD-ILD.[33,34] This disease is less common than IPF and CTD-ILD and has a completely different management strategy. Therefore, it should not be ignored in clinical practice.[35,36] Survival analysis showed that the prognosis of patients with IPAF was worse than that of patients with CTD-ILD. Patients with IPAF were predominantly female and younger than patients with IPF.[37] Diagnosis and classification of IPAF are based on serological, pulmonary function, imaging, and/or pathological findings. Here, we explored the value of evaluating KL-6 and SP-A levels in patients with IPAF.

HRCT can provide critical evidence for the diagnosis of IPAF, and the higher the degree of honeycombing, the worse the prognosis.[37] IPAF involves pleural and pulmonary interstitial dysfunction and is mainly manifested by restricted ventilation dysfunction and diffuse dysfunction. FVC, % predicted, and DLCO are the most commonly used indicators of pulmonary function in interstitial pulmonary disease,[9,38] and we found that FVC, % predicted, and DLCO were significantly decreased in the aggravation group at 52 weeks, when compared to these values at baseline ($P < .05$) and were negatively correlated with HRCT score. In contrast, FVC, % predicted, and DLCO did not differ significantly between the improvement and stable groups. Liang et al[39] stated that patients with IPAF suffered from systemic immune dysfunction, leading to pulmonary inflammatory factor infiltration and resulting in lung interstitial, alveolar, and pulmonary vascular damage. Therefore, combined with the imaging findings, we considered that patients with IPAF, under the circumstances of autoimmune disorders, suffered from lung

Table 4

Autoimmune antibodies of patients with IPAF.

	IPAF patients, N [%]	*P*-value (r) KL-6	*P*-value (r) SP-A
Serological domain	54 [83.1]	.259 (0.142)	0.155 (−0.178)
ANA≥ 1:320	49 [75.4]	.152 (0.180)	0.341 (−0.120)
RF ≥2× the upper limit of normal	28 [43.1]	.719 (0.046)	0.704 (−0.048)
Anti-CCP positive	6 [9.2]	.452 (−0.095)	0.334 (−0.122)
Anti-dsDNA positive	1 [1.5]	.088 (−0.213)	0.110 (−0.200)
Anti-Ro (SSA) positive	4 [6.2]	.787 (−0.034)	0.387 (−0.109)
Anti-La (SSB) positive	1 [1.5]	.088 (−0.213)	0.110 (−0.200)
Anti-ribonucleoprotein positive	0	——	——
Anti-Smith positive	1 [1.5]	.088 (−0.213)	0.110 (−0.200)
Anti-topoisomerase (Scl-70) positive	3 [4.6]	.734 (−0.043)	0.734 (−0.043)
Anti-tRNA synthetase positive	5 [7.7]	.575 (−0.071)	0.305 (−0.129)
Anti-Jo-1	3 [4.6]	.734 (−0.043)	0.734 (−0.043)
Anti-KS	2 [3.1]	.881 (−0.019)	0.140 (−0.185)
Anti-PM-Scl positive	1 [1.5]	.088 (−0.213)	0.110 (−0.200)
Anti-MDA-5 positive	0	——	——

ANA = antinuclear antibodies, RF = rheumatoid factor.

interstitial damage, alveolar collapse, bronchial passive tractive dilation, alveolar wall thickening, and airway occlusion, which affected the lung function of the patients. In a longitudinal study of stable immune-associated interstitial pulmonary disease, Sandra et al[40] demonstrated that the age was lower the better the pulmonary function of female patients. However, this trend was not observed in our patient population. Based on our findings and those of a previous study by Xue et al,[21] we concluded that there may not be sufficient specificity for evaluating lung function in IPAF patients.

In a study of ILD, KL-6 and SP-A were found to be associated with disease progression and are important indicators in the subclasses IPF and CTD-ILD. However, there is not much research on KL-6 and SP-A in IPAF.[41,42] Here, we found that KL-6 and SP-A levels in the aggravation group of IPAF patients showed significant upward trends over time. Based on lung imaging findings and HRCT scores, immune dysfunction and the restructuring and pulmonary interstitial, alveolar, and bronchial injuries were more serious in the aggravation group of IPAF patients than in the improvement and stable groups. Lung tissue injury leads to type II alveolar regeneration and high level expression of KL-6 and SP-A, which enter the circulation and promote lung fibroblast proliferation and migration, resulting in the imaging manifestations of interstitial fibrosis, such as honeycomb lesions and ground glass shadows. In ILD studies by Wang et al[43] and Behndig et al,[44] KL-6 and SP-A were confirmed to be closely related to disease activity, that is, the degree of lung injury, and our analysis was consistent with the mechanism inferred from their research.

A correlation analysis of KL-6 and SP-A with lung function was conducted, which indicated that KL-6 and SP-A levels were significantly negatively correlated with DLCO in the aggravation group of patients with IPAF. It should be noted that a progressive decline in lung function may be more common in IPAF patients with aggravated disease, but it may also result in the improvement and stable groups.[45] In addition, in this study, FVC, % predicted, and DLCO were decreased in the aggravation group, and the values at 52 weeks differed significantly from those at baseline. In contrast, no such differences were observed in the stable and improvement groups. We concluded that lung function may decline more significantly in patients with exacerbation of IPAF and can reflect the degree of disease progression. However, in patients with nonaggravated disease, lung function tests may not have sufficient specificity. Therefore, in patients with IPAF, lung function is not rigorous enough to judge disease progression without judging disease severity. KL-6 and SP-A levels could reflect disease progression in the group of IPAF patients with aggravated disease, but additional studies are needed in groups of patients with stable disease and improvement.

Imaging results were quantified and scored, and a comparative analysis of the values at baseline and at 52 weeks showed that KL-6 and SP-A were positively correlated with the CT scores in the aggravation group. Wang et al[46] and AI-Salmi et al[47] proposed that serum KL-6 and SP-A were related to HRCT and could be used as indicators for evaluating the progression of ILD. This is consistent with the findings of this study in patients with IPAF.

Among the patients in this study, Raynaud phenomenon (18.5%) was the most common symptom, and it was significantly correlated with KL-6 levels, whereas distal digital fissuring and tip ulceration were seen in only 1 case each. Gianluca et al hypothesized that this may be because the latter 2 symptoms are highly specific to idiopathic dermatomyositis rather than IPAF.[36] Dysphagia and rash photosensitivity were not observed in this study. The specificity of clinical symptoms in IPAF is low, and some clinical manifestations of CTD-ILD are included in the classification criteria for IPAF; thus, the boundary between the two may not be clear.[48] The symptom specificity in patients with IPAF is low, and the changes in serum KL-6 and SP-A levels are not parallel to the clinical manifestations in the ATS/ERS classification standard.[7]

The detection of serological biological factors, such as the presence of ANA at a high titer of 1:320 or RF at twice the normal value (>60 IU/mL), are important diagnostic and classification criteria for IPAF.[34,49] In this study, 83.1% of IPAF patients showed positive serological manifestations of IPAF, with the highest positive rate for ANA (75.4%), followed by RF (43.1%), although patients were negative for most tested autoantibodies. This is consistent with the results of a CTD-ILD study by Zhang et al.[50] The statistical results of the study by Chartrand et al

showed that 91% of patients with IPAF were positive for at least 1 autoantibody, and ANA was the most common.[51] However, according to the results of this study, although a high positive rate of ANA was detected, it did not reflect the progression of IPAF, nor was it significantly correlated with KL-6 or SP-A. Notably, Oldham et al proposed that the survival rate of IPAF patients was significantly lower than that of CTD-ILD patients, and this was not correlated with the rate of ANA positivity.[30] Unfortunately, we did not track survival in this study.

The mechanism underlying the development of autoimmune interstitial lung diseases is not clear at present. At different stages of the disease, that is, aggravation, improvement, and stable, the levels of various autoimmune factors did not change.[52] To date, the correlation between IPAF and autoimmune factors is not clear.

5. Conclusion

Here, to the best of our knowledge, for the first time, we longitudinally evaluated the levels of KL-6 and SP-A, 2 popular markers, in IPAF. Imaging studies showed that the serum markers KL-6 and SP-A are associated with the progression of lung injury in IPAF and can be used to evaluate disease progression in patients with aggravation of IPAF. Therefore, we expect that evaluation of KL-6 and SP-A will help provide new targets for slowing the progression of IPAF and improving patient prognosis. Based on the IPAF classification criteria, we also found that the clinical signs and symptoms were not completely parallel to the changes in imaging, serology, and lung function, and thus lacked sufficient specificity. Therefore, we believe that some of the CTD-ILD symptoms and manifestations currently included in the classification criteria for IPAF need to be improved.

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References

[1] Graney BA, Fischer A. Interstitial pneumonia with autoimmune features. Ann Am Thorac Soc 2019;16:525–33.
[2] Kelly BT, Moua T. Overlap of interstitial pneumonia with autoimmune features with undifferentiated connective tissue disease and contribution of UIP to mortality. Respirology 2018;23:600–5.
[3] Wiertz IA, van Moorsel CHM, Vorselaars ADM, et al. Cyclophosphamide in steroid refractory unclassifiable idiopathic interstitial pneumonia and interstitial pneumonia with autoimmune features (IPAF). Eur Respir J 2018;51.
[4] Fernandes L, Nasser M, Ahmad K, et al. Interstitial pneumonia with autoimmune features (IPAF). Front Med (Lausanne) 2019;6:209.
[5] Lim JU, Gil BM, Kang HS, et al. Interstitial pneumonia with autoimmune features show better survival and less exacerbations compared to idiopathic pulmonary fibrosis. BMC Pulm Med 2019;19:120.
[6] Chernau AK, Leone PM, Swigris JJ. Interstitial pneumonia with autoimmune features and undifferentiated connective tissue disease. Semin Respir Crit Care Med 2019;40:271–7.
[7] Mejia M, Herrera-Bringas D, Perez-Roman DI, et al. Interstitial lung disease and myositis-specific and associated autoantibodies: Clinical manifestations, survival and the performance of the new ATS/ERS criteria for interstitial pneumonia with autoimmune features (IPAF). Respir Med 2017;123:79–86.
[8] Jearn LH, Kim TY. The chaos of serologic markers in interstitial pneumonia with autoimmune features can be corrected by the laboratory physician. Clin Chem Lab Med 2019;58:e-75–8.
[9] Fischer A. Interstitial pneumonia with autoimmune features. Clin Chest Med 2019;40:609–16.
[10] Lee JS, Fischer A. POINT: does interstitial pneumonia with autoimmune features represent a distinct class of patients with idiopathic interstitial pneumonia? Yes Chest 2019;155:258–60.
[11] Benyamine A, Heim X, Resseguier N, et al. Elevated serum Krebs von den Lungen-6 in systemic sclerosis: a marker of lung fibrosis and severity of the disease. Rheumatol Int 2018;38:813–9.
[12] Okamoto T, Fujii M, Furusawa H, et al. The usefulness of KL-6 and SP-D for the diagnosis and management of chronic hypersensitivity pneumonitis. Respir Med 2015;109:1576–81.
[13] Tanaka S, Hattori N, Ishikawa N, et al. Krebs von den Lungen-6 (KL-6) is a prognostic biomarker in patients with surgically resected nonsmall cell lung cancer. Int J Cancer 2012;130:377–87.
[14] Hu C, Wu C, Yang E, et al. Serum KL-6 is associated with the severity of interstitial lung disease in Chinese patients with polymyositis and dermatomyositis. Clin Rheumatol 2019;38:2181–7.
[15] Thorenoor N, Kawasawa YI, Gandhi CK, et al. Differential impact of co-expressed SP-A1/SP-A2 protein on AM miRNome; sex differences. Front Immunol 2019;10:1960.
[16] Ghaffarpour S, Foroutan A, Askari N, et al. SP-A and TLR4 localization in lung tissue of SM-exposed patients. Int Immunopharmacol 2019;80:105936.
[17] Volkmann ER, Tashkin DP, Kuwana M, et al. Progression of interstitial lung disease in systemic sclerosis: the importance of pneumoproteins Krebs von den Lungen 6 and CCL18. Arthritis Rheumatol 2019;71:2059–67.
[18] Lee JS, Lee EY, Ha YJ, et al. Serum KL-6 levels reflect the severity of interstitial lung disease associated with connective tissue disease. Arthritis Res Ther 2019;21:58.
[19] Martinovic Kaliterna D, Petric M. Biomarkers of skin and lung fibrosis in systemic sclerosis. Expert Rev Clin Immunol 2019;15:1215–23.
[20] McLean-Tooke A, Moore I, Lake F. Idiopathic and immune-related pulmonary fibrosis: diagnostic and therapeutic challenges. Clin Transl Immunology 2019;8:e1086.
[21] Xue M, Guo Z, Cai C, et al. Evaluation of the diagnostic efficacies of serological markers KL-6, SP-A, SP-D, CCL2, and CXCL13 in idiopathic interstitial pneumonia. Respiration 2019;98:534–45.
[22] Todd JL, Neely ML, Overton R, et al. Peripheral blood proteomic profiling of idiopathic pulmonary fibrosis biomarkers in the multicentre IPF-PRO Registry. Respir Res 2019;20:227.
[23] Kass DJ, Nouraie M, Glassberg MK, et al. Comparative profiling of serum protein biomarkers in rheumatoid arthritis-associated interstitial lung disease and idiopathic pulmonary fibrosis. Arthritis Rheumatol 2019;72:409–19.
[24] Yamakawa H, Hagiwara E, Ikeda S, et al. Evaluation of changes in the serum levels of Krebs von den Lungen-6 and surfactant protein-D over time as important biomarkers in idiopathic fibrotic nonspecific interstitial pneumonia. Respir Investig 2019;57:422–9.
[25] Vasconcelos V, Barroso J, Marques L, et al. Enhanced classification of interstitial lung disease patterns in HRCT images using differential lacunarity. Biomed Res Int 2015;2015:672520.
[26] Jiang Y, Luo Q, Han Q, et al. Sequential changes of serum KL-6 predict the progression of interstitial lung disease. J Thorac Dis 2018;10:4705–14.
[27] Man MA, Dantes E, Domokos Hancu B, et al. Correlation between transthoracic lung ultrasound score and HRCT features in patients with interstitial lung diseases. J Clin Med 2019;8.
[28] Zou J, Guo Q, Chi J, et al. HRCT score and serum ferritin level are factors associated to the 1-year mortality of acute interstitial lung disease

in clinically amyopathic dermatomyositis patients. Clin Rheumatol 2015;34:707–14.
[29] Lee CT, Oldham JM. Interstitial pneumonia with autoimmune features: overview of proposed criteria and recent cohort characterization. Clin Pulm Med 2017;24:191–6.
[30] Oldham JM, Adegunsoye A, Valenzi E, et al. Characterisation of patients with interstitial pneumonia with autoimmune features. Eur Respir J 2016;47:1767–75.
[31] Doishita S, Inokuma S, Asashima H, et al. Serum KL-6 level as an indicator of active or inactive interstitial pneumonitis associated with connective tissue diseases. Intern Med 2011;50:2889–92.
[32] Zheng P, Liu X, Huang H, et al. Diagnostic value of KL-6 in idiopathic interstitial pneumonia. J Thorac Dis 2018;10:4724–32.
[33] Vivero F, Campins F, Lancellotti D, et al. Autoimmune interstitial lung disease in Latin-America. Clin Immunol 2019;199:52–6.
[34] Novikov P, Shchegoleva E, Akulkina L, et al. Diagnostic pitfalls and treatment challenges in interstitial pneumonia with autoimmune features: comment on the article by Wilfong et al. Arthritis Rheumatol 2019;71:651–2.
[35] Wilfong EM, Lentz RJ, Guttentag A, et al. Interstitial pneumonia with autoimmune features: an emerging challenge at the intersection of rheumatology and pulmonology. Arthritis Rheumatol 2018;70: 1901–13.
[36] Sambataro G, Sambataro D, Torrisi SE, et al. Clinical, serological and radiological features of a prospective cohort of Interstitial Pneumonia with Autoimmune Features (IPAF) patients. Respir Med 2019;150: 154–60.
[37] Nascimento E, Baldi BG, Sawamura MVY, et al. Morphologic aspects of interstitial pneumonia with autoimmune features. Arch Pathol Lab Med 2018;142:1080–9.
[38] Caron M, Hoa S, Hudson M, et al. Pulmonary function tests as outcomes for systemic sclerosis interstitial lung disease. Eur Respir Rev 2018;27.
[39] Liang M, Jiang Z, Huang Q, et al. Clinical association of chemokine (C-X-C motif) ligand 1 (CXCL1) with interstitial pneumonia with autoimmune features (IPAF). Sci Rep 2016;6:38949.
[40] Chartrand S, Lee JS, Swigris JJ, et al. Clinical characteristics and natural history of autoimmune forms of interstitial lung disease: a single-center experience. Lung 2019;197:709–13.
[41] Takanashi S, Nishina N, Nakazawa M, et al. Usefulness of serum Krebs von den Lungen-6 for the management of myositis-associated interstitial lung disease. Rheumatology (Oxford) 2019;58:1034–9.
[42] Yamaguchi K, Iwamoto H, Sakamoto S, et al. Serial measurements of KL-6 for monitoring activity and recurrence of interstitial pneumonia with anti-aminoacyl-tRNA synthetase antibody: a retrospective cohort study. Medicine (Baltimore) 2018;97:e13542.
[43] Wang J, Huang Z, Xue M, et al. Evaluation of the clinical performance of the HISCL-5000 analyzer in the detection of Krebs von den Lungen-6 antigen and its diagnostic value in interstitial lung disease. J Clin Lab Anal 2019;34:e23070.
[44] Behndig AF, Mirgorodskaya E, Blomberg A, et al. Surfactant protein A in particles in exhaled air (PExA), bronchial lavage and bronchial wash - a methodological comparison. Respir Res 2019;20:214.
[45] Wuyts WA, Wijsenbeek M, Bondue B, et al. Idiopathic pulmonary fibrosis: best practice in monitoring and managing a relentless fibrotic disease. Respiration 2020;99:73–82.
[46] Wang Y, Chen S, Lin J, et al. Lung ultrasound B-lines and serum KL-6 correlate with the severity of idiopathic inflammatory myositis-associated interstitial lung disease. Rheumatology (Oxford) 2019;59:2024–9.
[47] Al-Salmi QA, Walter JN, Colasurdo GN, et al. Serum KL-6 and surfactant proteins A and D in pediatric interstitial lung disease. Chest 2005;127:403–7.
[48] Sambataro G, Sambataro D, Torrisi SE, et al. State of the art in interstitial pneumonia with autoimmune features: a systematic review on retrospective studies and suggestions for further advances. Eur Respir Rev 2018;27.
[49] Bahmer T, Romagnoli M, Girelli F, et al. The use of auto-antibody testing in the evaluation of interstitial lung disease (ILD)–A practical approach for the pulmonologist. Respir Med 2016;113:80–92.
[50] Zhang T, Yuan F, Xu L, et al. Characteristics of patients with primary Sjogren's syndrome associated interstitial lung disease and relevant features of disease progression. Clin Rheumatol 2020;39:1561–8.
[51] Chartrand S, Swigris JJ, Stanchev L, et al. Clinical features and natural history of interstitial pneumonia with autoimmune features: a single center experience. Respir Med 2016;119:150–4.
[52] Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: a comprehensive update. J Intern Med 2015;278:369–95.