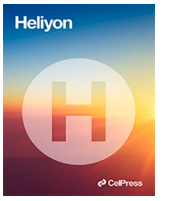
Heliyon 10 (2024) e27561

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Evaluating the diagnostic and therapeutic significance of KL-6 in patients with interstitial lung diseases

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ARTICLE INFO

Keywords:
KL-6
ILDs
Diagnosis
HRCT fibrosis score
LDH

ABSTRACT

Background: This study aimed to assess the diagnostic value of Krebs von den Lungen-6 (KL-6), Surfactant protein-A (SP-A), SP-D and molecular matrixmetalloproteinase-7 (MMP-7) in discriminating patients with interstitial lung diseases (ILDs) from disease control subjects.
Methods: Serum levels of KL-6, SP-A, SP-D and MMP-7 were measured in both the ILD and non-ILD (NILD) groups. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic potential of these markers and laboratory indices. High-resolution computed tomography (HRCT) fibrosis scores were determined, and their correlation with the serum markers was analyzed.
Results: Serum levels of KL-6 and MMP-7 were significantly elevated in the ILD group compared to the control group, while no significant differences were observed for SP-A and SP-D. ROC analysis of KL-6 demonstrated superior diagnostic accuracy, with a sensitivity of 76.36%, specificity of 91.07%, and an area under curve (AUC) of 0.902 (95%CI 0.866–0.945). These findings were consistent across an additional cohort. Correlation analysis revealed a link between KL-6 levels at initial diagnosis and HRCT fibrosis scores, indicating disease severity. Moreover, a negative correlation was found between KL-6 and pulmonary function indices, reflecting disease progression. Patients with increased 12-month HRCT fibrosis score showed higher lactate dehydrogenase (LDH) levels, with LDH exhibiting an AUC of 0.767 (95% CI: 0.520–0.927) as a predictor of progression.
Conclusions: Serum KL-6 detection proves to be a valuable tool for accurately distinguishing ILDs from control subjects. While KL-6 shows a correlation with HRCT fibrosis scores and a negative association with pulmonary function indices, its predictive value for ILDs prognosis is limited.
Trial registration: This study received retrospective approval from the Ethical Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (institutional review board ID: TJ-IRB20210331, date: 2021.03.30).

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https://doi.org/10.1016/j.heliyon.2024.e27561
Received 8 June 2023; Received in revised form 29 February 2024; Accepted 1 March 2024
Available online 8 March 2024

1. Background

Interstitial lung diseases (ILDs) represents a diverse group of pulmonary disorders characterized by varying patterns of inflammation and fibrosis within the lung interstitium. Damage to type II epithelial cells and diffuse infiltrates results in compromised as exchange. The disease course exhibits considerable variability among patients, and the overall prognosis is often unfavorable. Idiopathic pulmonary fibrosis (IPF) stands out as the most prevalent progressive form of ILDs, with a median survival of 3–5 years [1,2]. Therefore, timely intervention and the assessment of ILD severity play crucial roles in enhancing patient prognosis [3,4].

Previous studies have indicated that restricted pulmonary function, fibrous lesions evident on high-resolution computed tomography (HRCT) scans, and 6-min walking distance (6MWD) test are associated with poorer outcomes in patients with ILDs [5–7]. However, the utility of these parameters may be limited due to inadequate respiratory effort and poor coordination. In addition, repeated CT scans expose patients to higher levels of radiation. Thus, the development of reliable disease-specific biomarkers with diagnostic and prognostic utility could significantly impact disease management.

Krebs von den Lungen-6 (KL-6) is a high-molecular weight mucin-like glycoprotein expressed in the lungs by injured and regenerating alveolar type II cells [8,9]. Initially suggested as a serum biomarker for lung, breast and pancreatic cancer, KL-6 exhibited lower diagnostic accuracy than other tumor markers. Subsequently, it was proposed as a diagnostic marker for identifying ILDs and predicting responses to antifibrotic therapy [10]. Surfactant protein-A (SP-A) and SP-D, derived from type II pneumocytes, have been widely used as serum markers of ILDs in clinical practice in Japan [11,12]. Furthermore, molecular matrixmetalloproteinase-7 (MMP-7) has been identified as a potential diagnostic and prognostic markers for IPF [13]. Previous studies have demonstrated significantly elevated levels of MMP-7 in IPF patients compared with healthy controls, and its associated with impaired lung function [14]. Evaluating the prognostic values of these biomarkers in combination with HRCT for assessing disease severity is critical in ILD management [15,16]. However, the evaluation of KL-6, SP-A, SP-D and MMP-7 in conjunction with routine laboratory markers such as high-sensitivity C-reactive protein (hsCRP), erythrocyte sedimentation rate (ESR), white blood cell counts (WBCs) and lactate dehydrogenase (LDH) for discriminating of ILDs from a disease control group still needs further investigation.

This study aimed to compare the differences of KL-6, SP-A, SP-D, MMP-7 and laboratory markers between ILDs and disease control subjects, including cancer, bacterial infection and tuberculosis patients. Sensitivity, specificity and predictive values of these markers in discriminating ILDs from control subjects were performed. The inclusion of disease groups as control cohorts in this study expands the clinical utility. Additionally, we collected a subset of blood samples for validation. Pulmonary function Test (PFT) is the sensitive indicator for ILD superior to chest scan. The correlation between lung function and KL-6 is also discussed in this article. HRCT fibrosis scores were utilized to reflect disease severity [17], and their correlation with serum biomarkers was analyzed.

2. Methods

2.1. Subjects

This study retrospectively collected a total of 110 patients diagnosed with ILDs between June to December 2020. The control groups consisted of 49 lung cancer (LC) patients, 31 with bacterial pneumonia (BP), and 32 tuberculosis (TB) patients. To validate the performance of the detected serum biomarkers, an additional 23 ILDs and 62 non-ILDs patients were included. Among them, 20 had with lung cancer (CA), 20 had bacterial pneumonia (BP), and 22 had tuberculosis (TB), sourced from the Sino-French New City Branch of Tongji hospital. The cancer patients included in this study have not undergone radiation therapy or chemotherapy at the time of their initial diagnosis. For bacterial infection, a common inclusion criterion involves a positive sputum culture. In the case of tuberculosis (TB), typical inclusion criteria included a positive culture result from Xpert MTB/RIF testing. This study was approved by the ethical committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology.

2.2. Physiological testing

Pulmonary function tests (PFT) were performed using a standardized spirometry procedure at the initial diagnosis of ILDs. Forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1) and diffusion capacity for carbon monoxide (DL_{CO}) were assessed according to the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations [18].

2.3. HRCT fibrosis score

The findings of HRCT were evaluated using a semiquantitative scoring method outlined by Ooi et al. The HRCT fibrosis score was determined based on a previous published study [17]. Each lung was independently assessed in three zones (upper, middle and lower) for specific findings. The score for each zone was calculated by multiplying the percentage of the area according to the grading scale score. Subsequently, the average of six zone scores was calculate for each patient. The HRCT fibrosis scores were recorded at the initial diagnosis and after a 12-month interval using a similar approach.

2.4. Serum biomarker measurements

Venous blood samples were collected from the subjects at initial diagnosis. These samples were centrifuged at 3500 revolutions per

minute for 10 min to separate the sera, which were then stored at −80◦C until analysis, conducted within two months. Serum KL-6 concentration was measured using a Kangrun KL-6 assay (Guangzhou Kangrun Biotech Co.,Ltd) employing chemiluminescence analysis according to the manufacturer's instructions. Serum concentrations of KL-6 were quantified and expressed in U/mL. Additionally, each sera sample underwent analysis for SP-A, SP-D and MMP-7 using commercially available enzyme-linked immunosorbent assay kits following the protocol of the manufacturer (Cusabio Biotech Co.,Ltd). The serum levels were expressed in ng/mL for SP-A and MMP-7 and pg/mL for SP-D.

2.5. Statistical analysis

All values are expressed as mean ± standard deviation (SD) for continuous variables or percentages for categorical variables. Student's t-test or the Mann-Whitney *U* test was used to analyze continuous data. The receiver operating characteristic (ROC) curve analysis was performed to analyze the diagnostic value of each serum biomarkers in identifying ILDs. Correlation analysis using Spearman's rank correlation coefficients were performed for correlation analysis. A significant level of $p < 0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism 8.0 software and MedCalc (MedCalc software bvba).

3. Results

3.1. Baseline characteristics of the study population

The study included 110 patients diagnosed with ILDs and 112 disease controls, comprising 49 with CA, 31 with BP and 31 with TB. Among the ILDs cohorts, 48 individuals (43.64%) were male, while 62 (56.36%) were female. The basic characteristics of study participants were presented in Table 1. Notably, there were no significant differences observed in age between ILDs group and the control group. The mean values of lung function parameters and HRCT fibrosis scores in patients with ILDs were shown. The flowchart was shown in Supplementary Fig. 1 to illustrate the experimental design and method.

3.2. Laboratory test markers and serum levels of KL-6, SP-A, SP-D and MMP-7

The differences of laboratory markers between control groups (CA, BC and TB) and ILD group were compared. No significant differences were observed in white blood cell counts. The levels of hsCRP, ESR and LDH were notably lower in the CA group when compared with the ILD group, whereas in the BC group, markers for hsCRP and LDH were higher (Fig. 1A). Upon merging the three disease control groups into a non-ILD (NILD) group, only the levels of ESR and LDH displayed a decrease compared to those in the ILD group (Fig. 1B).

Serum KL-6 levels exhibited a significant increase in ILDs compared to each control group. SP-A and SP-D levels were higher in the CA group than in ILDs, likely attributed to their characterization as tumor biomarkers [10]. The levels of MMP-7 were higher in ILDs compared to patients with infections (BC and TB) (Fig. 1C). Overall, only KL-6 and MMP-7 were observed higher in patients with ILD than those of NILD controls (Fig. 1D).

Furthermore, upon categorizing ILD into connective tissue disease (CTD)-ILD and non-CTD-ILD subsets, the differences of KL-6, SP-A, SP-D, MMP-7 expression among CTD-ILD, non-CTD-ILD and NILD groups were conducted. Our results showed that no significant differrences were observed between the CTD-ILD and non-CTD-ILD groups (Fig. 2A–D).

3.3. Clinical value of KL-6, SP-A, SP-D and MMP-7 in the diagnosis of ILDs

Regarding the clinical value of KL-6, SP-A, SP-D and MMP-7 in diagnosing ILDs, ROC curve analysis was performed to evaluate their diagnostic potential, along with LDH and ESR (Fig. 3). The results showed that KL-6 levels distinctly discriminate between ILDs from NILDs groups, displaying an AUC of 0.902 (95% CI 0.866–0.945, $p < 0.001$), with a best cutoff value of 582.5U/mL, sensitivity of

Table 1
The basic characteristics of subjects.

	IPF	CA	BP	TB
Subjects (n)	110	49	31	32
Age, years	57.37 ± 13.77	55.57 ± 10.86	57.1 ± 17.77	53.19 ± 16.32
Sex, M/F	48/62	30/19	21/10	18/14
Spirometry				
FVC, L	2.155 ± 0.562	–	–	–
%FVC, %	76.42 ± 25.13	–	–	–
FEV1/FVC, %	75.14 ± 25.71	–	–	–
% DL_{CO}, %	54.50 ± 16.11	–	–	–
HRCT score	170.3 ± 52.80	–	–	–

IPF: idiopathic pulmonary fibrosis; CA: cancer; BP: bacterial pneumonia; TB: tuberculosis; FVC = forced vital capacity; FEV1: forced expiratory volume in first second; DL_{CO} = diffusing capacity for carbon monoxide; HRCT: high-resolution CT.

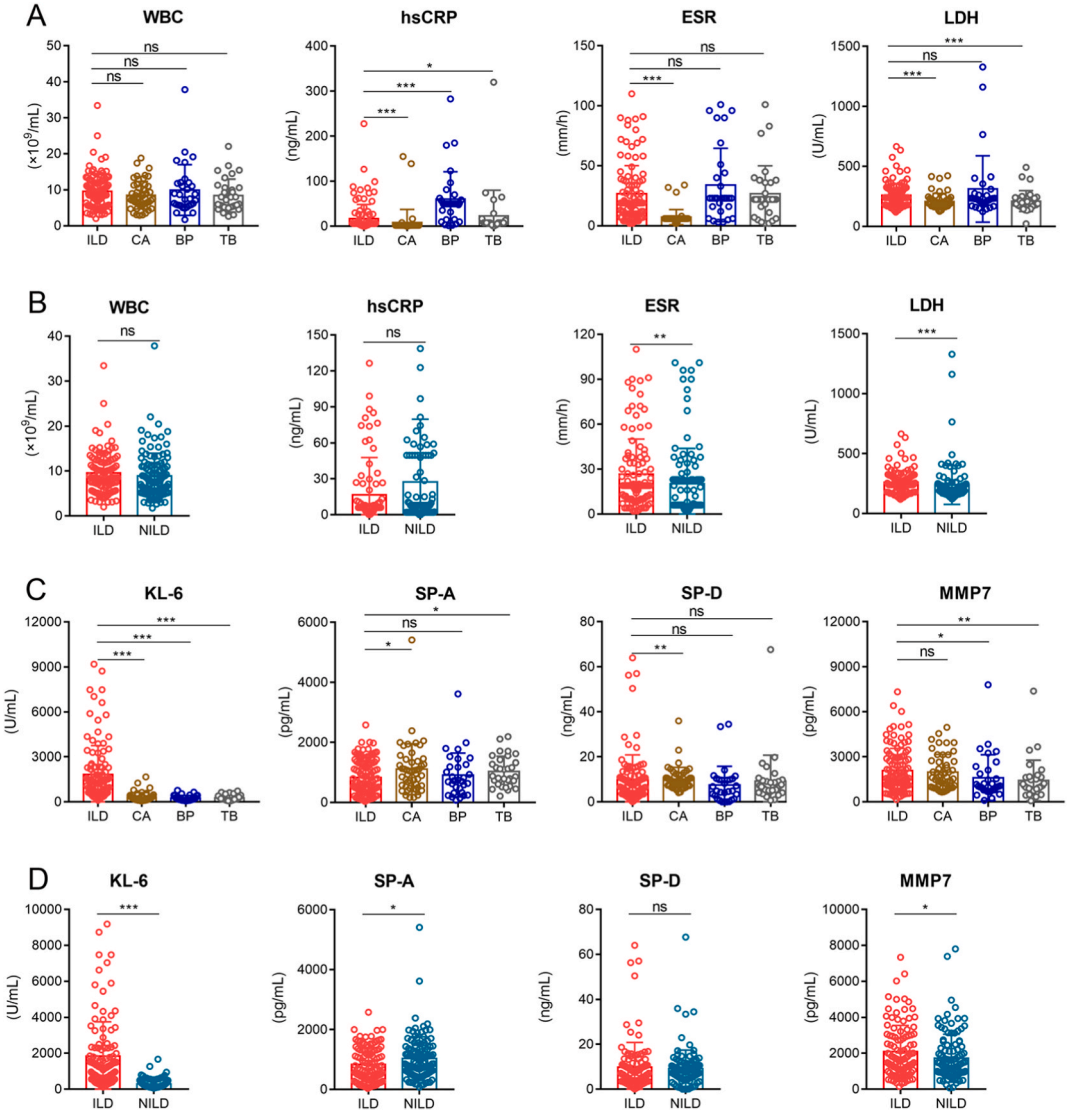

Fig. 1. The results of laboratory features and serum markers in ILD patients and control subjects. A total of 110 patients diagnosed with ILDs were included. The control non-ILD patients were also included, consisting of 49 lung cancer, 31 bacterial pneumonia and 32 tuberculosis. (A) The results of white blood cell count (WBC), hsCRP, ESR and LDH in different groups were shown. (B) The results of WBC, hsCRP, ESR and LDH in ILD and NILD groups were shown. (C) The serum levels of KL-6, SP-A, SP-D and MMP-7 in ILD patients and control patients were shown. (D) The serum levels of KL-6, SP-A, SP-D and MMP-7 in ILD and NILD groups were shown. ILD: interstitial lung diseases, NILD: non-interstitial lung diseases.

76.36% and specificity of 91.07%. In contrast, the AUC for other markers were all less than 0.7: LDH (AUC = 0.651, 95% CI = 0.594–0.722), ESR (AUC = 0.624, 95% CI = 0.554–0.685), SP-A (AUC = 0.580, 95% CI = 0.519–0.653), SP-D (AUC = 0.534, 95% CI = 0.463–0.600), MMP-7 (AUC = 0.574, 95% CI = 0.513–0.647) (Table 2). The diagnostic value of these markers in CTD-ILD and non-CTD-ILD were analyzed, ROC curve analysis suggested that KL-6 could be used as superior marker to diffentiate both CTD-ILD and non-CTD-ILD from NILD group (Fig. 4A–C).

Moreover, the validation of KL-6 in additional cohort of 23 ILDs and 62 non-ILDs patients revealed its robust performance, exhibiting an AUC of 0.845 (95% CI: 0.750–0.914). with a sensitivity of 73.91% and specificity of 87.10% (Fig. 5A–C). Subsequent correlation analyses demonstrated a significant association between KL-6 levels and both PFT and HRCT fibrosis scores at the initial diagnosis (Fig. 6A and B).

3.4. LDH could be used to predict the prognosis of ILD patients

The HRCT fibrosis score was monitored in the following 12 months in 19 ILD patients. Based on the variation in HRCT fibrosis score from baseline to follow up, patients were categorized as having stable or progressive disease [17,19]. Among the participants, 11

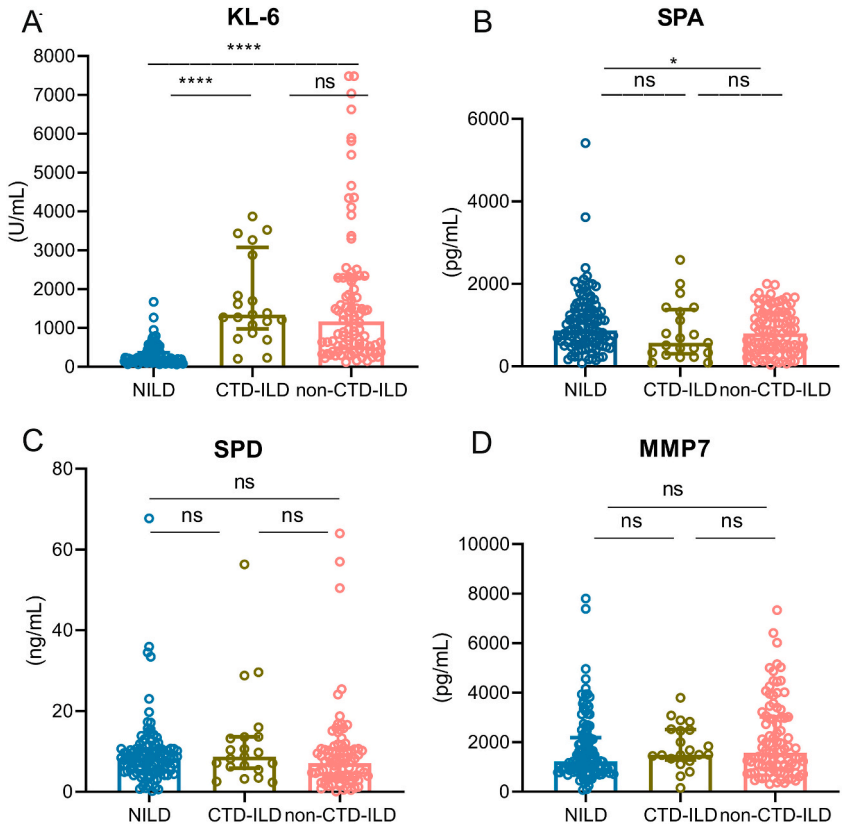

Fig. 2. The results of KL-6, SP-A, SP-D and MMP-7 between CTD-ILDs and non-CTD-ILDs. The serum levels of (A) KL-6, (B) SP-A, (C) SP-D and (D) MMP-7 in CTD-ILD and non-CTD-ILD groups were shown. CTD: connective tissue disease.

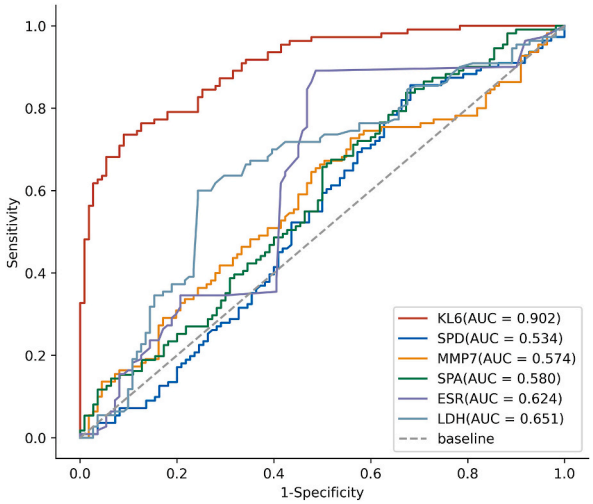

Fig. 3. ROC curve analysis in serum markers to distinguishing ILD from control subjects.

exhibited decreased or stable HRCT score, while the remaining 8 showed an increase trend. Analysis of serum markers within subgroups of patients with stable and progressive disease were conducted. The comparison of KL-6, SP-A, SP-D and MMP-7 levels at baseline indicated no significant difference between these subgroups (Fig. 7A). However, LDH levels were notably higher in patients demonstrating an increased change in HRCT fibrosis score (Fig. 7B). The evaluation of LDH as a predictor for 12 months progression yielded an AUC of 0.767 (95% CI: 0.520–0.927) (Fig. 7C).

Table 2
ROC curve analysis of six markers in distinguishes IPF patients from disease control subjects.

Variable	AUC	SE	95% CI	Cutoff value	Sensitivity	Specificity	*p* value
KL-6	0.902	0.019	0.866-0.945	582.5	76.36	91.07	<0.001
LDH	0.651	0.037	0.594-0.722	236.0	61.82	75.89	<0.001
ESR	0.624	0.039	0.554-0.685	6.000	89.09	50.89	0.002
SP-A	0.580	0.038	0.519-0.653	689.1	51.38	66.96	0.023
MMP7	0.574	0.039	0.513-0.647	1090	74.31	43.75	0.036
SP-D	0.534	0.040	0.463-0.600	4.000	31.78	85.59	0.421

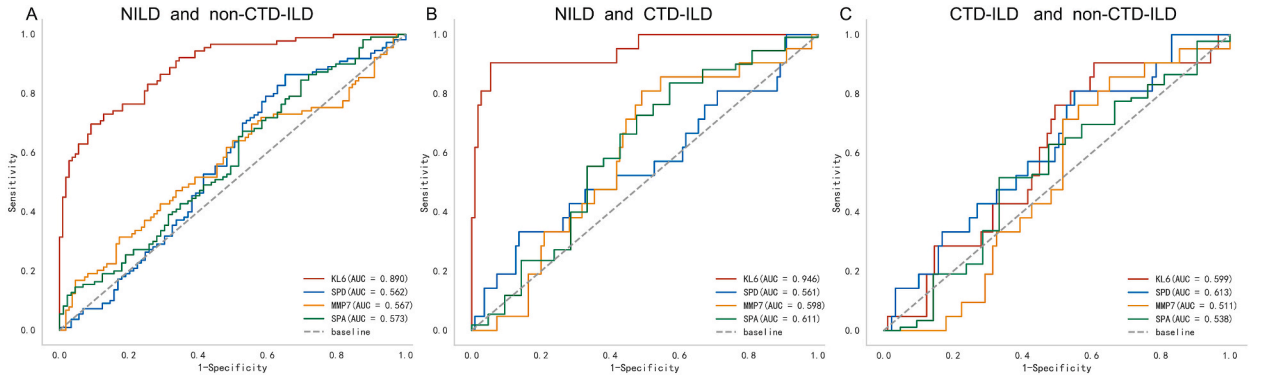

Fig. 4. Receiver operating characteristic (ROC) curve analysis in serum markers (KL-6, SP-A, SP-D, MMP-7) to distinguishing ILD from connective tissue disease non-CTD-ILD (A) and CTD-ILD (B) subsets; non-CTD-ILD and CTD-ILD (C)subsets.

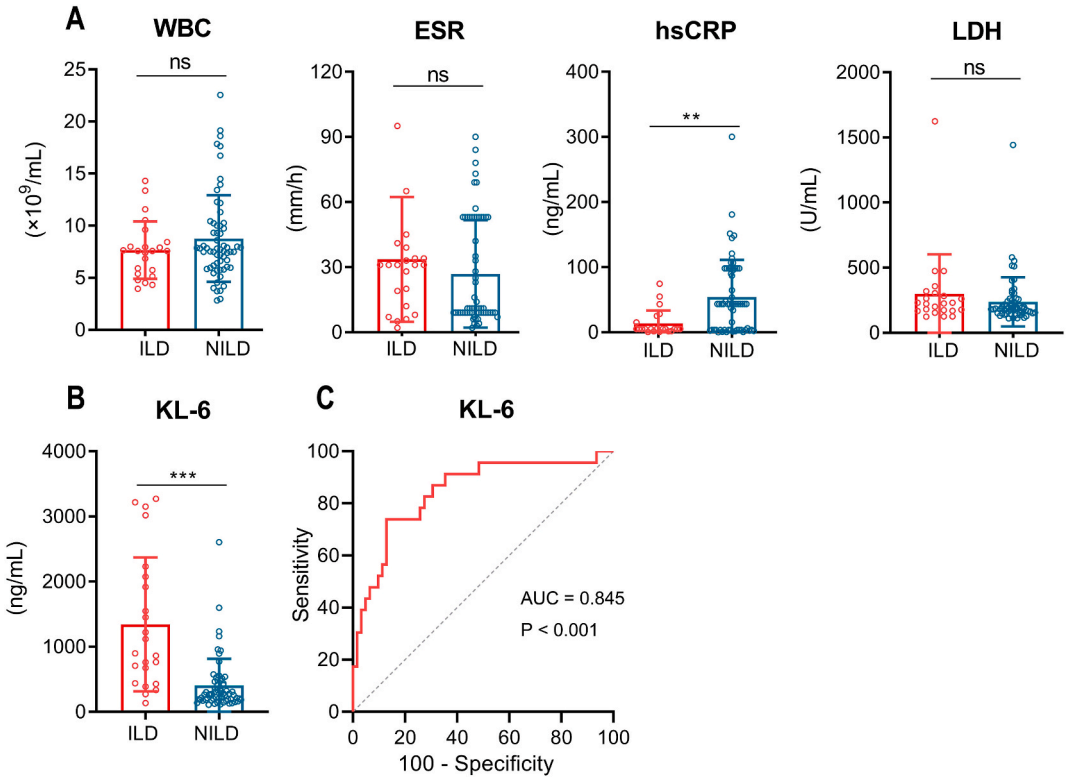

Fig. 5. The results of laboratory indicators and KL-6 in validation groups. (A) The results of WBC, hsCRP, ESR and LDH in ILD and NILD groups were shown. (B) KL-6 level in ILD and NILD groups were shown. (C) ROC curve analysis of KL-6 to distinguish ILD from NILD patients.

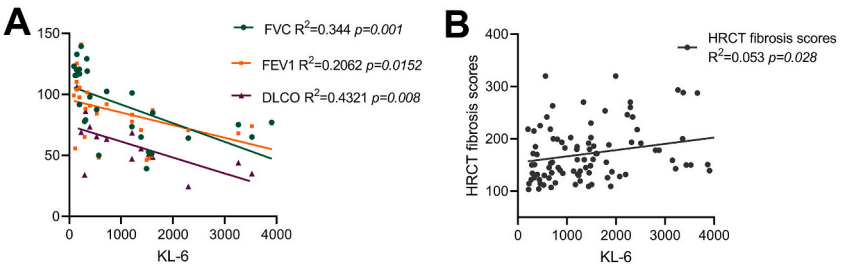

Fig. 6. Correlation analysis between KL-6 and pulmonary function. (A) Correlation between KL-6 and HRCT fibrosis score. (B) Correlation between KL-6 and pulmonary function parameters.

4. Discussion

ILDs is a common pulmonary manifestation with symptoms of cough, dyspnea, abnormal gas exchange, limited ventilation disorder, and respiratory failure, which can lead to clinical fatalities upon disease progression. In recent years, the incidence of ILDs in China has been gradually increasing, contributing to poor prognosis and high mortality rates. Diagnosis and evaluation involve pulmonary function tests, biopsy and laboratory indicators [20]. However, biopsy's invasiveness restricts its use, and early-stage ILDs might manifest atypically on HRCT. Laboratory indicators such as CRP, ESR and WBC lack specificity and sensitivity for diagnosing and assessing ILD activity. Therefore, it is critical to explore new serum markers with higher diagnostic accuracy.

In the present study, we compared KL-6, SP-A, SP-D, and MMP-7 levels in ILD patients and disease control groups (including cancer, bacterial infection and tuberculosis patients). KL-6, mainly expressed on type II alveolar epithelial cells, increases in ILD due to significant proliferation and damage to the alveolar basement membrane. SP-A and SP-D, crucial in lung innate immunity, were observed to vary among groups [21]. Our finding indicated significant elevation in KL-6 and MMP-7 levels in ILD patients, but not in SP-D, possibly due to increased SP-A and SP-D in the cancer control group, consistent with previous reports [22].

KL-6 is well-studied as a specific ILD marker [9,23]. Our study confirmed KL-6's superior diagnostic capability for ILDs, exhibiting a sensitivity of 76.36% and specificity of 91.07%. Similar results were also observed in the validation group. Previous studies highlighted elevated KL-6, SP-A, SP-D and MMP-7 levels in IPF [24,25]. However, our results showed poor performance of SP-A, and SP-D in differentiating ILDs from controls, possibly due to our study's disease-focused control subjects rather than healthy controls. Additionally, ILD was categorized into CTD-ILD and non-CTD-ILD groups, and we compared them with disease control groups, evaluating KL-6, SP-A, SP-D, and MMP-7 to discern differences in distinguishing between CTD-ILD and non-CTD-ILD. Experimental results confirmed that these markers showed no significant differences in ILD subgroups.

KL-6 inversely correlated with lung function and exercise capacity and might be useful for predicting the prognosis of ILDs [24,26]. KL-6 could also predict the incidence of acute exacerbation [26,27], which are the most common death in ILD patients. However, other studies proposed that sequential change of KL-6 could predict the progression of ILDs but not the baseline level [16,27]. Furthermore, the association of serum markers and HRCR fibrosis score were analyzed and our results suggested that only KL-6 was correlated with HRCT fibrosis score at the initial diagnosis. Therefore, detection of KL-6 has critical value for diagnosing ILDs and evaluating the severity of disease.

The prognosis effective of serum markers was assessed according to the change of HRCT fibrosis score and revealed that only LDH level was significantly elevated in progressive patients than stable or improved patients. Elevated LDH has been linked to poor outcomes in patients with honeycombing present on CT results, suggesting its potential as a predictive marker for ILD prognosis [28]. However, our study had limitations due to sample size, and lack of consideration for disease severity and treatment. Monitoring lung function over follow-ups was absent, and prognosis relied on HRCT fibrosis score changes. Larger longitudinal studies are necessary for robust conclusions.

5. Conclusions

Our study underscores the high diagnostic accuracy of serum KL-6 in distinguishing ILDs from control subjects. However, while KL-6 correlates with HRCT fibrosis scores and pulmonary function, its prognostic potential for ILDs might be limited.

Ethics approval and consent to participate

The studies were performed in accordance with the Declaration of Helsinki and approved by the ethical committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology. Written informed consent to participate in this study was provided by the participants. Written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article. All methods were carried out in accordance with relevant guidelines and regulations.

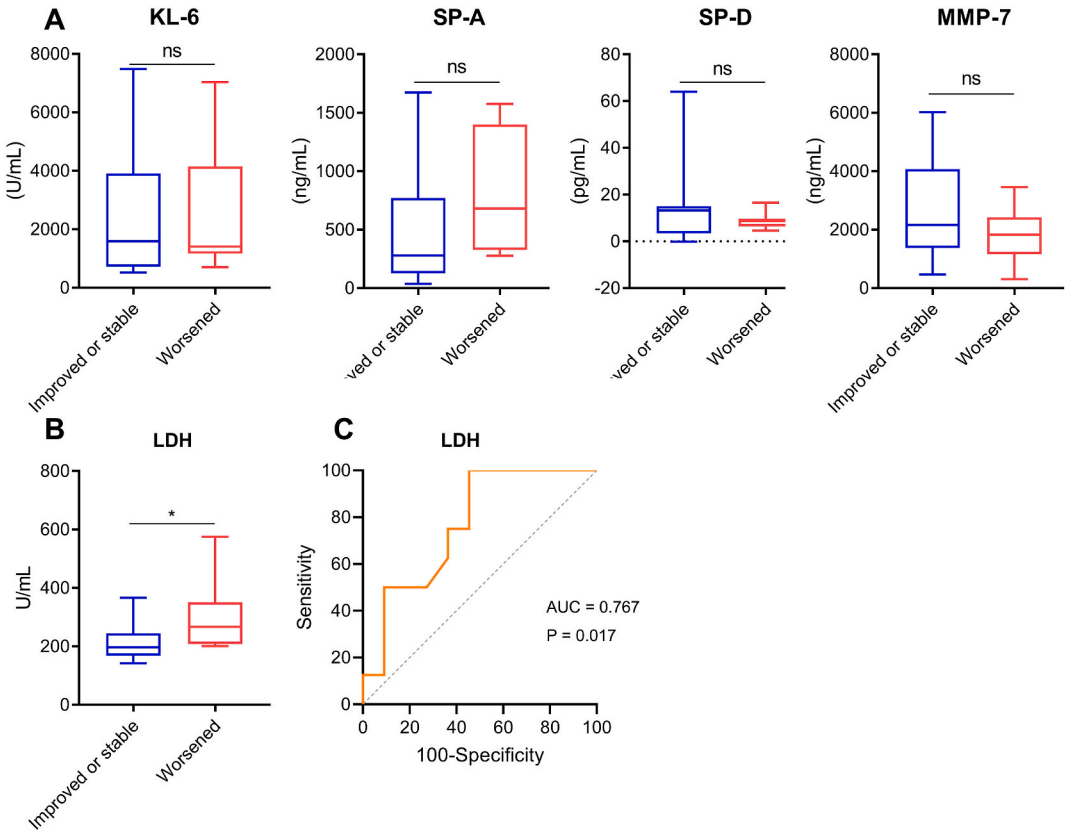

Fig. 7. Comparison of serum markers according to change of HRCT fibrosis score. HRCT fibrosis score was monitored in the following one year from the initial diagnosis. Patients with ILD were divided into improved/stable and worsened according to the change of HRCT fibrosis score. (A) The differences of KL-6, SP-A, SP-D and MMP-7 were compared between improved/stable and worsened subgroups. (B) The level of LDH between the two subgroups were shown. (C) ROC curve analysis to predict the prognosis of ILD patients.

Consent for publication

Not applicable.

Data availability statement

Data associated with this study can been found online at 10.6084/m9.figshare.25304047.

Funding

This study was funded by the National Mega Project on Major Infectious Disease Prevention (2017ZX10103005-007). This funding body funded the purchase of reagents in this study.

CRediT authorship contribution statement

Ting Wang: Writing – original draft. **Yihao Yao:** Data curation. **Yun Wang:** Data curation. **Wei Wei:** Data curation. **Botao Yin:** Methodology. **Min Huang:** Methodology. **Peihong Yuan:** Methodology. **Rujia Chen:** Methodology. **Feng Wang:** Writing – review & editing, Formal analysis. **Shiji Wu:** Writing – review & editing, Formal analysis. **Hongyan Hou:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27561.

List of abbreviations

ILDs	Interstitial lung diseases
IPF	Idiopathic pulmonary fibrosis
HRCT	high-resolution computed tomography
6MWD	6-min walking distance
KL-6	Krebs von den Lungen-6
SP-A	Surfactant protein-A
MMP-7	molecular matrixmetalloproteinase-7
hsCRP	C-reactive protein
ESR	erythrocyte sedimentation rate
WBCs	white blood cell counts
LDH	lactate dehydrogenase
CA	cancer
BP	bacterial pneumonia
TB	tuberculosis
FVC	Forced vital capacity
FEV1	forced expiratory volume in 1 s
DL_{CO}	diffusion capacity for carbon monoxide
SD	standard deviation
ROC	receiver operating characteristic
NILD	non-ILD
CTD	connective tissue disease

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