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Original Article

Association between serum Krebs von den Lungen-6 (KL-6), Carcinoma antigen 15-3 (CA15-3), and connective tissue disease-related interstitial pneumonia (CTD-ILD)

Zijun Guo[1*], Peiyan Zheng[1*], Haisheng Hu[1], Huimin Huang[1], Nili Wei[1], Wenting Luo[1], Xi Chen[1], Ning Li[2*], Baoqing Sun[1*]

[1]Department of Allergy and Clinical Immunology, Guangzhou Institute of Respiratory Health, State Key Laboratory of Respiratory Disease, National Clinical Research Center for Respiratory Disease, First Affiliated Hospital of Guangzhou Medical University, Guangzhou 510120, Guangdong, China; [2]Department of Clinical Laboratory, First Affiliated Hospital of Guangzhou Medical University, Guangzhou, China. [*]Equal contributors.

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Abstract: We investigated the association between Krebs von den Lungen-6 (KL-6), Carcinoma antigen 15-3 (CA15-3), and the severity of connective tissue disease-related interstitial pneumonia (CTD-ILD) to explore their significance in CTD-ILD diagnosis and disease management. 199 patients were divided into CTD group (43 cases) and CTD-ILD group (156 cases). Serum KL-6 and CA15-3 levels and clinical manifestations were used to conduct a cross-sectional analysis. Data was obtained from 57 CTD-ILD patients before and after treatment, after which they were divided into the progression group, remission group, and stabilization group, A longitudinal analysis was then conducted. The levels of serum KL-6 and CA15-3 in the CTD-ILD group were significantly higher than those in the CTD group ($P < 0.01$). If joint criteria of KL-6 ≥ 397.50 U/ml or CA15-3 ≥ 19.73 ng/mL were used in the diagnosis of CTD-ILD, the positive predictive value reached 95.61%, in which case the sensitivity and specificity were 82.57% and 82.75%. The horizontal analysis showed that the levels of KL-6 and CA15-3 in CTD-ILD patients were negatively correlated with lung function (percentage of predicted forced vita capacity (FVC% pred), percentage of predicted forced expiratory volume in 1 s (FEV1% pred), percentage of predicted total lung capacity (TLC% pred), percentage of predicted diffusion lung capacity for carbon monoxide (DLCO% pred)) ($P < 0.05$). Longitudinal analysis showed that the changes in KL-6 (ΔKL-6) and CA15-3 (ΔCA15-3) were negatively correlated with the changes in FVC% pred (ΔFVC% pred) and FEV1% pred (ΔFVC% pred) before and after treatment in 57 CTD-ILD patients. In addition, the levels of KL-6 and CA15-3 in the progression group increased along with the deterioration of the disease.Serum KL-6 and CA15-3 was positivly correlated with the severity of CTD-ILD The combined detection of serum KL-6 and CA15-3 can improve the diagnosis of CTD-ILD. Dynamic monitoring of changes in KL-6 and CA15-3 levels were conducive for effective management and monitoring of this disease.

Keywords: KL-6, CTD-ILD, lung function, CA15-3, diagnosis

Introduction

Connective tissues disease (CTD) is a group of autoimmune diseases that affect multiple systemic connective tissues. The lung is one of the most commonly affected organs, with interstitial lung disease (ILD) being one of the most prominent manifestations [1]; and the major causes of CTD death [1, 2]. CTD-ILD diagnosis depends mainly on clinical manifestations, chest imaging, and lung biopsy. Lung biopsy is the gold standard, but it involves a traumatic examination, and its penetration rate is low. High resolution computerized tomopraphy (HRCT) and other imaging tests are not appropriate for short-term dynamic monitoring of patients with radioactive injury. Pulmonary function testing requires a high degree of patient fit, and is thus not suitable for patients with severe cases of the disease. Therefore, it is important to find simple and easy methods for diagnosing connective tissue-related inter-

stitial pneumonia (CTD-ILD), assessing its severity and monitoring its activity. Krebs von den Lungen-6 (KL-6) is a 1 million kD glycoprotein antigen [3], and was initially used as a tumor marker. A follow-up study found that KL-6 can regulate the secretion of transform growth factor-β (TGF-β), and that it has chemotactic and anti-apoptotic effects on fibroblasts [4]. KL-6 was found to be strongly expressed in atypical and/or regenerated type II lung cells in lung tissue sections of ILD patients. Recently, it has been used as a marker of interstitial pneumonia in the capacity of a diagnostic indicator [4-8].

There are some known serum markers associated with ILD, such as Carcinoma antigen 15-3 (CA15-3). Stefania Celeste et al found that the level of CA15-3 was positively correlated with the CT fibrosis score in ILD patients [9], and that it could be used as an indicator to assess the degree of fibrosis in ILD. However, previous studies have only separately explored the diagnostic value of KL-6 or CA15-3 in ILD. In fact, there is a lack of studies that have investigated both KL-6 and CA15-3 in the diagnosis of ILD, or provided an assessment of its severity and disease monitoring, particularly in CTD-ILD cases.

Therefore, this study conducted a comprehensive analysis of CTD-ILD, with regards to clinical and laboratory tests and lung function assays. Our goal was to investigate the correlation between serum markers KL-6 and CA15-3 and the laboratory test results, and thus determine their significance in the diagnosis of CTD-ILD and disease management.

Material and methods

Ethics

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangzhou Medical University. The ethical number is as follows: gyfyy-2016-73. For all patients who were enrolled in the study. The informed consent form was signed by the patient himself or the legal guardian in duplicate.

Subjects

This is a retrospective study using the clinical information base of the First Affiliated Hospital of Guangzhou Medical University. We screened 199 patients with CTD from April 2014 to January 2017, including 43 patients with CTD alone (CTD group) and 156 CTD patients with ILD (CTD-ILD group). The patients' gender, age, laboratory tests (KL-6, CA15-3, lactate dehydrogenase (LDH), arterial partial pressure of oxygen (PaO_2), pathologic biopsy, high resolution computerized tomopraphy (HRCT), and lung function (percentage of predicted forced vita capacity (FVC% pred), percentage of predicted forced expiratory volume in 1 s (FEV1% pred), percentage of predicted vital capacity (VC% pred), percentage of predicted total lung capacity (TLC% pred), percentage of predicted diffusion lung capacity for carbon monoxide (DLCO% pred) were collected.

CTD diagnostic criteria

The following CTD diagnostic criteria were used: Polymyositis/Dermatomyositis (PM/DM) complied with the Bohan and Peter diagnostic criteria in 1975 [10]; Rheumatoid arthritis (RA) was in line with the RA diagnostic criteria of American Rheumatism Association in 1987 [11]; Systemic lupus erythematosus (SLE) was consistent with the Systemic lupus erythematosus diagnostic criteria of the American College of Rheumatology in 1997 [12]; Systemic sclerosis (SSc) was in line with the SSc diagnostic criteria of American Rheumatism Association in 2013 [13]; and Sjogren's syndrome (SS) was in line with the Classification criteria for Sjogren's syndrome of American-European Consensus Group in 2002 [14].

ILD inclusion criteria

The comprehensive diagnosis of ILD involved a combination of clinical symptom analysis, imaging, pulmonary function tests, and pathology. If the patients presented with any of the following at a rate of 3 of 5 or more, they would be diagnosed with pulmonary interstitial disease:

(1). Steady state or post-activity shortness of breath; (2). Velcro-like breathing sounds; (3). Pulmonary interstitial lesions were consistent with clinical symptoms (HRCT), such as lung peripheral appearance of honeycomb shadow, ground glass opacity, lobular interval thickening, or a variety of performance synthesis; (4). Pulmonary function tests showed obstructive ventilatory and/or pulmonary ventilation dysfunction (FVC% pred , FEV1% pred, TLC% pred,

Table 1. The characteristics of 199 CTD patients

Characteristic	CTD-ILD	CTD	*P* value
N (M/F)	156 (46/110)	43 (7/36)	0.12
Age	54.9±11.4	56.1±13.5	0.59
CA153 (ng/mL)**	26.21 (5.13-177.9)	12.40 (6.17-30.03)	<0.01
KL-6 (U/mL)**	948 (148-5416)	232 (69-1236)	<0.01
FVC% pred*	70.61±21.35	87.25±16.74	0.02
FEV1% pred	70.52±21.28	82.55±15.68	0.08
FEV1%/FVC (%)	77.55±21.81	79.37±11.61	0.94
TLC% pred*	69.98±26.28	85.17±7.88	0.05
VC% pred*	69.21±23.31	84.51±7.50	0.04
DLco% pred*	54.41±19.67	69.95±20.01	0.02
LDH (IU/L)**	252.65±99.86	203.56±58.39	<0.01
PaO_2 (mmHg)	90.26±21.11	103.10±9.89	0.65

*The independent samples t test was used to compare lung function, LDH, and PaO_2 between the CTD-ILD and CTD group; Mann-Whitney U test was used to compare the levels of KL-6 and CA15-3. FVC% pred, percentage of predicted forced vita capacity; FEV1% pred, percentage of predicted forced expiratory volume in 1 s; VC% pred, percentage of predicted vital capacity; TLC% pred, percentage of predicted total lung capacity; DLCO% pred, percentage of predicted diffusion lung capacity for carbon monoxide; LDH, Lactate dehydrogenase; PaO_2, arterial partial pressure of oxygen. *P < 0.05, **P < 0.01.

VC% pred, DLco% pred); (5). Pulmonary pathology was in line with European Society Diagnostic Criteria in 2002 [15].

Exclusion criteria

The patients who presented with the situation below here were excluded from the research.

(1). Malignant tumors; (2). Infections, occupational, drug, genetic,and environmental factor-related pulmonary lesions; (3). Congenital heart disease, heart failure; (4). Pulmonary vein occlusion; (5). Pregnant.

ILD activity assessment

Our 156 CTD-ILD patients included 57 patients who underwent KL-6 analysis, HRCT, and pulmonary function tests before and after treatment. The "before treatment" data were the first records of patients before the initial diagnosis, and the "after treatment" data were collected from patients after more than three months of glucocorticoids, immunosuppressive agents, and other regulatory treatments. Based on the clinical manifestations, and imaging and lung function tests, the 57 patients were divided into 3 groups: the progression group (16 cases), remission group (21 cases), and stabilization group (20 cases).

The progression group findings were as follows: 1. Patients with dyspnea symptoms deteriorated; 2. The ground glass opacity on HRCT appeared or progressed; 3. FVC% pred decreased by more than 10% and/or DLco% pred decreased by more than 10%, and/or arterial blood gas analysis progressively deteriorated, while PaO_2 decreased more than 10 mmHg.

The remission group findings were as follows: 1. Patients with dyspnea exhibited easing of symptoms; 2. The ground glass opacity on HRCT exhibited no change or decreased; 3. FVC% predicreased by more than 10% and/or DLco% pred increased by more than 10% and/or arterial blood gas analysis progressively eased, PaO_2 increased more than 10 mmHg.

The stabilization group findings were as follows: 1. Patients with dyspnea exhibited eased or smoothed symptoms; 2. The ground glass opacity on HRCT exhibited no change; 3. FVC% pred fluctuation did not exceed 10% and/or% DLco fluctuation did not exceed 10% and/or arterial blood gas analysis exhibited no change, while PaO_2 fluctuation did not exceed 10 mmHg.

Statistical analysis

Data with a normal distribution are expressed as mean ± SD, while data with a non-normal distribution are expressed as median (minimum, maximum). Count data are expressed as ratios (%). The independent samples t test or Mann-Whitney U test was used to compare the data between the CTD-ILD and CTD group. The diagnostic effects of KL-6 and CA15-3 were then evaluated by calculating the area under the curve (AUC) of the receiver-operating characteristic curve (ROC). The change of data in the 57 CTD-ILD patients before and after treatment is expressed by "Δ". The correlation analysis involved either the Pearson correlation or Spearman's rank correlation coefficient. The ANOVA test or Kruskal-Wallis H test was used to compare the data among the progression group (16 cases), remission group (21 cases), and stabilization group (20 cases). The Paired-t sam-

Table 2. Correlation between KL-6, CA15-3, and pulmonary function and other laboratory data in 156 CTD-ILD patients

r_s	KL-6	Ca15-3
FVC% pred	-0.37**	-0.37**
FEV1% pred	-0.24*	-0.25*
FEV1%/FVC (%)	0.29	0.17
TLC% pred	-0.32*	-0.24*
VC% pred	-0.16	-0.21
DLco% pred	-0.41**	-0.36**
LDH (IU/L)	0.50**	0.36**
PaO_2 (mmHg)	-0.14	-0.13
CA15-3 (ng/mL)	0.83**	--

*Used Spearman's rank correlation coefficient for correlation analysis. FVC% pred, percentage of predicted forced vita capacity; FEV1% pred, percentage of predicted forced expiratory volume in 1 s; VC% pred, percentage of predicted vital capacity; TLC%pred, percentage of predicted total lung capacity; DLCO% pred, percentage of predicted diffusion lung capacity for carbon monoxide; LDH, Lactate dehydrogenase. *P < 0.05, **P < 0.01.

ples t test or Wilcoxon test was used to compare the data before and after treatment in the progression group, remission group, and stabilization group. The Chi-squared test was used for the statistical analysis of count data. We considered P < 0.05 to represent statistical significance in all analyses. All data were analyzed with SPSS 19.0 software and GraphPad Prism 5.0 for statistical mapping.

Results

Serum KL-6 and CA15-3 were positivly correlated with the severity of CTD-ILD

Demographic and clinical characteristics of the patients are shown in **Table 1**. In total, 199 patients (53 males and 146 females) were investigated. 43 of them were diagnosed as having connective tissue disease alone (CTD group), and their average age was 56.0 ± 13.5 years old. Among these patients, there were 2 patients with PM/DM, 21 with RA, 4 with SLE, 1 with SSc, 6 with SS, 5 with mixed CTD (MCTD), and 4 with undifferentiated CTD (UCTD). The other 156 patients presented with CTD complicated with interstitial pneumonia (CTD-ILD group), and their average age was 54.9 ± 11.4. Among them, there were 48 patients with DM/PM, 29 with RA, 3 with SLE, 16 with SSc, 15 with SS, 7 with MCTD, and 38 with UCTD.

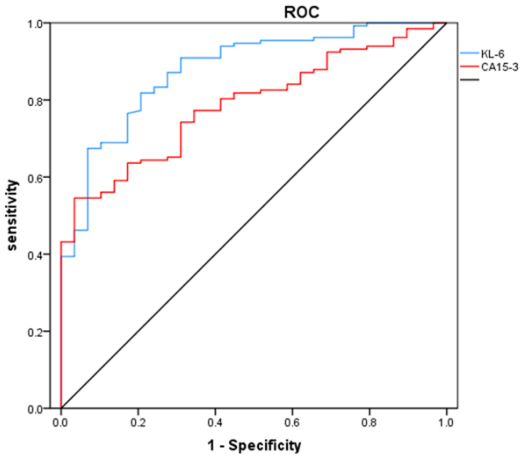

Figure 1. The ROC curves for KL-6 and CA15-3 with regards to their diagnostic effects in CTD-ILD. ROC curves of serum KL-6 and CA15-3 in 156 patients with CTD-ILD and 43 patients with CTD were analyzed. KL-6 > 479.50 U/ml on the ROC curve was used as the optimal cut-off point by the Youden Index method (0.64), the area under the curve of CTD-ILD was 0.89 (95% CI: 0.84~0.92, P < 0.01). The ROC curve for CA15-3 and the area under curve was 0.787 (95% CI: 0.71 ~ 0.98, P < 0.01). The optimal cutoff point was 19.73 ng/mL, the sensitivity and specificity were 63.60% and 82.76%.

The level of serum KL-6 in the CTD-ILD group was significantly higher than in the CTD group [948 (148-5416) vs 232 (69-1236) U/ml, Z = -7.85, P < 0.01]. The level of CA15-3 in the CTD-ILD group was significantly higher than in the CTD group [26.21 (5.13-177.9) vs 12.40 (6.17-30.03) ng/mL, Z = -4.54, P < 0.01]. KL-6 and CA15-3 levels in CTD-ILD patients were negatively correlated with FVC% pred, FEV1% pred, TLC% pred and DLco% pred, while LDH levels and KL-6 (r = 0.50, P < 0.05) and CA15-3 (r = 0.36, P < 0.05) had a positive correlation. In addition, the level of KL-6 was strongly correlated with the level of the tumor marker CA15-3 (r = 0.83, P < 0.05). Details are shown in **Table 2**.

The combined detection of serum KL-6 and CA15-3 improved the diagnosisof CTD-ILD.

The ROC curves of serum KL-6 and CA15-3 levels in 156 patients with CTD-ILD and 43 patients with CTD were analyzed. We considered KL-6 > 479.50 U/ml on the ROC curve to be the optimal cut-off point using the Youden Index method (0.64). The results showed that the

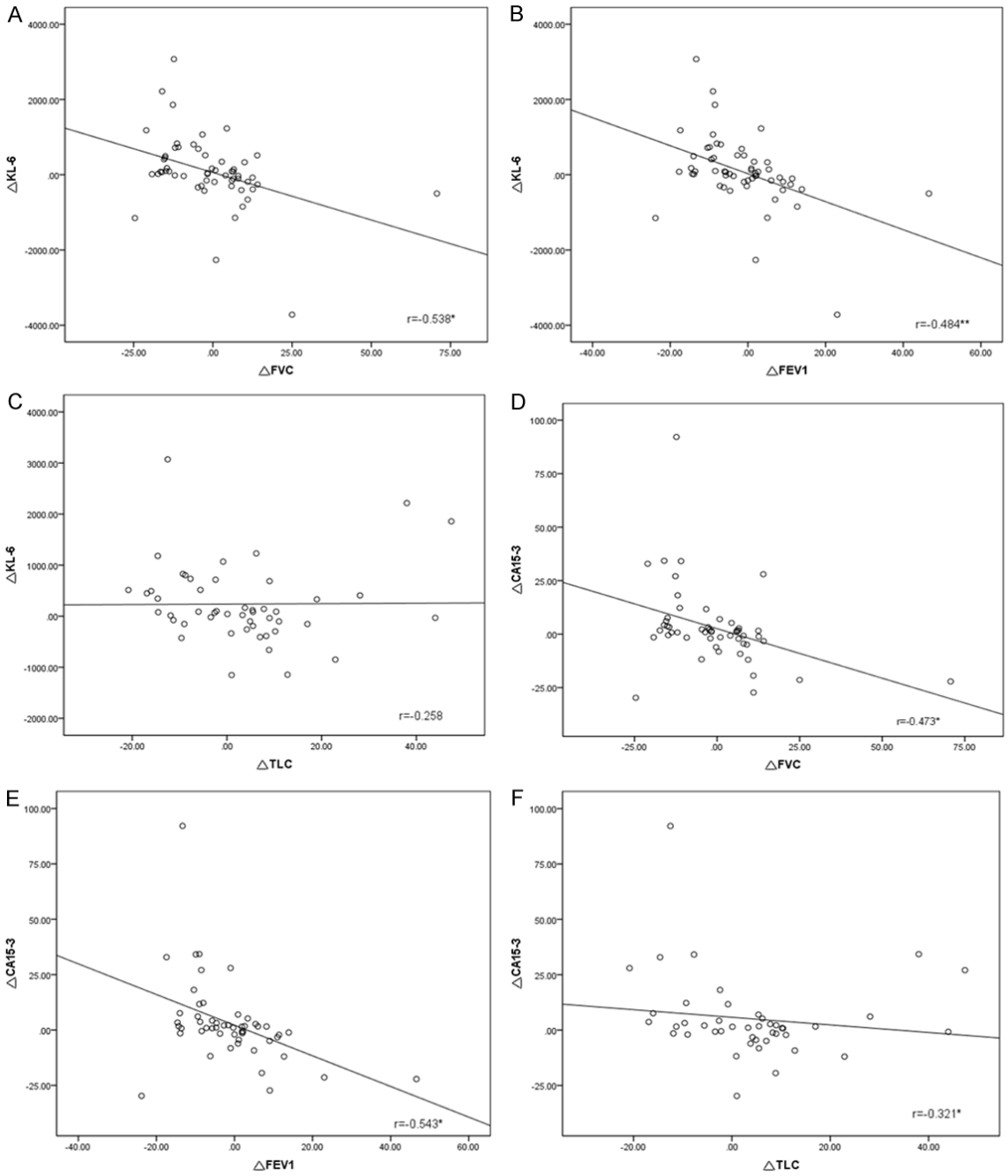

Figure 2. Association of the changes in KL-6 and CA15-3 and the changes in pulmonary function in patients with CTD-ILD before and after treatment. Spearman's rank correlation coefficient was used for Correlation analysis in 57 CTD-ILD patients. $P < 0.05$, $^{**}P < 0.001$.

area under the curve of CTD-ILD was 0.89 (95% confidence interval was 0.84 ~ 0.92, $P < 0.01$). If clinical sensitivity needs to be improved, 397.50 U/ml can be chosen as a cut-off point, in which case the sensitivity and specificity become higher than 80.00%, and the positive predictive value reaches 94.70% (**Figure 1**).

The ROC curve for CA15-3 with regards to its diagnostic effects in CTD-ILD was analyzed. The area under the curve of CTD-ILD was only 0.79 (95% CI: 0.71-0.98, $P < 0.01$). The optimal cutoff point was 19.73 ng/mL, and the sensitivity and specificity were 63.60% and 82.76% respectively, with the sensitivity being low

Table 3. Correlation between changes in KL-6, and CA15-3, and changes in pulmonary function and other laboratory data in 57 CTD-ILD patients

r_s	ΔKL-6	ΔCA15-3
ΔLDH (IU/L)	0.24	0.25
ΔPaO_2 (mmHg)	0.16	0.27
ΔFVC% pred	-0.54**	-0.47**
ΔFEV1% pred	-0.48**	-0.54**
ΔTLC% pred	-0.26	-0.31*
ΔVC% pred	-0.28	-0.28
ΔDLCO% pred	-0.18	-0.15
ΔCA15-3 (ng/mL)	0.85**	--

*Used Spearman's rank correlation coefficient for correlation analysis. The change in data for the 57 CTD-ILD patients before and after treatment is expressed as "Δ". *P < 0.05, **P < 0.01.

(**Figure 1**). However, if combined with KL-6 levels for CTD-ILD diagnosis, then 95.61% of CTD patients will present with KL-6 ≥ 397.5 U/ml or meet the CA15-3 ≥ 19.73 ng/mL criteria, and thus can accurately be diagnosed as CTD-ILD. In this case, the sensitivity and specificity are 82.57% and 82.75%, respectively, And the positive predictive value can reach 95.61%, that is, 82 of 100 patients can be diagnosed correctly, which is quite sensitive.

The change in KL-6 and CA15-3 were negetivly correlated with the change in pulmonary function in CTD-ILD patients

Longitudinal analysis showed that the change in KL-6 (ΔKL-6) was negatively correlated with the changes in FVC% pred (ΔFVC% pred) (r = -0.54, P < 0.01) and FEV1% pred (ΔFEV1% pred) (r = -0.48, P < 0.01) before and after treatment in 57 CTD-ILD patients (**Figure 2A**, **2B**). ΔFVC% pred (r = -0.47, P < 0.05), ΔFEV1% pred (r = -0.51, P < 0.05), ΔTLC% pred (r = 0.31, P < 0.05), and ΔCA15-3 also showed a negative correlation (**Figure 2D-F**) (**Table 3**).

Dynamic monitoring of KL-6 and CA15-3 assessed dieases activity in CTD-ILD patients

57 CTD-ILD patients who underwent KL-6, HRCT, and lung function analysis before and after treatment were divided into three groups: the progression group (16 cases), remission group (21 cases), and stabilization group (20 cases). There was no significant difference in age (F = 2.60, P = 0.09), KL-6 levels (F = 2.38, P = 0.31), and CA15-3 levels (F = 1.86, P = 0.40) among the three groups (**Table 4**).

In the progression group, the levels of KL-6 after treatment [1351.5 (359-5277) U/ml] were higher than before treatment [927 (164-4737) U/ml, Z = -3.52, P < 0.01]. The levels of KL-6 in the remission group after treatment [818 (186-2758) U/ml] were lower than before treatment [1505 (157-5416) U/ml, Z = -4.47, P < 0.01); and there was no significant change in patients in stable condition (P > 0.05) (**Figure 3A-C**). The changes in CA15-3 in the 3 groups were similar to those exhibited for KL-6. The levels of CA15-3 in the progression group were found to increase with the deterioration of the disease [37.71 (11.01-73.52) vs 30.23 (6.12-64.29) ng/mL, Z = -2.84, P < 0.01], while the levels of CA15-3 in the remission group decreased with the amelioration of the disease [(19.33 (7.60-55.38) vs. 28.96 (10.46-147.50) ng/mL, Z = -3.52, P < 0.01] (**Figure 4A-C**).

Discussion

Previous studies suggest that the initial serum KL-6 levels are associated with long-term survival in idiopathic pulmonary fibrosis and connective tissue-related interstitial pneumonia [16-18]. The level of serum KL-6 in patients with pulmonary alveolar proteinosis changes with the activity of ILD [19], indicating that KL-6 is a serum marker of ILD diagnosis and disease activity. In addition, some studies have suggested that CA15-3 can be used as an indicator of the degree of ILD fibrosis and the severity of ILD [20, 21]. However, there is no study yet that has conducted the joint investigation of KL-6 and CA15-3 in CTD-ILD diagnosis and dynamic monitoring of disease activity.

Our study showed that serum KL-6 and CA15-3 levels in patients with CTD-ILD were significantly higher than those in patients with simple connective tissue disease. At a KL-6 cutoff value of 479.5U/ml for diagnosis of CTD patients with ILD, the sensitivity was 75.64% and the specificity was 88.37%, which was consistent with previous studies [22, 23]. The cutoff value of CA15-3 for CTD-ILD was 19.73 ng/mL, and the sensitivity was 63.60% and the specificity was 82.76%. The diagnostic value of KL-6 for ILD is superior to that of CA15-3. When combining KL-6 ≥ 397.5 U/ml or CA15-3 ≥ 19.73 ng/mL

Table 4. The characteristics of 57 CTD-ILD patients in the progression group, remission group, and stabilization group

Characteristic	Progression	Remission	Stabilization	*P* value
N (F)	16 (10)	21 (16)	20 (10)	0.22
Age	54.8 ± 10.7	48.6 ± 9.2	48.9 ± 8.5	0.09
Categorization (%)				0.80
SSc-ILD	1 (6.25)	2 (9.50)	0 (0.00)	
SLE-ILD	0 (0.00)	1 (4.80)	0 (0.00)	
RA-ILD	2 (12.50)	2 (9.50)	5 (25.00)	
SS-ILD	1 (6.25)	1 (4.80)	2 (10.00)	
DM/PM-ILD	8 (50.00)	10 (47.60)	10 (50.00)	
MCTD/UCTD-ILD	4 (25.00)	5(23.80)	3 (15.00)	
CA153 (ng/mL)	30.23 (6.12-64.29)	28.96 (10.46-147.50)	24.24 (5.82-90.99)	0.40
KL-6 (U/mL)	927 (164-4737)	1505 (157-5416)	692 (163-4553)	0.31
FVC% pred	73.64 ± 16.51	62.94 ± 18.69	68.71 ± 27.38	0.45
FEV1% pred	71.51 ± 17.48	65.93 ± 19.85	68.03 ± 25.4	0.83
FEV1%/FVC	79.88 ± 11.77	86.77 ± 7.30	85.16 ± 9.25	0.10
TLC% pred	76.70 ± 13.26	72.59 ± 25.62	67.55 ± 20.62	0.42
VC% pred	78.28 ± 16.46	68.94 ± 19.30	62.81 ± 23.82	0.14
DLco% pred	53.66 ± 15.20	46.68 ± 14.57	57.10 ± 21.54	0.19
LDH (IU/L)	253.67 ± 77.32	272.53 ± 113.03	268.76 ± 114.11	0.85
PaO_2 (mmHg)	96.71 ± 23.25	93.66 ± 21.47	87.37 ± 15.10	0.45

The ANOVA H test was used to compare lung function, LDH, and PaO_2 among the progression group, remission group, and stabilization group. The Kruskal-Wallis test was used to compare the levels of KL-6 and CA15-3 among the 3 groups. SSc, systemic sclerosis; SLE, systemiclupus erythematosus; RA, rheumatoid arthritis; SS, primary Sjögren syndrome; DM / PM, dermatomyositis / polymyositis; MCTD, mixed CTD; UCTD, undifferentiated CTD. *P < 0.05,**P < 0.01.

criteria, the positive predictive value of CTL-ILD was 95.61%, and the sensitivity and specificity were 82.57% and 82.75%, respectively. The combined diagnostic value is superior to the value of KL-6 or CA15-3 alone in the diagnosis of ILD. Previous studies have only been able to compare the differences between CA15-3 and/or KL-6 between ILD patients and controls [20, 21]. Our study was the first to propose that combining KL-6 and CA15-3 levels could be used to improve diagnosis of CTD-ILD.

The monitoring of lung function is important in assessing the severity of disease in patients with ILD, and several studies have found that KL-6 levels are negatively correlated with lung function in CTD-ILD patients [19, 23-26]. Previous studies of CA15-3 focused on the diagnostic efficacy of ILD [20, 21, 27, 28], and explored the association of CA15-3 with pulmonary function [9], or monitored the changes in CA15-3 before and after lung transplantation in ILD patients [29]. The cases included in these studies were limited, and did not focus on the association between the change in lung function and CA15-3 levels in ILD, so they do not constitute strong evidence. In this study, we found that KL-6 and CA15-3 levels were negatively correlated with FVC, FEV1, TLC, and DLco in 156 patients with CTD-ILD. The changes in KL-6 before and after treatment were negatively correlated with ΔFVC% pred and ΔFEV1% pred, while the changes in CA15-3 before and after treatment were negatively correlated with ΔFVC% pred, ΔFEV1% pred, andΔTLC% pred. This means that the clinical combination of KL-6 and CA15-3 can be used to assess the severity of lung injury and disease development in these patients.

In addition, serum KL-6 and CA15-3 levels can be used as dynamic disease tracking markers in CTD-ILD. 57 patients were divided into the progression group, remission group, and stabilization group based on their clinical manifestations. In the progression group, the levels of KL-6 and CA15-3 increased with the deterioration of the disease. In the remission group, the levels of KL-6 and CA15-3 decreased with the improvement of the disease, thus the dynamic

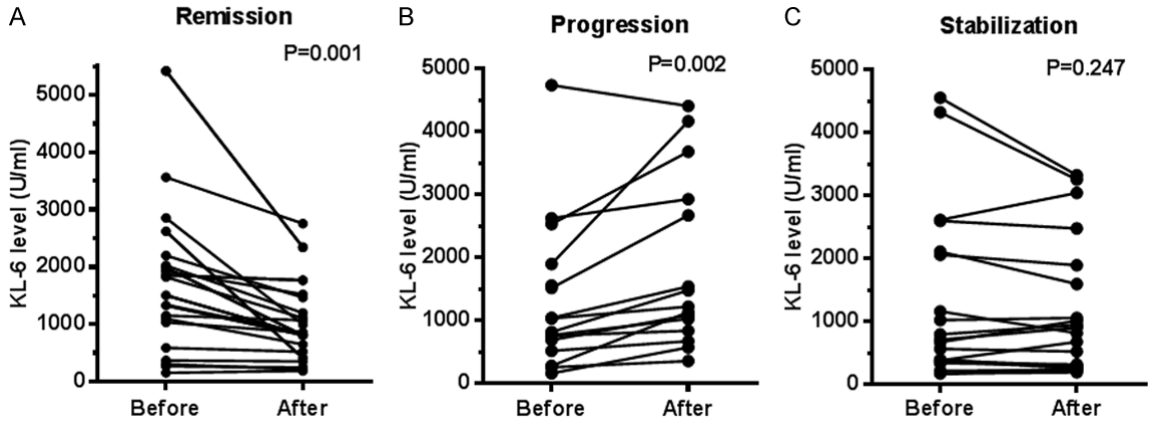

Figure 3. The change in KL-6 in the progression group, remission group, and stabilization group before and after treatment. *Wilcoxon test was used to compare the KL-6 levels before and after treatment in the progression group (16 cases), remission group (21 cases), and stabilization group (20 cases).The level of KL-6 after treatment in progression group was higher than before treatment [1351.5 (359-5277) vs. 927 (164-4737) U/ml, Z = -3.52, P < 0.01]. The level of KL-6 after treatment in the remission group was lower than before treatment [818 (186-2758) vs.1505 (157-5416) U/ml, Z = -4.47, P < 0.01)]; and there was no significant change in patients in stable condition [1280 (163-4553) vs.692 (163-4553) U/ml, Z = -0.75, P = 0.46].

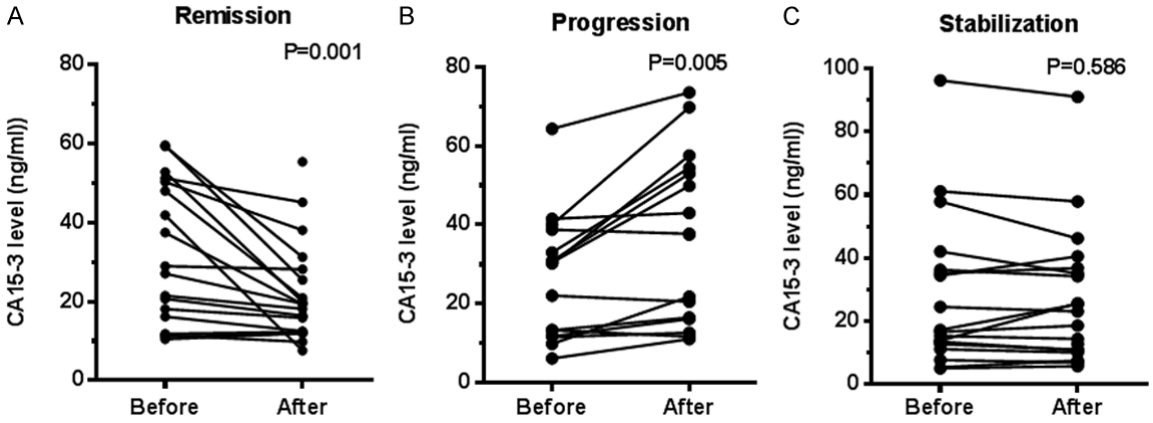

Figure 4. The change in CA15-3 in the progression group, remission group, and stabilization group before and after treatment. *Wilcoxon test was used to compare the level of CA15-3 before and after treatment in the progression group (16 cases), remission group (21 cases), and stabilization group (20 cases). The level of CA15-3 after treatment in progression group was higher than before treatment [37.71 (11.01-73.52) vs. 30.23 (6.12-64.29) ng/mL, Z = -2.84, P < 0.01]. The level of KL-6 after treatment in the remission group was lower than before treatment [(19.33 (7.60-55.38) vs. 28.96 (10.46-147.50) ng/mL, Z = -3.52, P < 0.01]; and there was no significant change in patients in stable condition [24.24 (5.82-90.99) vs. 16.93 (5.13-96) ng/mL, Z = -0.54, P = 0.59].

continuous detection of KL-6 and CA15-3 levels can be used to monitor changes in the patient›s condition. Previous studies of KL-6 focused more on its diagnostic value in ILD and whether baseline KL-6 levels could be used in the assessment of patient outcomes. The level of basal KL-6 in patients with progressive deterioration of CTD-ILD was higher than in patients with steady state disease and was significantly associated with prognosis [30-33]. Yanaba K et al have found that KL-6 levels in patients with systemic sclerosis and concurrent ILD activity are higher than those with ILD at rest. KL-6 levels greater than 1000 U/ml in patients with systemic sclerosis were associated with ILD activity [19]. It has also been found that CA15-3 levels are associated with survival in patients [9], and that their dynamic changes before and after lung transplantation in ILD patients are associated with post-transplant recovery [29]. Our study found that the dynamic monitoring of KL-6 and CA15-3 levels can reveal recent

dynamic changes in ILD, and that these compounds can be used as simple markers which are easy to detect. Unfortunately, this study is an assessment of recent dynamic changes of CTD-ILD disease, with no long-term follow-up and statistical survival rates, which may result in differences in group and disease assessment. However, our results show that the levels of KL-6 and CA15-3 change along with the activity of CTD-ILD, and can therefore directly reflect changes in recent disease activity.

Previous studies have shown that KL-6 is highly correlated with CA15-3 in ILD patients and normal subjects [20, 34]. This study also found that KL-6 and CA15-3 had a highly positive correlation in CTD-ILD patients, and that ΔKL-6 changes and ΔCA15-3 changes were also positively correlated. KL-6 and CA15-3 are present at different positions of the high-quality glycoprotein MUC1, which is distributed in different epithelial cells, including type II lung cells [35]. The levels of KL-6 and CA15-3 in CTD-ILD patients were significantly higher than those of patients with CTD alone. The increase in serum KL-6/MUC1 levels in patients with ILD is the result of an increase in KL-6/MUC1 production through regeneration of alveolar type II pneumocytes and/or enhancement of permeability following destruction of the alveolar-capillary barrier in the affected lung [4]. In our study, the specificity of KL-6 in lung tissue was significantly higher than that of CA15-3, but the mechanisms of tissue specificity remain unclear [35].

There are some shortcomings to this study. First, 156 patients with CTD-ILD and 43 patients with simple CTD were included in the study. Although these numbers are sufficient to investigate the relationship between serum KL-6 and CA15-3 levels and CTD-ILD, the sample size is relatively small, especially for subgroup analysis and longitudinal studies. If the sample size is increased, the results would be more representative. Secondly, the treatment of patients did not involve unified intervention to reduce the impact of confounding factors. Therefore, more research studies with larger sample sizes are still needed to overcome these specific problems, in order to further verify our results.

Conclusion

Serum KL-6 and CA15-3 were positivly correlated with the severity of CTD-ILD. The combined detection of serum KL-6 and CA15-3 can Improve diagnosis of CTD-ILD. The dynamic monitoring of changes in KL-6 and CA15-3 levels were conducive for effective management and monitoring of this disease.

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Disclosure of conflict of interest

None.

Address correspondence to: Baoqing Sun and Ning Li, First Affiliated Hospital of Guangzhou Medical University, 151 Yanjiangxi Road, Guangzhou 510-120, Guangdong, China. Tel: +86 20 8306 2865; Fax: +86 20 8306 2729; E-mail: sunbaoqing@vip.163.com (BQS); 13434180887@139.com (NL)

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