Diagnostic Value of Nanopia® KL-6 in Chinese Patients with Interstitial Lung Disease: A Prospective Multicenter Observational Study

Bingpeng Guo
The First Affiliated Hospital of Guangzhou Medical University
Qian Han
The First Affiliated Hospital of Guangzhou Medical University
Wenjie Wang
Harbin Medical University
Ziyi Zhang
The First Affiliated Hospital of Guangzhou Medical University
Baoqing Sun
The First Affiliated Hospital of Guangzhou Medical University
Jian Kang
The First Affiliated Hospital of China Medical University
Yabin Zhao
The First Affiliated Hospital of China Medical University
Zuojun Xu
Peking Union Medical College Hospital, Chinese Academy of Medical Sciences
Hui Huang
Peking Union Medical College Hospital, Chinese Academy of Medical Sciences
Nanshan Zhong
The First Affiliated Hospital of Guangzhou Medical University
Qun Luo (luoqunx@163.com)
The First Affiliated Hospital of Guangzhou Medical University

Research Article

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Abstract

Background: Krebs von den Lungen-6 (KL-6) is considered a sensitive biomarker for diagnosis of interstitial lung disease (ILD). We aimed to evaluate the diagnosis value of Nanopia® KL-6 (SEKISUI MEDICAL CO., LTD., Tokyo, Japan) in a Chinese cohort of patients with ILD.

Methods: Totally 451 patients were enrolled in our multicenter study, including 166 (36.8%) ILD patients, 210 (46.6%) non-ILD patients and 75 (16.6%) health controls. All ILD patients underwent high-resolution computed tomography (HRCT) followed by pulmonary function test (PFT). Serum KL-6 concentrations were measured by latex particle enhanced turbidimetric immunoassay (LTIA).

Results: KL-6 serum concentrations were significantly higher in ILD patients (911 U/ml, IQR 477-1790) than in non-ILD patients (225 U/ml, IQR 166-323) and health controls (196 U/ml, IQR 153-230, $p<0.0001$). Serum KL-6 higher than 435.5 U/ml appeared as the optimal cut-off value associated with ILD. KL-6 concentrations were inversely correlated with forced vital capacity (FVC) (rho=-0.515, $p<0.001$), total lung capacity (TLC) (rho=-0.563, $p<0.001$) and diffuse lung capacity of carbon monoxide (DLco) (rho=-0.544, $p<0.001$). ILD patients with more severe characteristics of HRCT including ground glass opacity, reticular pattern or honeycombing had significantly higher serum KL-6 levels. In the subgroup of ILD patients, serum KL-6 concentrations were higher in idiopathic interstitial pneumonia (IIP) patients (1024U/ml, IQR 697-2112.25) than in other ILD patients (743U/ml, IQR 702.75-2058.25, p<10-4). ILD and retained smoking were independent factors associated with higher KL-6 levels in multivariate analysis.

Conclusions: Our study confirms that KL-6 is a credible biomarker for the diagnosis of ILD in a Chinese cohort of patients. High serum KL-6 concentration should call attention to physicians to assess ILD with HRCT and PFT.

Introduction

Interstitial lung diseases (ILDs) are a broad, heterogeneous group of pulmonary disorders that encompass more than 200 acute or chronic diseases with various degrees of inflammation or fibrosis(1, 2). ILDs are broadly divided into four categories: ILDs with known causes, idiopathic interstitial pneumonia (IIP) of unknown causes, granulomatous ILD, and rare forms of ILDs. It has been reported that the incidence of ILDs was between 3.6 and 32/100,000 people(3–5). In the subtype diagnosis of ILDs, idiopathic pulmonary fibrosis (IPF) and connective tissue disease (CTD) associated with ILD are the most common in China(6).

The ILDs diagnosis approach needs multidisciplinary discussion, which involves examination of clinical aspects, serological testing associated with CTD, high-resolution computed tomography (HRCT) and pulmonary function tests (PFT). Although forced vital capacity (FVC) and diffusing capacity of the lungs for carbon monoxide (DLco) are strongly correlated with disease severity and prognosis, but they are not always performed in severe or advanced-stage ILDs patients. Moreover, one of main concerns for CT

screening is repeated radiation exposure, with the risk of inducing malignancies in individuals. Therefore, serological biomarkers with high sensitivity and specificity for ILDs are needed.

Krebs von den Lungen-6 (KL-6) is a high molecular weight mucin-like glycoprotein, and secreted by type II alveolar pneumocytes and bronchial epithelial cells in response to cellular destruction and regeneration, resulting in an increased concentration in serum(7). KL-6 concentration elevates in over 70% of ILDs, such as IPF, CTD-ILD, hypersensitivity pneumonitis, sarcoidosis, and radiation pneumonitis(8). The National Health Insurance system of Japan has routinely used KL-6 as a diagnostic biomarker in ILDs since 1999(7). Recently, we have found that KL-6 concentrations in IIPs were significantly higher than in non-IIP patients which means that KL-6 is a valuable diagnostic biomarker(9). However, this was a retrospective study with limited information and the data of post treatment of KL-6 was only observed in a single center, which may not be representative.

KL-6 concentration can be measured by ELISA, electrochemiluminescence immunoassay, and latex particle-enhanced turbidimetric immunoassay (LTIA)(10). Clinical laboratories can use automated analyzers to perform the Nanopia® KL-6 kit assay (SEKISUI MEDICAL CO.LTD., Tokyo, Japan). LTIA is faster and more convenient than ELISA Method. One study determined good precision, linearity, and correlation between LTIA and ELISA(10).

Therefore, we aimed to evaluate the diagnostic value of serum KL-6 concentrations measured by Nanopia KL-6 kit assay in Chinese cohort of ILD patients. Moreover, we identified possible correlations between KL-6 concentrations and clinical results obtained performing HRCT and PFT.

Methods

Study design

This was a prospective, single-blinded, multicenter observational clinical trial performed at three of the largest ILDs specific clinics in China, including the First Affiliated Hospital of Guangzhou Medical University, the First Affiliated Hospital of China Medical University and Peking Union Medical College Hospital. The Ethical approval was obtained by the Institutional Ethics Committee of each hospital. Written informed consent was obtained from each individual before enrolment at the time of their diagnosis.

Patients

Three individual groups were included in this study: (a) ILD group (IIP, CTD-ILD, sarcoidosis, pulmonary alveolar proteinosis (PAP) and hypersensitivity pneumonia (HP)); (b) non-ILD group (other pulmonary diseases including community-acquired pneumonia (CAP), bronchiectasis, pulmonary tuberculosis, asthma, chronic obstructive pulmonary disease (COPD), asthma, pneumonomycosis, CTD without ILD); (c) healthy individuals with peripheral blood oxygen saturation over 95% at rest without abnormal chest

X-ray within four weeks before recruitment. Patients were eligible to participate in the trial if they were 18 years of age or older and had received a diagnosis of ILD or other types of pulmonary diseases. The diagnostic criteria for IIP, CTD-ILD, sarcoidosis, HP, PAP, and other types of pulmonary diseases were based on the internationally accepted guidelines(1,2,11-20). Patients who were diagnosed with malignant tumor disease or had a history of hemodialysis were excluded. Pregnant and lactating women were also excluded.

Clinical Information Collection

The following data were recorded in ILDs group and non-ILD group: gender, age, body mass index (BMI), clinical manifestations, medical history, oxygen therapy history, smoking history, and serologic examination including lactate dehydrogenase (LDH), C - reactive protein (CRP).

HRCT and PFT were performed in all patients. Lung volume including FVC, total lung capacity (TLC) and single breath DLco corrected for hemoglobin levels were expressed as percentage of the predicted value. The extent of HRCT abnormalities was determined by visually estimating the percentage of lung involvement including ground glass opacity (GGO), reticular pattern and honeycombing using a semiquantitative scoring system. The score of HRCT were regarded on a scale of 0-5 as follows: 0-none; 1-less than 5%; 2-5%-24%; 3-25%-49%; 4-50%-74%; 5-over 75%.

As for healthy controls, the information of gender, age and PFTs parameters including vital capacity (VC) was included.

Lung disease severity was measured on the scale of I to IV according to ILD-GAP model (TableS1), which accurately predicts the severity and mortality of ILD(21).

Laboratory measurements

We collected blood (4ml) from each participant using standardized operating procedures. Serum were separated by centrifugation and all specimens were stored at -80℃ until assayed. All samples were processed within 2 hours. Serum KL-6 concentration was measured using the Nanopia® KL-6 (SEKISUI MEDICAL CO.LTD., Tokyo, Japan)

Statistical analysis

Quantitative variables were described as means ± standard deviations or median and interquartile ranges (IQR) (first quartile- third quartile) according to their distribution. Qualitative variables were described as numbers and percentages.

Serum KL-6 concentrations and other clinical characteristic parameters were conducted among different groups. Parametric data was analyzed using a t-test and non-parametric date was analyzed using the Wilcoxon rank sum test. Receiver operating characteristic curves (ROC) were established to define the best threshold value for KL-6 to discriminate patients with and without ILD, according to the Youden methods which allows the maximization of both specificity and sensitivity. The area under the curve and its 95% confidence interval was analyzed. Sensitivity, specificity, positive and negative predictive values associated with the identified threshold were also analyzed. A multivariate liner regression was performed according to the results of the univariate analysis ($p<0.20$) and the pre-identified factors known to be associated with KL-6.

All statistical analyses were performed using SPSS 22.0 (SPSS, Inc., Chicago, IL, USA) and Prism 5.0 statistical software (GraphPad, Inc., La Jolla, CA, USA). All tests were two sided. A value of $p<0.05$ was considered as significant.

Results

Demographic and clinical characteristics of the study population are presented in Table 1. The study comprised 451 individuals, including 166 (36.8%) ILD patients, 210 (46.6%) non-ILD patients and 75 (16.6%) health controls. No significant difference concerning the age ($p = 0.53$), gender ($p = 0.70$) and CRP ($p = 0.263$) was observed between these three groups. Compared to non-ILD group, ILD group had a significantly higher BMI ($p < 10 - 4$). Compared to health controls, although former or current smoker and LDH were significantly higher in ILD group and non-ILD group, $p = 0.048$ and $p < 10 - 4$, respectively, there were no significant difference between ILD group and non-ILD group, $p = 0.803$ and $p = 0.458$, respectively.

Table 1
Demographic and clinical characteristic of the study population

	ILD (n = 165)	Non-ILD (n = 211)	Health individual(n = 75)	P value
Male –no. (%)	86(52.1)	114(54.0)	36(48.0)	0.70
Age-yr	56.0 ± 18.9	55.1 ± 19.2	53.2 ± 17.4	0.539
BMI	24.7 ± 3.7	22.2 ± 4.4	23.6 ± 3.9	< 0.001
Former or current smoker- no. (%)	63(38.2)	82(38.8)	8(10.7)	< 0.001
CRP(mg/L)	1.02(0.32,3.55)	1.03(0.24,2.51)	0.46(0.1,1.94)	0.263
KL-6(U/L)	911(477,1790)	225(166,323)	196(153,230)	< 0.001*
LDH (IU/L)	246.7 ± 69.7	209.5 ± 94.6	189.6 ± 36.0	< 0.001

KL-6 serum concentrations were significantly higher in ILD patients (911 U/ml, IQR 477–1790) than in those without ILD (225 U/ml, IQR 166–323) and health individuals (196 U/ml, IQR 153–230, $p < 10 - 4$) (Fig. 1). In the subgroup of ILD patients, KL-6 serum concentrations were significantly higher in IIP patients (1201U/ml, IQR 697-2112.25) than in CTD-ILD patients (1024 U/ml, IQR 635-1789.5) and other ILD patients (1004.74, IQR 210.75-1004.75, $p < 10 - 4$) (Fig. 2). The serum KL-6 concentration in CTD-ILD (921.00U/ml, IQR 443–1669.00) were higher than CTD without ILD patients (199.50U/ml, IQR 147.75–322.00, $p < 10 - 4$) (Data not shown).

According to the ROC analysis, the area under the ROC curve was 0.892 [95% confidence interval (CI): 0.856–0.927, $p < 10 - 4$]. The optimal cut-off value of serum KL-6 to discriminate the presence of ILD was 435.5 U/ml, with sensitivity of 78.7%, specificity of 92.7%, indicating the relatively high diagnostic value of the KL-6 assay. (Fig. 3)

Patients with FVC < 80% (1492.5, IQR 812.5-2115.5) had significantly higher serum KL-6 than patients with FVC > = 80% (614, IQR 337–1019, $p < 10 - 4$). Patients with TLC < 80% had significantly higher KL-6 serum concentrations (1071.5, IQR 697–1929) than patients with TLC > = 80% (484, IQR 331–825, $p < 10 - 4$). Patients with DLCO < 50% (1330, IQR 866–2233) also had significantly higher KL-6 concentration than DLCO > = 50% (697, IQR 338–1390, $p = 0.0004$) (Fig. 4). Altogether, KL-6 serum levels were inversely correlated with FVC (rho=-0.515, $p < 0.001$), TLC (rho=-0.563, $p < 0.001$) and DLco (rho=-0.544, $p < 0.001$) (Fig. 5).

KL-6 concentrations positively increased according to the lung disease severity based on the radiological characteristic, GGO ($p < 0.001$), reticular pattern ($p < 0.001$) and honeycombing ($p = 0.002$) (Fig. 6).

Importantly, a multivariate analysis, including the following variables:BMI, age at KL-6 analysis, gender, presence of an ILD, smoking state, only ILD and retained smoking positivity as independent factors associated with KL-6 (both $p < 0.001$) (data not shown).

KL-6 serum concentrations positively increased according to the lung disease severity using the ILD-GAP model ($p = 0.003$) (Fig. 7). Serum KL-6 concentration differentiated between ILD-GAP stage I and III ($p < 0.001$), ILD-GAP stage I and IV ($p = 0.032$) and ILD-GAP stage II and III ($p < 0.001$).

Discussion

In our study, we found that serum concentration of KL-6 is a useful marker for differential diagnosis of ILD from non-ILD patients. In addition, we showed that KL-6 is a severity marker of functional pulmonary involvement, with higher levels in more severe ILD patients. To our knowledge, this is the first prospective study evaluating KL-6 concentration measured by Nanopia assay in Chinese cohort.

Serum KL-6 concentrations lower than 435.5 U/ml were able to differentiate the absence of ILD. However, in Miyazaki et al. study, 500 U/ml was reported as the cut-off value for a specific condition and needs to be drawn based on a comparison with adequate control(22). KL-6 concentration might be affected by various conditions, such as lung cancer, age, sex, gender. We found that ILD and smoking state were independent associated with KL-6 concentration by multivariate analysis.

The results of our study were consistent with the previous studies that serum concentration of KL-6 correlated with overall degree of lung involvement and presence of typical higher serum KL-6 concentration were found in IPF with more abnormal involvement such as honeycombing and reticular pattern(10, 23). In Bonella et al. study, they found that serum KL-6 concentration was correlated with fibrosis score on HRCT in patients with systemic sclerosis associated ILD (SSc-ILD) (24).

We also found that serum KL-6 concentrations are much higher in CTD patients with ILD than without ILD. This result is essential for CTD patients to have a noninvasive biological marker, which could indicate patients with CTD during following up. Because deciding when and how long to treat CTD patients with ILD and without ILD is quite different in clinical practice. Benyamine et al. found that KL-6 was an independent factor associated with ILD in SSc patients($p = 0.0007$)(25). However, according to ROC analysis, the optimal cut-off value of serum KL-6 concentration in Benyamine et al. study was 872U/ml, higher than our study(25).

There are several studies reporting that KL-6 concentration was correlated with the level of FVC, DLco and TLC, which is similar to our study. Bonella et al. found that declined FVC and DLco were correlated with increased serum KL-6 concentrations in SSc-ILD patients(24).

There are many methods used for measuring KL-6 concentrations, such as ELISA, LTIA and electrochemiluminescence immunoassays. Cho et al. found that the result of KL-6 concentration measured by ELISA and Nanopia were strongly correlated(10). However, the concentration of KL-6

measured by ELISA kit were lower than Nanopia assays due to the heterophile antibodies or rheumatoid factor(26). Therefore, compared with ELISA kit, LTIA would provide more accurate results.

This study had a few limitations. First, the number in ILD-GAP stage IV patients was rather low (4/165). Because many of the ILD-GAP stage IV were not able to perform the PFTs or chest HRCT, so we could not enroll them in our study.

Conclusion

Serum KL-6 concentration is a sensitivity biomarker to differentiate ILD patients from non-ILD patients. KL-6 levels had a negative correlation with PFT parameters and extent of CT scans semiquantitatively. Serum KL-6 could be a clinically useful biomarker in screening and evaluating ILD.

Abbreviations

KL-6: Krebs von den Lungen-6; ILD: interstitial lung disease; HRCT: high-resolution computed tomography; PFT: pulmonary function test; LTIA: latex particle enhanced turbidimetric immunoassay; FVC: forced vital capacity; TLC: total lung capacity; DLco: diffuse lung capacity of carbon monoxide; IIP: idiopathic interstitial pneumonia; IPF: idiopathic pulmonary fibrosis; CTD: connective tissue disease; PFT: pulmonary function tests; PAP: pulmonary alveolar proteinosis; HP: hypersensitivity pneumonia; CAP: community-acquired pneumonia; COPD：chronic obstructive pulmonary disease；LDH：lactate dehydrogenase；CRP：C - reactive protein；GGO： ground glass opacity；VC：vital capacity；ROC： Receiver operating characteristic curves；SSc: systemic sclerosis associated;

Declarations

Author Contributions

The study was designed by NZ and QL. QH, JK, YZ, ZX, BS and HH recruited patients. WW, ZZ, BG analyzed data. BG, WW, ZZ wrote the first draft of the manuscript. QH revised it. All authors critically revised the manuscript for intellectually important content. All authors gave final approval for the publication of the work, and all accepted responsibility for the integrity of the work.

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Availability of data and materials

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

Ethics approval and consent to participate

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The research was conducted in accordance with the ethical principles of the World Medical Association Declaration of Helsinki. After the subjects (or their parents or guardians) have signed written informed consent, the research protocol has been approved by the Human Research Committee of the Institute. Approval Number: The First Affiliated Hospital of Guangzhou Medical University [2014]06, the First Affiliated Hospital of China Medical University [2014]066, the Peking Union Medical College & Chinese Academy of Mediical Sciences HS2014005.

Consent for publication

Not applicable

Competing Interest

All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

Ethical Approval Number

The First Affiliated Hospital of Guangzhou Medical University [2014]06,

The First Affiliated Hospital of China Medical University [2014]066, Peking Union Medical College & Chinese Academy of Medical Sciences KS2014026.

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Figures

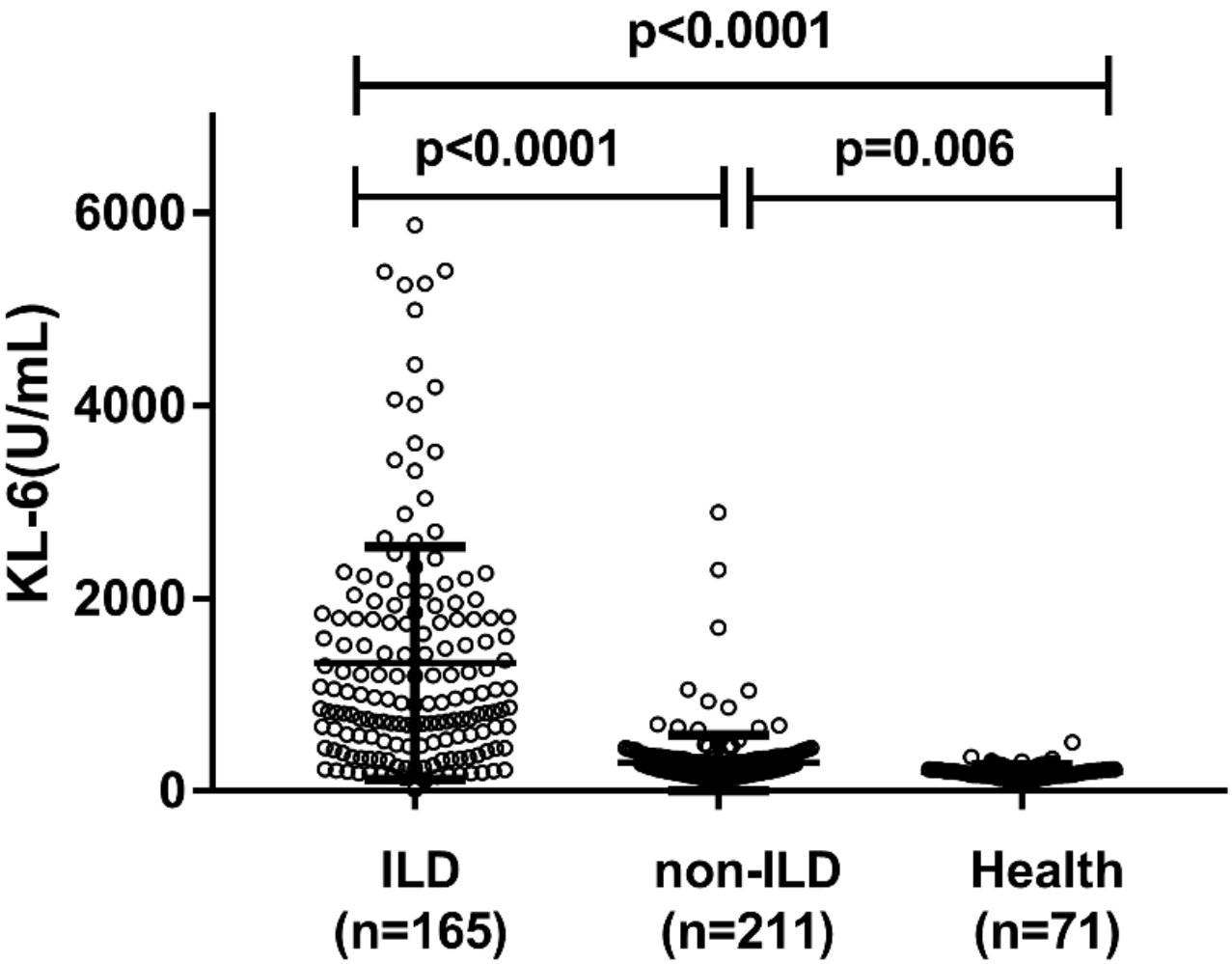

Figure 1

Distribution of serum KL-6 concentration in ILD group, non-ILD group and health controls Scatter plot graphs showing the distribution of baseline serum KL-6 concentration in ILD group (n=165), non-ILD group (n=211) and healthy group (n=71). Results were depicted as median ± interquartile range. ILD, interstitial lung disease

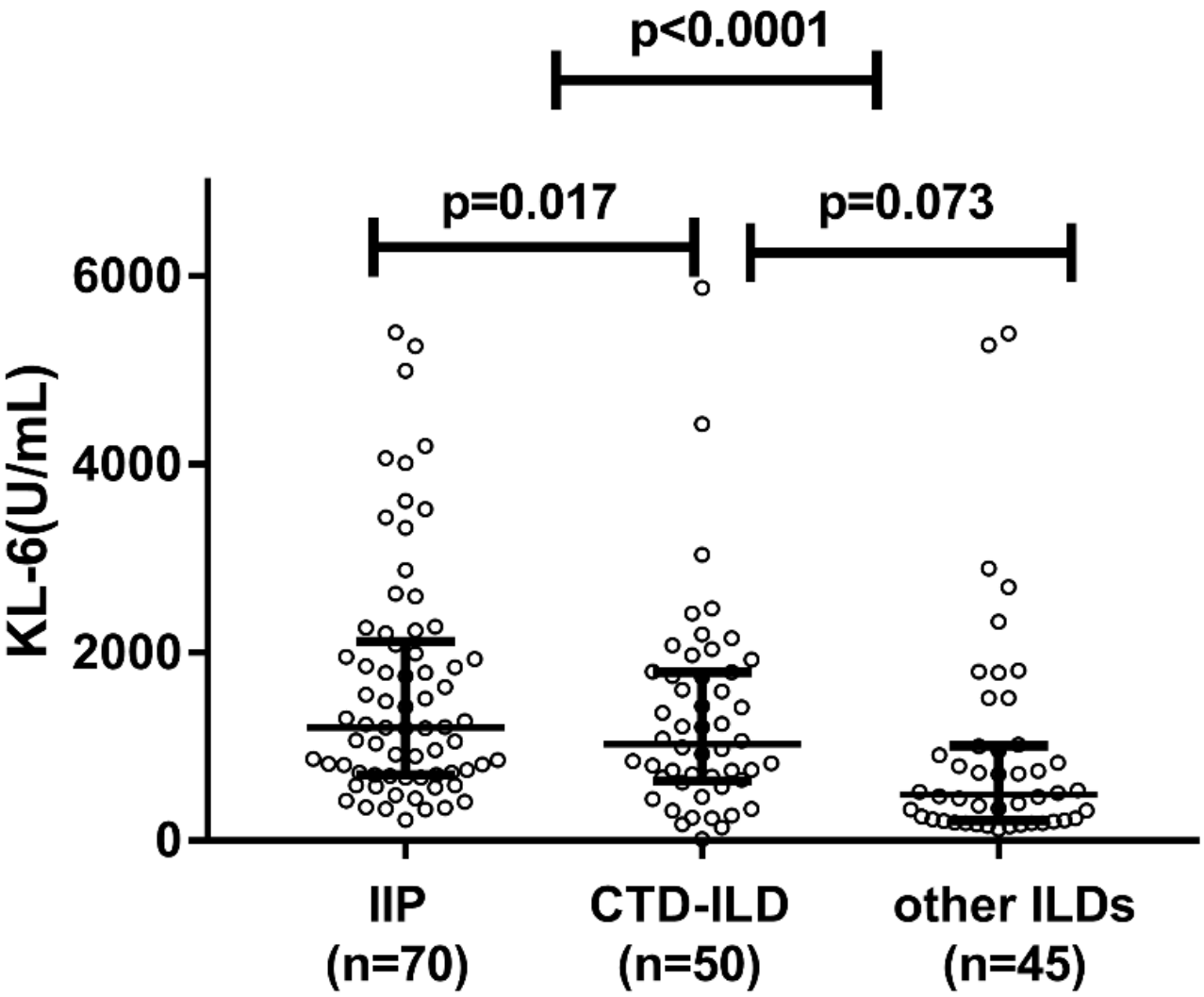

Figure 2

Distribution of serum KL-6 concentration in ILD group Scatter plot graphs showing the distribution of baseline serum KL-6 concentration in IIP group (n=70), CTD-ILD group (n=50) and other ILD group (n=45). ILD, interstitial lung disease; IIP, idiopathic interstitial pneumonia; CTD, connective tissue disease.

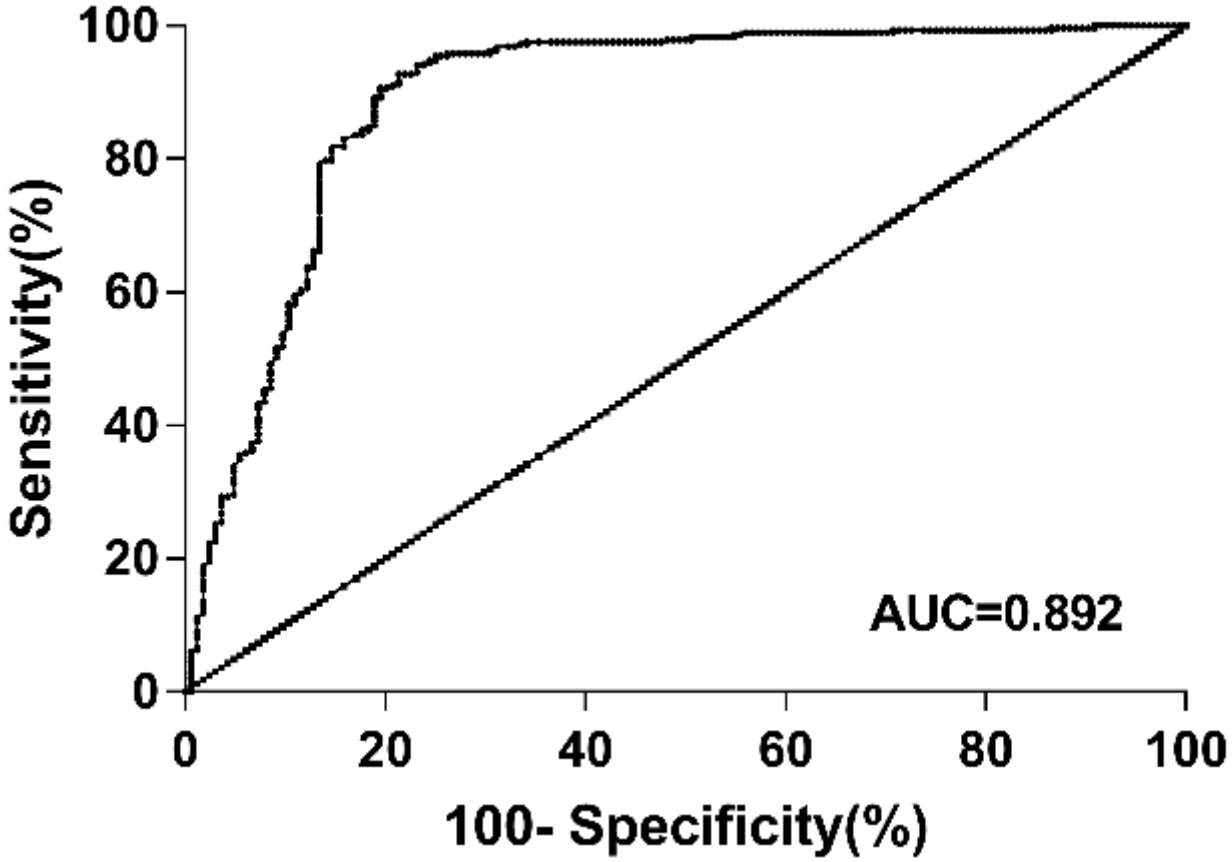

Figure 3

Receiver operating characteristic curve (ROC) analysis. Sensitivity and specificity of KL-6 level to discriminate for the presence of ILD from non-ILD and healthy controls was optimal for a cut-off value of 435.5 U/ml. ILD, interstitial lung disease

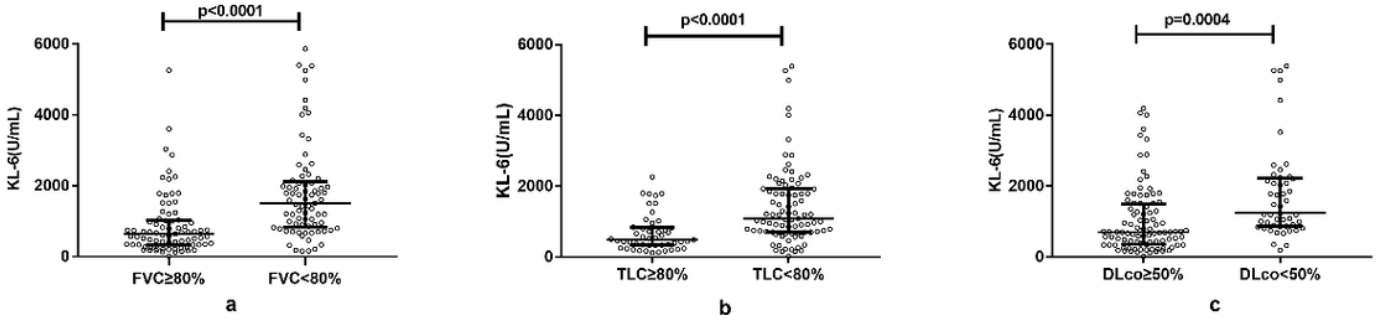

Figure 4

Serum KL-6 concentration dependent on the severity of FVC, TLC and DLco. Scatter plot graphs showing the distribution of serum KL-6 concentration dependent on FVC (>=80% VS <80%), TLC (>=80% VS <80%)

and DLco (>=50% VS <50%). FVC, forced vital capacity, TLC, total lung capacity, DLco, diffuse lung capacity of carbon monoxide

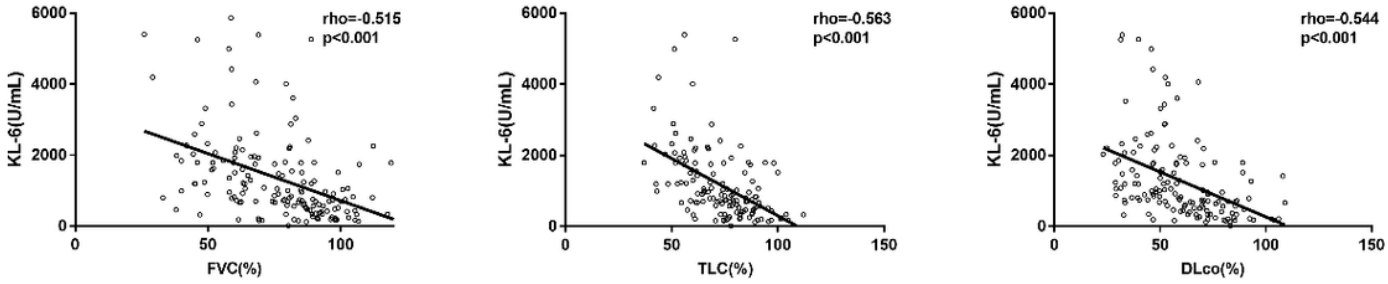

Figure 5

a. correlation between KL-6 serum concentration and FVC in ILD patients; b. correlation between KL-6 serum concentration and TLC in ILD patients; c. correlation between KL-6 serum concentration and DLco in ILD patients. All the spearman correlation coefficients are shown (rho). FVC, forced vital capacity, TLC, total lung capacity, DLco, diffuse lung capacity of carbon monoxide.

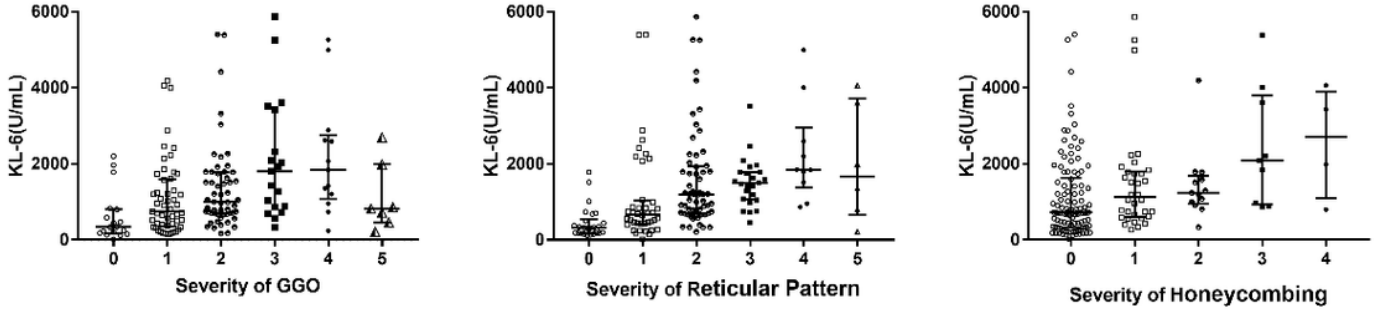

Figure 6

KL-6 concentrations depending on severity of HRCT including ground glass opacity (GGO), reticular pattern and honeycombing. The score of HRCT was established on a scale of 0-5 as follows: 0-none; 1-less than 5%; 2-5%-24%; 3-25%-49%; 4-50%-74%; 5-over 75%. Importantly, a multivariate analysis, including the following variables:BMI, age at KL-6 analysis, gender, presence of an ILD, smoking state, only ILD and retained smoking positivity as independent factors associated with KL-6 (both p<0.001) (data not shown).

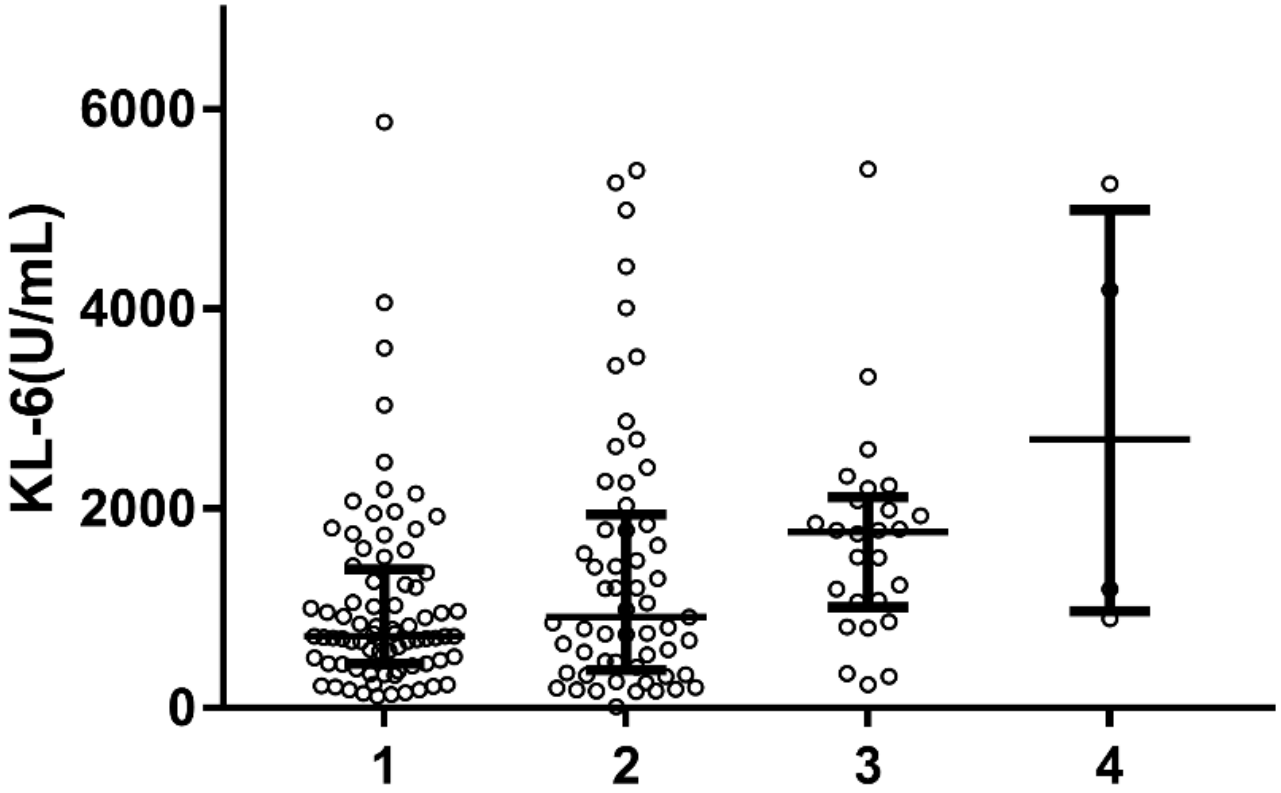

Figure 7

KL-6 concentrations (U/ml) depending on the severity of lung diseased based on the scale of ILD-GAP model. 1: ILD-GAP Index 0-1; 2: ILD-GAP Index 2-3; 3: ILD-GAP Index 2-3; 4: ILD-GAP Index >5