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Krebs von den Lungen-6 as biomarker of the new progressive fibrotic phenotype of interstitial lung disease

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ABSTRACT

Background: Novel progressive fibrotic phenotype has recently been proposed characterized by progressive and inexorable worsening of the disease. Krebs von den Lungen-6 (KL-6) has been proposed as fibrotic-ILD biomarker. We aimed to assess the role of KL-6 in fibrotic-ILD and the progressive phenotype in accordance with serial serum KL-6. Methods: 107 patients were enrolled in the study (median age,IQR, 65(54−71)y/o) followed at respiratory diseases and rheumatology units of University of Siena. Thirty-five had diagnoses of IPF, 18 sarcoidosis, 10 PLCH, 5 LAM, 24 fibrotic HP(fHP), 13 RA (4/13 RA-ILD) and 22 SSc (18/22 SSc-ILD). Serial serum samples were collected before therapy (t0) and 24 months later (t1) from IPF, SSc- and RA-ILD patients. Twenty-two healthy controls (HC) were enrolled. Serum samples were assayed for KL-6 concentrations (Fujirebio Europe, Gent, Belgium). Results: Higher KL-6 concentrations were reported in IPF, fHP and SSc-ILD patients than HC ($p<0.0001$). KL-6 cut-off value of 885 U/mL identified fibrotic-ILD patients. Logistic regression analysis indicated KL-6 ($p=0.004$) and smoking-habit ($p=0.005$) affected the ILD diagnosis. The decision tree model showed KL-6>1145 U/mL, DLco≤60.15 %, FVC≤86 % to classify 86 % IPF patients. Inverse correlation between T0-KL-6 and T1-FVC%($r=-0.314$, $p=0.046$) and T1-DLco%($r=-0.327$, $p=0.038$) in the progressive group. Conclusion: KL-6 proved to be a reliable marker for diagnosis and prognosis of fibrotic ILD patients with predictive value in progressive fibrotic patients and a useful marker to identify the new and similar progressive phenotype of IPF and SSc-ILD patients assessing the functional progression in accordance with serial serum KL-6 measurements.

1. Introduction

Among idiopathic interstitial lung disease (ILD), the most common is idiopathic pulmonary fibrosis (IPF), characterized by a progressive clinical course, sometimes interrupted by high mortality events, defined as "acute exacerbation" (Agustí et al., 1991; Ayed et al., 2017). In the last decades, it has been reported that also patients with non-IPF ILDs, such as fibrotic hypersensitivity pneumonitis (HP) or connective tissue diseases associated with ILD (CTD-ILD: Rheumatoid Arthritis (AR)-ILD, Systemic Sclerosis (SSc)-ILD), can show a progressive worsening of the disease regardless of treatments (Buschulte et al., 2024; Wang et al., 2022). Moreover, many recent studies have suggested the presence of common pathogenetic pathways between IPF and non-IPF ILDs, like shortening of telomeres, epithelial cell dysfunction and immune dysregulation (d'Alessandro et al., 2021a; Inoue et al., 2020). As a result, a new progressive fibrotic phenotype has recently been proposed that encompasses all patients with ILD (both idiopathic and non-idiopathic) who generally show a progressive deterioration of their condition (Cottin et al., 2022). Therefore, identification of prognostic biomarkers for ILDs is more compelling than ever. In IPF, many biomarkers have

Abbreviations: HRCT, high resolution computed tomography; ILD, interstitial lung disease; RA, rheumatoid arthritis; SSc, systemic sclerosis; KL-6, Krebs von den Lungen-6; LAM, lymphangioleiomyomatosis; PLCH, pulmonary Langerhans cell histiocytosis; HP, hypersensitivity pneumonitis.

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shown to have prognostic significance, but their utility in the clinical practice to predict outcome and answer to therapy is limited and no biological marker has been proven in non-IPF disease (Zhu et al., 2024; Drakopanagiotakis et al., 2023). Anyway, up to now, there is no single validated biological marker that can be routinely used in the clinical management of ILD patients: many studies support the prognostic value of serum Krebs von den Lungen-6 (KL-6) (Castellví et al., 2022).

In humans, the MUC1 gene encodes KL-6, a mucin that is located on chromosome 1. A higher level of KL-6 is expressed in injured or regenerating pneumocytes and is involved in cell proliferation, growth, and apoptosis (Naderi and Rahimzadeh, 2022; Stockhammer et al., 2023). The pathophysiologic role of type 2 pneumocytes' epithelial mucins has not yet been fully clarified (Ishikawa et al., 2012). As a result of the repair of injured alveolar epithelium, Type 2 pneumocytes are recruited to cover the space left by damaged Type 1 pneumocytes when they desquamate from the basement membrane (González-López and Albaiceta, 2012). It has been demonstrated that patients suffering from IPF produce a greater amount of TGF-beta, and TGF-beta acts as a mitogen for fibroblasts, suggesting that Type 2 pneumocytes may be responsible for the formation of fibrosis (Kim et al., 2018).

Japanese Health Insurance Program approved KL-6 in 1999 as a diagnostic marker of ILD. KL-6 levels in serum have been detected using the Chemiluminescent Enzyme ImmunoAssay (CLEIA) system in normal Japanese clinical settings, but not in Western European countries, such as Italy (d'Alessandro et al., 2021a, 2022).

To distinguish between fibrotic ILD and healthy subjects, researchers suggested a serum cut-off value of 465 U/mL in 2002 (Ohnishi et al., 2002).

According to a recent study, KL-6 is a reliable prognostic biomarker that provides insight into the response of IPF patients to antifibrotic treatment. Forced vital capacity (FVC) percentages and KL-6 values of IPF patients treated with nintedanib for 12 months remain stable (d'Alessandro et al., 2021a).

Many authors have found that patients with sarcoidosis have higher serum KL-6 concentrations than healthy controls. Janssen et al. proposed KL-6 as marker for assessing the severity of sarcoidosis distinguishing patients from healthy controls (Janssen et al., 2003). Interestingly, Bergantini et al. found that serum KL-6 concentrations were proportionally higher in patients with fibrotic phases of sarcoidosis than in those with other radiological stages (Bergantini et al., 2019a).

Currently, there are very few data available on KL-6 in patients with hypersensitivity pneumonitis (HP). Literature data available on KL-6 in HP patients concern to the fibrotic patterns, since this biomarker was proposed to identify the interstitial lung involvement. Okamoto et al. demonstrated the usefulness of serum KL-6 concentrations of 1500 U/mL to diagnose and manage chronic HP patients during episodes of acute exacerbation (Okamoto et al., 2015).

Only two studies investigated the role of KL-6 in the diagnosis of miscellaneous cystic ILD: lymphangioleiomyomatosis (LAM) and pulmonary Langerhans cell histiocytosis (PLCH). The higher KL-6 concentrations in PLCH patients than healthy controls revealed the utility of this protein to identify the alveolar damage typical of this rare interstitial lung disease (d'Alessandro et al., 2020a). Higher KL-6 concentrations in LAM patients than healthy controls suggested that this measure could be prognostic of functional severity in LAM patients (D'Alessandro et al., 2022).

KL-6 has shown the strongest sensibility and accuracy for ILD diagnosis in CTD, mainly AR- and SSc-ILD. It has been reported the efficacy of baricitinib to reduce the serum KL-6 concentrations in RA patients, including a subgroup with interstitial lung impairment (d'Alessandro et al., 2020b).

Literature data demonstrated the ability of KL-6 to discriminate between disease severity and pulmonary functional decline with quantitative HRCT scores of lung involvement (d'Alessandro et al., 2021b; Stock et al., 2021). The aim of the present study was to assess the role of KL-6 as diagnostic marker of fibrotic ILD and predictor of the fibrotic progression. At the moment of diagnosis and before any pharmacological treatment, the primary aim was to compare KL-6 concentrations in fibrotic, idiopathic or secondary to autoimmune disorders, and non-fibrotic ILD. The secondary aim was to characterize and compare the progressive fibrotic phenotype assessing the functional progression in accordance with serial serum KL-6 concentrations.

2. Material and methods

2.1. Patients

The study prospectively and consecutively enrolled 107 patients (median age *IQR*, 65 (54–71) years) who were being diagnosed and monitored at the Rheumatology Unit at Siena University Hospital and at the Sarcoidosis and Interstitial Lung Disease Referral Center at Siena.

According to American thoracic society/European respiratory society (ATS/ERS) diagnostic guidelines (Raghu et al., 2022; Hunninghake et al., 1999; Raghu et al., 2020), thirty-five had diagnoses of IPF (median age *IQR*, 69 (63–76) years; 26 males; 82 % smokers), 18 Scadding stage II sarcoidosis (median age *IQR*, 53 (48–63) years; 2 males; 55 % smokers), 10 PLCH (median age *IQR*, 55 (51–68) years; 3 males; 90 % smokers), 5 LAM (median age *IQR*, 43 (Kumánovics et al., 2014; Salazar et al., 2018; Torrisi et al., 2019; Mattoo and Pillai, 2021; Karampitsakos et al., 2023) years; one male; 80 % smokers), 24 fibrotic HP (median age *IQR*, 70 (65–74) years; 13 males; 54 % smokers). International criteria were used for multidisciplinary discussions, and international ATS/ERS guidelines were followed for diagnosing (Hunninghake et al., 1999; Raghu et al., 2018). Exclusion criteria were:

- patients with a follow-up inferior to 24 months;
- fibrotic HP patients without any histological confirmation diagnosis;
- patients with active malignant diseases and acute lung infections;
- PLCH patients without similar predominantly nodular radiological HRCT patterns;
- sarcoidosis patients with Lofgren syndrome, acute disease onset or spontaneous resolution of disease.

Among rheumatologic diseases, thirteen patients had diagnoses of RA (median age *IQR*, 66 (59–68) years; 2 males; 53 % smokers) based on American College of Rheumatology criteria and 4 out of 13 had ILD involvement (van den Hoogen et al., 2013; Smolen et al., 2017). Twenty-two had diagnoses of SSc (median age *IQR*, 64 (57–68) years; 4 males; 37 % smokers) according to international criteria. Eighteen out of 22 had ILD involvement and 10 out of 18 revealed radiological diagnosis of PPFE. Diagnosis of ILD was made according to ATS/ERS guidelines.

A chest high resolution computed tomography (HRCT) and lung function tests were administered to all patients as part of the diagnostic work-up for ILD. For each patient, peripheral blood sampling was collected for immunological analysis at the moment of diagnosis.

Serial serum samples were collected before therapy started (t0) and 24 months later (t1) from patients with IPF, SSc- and RA-ILD. We excluded IPF, SSc- and RA-ILD patients with a follow-up inferior to 24 months. LFT parameters were repeated according to our center's follow-up protocol and performed in accordance with ATS/ERS guidelines (Culver et al., 2017).

Medical records were reviewed for demographic and clinical information, including comorbidities, family history, lung function parameters, and radiological findings. This data was entered into an electronic database for statistical analysis.

We also enrolled 22 healthy controls (median age (IQR) 54 years (42−60). All of them had normal lung function test parameters and no history of concomitant pathologies.

According to the Declaration of Helsinki, the study has been approved by the regional ethical review board of Siena, Italy (C.E.A.V.S.E. Markerlung 17431 and RHELABUS 22271).

2.2. Lung function test

An ATS/ERS standard plethysmograph with corrections for temperature and barometric pressure was used to measure lung function as per ATS / ERS standards. The forced expiratory volume in the first second (FEV1) and the lung diffusing capacity for carbon monoxide (DLCO) were measured.

2.3. Definition of progressive fibrosing phenotype

Progressive phenotype of ILD (PF-ILD) is defined as developing any of the following within 2 years of diagnosis despite treatment: a decline in FVC by 10 %; a decline in FVC by 5 %, with a 15 % decline in DLCO, or with worsened symptoms or radiological appearances, or a decrease in FVC of 5 %, with an increase in the extent of fibrosis with worsening symptoms (Flaherty et al., 2019).

2.4. KL-6 assay

According to previous studies, serum samples were collected from all patients, and Krebs von den Lungen-6 (KL-6) concentrations were determined by KL-6 reagent assays (Fujirebio Europe, Belgium) based on serum samples from all patients (Bergantini et al., 2019b). By measuring the change in absorbance, KL-6 concentrations, expressed in Units per mL, were determined by agglutination of sialylated carbohydrate antigen by antigen-antibody reaction in samples.

2.5. Statistical analysis

A median is the value between the five quartiles (IQR) or the mean minus the standard deviation. We performed a one-way ANOVA (Kruskal-Wallis test) and Dunn test for multiple comparisons using nonparametric methods. The comparison between two groups was performed through non-parametric Mann-Whitney U-test analysis. The Chi-squared test was used for categorical variables, as appropriate.

Patients were stratified according to diagnosis: IPF, fibrotic HP, LAM, PLCH, sarcoidosis, RA, RA-ILD, SSc, SSc-ILD at the time of diagnosis (T0).

In order to discriminate fibrotic ((IPF, fibrotic HP, RA-ILD, SSc-ILD)) and non-fibrotic ILDs (LAM, PLCH, sarcoidosis, RA, SSc) according to KL-6 concentrations, we constructed a regression decision tree based on the Gini criterion. Binary classifier accuracy was assessed to create a series of test/training partitions by utilizing a confusion matrix. The best thresholds for each binary classifier based on specificity and sensitivity were determined through Youden's J method.

A multinomial logistic regression analysis was performed to model the probability of patients' diagnosis versus HC group given the values of a set of quantitative (age and KL-6) and/or qualitative (gender smoking habit) descriptive variables.

During the research, we formed a group of patients with non-fibrotic involvement (LAM, PLCH, sarcoidosis, RA, SSc) with the aim of investigating potential binary classifiers for the diagnosis of fibrotic patients (IPF, fibrotic HP, SSc-ILD, RA-ILD). In order to determine the best clustering variables based on the Gini criterion for fibrotic and non-fibrotic lung involvement, we constructed a regression decision tree to determine the optimal clustering variables. A confusion matrix was used to evaluate the accuracy of potential binary classifiers by creating a series of test/training partitions.

Progressive (IPF, SSc-ILD) and non-progressive (RA-ILD) patients were identified according to fibrotic progression at HRCT and LFT parameters performed during follow-up period.

In an exploratory analysis, supervised Principal Component Analysis (PCA) was used to determine trends in immunological properties (KL-6) and clinical features (FEV1, FVC, and DLco) using a two-dimensional representation of the multidimensional data set.

In order to determine the best clustering variables for progressive versus non-progressive patients in order to improve KL-6's predictive prognostic role, a regression decision tree based on the Gini criteria was constructed to determine the best clustering variables. To assess the accuracy of potential binary classifiers using a confusion matrix, a series of test/training partitions were constructed. Youden's J method was used to select the best thresholds for each binary classifier based on its specificity and sensitivity.

An analysis of correlations was carried out using the Spearman test. It was considered statistically significant if the p value was less than 0.05. GraphPad Prism 9.3 and XLSTAT 2021 were used for statistical analysis.

3. Results

3.1. Demographic analysis

Table 1 reported the baseline demographic and clinical data of our patients.

Our study showed that in both IPF and fibrotic HP populations, males were more prevalent, while females were more prevalent in sarcoidosis, LAM, PLCH, RA, and SSc patients. Former smokers were more prevalent in IPF and PLCH patients. Functional parametersrevealed mild restrictive deficits among IPF, PLCH, and fibrotic HP patients, as well as a moderate reduction in DLco. Most patients were between 50 and 70 years of age and were younger in sarcoidosis than fibrotic HP and IPF ($p=0.0162$ and $p=0.0045$, respectively) as well as in LAM than IPF patients ($p=0.0405$).

KL-6 concentrations in ILD and HC groups Comparative analysis (Fig. 1) showed KL-6 concentrations above 500 U/mL in patients than controls. In particular, higher KL-6 concentrations were reported in IPF, fibrotic HP and SSc-ILD patients than HC ($p<0.0001$).

In order to better classify patients than controls according to cut-off values of KL-6 concentrations, a regression tree analysis was performed. The model (figure s1) showed KL-6 >885 U/mL for 97 % of IPF patients, 89 % of SSc-ILD patients and 62 % of fibrotic HP patients. KL-6 concentrations ≤ 574 classified 100 % of RA, 95 % of controls, 80 % of LAM, 70 % of PLCH, 55 % of sarcoidosis, 50 % of SSc and RA-ILD patients.

A multinomial logistic regression analysis was performed to model the probability of patients' diagnosis versus HC group given the values of a set of quantitative (age and KL-6) and/or qualitative (gender and smoking habit) descriptive variables.

Chi2 was associated with the Log ratio (L.R.) in the goodness-of-fit statistics <0.0001 (figure s2). The Type II analysis indicated that KL-6 concentrations ($p=0.004$) and smoking habit ($p=0.005$) were the most important variables that affected the diagnosis of ILD based on the probability associated with the Chi-square tests.

Using a decision tree model (cross-validated by confusion matrix) was used to identify the best clustering variables for ILD diagnoses. The model obtained (figure s3) using functional parameters and KL-6 concentrations cut-off values showed a classification for 70 % of IPF patients with KL-6 >1145 U/mL, DLco ≤60.15 % and FVC ≤86 %. While 67 % of SSc-ILD patients with KL-6 >1145 U/mL, DLco >60.15 % and FVC >74.2 %.

3.2. KL-6 concentrations in progressive and non-progressive patients

To improve the prognostic role of KL-6, ILD patients were stratified according to progressive fibrotic phenotype. IPF, RA-ILD and SSc-ILD patients with a progressive phenotype had higher T0 KL-6 concentrations than non-progressive patients. However, KL-6 values were greater over the time.

Inverse correlations between T0 KL-6 serum concentrations and T1 FVC and T1 DLco percentages were found ($r=-0.314$, $p=0.046$ and $r=-0.327$, $p=0.038$, respectively).

Assessing the rate of variation (T1-T0) of FEV1, FVC and DLco percentages, patients who showed progressive phenotype reported

Table 1
Demographic and clinical baseline characteristics of patients enrolled in the study stratified according to diagnosis. Abbreviations: IPF, idiopathic pulmonary fibrosis; fHP, fibrotic HP; LAM, lymphangioleiomyomatosis; PLCH, pulmonary Langerhans cell histiocytosis; RA, rheumatoid arthritis; ILD, interstitial lung disease; SSc, systemic sclerosis; FVC, forced vital capacity; FEV1, forced expiratory volume in the first second of expiration; DLco, carbon monoxide diffusing capacity; IQR, interquartile range.

Parameters	RA (n=9)	fHP (n=24)	LAM (n=5)	PLCH (n=10)	SSC (n=4)	SSC ILD (n=18)	Sarcoidosis (n=18)	IPF (n=35)
Age, median (interquartile range)	65 (58–67)	70 (65–74)	43 (41–45)	55 (51–68)	63 (56–64)	65 (61–67)	53 (48–63)	69 (63–76)
Gender								
male	0	13	1	3	2	2	2	26
female	9	11	4	7	2	16	16	9
Smoking habit								
never	4	11	1	1	2	12	8	7
former	5	13	4	9	2	6	10	28
Pulmonary function test parameters, median (standard deviation)								
FVC %	110.3 (12.4)	72.1 (18.4)	105.3 (35.3)	100.2 (24.8)	103.2 (20.5)	105.5 (17.0)	103.2 (17.0)	79.3 (22.0)
DLco %	74.3 (18.2)	52.5 (23.1)	72.3 (30.4)	51.5 (15.2)	96.0 (15.1)	72.8 (15.7)	75.4 (13.6)	43.0 (15.2)
FEV1 %	98.1 (13.3)	72.7 (20.9)	86.0 (38.9)	83.3 (23.8)	99.4 (29.4)	101.1 (16.4)	93.1 (13.8)	79.6 (20.5)

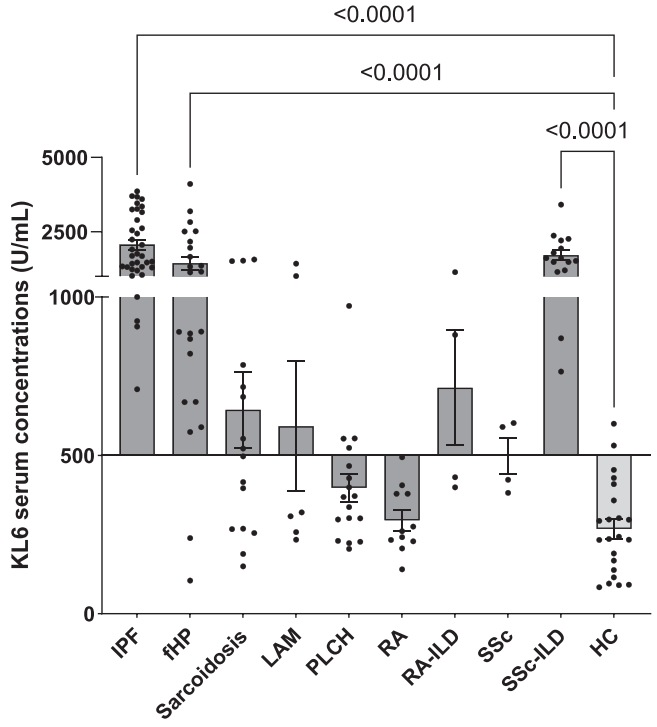

Fig. 1. Comparative analysis of serum KL-6 concentrations in different ILD. KL-6 concentrations were higher in IPF, fHP and SSc-ILD than HC group ($p<0.0001$). Abbreviations: IPF, idiopathic pulmonary fibrosis; fHP, fibrotic HP; LAM, lymphangioleiomyomatosis; PLCH, pulmonary Langerhans cell histiocytosis; RA, rheumatoid arthritis; ILD, interstitial lung disease; SSc, systemic sclerosis; HC, healthy controls; KL-6, Krebs von den Lungen-6.

pulmonary functional decline of such parameters over the time (table s1).

Progressive patients (IPF and SSc-ILD) had pulmonary functional decline and greater KL-6 concentrations over the time in respect with non-progressive patients (Fig. 2).

The PCA plot (Fig. 3) distinguished the progressive disease clusters: progressive and non-progressive patients showed that they separated on the basis of ΔKL-6, ΔFEV1, ΔFVC, ΔDCco. The first and second components explained 56.39 % and 20.53 % of the total variance.

To identify the best cut-off values to cluster progressive and non-progressive patients, a regression tree analysis was performed. The model (Fig. 4) showed $\Delta KL\text{-}6 \leq -40.03$ for 100 % of RA-ILD non-progressive patients. $\Delta KL\text{-}6 > -40.03$, $\Delta FVC > -6.81$ and $\Delta DLco > -1.68$ cluster for 82 % of SSc-ILD non-progressive patients and $\Delta DLco \leq -1.68$ for 80 % of IPF non-progressive patients. On the other hand, $\Delta KL\text{-}6 > -40.03$, $\Delta FVC \leq -6.81$ and $\Delta DLco > -10.55$ cluster for 75 % of IPF progressive patients and $\Delta DLco \leq -10.55$ for 80 % of SSc-ILD progressive patients.

4. Discussion

In the present study, serum KL-6 concentrations were evaluated in IPF, fibrotic HP, LAM, PLCH, sarcoidosis, RA and SSc with and without ILD and compared with healthy volunteers.

The highest KL-6 concentrations were reported in IPF, fibrotic HP and SSc-ILD patients than HC. From logistic regression analysis, the variables that most influences the ILD diagnosis was the KL-6 concentrations and smoking habit.

The fibrotic progression was evaluated in our cohort through radiological features and LFT parameters. Pulmonary functional decline and greater KL-6 concentrations over the time were identified in progressive than non-progressive patients.

There was an inverse correlation between serum KL-6 concentrations at T0 and the percentages of FVC and DLco at T1 in the progressive group compared to the non-progressive group.

Studies of immunohistochemistry have demonstrated that KL-6 is highly expressed on Type 2 pneumocytes, although it is not yet clear whether the elevated concentrations of KL-6 in sera from patients with ILD represent exclusively lower respiratory origins (Ohtsuki et al., 2007).

As the most common fibrotic ILD, IPF, more than 400 publications have been published concerning KL-6's role in the treatment of ILD patients (d'Alessandro et al., 2021a; Ohnishi et al., 2002; Bergantini et al., 2019b; Ohtsuki et al., 2007; A Y et al., 2006; Arai et al., 2001). Although no specific differential diagnostic cut-off value of KL-6 was proposed to differentiate several types of fibrotic ILD, KL-6 was demonstrated as useful marker for predictive prognosis and response to therapy in IPF patients (d'Alessandro et al., 2021a).

In this study, it has been observed that serum KL-6 concentrations identified fibrotic patients and in line with Ishii et al., who demonstrated high baseline KL-6 values related to significant worse survival, our cohort showed increased T0 and T1 KL-6 serum concentrations in progressive than non-progressive patients. Moreover, higher KL-6 values were correlated with FVC and DLco decline in progressive patients pointing out the potential of KL-6 in serum for predicting functional disease progression.

As a result of acute exacerbations of IPF, serum levels of KL-6 have been reported to increase. Moreover, a recent study showed that increasing serum KL-6 levels is associated with a rapid decrease in predicted FVC, as well as lower survival rates for patients with IPF who

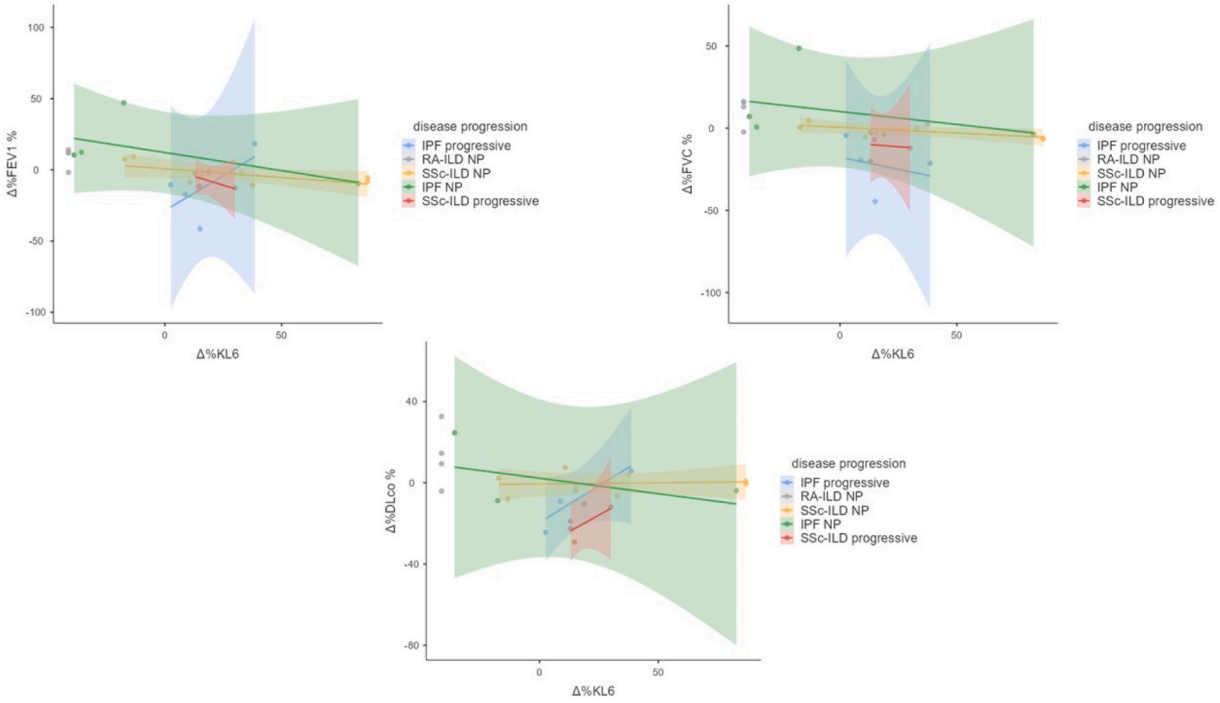

Fig. 2. Pulmonary functional decline and greater KL-6 concentrations over the time in progressive patients rather than non-progressive including RA-ILD, SSc-ILD and IPF. Abbreviations: IPF, idiopathic pulmonary fibrosis; RA, rheumatoid arthritis; ILD, interstitial lung disease; SSc, systemic sclerosis; KL-6, Krebs von den Lungen-6.

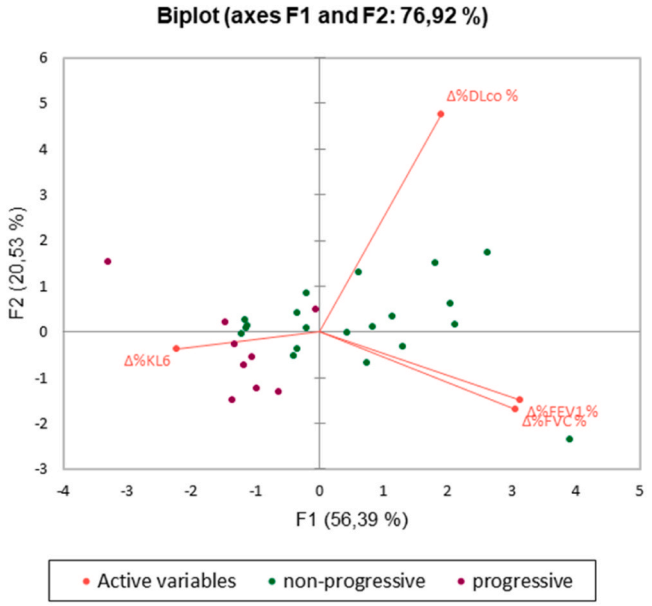

Fig. 3. Principal component analysis distinguished the progressive disease clusters on the basis of ΔKL-6, ΔFEV1, ΔFVC, ΔDCco with a total variance of 76.92 %.

have higher levels of KL-6 (Jiang et al., 2018). As a result, secreted KL-6 is considered a useful biomarker for predicting IPF outcome and evaluating disease activity.

There has been a good correlation between KL-6 and pulmonary function tests and quantitative HRCT scores of lung involvement in SSc studies, which found that it had a good ability to stage disease severity (Bonella et al., 2011; Kumánovics et al., 2014; Salazar et al., 2018). The serum KL-6 level in SSc patients was investigated as a marker of disease activity and was correlated with the presence of pleuroparenchymal fibroelastosis, which was a worse prognostic indicator (d'Alessandro et al., 2021b).

We can speculate that high values of KL-6 in sera from fibrotic and progressive patients (IPF and SSc-ILD) are derived from activated regenerating Type 2 pneumocytes and the presence of alveolar epithelial damage suggesting the potential of KL-6 values along with LFT parameters to identify a new and similar progressive phenotype in IPF ILD and non-IPF ILD patients.

In the last decades, it has been reported that also patients with non-IPF ILD (such as SSc-ILD patients) can show a progressive worsening of the disease regardless of treatments (Torrisi et al., 2019). Moreover, many recent studies have suggested the presence of common pathogenetic pathways between IPF and non-IPF ILD, like shortening of telomeres, epithelial cell dysfunction and immune dysregulation (Mattoo and Pillai, 2021; Karampitsakos et al., 2023; Ma et al., 2022; Wang and Young,.).

Our study shines a spotlight on the potential of KL-6 to identify a progressive fibrotic phenotype along with pulmonary functional parameters.

Although our findings confirmed the prognostic role of peripheral KL-6 values from fibrotic ILD patients and demonstrated the clinical usefulness to add their measurements along with pulmonary functional parameters at the time of diagnosis for predicting progressive patients, our study has some limitations. First, the limited number of patients affected by orphan rare lung diseases (e.g. LAM and PLCH). Second, serial serum samples of fibrotic and non-fibrotic ILD patients in order to validate the utility of KL-6 as marker for monitoring and response to treatment.

The results of our study provide insights into the diagnostic as well as prognostic role of KL-6 in patients with fibrotic ILD, and multicentric prospective studies would be useful for validating these findings in other patients with fibrotic ILD.

5. Conclusion

The development of numerous anti-fibrotic therapies has been a

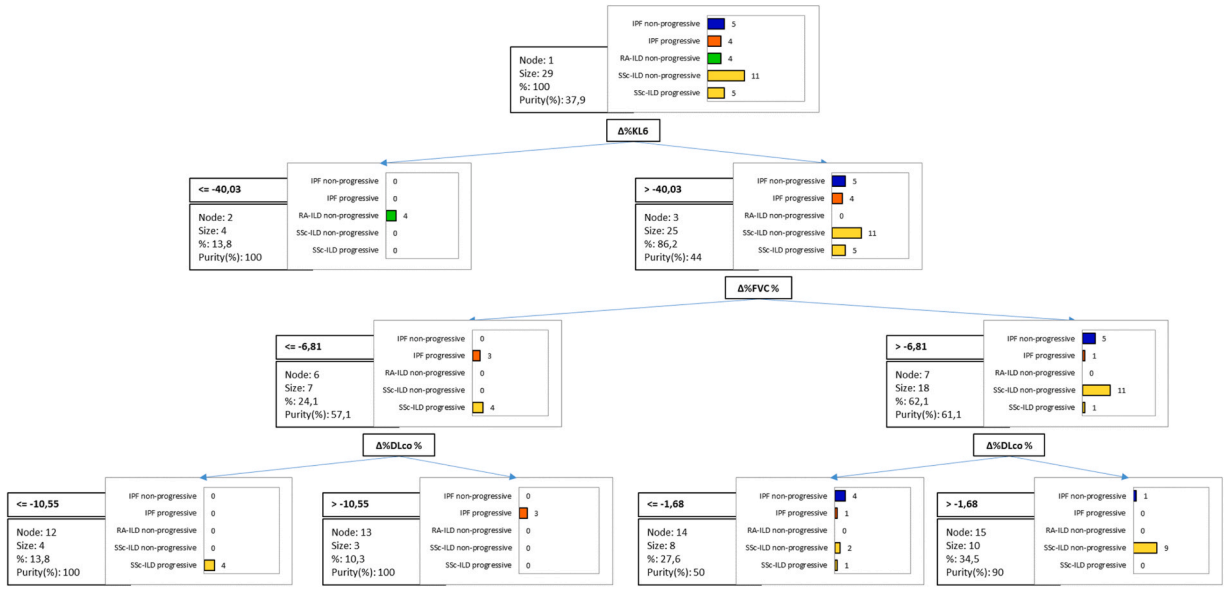

Fig. 4. Decision tree model of progressive vs non-progressive patients using ΔKL-6, ΔFVC and ΔDLco. The model showed ΔKL-6 ≤ -40.03 for 100 % of RA-ILD non-progressive patients. ΔKL-6 > -40.03, ΔFVC > -6.81 and ΔDLco > -1.68 cluster for 82 % of SSc-ILD non-progressive patients and ΔDLco ≤ -1.68 for 80 % of IPF non-progressive patients. On the other hand, ΔKL-6 > -40.03, ΔFVC ≤ -6.81 and ΔDLco > -10.55 cluster for 75 % of IPF progressive patients and ΔDLco ≤ -10.55 for 80 % of SSc-ILD progressive patients. Abbreviations: IPF, idiopathic pulmonary fibrosis; RA, rheumatoid arthritis; ILD, interstitial lung disease; SSc, systemic sclerosis; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity; FEV1, forced expired volume in 1 sec; DLco, diffusing capacity of the lungs for carbon monoxide.

result of continuous advancements in our understanding of the pathophysiology of IPF. Currently, only two treatments (pirfenidone and nintedanib) have been approved, and both have limited efficacy. KL-6 proved to be a reliable marker for diagnosis and prognosis of fibrotic ILD patients with predictive value in progressive fibrotic patients and a useful marker to identify the new and similar progressive phenotype of IPF and SSc-ILD patients assessing the functional progression in accordance with serial serum KL-6 measurement. Further studies will be performed to validate KL-6 as biomarker predictive of response to antifibrotic treatment. Multicentre prospective studies on a larger sample cohort over a longer follow-up will corroborate our findings.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Local Ethics Committee of Siena University Hospital (C.E.A.V.S.E.) (protocol code Markerlung 17431 and RHELABUS 22271).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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CRediT authorship contribution statement

Elena Bargagli: Writing – original draft, Validation, Supervision, Investigation. **Bruno Frediani:** Writing – original draft, Validation, Supervision, Investigation. **Maria Antonietta Mazzei:** Writing – original draft, Visualization, Investigation, Data curation. **Francesca Bellisai:** Writing – original draft, Supervision, Investigation. **Enrico Selvi:** Writing – original draft, Supervision, Investigation. **Paolo Cameli:** Writing – original draft, Validation, Supervision, Data curation. **Miriana d'Alessandro:** Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Edoardo Conticini:** Writing – original draft, Supervision, Project administration, Investigation, Data curation. **Laura Bergantini:** Writing – original draft, Methodology, Investigation.

Declaration of Competing Interest

The authors have declared that no conflict of interest exists.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tice.2024.102516.

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