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Baseline KL-6 predicts increased risk for acute exacerbation of idiopathic pulmonary fibrosis

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Summary
Background: Acute exacerbation (AE) is a major cause of death in idiopathic pulmonary fibrosis (IPF). However, little is known about sensitive biomarkers for predicting AE. The aim of our study was to investigate the significance of KL-6 and CC-Chemokine Ligand 18 (CCL18) as predictors for AE of IPF.
Methods: We prospectively collected a total of 77 patients with IPF. Serum levels of KL-6 and CCL18 were measured by ELISA. The correlation between baseline serum levels of the markers and the incidence of AE was evaluated.
Results: Thirteen (17%) patients experienced AE during follow-up. Baseline serum KL-6 levels were significantly higher in patients who developed AE than in patients with stable IPF ($p < 0.0001$), whereas serum CCL18 levels showed no difference between these groups ($p = 0.13$). At a cut-off level of 1300 U/mL for KL-6, the sensitivity, specificity, accuracy and likelihood ratio to predict AE were 92%, 61%, 66% and 2.36, respectively. In the Kaplan

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—Meier analysis, patients with baseline serum KL-6 level ≥1300 U/mL experienced earlier onset of AE ($p = 0.002$), whereas CCL18 showed no predictive value ($p = 0.11$). In the multivariate analysis, baseline serum KL-6 (both continuous and at a cut-off level of ≥1300 U/mL) was an independent predictive factor for AE after adjustment for age, sex, smoking history and %vital capacity (hazard ratio = 1.001, 18.8; $p = 0.010$, 0.008, respectively).
Conclusions: Baseline serum KL-6 level is a sensitive predictor for the onset of AE in IPF.

List of abbreviations

$A-aD_{O_2}$	alveolar-arterial oxygen gradient
AE	acute exacerbation
AE-IPF	acute exacerbation of idiopathic pulmonary fibrosis
ALAT	Latin American Thoracic Association
ATS	American Thoracic Society
BALF	bronchoalveolar lavage fluid
CCL18	CC-chemokine ligand 18
CI	confidence interval
$\%D_{LCO}$	diffusion lung capacity for carbon monoxide
ELISA	enzyme-linked immunosorbent assay
ERS	European Respiratory Society
HMGB1	high mobility group box 1
HR	hazard ratio
HRCT	high-resolution computed tomography
hTERT	telomerase reverse transcriptase
ICAM	intercellular adhesion molecule
IIPs	idiopathic interstitial pneumonias
IL	interleukin
IPF	idiopathic pulmonary fibrosis
IQR	interquartile range
JRS	Japanese Respiratory Society
MCP-1	monocyte chemotactic protein-1
MMP	matrix metalloproteinase
$PaCO_2$	partial pressure of carbon dioxide
PAI-1	plasminogen activator inhibitor-1
PARC	pulmonary activation-related chemokine
ROC	receiver operating characteristic
SD	standard deviation
SP-D	surfactant protein-D
TGF-β	transforming growth factor-β
TLC	total lung capacity
%TLC	total lung capacity % predicted
VC	vital capacity
%VC	vital capacity % predicted

Background

Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung disease of unknown etiology with a median survival of 3—5 years [1,2]. Although the clinical course of IPF is usually chronic, some patients may experience acute respiratory worsening, termed acute exacerbation (AE) [1,3,4].

AE of IPF (AE-IPF) is defined by acute worsening of symptoms, the presence of new ground glass abnormalities on computed tomography of the chest, and the absence of identifiable causes including infection, left heart failure or pulmonary embolism [3]. The prognosis of AE-IPF is poor, usually leading to death within a few weeks to months [1,3,4]. The precise incidence, risk factors and outcomes of AE-IPF remain unknown [3]. Although sensitive biomarkers predicting AE are necessary for early interventions, little is known about sensitive biomarkers predicting AE-IPF.

Serum levels of KL-6 and CC-chemokine ligand 18 (CCL18) are potential biomarkers to predict prognosis of IPF. KL-6, a complex sialo-carbohydrate glycoprotein present in the human MUC1 mucin, is a sensitive biomarker for type II alveolar epithelial proliferation and/or injury [5]. Serum levels of KL-6 are increased in patients with various interstitial lung diseases including IPF [5,6]. Serial changes of serum KL-6 have been shown to predict the short-term prognosis of rapid deterioration in IPF [7].

CCL18, a CC-chemokine produced by human myeloid cells, is secreted mainly by the M2 phenotype of alveolar macrophages activated by Th2 cytokines and interleukin (IL)-10 [8]. CCL18 plays an important role in the fibroblast proliferation and collagen production in IPF [8]. Serum CCL18 levels have been reported to be associated with change in lung function and survival in IPF [9].

The aim of the present study was to investigate the significance of serum KL-6 and CCL18 as predictive biomarkers for AE-IPF. Some of the results of these studies have been previously reported in the form of an abstract [10].

Methods

Study subjects

The subjects consisted of 77 consecutive patients diagnosed as having IPF according to the official American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese Respiratory Society (JRS)/Latin American Thoracic Association (ALAT) statement [11] who were admitted to Ruhrlandklinik (Essen, Germany) between 2006 and 2008. All patients were reviewed for the current study to validate the diagnosis using the current criteria, and 71 (92%) patients were classified into definite IPF according to the current guidelines. The IPF diagnosis in 6 (8%) patients was confirmed by the histopathological examinations of surgical lung biopsy. The control group for serum KL-6 levels consisted of 155 age- and sex-matched healthy individuals. All subjects enrolled were Caucasians.

All patients were prospectively enrolled, and serum samples were obtained from every patient at the

enrollment. Serum samples were obtained at the first diagnosis in 22 (29%) patients and during follow-up in 55 (71%) patients. Samples were stored at −80 °C until analyses of KL-6 and CCL18. Serum levels of KL-6 and CCL18 were measured by commercially available enzyme-linked immunosorbent assay (ELISA) (Eitest KL-6 ELISA kit, EIDIA Co., Ltd., Tokyo, Japan; Human CCL18/PARC Quantikine ELISA Kit, R&D Systems, MN). All measurements were performed in duplicate, and the data were expressed as the mean value. The correlation between baseline serum levels of these biomarkers and the incidence of AE was evaluated.

The study was approved by the Institutional Review Board of Ruhrlandklinik (IRB 06-3170) and Hiroshima University Hospital (IRB 326) and conducted in accordance with the ethical standards established in the Helsinki Declaration of 1975. Written informed consent was obtained from each participant in this study.

Pulmonary function tests

Pulmonary function variables were analyzed by using spirometry (ZAN 400 Sniff, ZAN Messgeraete GmbH, Kerpen Sindorf, Germany) according to the ATS/ERS recommendation [12]. Arterial blood gas was analyzed by using ABL 800 Flex (Radiometer GmbH, Brønshøj, Denmark). Values were expressed as percentages of predicted normal values.

Definition of AE

Definite AE was defined as previously described [3]. In brief, patients had a previous or concurrent diagnosis of IPF and presented with unexpected worsening of dyspnea within 30 days and newly developed bilateral ground-glass abnormalities on high-resolution computed tomography (HRCT) with no evidence of pulmonary infection by endotracheal aspirate or bronchoalveolar lavage fluid (BALF). Suspected AE was defined as above, but data on endotracheal aspirate or BALF examination were missing. Based on the findings of CT, electrocardiogram, echocardiography and the repeated culture of respiratory aspirates, patients with left heart failure, pulmonary embolism, or identifiable cause of acute lung injury were excluded from definite and suspected AE. Among the 77 patients enrolled, 4 (5%) patients experienced a definite AE, and 9 (12%) patients experienced a suspected AE. For the purpose of data analysis, both definite and suspected AE were included as cases of AE [13].

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Comparison of non-normally distributed variables between groups was done with the Mann—Whitney's *U* test. Comparison of categorical variables between two groups was done with the chi-square test. Correlation between 2 groups was analyzed with linear regression analysis. The probability of AE was estimated with the Kaplan—Meier method, and the differences in AE-free rates were evaluated by log-rank test. Multivariate analysis of predictive factors for AE-IPF was done using the Cox regression hazard model. The predictive value for AE-IPF of each marker was evaluated by the C statistics. All statistical analyses were done using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL). Differences were considered statistically significant when the p-value was <0.05.

Results and discussion

Patient characteristics

There were 58 males and 19 females with a mean age of 69 ± 8 years. There were 34 never smokers, 32 former smokers and 11 current smokers, respectively. No significant differences were observed in sex and age distribution and therapeutic regimens between the groups (Table 1). The mean follow-up period was 3.0 ± 2.1 years (median 2.7 years, interquartile range (IQR) 1.1—4.4 years). Among the 77 patients with IPF, 4 (5%) patients experienced a definite AE, and 9 (12%) patients experienced a suspected AE (Additional file 1). The median duration before the onset of AE-IPF was 33 months (IQR 13—53 months). The 1-yr incidence of AE-IPF was 7%.

Patients with AE-IPF had a higher proportion of smokers, a lower vital capacity (VC) and a lower total lung capacity (TLC) compared to those without ($p = 0.031$, $p = 0.003$, $p = 0.048$, respectively, Table 1). There were no significant differences in the delay between the time of first symptoms until KL-6 measurement (median 44 months (IQR 31—60 months), 33 months (IQR 11—65 months), respectively; $p = 0.65$) and in the delay between the time of IPF

Table 1 Baseline characteristics of the enrolled IPF patients with/without AE-IPF.

	Without AE-IPF	With AE-IPF	p-Value[a]
n	64	13	
Age, yr	70 ± 8	67 ± 5	0.14
Sex, male/female	47/17	11/2	0.50
Smoking, current/non	9/58	2/11	0.90
Baseline %VC, %	68 ± 15	54 ± 17	0.003
Baseline %TLC, %	64 ± 12	55 ± 10	0.048
Baseline $\%D_{LCO}$, %	44 ± 14	43 ± 10	0.87
Baseline A-aD_{O2}, mmHg	29 ± 10	30 ± 17	0.73
Delay between first symptoms and KL-6 measurement, months	69 ± 169 33 (11—65)	41 ± 26 44 (31—60)	0.65
Delay between IPF diagnosis and KL-6 measurement, months	30 ± 45 12 (0—40)	31 ± 20 29 (21—43)	0.17
Therapy, *n* (%)			
- Prednisolone	50 (78)	12 (92)	0.44
- Azathioprine	46 (72)	10 (77)	>0.99
- N-acetylcysteine	45 (70)	10 (77)	0.75
- Cyclophosphamide	6 (9)	2 (15)	0.62
- Others	2 (3)	2 (15)	0.13

Data are expressed as mean ± standard deviation or median (interquartile range).
IPF, idiopathic pulmonary fibrosis.
AE-IPF, acute exacerbation of idiopathic pulmonary fibrosis.
[a] Mann—Whitney's *U* test or chi-square test.

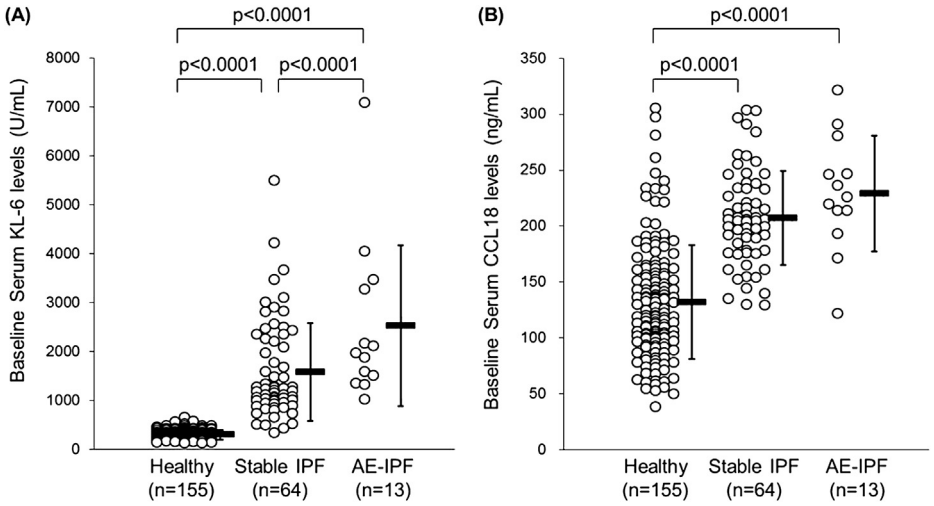

Figure 1 Distribution of baseline serum levels of KL-6 and CCL18. Scatter plot graphs showing the distribution of baseline serum levels of KL-6 (A) and CCL18 (B) in healthy subjects ($n = 155$), patients without AE-IPF ($n = 64$) and patients with AE-IPF ($n = 13$). IPF, idiopathic pulmonary fibrosis; AE-IPF, acute exacerbation of idiopathic pulmonary fibrosis.

diagnosis until KL-6 measurement (median 29 months (IQR 21—43 months), 12 months (IQR 0—40 months), respectively; $p = 0.17$) between the patients with AE-IPF and without AE-IPF (Table 1).

Baseline serum KL-6/CCL18 levels

The distributions of baseline serum KL-6 and CCL18 levels are shown in Fig. 1. Serum KL-6 levels were significantly higher in patients with AE-IPF than in those without or in healthy subjects (patients with AE-IPF, 2528 ± 1645 U/mL; patients without AE-IPF, 1584 ± 1000 U/mL; healthy subjects, 299 ± 97 U/mL; $p < 0.0001$, $p < 0.0001$, respectively). Serum CCL18 levels were also significantly higher in patients with AE-IPF than in healthy subjects, however, no significant difference was observed between patients with and without AE-IPF (patients with AE-IPF, 229 ± 52 ng/mL; patients with stable IPF, 207 ± 42 ng/mL; healthy subjects, 132 ± 51 ng/mL; $p = 0.13$, $p < 0.0001$, respectively).

Correlation with pulmonary function variables

The correlation between baseline serum KL-6 levels and pulmonary function variables are shown in Fig. 2. TLC% predicted (%TLC) and diffusion lung capacity for carbon monoxide (%D_{LCO}) were inversely correlated with serum KL-6 levels ($r = -0.408$, $p = 0.001$; $r = -0.263$, $p = 0.042$, respectively; Fig. 2(B and C). VC% predicted (%VC) and alveolar-arterial oxygen gradient (A-aD_{O2}) showed no correlation with serum KL-6 levels Fig. 2(A and D).

Predictive value for acute exacerbation

Receiver operating characteristic (ROC) curve analysis was used to evaluate the sensitivity, specificity and accuracy of serum KL-6 and CCL18 levels for predicting AE-IPF. The larger area under the curve was found for serum KL-6 with 0.736 (95% confidence interval (CI), 0.61 to 0.85; $p = 0.008$) compared with serum CCL18 with 0.651 (95%CI, 0.48 to 0.82; $p = 0.09$). When the cut-off levels were set at the closest point to 100% sensitivity and 100% specificity, the levels for predicting AE-IPF were 1300 U/mL for KL-6 (sensitivity, 92%; specificity, 61%; accuracy, 66%; likelihood ratio, 2.36, respectively) and 212 ng/mL for CCL18 (sensitivity, 77%; specificity, 64%; accuracy, 66%; likelihood ratio, 2.14, respectively).

Correlation with acute exacerbation

The Kaplan—Meier analysis showed that higher serum KL-6 was associated with a shorter follow-up period before the onset of AE-IPF ($p = 0.002$, Fig. 3A). In contrast, serum CCL18 showed no correlation with the follow-up period before AE-IPF ($p = 0.11$, Fig. 3B). There was no correlation between baseline serum KL-6 and time to AE-IPF ($r = -0.31$, $p = 0.30$). However, if the patients were divided into 2 groups with a cut-off level of 1300 U/mL for KL-6, the patients with higher KL-6 levels experienced earlier onset of AE-IPF than the patients with lower KL-6 levels (median 29 months (IQR 7—41 months), 44 months (IQR 22—70 months), respectively; $p = 0.013$). There were no significant differences in the delay between the time of first symptoms until KL-6 measurement (median 35 months (IQR 15—56 months), 33 months (IQR 8—72 months), respectively; $p = 0.74$) and in the delay between the time of IPF diagnosis until KL-6 measurement (median 9 months (IQR 0—49 months), 21 months (IQR 2—35 months), respectively; $p = 0.74$) between the patients with higher and lower baseline KL-6 levels.

In the univariate survival analysis, %VC (continuous) (hazard ratio (HR), 0.93, 95%CI, 0.89—0.97; $p = 0.001$), serum KL-6 levels (continuous) (HR, 1.001, 95%CI, 1.000—1.001; $p = 0.002$) and serum KL-6 levels $\geq$1300 U/mL (HR, 12.1, 95%CI, 1.52—100; $p = 0.018$) were associated with the risk of AE-IPF, whereas no correlations were found between the risk of AE-IPF and age, sex, smoking, %D_{LCO}

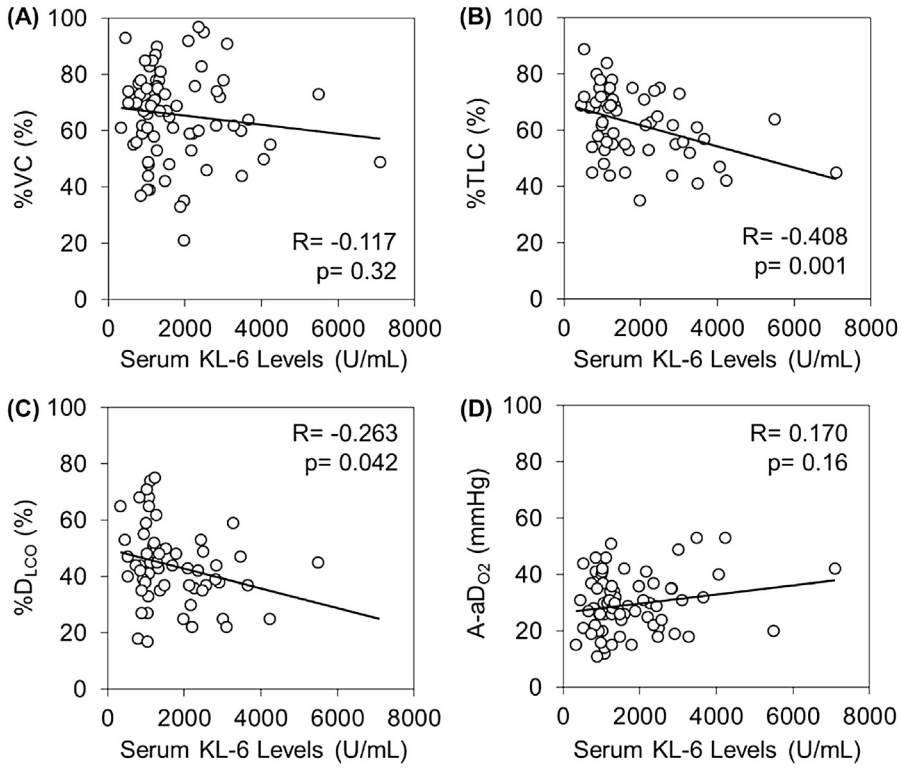

Figure 2 Correlation between KL-6 and pulmonary function variables. Scatter plot graphs showing the correlation of KL-6 with %VC (A), %TLC (B), $\%D_{LCO}$ (C) and A-aD_{O2} (D). VC, vital capacity; TLC, total lung capacity; D_{LCO}, Diffusion lung capacity for carbon monoxide; A-aD_{O2}, alveolar-arterial oxygen gradient.

and serum CCL18 levels (Table 2). In the multivariate survival analysis, %VC (continuous), serum KL-6 levels (continuous) and serum KL-6 levels $\geq$1300 U/mL were independently associated with the risk of AE-IPF (%VC: HR, 0.92, 95%CI, 0.87—0.97, $p = 0.004$; KL-6 (continuous): HR, 1.001, 95%CI, 1.000—1.001, $p = 0.010$; KL-6 $\geq$ 1300 U/mL: HR, 11.8, 95%CI, 1.43—97.8, $p = 0.022$) after adjustment for age, sex, smoking history and the use of triple therapy (prednisone, azathioprine and N-acetylcysteine).

In our cohort, the C statistic for predicting AE-IPF significantly increased when KL-6 and %VC were separately incorporated into a model with covariates (C statistic, 0.708, 0.708; 95%CI, 0.544—0.872, 0.549—0.868; $p = 0.021$, 0.021, respectively) (Table 3). However, the addition of CCL18 to the model with covariates did not improve the C statistic for predicting AE-IPF. When the combination of KL-6 and %VC were incorporated into a model with covariates, the C statistic was highest (C statistic, 0.747; 95%CI, 0.575—0.919; $p = 0.006$).

Discussion

This study showed that baseline serum KL-6 levels were significantly higher in patients who experienced an AE-IPF during the follow-up compared to those who remained free from an AE-IPF. There was no significant difference in serum CCL18 levels between patients with AE-IPF and those without. Baseline serum KL-6 levels were inversely correlated with %TLC and $\%D_{LCO}$. In the multivariate analysis, baseline serum KL-6 level (both as continuous value and at the cut-off levels of $\geq$1300 U/mL) and decreased %VC were independent risk factors for the development of AE-IPF. KL-6 is the first biomarker that demonstrated a predictive value for the risk of AE in patients with IPF.

Collard et al. recently reported that suspected AE-IPF were clinically indistinguishable from definite AE-IPF, and were associated with a similarly high risk of disease progression and short-term mortality in IPF [13]. In their cohort of 180 IPF patients, 4 (2%) cases were classified as definite AE-IPF and 14 (8%) as suspected AE-IPF. Thus, the majority of events in that trial were only suspected and not definite AE-IPF. In our cohort of 77 IPF patients, 4 (5%) cases were classified as definite AE-IPF and 9 (12%) as suspected AE-IPF. The proportions of definite and suspected AE-IPF were similar to their study. Because the enrollment of both definite and suspected AE-IPF appears to be useful as outcome measures in clinical trials, we have adopted these enrollment criteria in our study.

Previous studies have reported several clinical, physiological and radiological factors associated with mortality in IPF. The factors include age, sex, smoking history, duration of symptoms, responsiveness to therapy, histopathologic findings, HRCT findings [14], 6-min walking test and baseline and decline in D_{LCO} and FVC [15]. Genetic factors may be important in the manifestation of in IPF. Previous studies reported that mutations in MUC5B gene [16], surfactant protein genes [17], erythrocyte complement receptor 1 [18] and telomerase reverse transcriptase (hTERT) [19] were

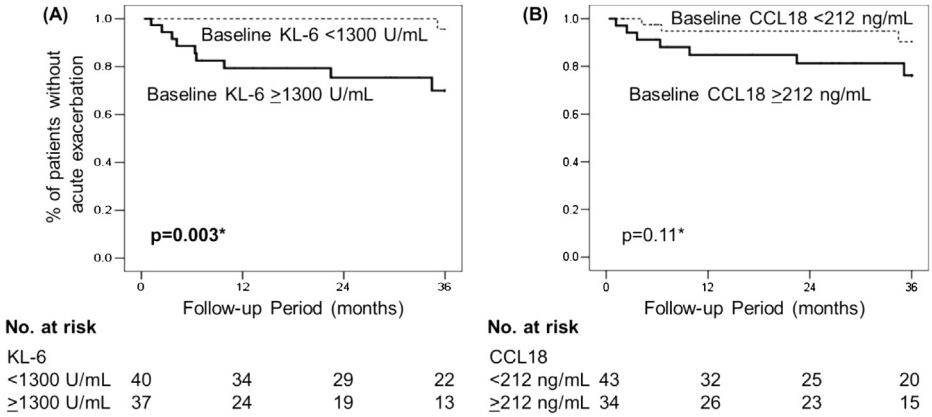

Figure 3 Kaplan—Meier analysis for the onset of acute exacerbation of IPF. Patients with baseline serum KL-6 level ≥1300 U/mL showed shorter duration before the onset of acute exacerbation compared with patients with baseline serum KL-6 level <1300 U/mL (A), whereas CCL18 showed no statistical impact on the prediction of acute exacerbation (B).

associated with IPF. However, these markers were associated only with the mortality in IPF, and none of these markers have shown to be associated with the risk of AE-IPF [16—19].

Serum levels of several biomarkers are increased in AE-IPF, which are likely involved in the pathogenesis of AE-IPF. Collard et al. demonstrated that serum levels of KL-6, surfactant protein-D (SP-D), von Willebrand factor, IL-6, total protein C, thrombomodulin and plasminogen activator inhibitor-1 (PAI-1) were significantly higher in AE-IPF than in stable IPF [20]. Other biomarkers including high mobility group box 1 (HMGB1), monocyte chemotactic protein-1 (MCP-1), soluble ST2 protein [21], α-defensin [22], or CCNA2 gene [22] might be candidates for predicting the risk of AE-IPF.

Increased levels of biomarkers may be predictive of survival in AE-IPF. Richards et al. demonstrated in 241 patients with stable IPF that high plasma levels of matrix metalloproteinase (MMP)-7, intercellular adhesion molecule (ICAM)-1 and IL-8 predicted poor overall survival [23]. Korthagen et al. have shown in 85 patients with stable IPF that high serum and BALF levels of YKL-40 predicted poor survival [24]. Tachibana et al. demonstrated in 19 patients with AE-IPF that increased serum IL-7, an inhibitor of transforming growth factor-β (TGF-β) production and fibroblast signaling, was associated with better prognosis in AE-IPF [25]. Although these biomarkers might be candidates as predictors for AE-IPF, no biomarker has clearly shown a predictive utility for the onset of AE-IPF.

In this study, we focused on the utility of KL-6 and CCL18 as candidates of predictive biomarkers for the onset of AE-IPF, because both biomarkers have shown diagnostic and prognostic utility in stable IPF [9,26]. Increased serum KL-6 levels ≥1000 U/mL was found to be predictive of poor prognosis in stable IPF [26]. Yokoyama et al. demonstrated in 14 patients with AE-IPF that changes in serum KL-6 levels predicted the response to treatment [7]. Fujimoto et al. showed in 60 patients with AE-IPF that increased partial pressure of carbon dioxide ($PaCO_2$), increased serum KL-6

Table 2 Univariate and multivariate analyses for the risk of AE-IPF.

Variables	β	HR	(95%CI)	*p*-Value
Univariate analysis				
Age (continuous)	−0.03	0.98	(0.90 — 1.05)	0.51
Male sex	0.95	2.58	(0.33 — 20.4)	0.37
Current smoker	0.38	1.46	(0.31 — 6.90)	0.63
Use of triple therapy	0.43	1.53	(0.43 — 5.43)	0.51
%VC (continuous)	−0.07	0.93	(0.89 — 0.97)	0.001
$\%D_{LCO}$ (continuous)	−0.01	0.99	(0.92 — 1.07)	0.83
KL-6 (continuous)	0.00	1.00	(1.00 — 1.00)	0.002
KL-6 ≥1300 U/mL	2.49	12.1	(1.52 — 100)	0.018
CCL18 (continuous)	0.01	1.01	(0.99 — 1.02)	0.47
CCL18 ≥212 ng/mL	1.07	2.92	(0.76 — 11.4)	0.12
Multivariate analysis[a]				
%VC (continuous)	−0.08	0.92	(0.87 — 0.97)	0.004
KL-6 (continuous)	0.00	1.00	(1.00 — 1.00)	0.009
KL-6 ≥1300 U/mL	2.47	11.8	(1.43 — 97.8)	0.022

HR, hazard ratio; CI, confidence interval; VC, vital capacity. DLCO, diffusion lung capacity for carbon monoxide.
[a] Adjusted for age, sex, smoking history and the use of triple therapy(prednisone, azathioprine and N-acetylcysteine).

Table 3 C statistic for Cox regression models predicting AE-IPF.

Biomarkers	C statistic	(95%CI)	*p*-Value
Covariates[a] only	0.649	(0.460 — 0.839)	Referent
Covariates[a] plus%VC	0.811	(0.656 — 0.965)	0.002
Covariates[a] plus KL-6	0.770	(0.625 — 0.914)	0.006
Covariates[a] plus CCL18	0.663	(0.505 — 0.820)	0.10
Covariates[a] plus%VC and KL-6	0.854	(0.701 — 1.006)	0.0003

[a] Covariates included age (continuous variable), sex, smoking history and the use of triple therapy (prednisone, azathioprine and N-acetylcysteine).

level and severer HRCT score were correlated with poor survival. In the multivariate analysis, however, only HRCT score was an independent prognostic factor for AE-IPF [14]. Prasse et al. demonstrated in 72 patients with IPF that baseline serum CCL18 levels predicted the change in %TLC and %FVC at 6-month follow-up, and baseline serum CCL18 $>$ 150 ng/mL was an independent predictor of poor prognosis with the HR of 8.0 [9]. In this study, we showed for the first time that baseline serum KL-6 level $\geq$1300 U/mL is an independent predictive biomarker for the onset of AE-IPF, whereas CCL18 was not able to predict the onset of AE-IPF. Although the mechanisms of increased serum CCL18 levels in patients with IPF have not been well known, the excessive production of CCL18 and/or the change in macrophage activation might be associated with the increase in serum CCL18 levels [9]. CCL18 appears to be a sensitive biomarker predicting the chronic progression of IPF and the subsequent survival; however, CCL18 does not seem to be sensitive to predict acute worsening of IPF. This discrepant finding of CCL18 might be associated with the different mechanisms involved in chronic progression of IPF and in AE-IPF.

High sensitivity (92%) and low specificity (61%) of KL-6 with a cut-off level of 1300 U/mL for predicting AE-IPF indicate that high KL-6 is predictive of high risk of AE-IPF, although low KL-6 is not predictive of low risk of AE-IPF. Therefore, clinicians should most carefully monitor the clinical course and pulmonary dysfunction of the patients, and should prepare for prompt treatment including high-dose corticosteroid therapy in patients with higher baseline KL-6 levels. However, clinicians should also be aware that serum KL-6 level with a cut-off level of 1,300 U/mL shows a high false-positive rate (39%).

The mechanisms of the increase in serum KL-6 levels are thought to include an overexpression of KL-6 by regenerating alveolar type II pneumocytes, and/or increased permeability following disintegration of the alveolar-vessel barrier [27]. The epitope of KL-6 monoclonal antibody involves sulfate and sialic acid residues, which may be regulated by Gal6ST gene [28]. Collard et al. have suggested that the rapid alteration in epithelial cell integrity, cellular inflammation, cytokines, MMPs, and coagulation components may be involved in the pathogenesis of AE-IPF [3]. An acute direct stress to the lung leads to diffuse alveolar damage with a subsequent acceleration of the fibroproliferation in AE-IPF. The severity and rapidity of diffuse alveolar damage are likely to be correlated with the subsequent increase in serum KL-6 level. The purified KL-6 molecule has chemotactic, proliferative and anti-apoptotic effects on fibroblasts *in vitro* [29]. Persistent high concentration of KL-6 in the alveolar space is highly likely to evoke a high potential for fibrosis. By stimulating the alveolar epithelial cells and/or fibroblasts by yet unknown triggers in AE-IPF, KL-6 may contribute to accelerated epithelial damage, leading to fibrosis. Whether KL-6 levels are related to bronchiolar hyperplasia or honeycomb changes on HRCT is an open question and need to be addressed in future studies.

In this study, we have defined the cut-off level for KL-6 as 1300 U/mL, whereas most of the previous studies defined the cut-off level for KL-6 as 1000 U/mL [26]. The relatively higher cut-off level for KL-6 compared with previous studies is likely to be associated with a genetic difference [30]. The distribution of the rs4072037 genotype, which is associated with the range of serum KL-6 levels, is different in Caucasian and Japanese cohorts, leading to higher normal values in Caucasian than in Japanese subjects. The patients enrolled in this study were Caucasians, whereas most of the previous studies enrolled Japanese patients.

A limitation is the small number of patients enrolled. Larger, potentially multicenter prospective studies are needed to validate the results of our study. The other limitation is the lack of data in the chronologic changes of KL-6 and CCL18 levels. Longitudinal studies are also important to validate our results.

Conclusions

In conclusion, the present study suggests that baseline serum KL-6 level (both as continuous value and at the cut-off level of $\geq$1300 U/mL) is a sensitive predictor for the onset of AE-IPF. Future studies are necessary to confirm the clinical utility of baseline KL-6 measurement for the prediction of AE in patients with IPF.

Authors' contributions

SO carried out the patient collection, made the data base, carried out the statistical analyses and drafted the manuscript. NI and NK conceived the study and helped to draft the manuscript. YH and FB measured the serum concentrations of biomarkers, collected clinical data, and helped to draft the manuscript. NH, NH and KT participated in the design of the study and coordination, and helped to draft the manuscript. JG and UC participated in the design of the study, interpreted the data and the statistical analyses and helped to draft the manuscript. All authors read and approved the final manuscript.

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Conflict of interest statement

Nobuoki Kohno has a royalty income concerning the discovery and the clinical application of KL-6. However, he has no significant conflicts of interest on the theme discussed in this article. Other authors have no financial support. No significant conflicts of interest exist with any companies/organizations whose products or services may be discussed in this article.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.rmed.2014.04.009.

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