Tian *et al.*
European Journal of Medical Research (2025) 30:20
https://doi.org/10.1186/s40001-024-02256-x

European Journal of Medical Research

RESEARCH

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Nomogram model using serum Club cell secretory protein 16 to predict prognosis and acute exacerbation in patients with idiopathic pulmonary fibrosis

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Abstract

Background Idiopathic pulmonary fibrosis (IPF) is a progressive fibrotic lung disease with poor prognosis, nomogram model for its prognosis and acute exacerbation was constructed.

Methods Two hundred and sixty eight patients with IPF were grouped with different severity according to fibrosis area, serum Club cell secretory protein 16(CC16) was compared between these groups. All patients were randomly divided into training and testing sets. COX regression and LASSO algorithm were used to screen featured characteristics. Then nomogram models were constructed, ROC curve, calibration curve and decision curve analysis(DCA) were conducted to evaluate the performance of model. Expression of CC16 were detected in fibrotic human lung tissues, bronchoalveolar lavage fluid (BALF) and Bleomycin(BLM)-treated mouse lung tissues and serums.

Results Serum CC16 gradually increased with the severity of fibrosis, and was especially high in AE-IPF group. CC16 and diffusion capacity for carbon monoxide (DLCO) were screened as characteristic variables to construct nomogram model for IPF prognosis. The survival was significantly lower in high-risk group scored by the model. The area under ROC curves(AUCs) for 1-year and 2-year mortality prediction were 0.866 and 0.916, respectively. This model performed better than gender-age-physiology (GAP) index for predicting 2-year and 3-year mortality. Another nomogram model for acute exacerbation of IPF based on CC16, Krebs von den Lungen-6(KL-6) and DLCO was developed, the AUC was 0.815. Expression of CC16 obviously up-regulated in fibrotic lung tissues, BALF and BLM-treated mice lung tissues and serums.

Conclusions The nomogram model based on CC16 performed good predictive ability for prognosis and acute exacerbation of IPF.

Keywords Idiopathic pulmonary fibrosis (IPF), Acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF), Club cell secretory protein 16 (CC16), Prognostic model, Nomogram

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Background

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive and fatal fibrotic interstitial lung disease with unknown etiology and few treatment options, and the median survival time after diagnosis is 3–5 years [1]. The major pathogenesis of IPF involves aberrant injury and repair of alveolar epithelial cells (AEC) which can initiate fibroblast proliferation, migration, differentiation into myofibroblast and promote extracellular matrix deposition in lung [2]. The diagnosis of this deadly disease mainly relies on imaging tests, such as high-resolution computed tomography (HRCT) and biopsy [3]. Early prediction of the prognosis and acute exacerbation of IPF patients is particularly important for guiding timely antifibrotic therapy which can help to improve the prognosis of the patients.

Club cell secretory protein 16 (CC16) also known as uteroglobin, a member of the secretoglobin family and 16kDa homodimeric protein, is encoded by the SCGB1A1 gene, and can be detected in airways, sputum, circulation and urine [4]. In human, CC16 is secreted predominantly by Club cells and non-ciliated bronchiolar epithelial cells, which is located in the distal bronchiolar epithelium [5]. In mice, 50–70% of total airway epithelial cells are non-ciliated cells and > 95% of non-ciliated cells are Club cells [6]. For its large area distribution in lung, CC16 may have various physiological functions to maintain normal lung function and altered CC16 may be correlated with multiple pulmonary diseases.

Previous studies have demonstrated that CC16 plays important role in anti-oxidative stress, anti-chemotaxis and anti-inflammatory, and possesses an immunomodulatory properties [7]. Normal level of CC16 has been proved to maintain normal lung function and lung structure. Expression of CC16 differs among different pulmonary diseases. Decreased CC16 has been regarded as a potential biomarker in chronic obstructive pulmonary diseases(COPD) [8], asthma [9], obstructive sleep apnea (OSA) [10], but it is up-regulated in acute respiratory distress syndrome (ARDS) [11], COVID-19 [12] and silicosis [13, 14]. Our previous proteomics study revealed that protein expression of CC16 was significantly elevated in lung tissues of IPF patients [15]. Previous studies reported that CC16 levels from serum, bronchoalveolar lavage fluid (BALF) and sputum were significantly increased in IPF compared with non-IPF interstitial lung diseases (ILD) including chronic hypersensitivity pneumonitis (CHP) and connective tissue diseases associated ILD (CTD-ILD) [16, 17]. And serum CC16 could help to distinguish IPF from non-IPF ILD, demonstrating that changes of CC16 may participate in pathogenesis of IPF [17]. According to previous researches, increased CC16 may be involved in the pathogenesis of IPF and may also serve as a potential biomarker for IPF.

Several studies have focused on building clinical models to identify IPF and AE-IPF and to predict the prognosis. A nomogram model included gender, DLCO and CTD to predict the mortality risk of IPF patients in a Singaporean cohort [18]. An AE-IPF nomogram prediction model based on history of occupational exposure, diabetes mellitus (DM), essential hypertension (EH) and DLCO was developed to predict the occurrence of AE-IPF [19]. In previous studies, PaO2/FiO2, CRP, Ang-2, HMGB1 and CC16 were used to construct a predictive nomogram model that can enhance ARDS diagnosis [20]. Our research aimed to construct a prognostic and acute exacerbation prediction model for IPF patients based on serum CC16, so as to timely identify IPF patients with high-risk of death and acute exacerbation in clinical practice.

Methods

Study population

A total of 268 patients who were diagnosed with IPF between 2017 and 2020 at Nanjing Drum Tower Hospital were enrolled in this study. Serums of 48 normal persons from the medical examination department of the hospital were collected as control group. And BALF from 23 IPF patients and 14 patients with benign lung nodule as control group were collected to examine the level of CC16. Six human lung tissues of IPF and six normal healthy lung tissues were obtained from the Department of Lung Transplantation, Wuxi People's Hospital. Written informed consent was obtained from the participants, and the study was officially approved by the Ethics Committee of Nanjing Drum Tower Hospital of Medical School of Nanjing University (No. 31/93, 84/93, 29/01).

Definition of groups

Two hundred and sixty eight patients were classified as mild ($n=39$), moderate ($n=91$), severe ($n=138$) group assessed by lung fibrosis area in HRCT. 57 out of 268 patients were identified into AE-IPF group, and 211 patients were divided into IPF group. 43 out of 211 patients (about 20.4%) took pirfenidone and 9 patients (about 4.3%) took nintedanib in IPF group. In AE-IPF group, 6 patients (about 10.5%) took pirfenidone and 1 patient (about 1.8%) took nintedanib. All diagnosis of IPF and extent of fibrosis were according to the official criteria [3, 21–23]. Mild patients present mainly subpleural reticulation, moderate patients present mainly subpleural honeycombing, severe patients present extensive lung honeycomb on HRCT, and the diagnosis of AE-IPF was made strictly according to official criteria.

Enzyme-linked immunosorbent assay (ELISA)

The concentrations of CC16 in serum and BALF were measured via sandwich-type ELISA following the manufacturer's instructions. Human serum was diluted fivefold, human BALF was diluted 500 fold and mouse serum was diluted tenfold for ELISA test. ELISA kit for human CC16 was purchased from R&D systems (Catalog Number DUGB00), mouse CC16 ELISA kit purchased from Novus biologicals (Catalog Number NPB2-75184). Human serum KL-6 was detected by serum KL-6 quantitative detection kit purchased from Japan Sekisui Medical Company (Product name Sialylated carbonhydrate antigen KL-6 Kit, Catalog Number 202138-005) with MODULAR P800 automatic biochemical analyzer manufactured by Roche, Switzerland.

BLM-induced pulmonary fibrosis in mice

Six to eight-week-old male SPF C57BL/6 mice were randomly divided into control group and treatment group, 50 μl of 0.9% saline and 50 μl of 5 mg/kg BLM were intratracheally injected, respectively. All mice were sacrificed at 7, 14 and 21 days after treatment, mice blood and lungs were collected for subsequent experiments. All mice were fully anesthetized with isoflurane before injection and sacrifice. The induction concentration of isoflurane was about 4% and the maintain concentration was about 1.5%. All mice were treated according to the protocols approved by the Ethics Committee for Animal Research of Medical School of Nanjing University.

Western Blot

Whole protein samples were separated on SDS-PAGE gels and transferred to PVDF membranes, then it was blocked with 5% nonfat milk and incubated with primary antibody at room temperature for 4 h. Incubated with HRP-conjugated secondary antibody for one hour at room temperature, and protein expression was detected using electrochemical luminescence method. Antibody against CC16 were purchased from Santa Cruz Biotechnology (USA). Primary antibodies against β-actin and Collagen 1α were purchased from Abcam (United Kingdom).

Construction and validation of CC16-based prognostic signature

Two hundred and eleven patients diagnosed with IPF were randomly partitioned into distinct training set ($n=140$) and testing set ($n=71$) at a ratio of 2:1. In the training set, 13 factors (gender, age, smoker, CC16, SpO_2, PaO_2, oxygenation index (OI), forced vital capacity (FVC), forced vital capacity in the first second (FEV1), DLCO-SB, white blood cell (WBC), C-reactive protein (CRP), and lactic dehydrogenase (LDH) were included in analysis. To establish a model for predicting overall survival (OS) in IPF, we conducted a univariate COX regression analysis based on the factors mentioned above. Variables who met the criteria of P values less than 0.1 in COX analysis were selected for least absolute shrinkage and selection operator (LASSO)-penalized Cox regression analysis using R package "glmnet". LASSO regression was performed using a tenfold cross validation. Based on the results of the LASSO regression, risk scores were calculated using the following formula: risk score = variable 1 * coefficient 1 + variable 2 * coefficient 2 + ... + variable n * coefficient n + constant. Patients in the training set were separated as low- and high-risk groups based on the median risk score. The differences in OS between the two groups were compared with a Kaplan–Meier (KM) survival analysis. Moreover, time-dependent ROC curves were constructed with R package "timeROC", and AUCs were calculated. The established prognostic model was visualized through nomograms. By using the R package "rms", the calibration curves were plotted to validate the accuracy of the nomogram. The model's clinical utility was evaluated based on the decision curve analysis (DCA). Moreover, we compared our model with gender-age-physiology (GAP) index. The prognostic model was further examined in our testing set. Each patient was given a risk score according to the model and separated into low- and high-risk group. The Kaplan–Meier plotter, timeROC curves and AUCs, and DCA were used to assess the model performance.

Construction of CC16-based diagnostic model for AE-IPF

The ROC curves and AUCs of CC16, KL-6 and DLCO-SB were analyzed in IPF patients with acute exacerbation. Then a predictive model for acute exacerbation of IPF was constructed based on above three factors using univariable and multivariable logistic regression analyses. The model was displayed as a nomogram, and calibration curves and DCA analysis were performed to evaluate its performance.

Statistical analysis

Comparison of quantitative data between different groups were performed by Kruskal–Wallis or Mann–Whitney U test according to data distribution. Comparison of qualitative data between different groups were conducted by Chi-square test. Quantitative data was shown as median (IQR) and qualitative data was presented as numbers (percentages) in Table. All statistical analyses were performed by IBM SPSS Statistics (Version 26.0) and R (version 4.1.2). P value less than 0.05 were considered statistically significant difference.

Results

Correlation between CC16 concentration and clinical characteristics

The clinical characteristics of the 268 patients of IPF and control group were shown in Table 1. Serum CC16 was increased in IPF group (Fig. 1A), and it was significantly higher in AE-IPF group than IPF group (Fig. 1B). Serum CC16 was gradually elevated with severity of pulmonary fibrosis, and they were especially higher in severe and AE-IPF group (Fig. 1C, D). Heatmap indicated that heavy lung fibrosis mainly located at area of high CC16 (Fig. 1E). Correlation analysis showed that FVC, FEV1, DLCO-SB were negatively related with level of serum CC16 (Fig. 1F). Serum CC16 was significantly elevated in patients with age > 60 years (Fig. 1G), but it did not differ significantly between different gender and smoking group (Fig. 1H, I). These results indicated that CC16 was significantly elevated in IPF and correlated with the poor lung function and severity of pulmonary fibrosis.

Prognostic implication of CC16

The minimum P value when CC16 difference was most significant in IPF patients was used to divide patients into CC16 Low group ($n=84$) and High group ($n=127$). Then Kaplan–Meier survival analysis showed that overall survival of High group was significantly lower than Low group (Fig. 2A). But the survival had no significant difference between Low and High group in patients with AE-IPF (Fig. 2B). Combining the minimum *P* value of CC16 with clinical characteristics, Kaplan–Meier analysis showed that overall survival of IPF mainly influenced by level of CC16 rather than age, gender and smoke status (Fig. 2C–E). From these results, level of serum CC16 was closely related to the prognosis of IPF patients.

Prognostic model construction based on CC16

Two hundred and eleven patients of IPF were randomly divided into training set ($n=140$) and testing set ($n=71$). Through univariate Cox regression analysis, 11 among the 13 factors met the criteria of $p<0.1$

Table 1 Clinical characteristics of different groups

Variables	Control (*n* = 48)	IPF (*n* = 211)	AE-IPF (*n* = 57)	*P*-value	Mild (*n* = 39)	Moderate (*n* = 91)	Severe (*n* = 138)	P-value
Demographic data								
Male, *n* (%)	29 (60.4)	182 (86.3)	49 (86.0)	< 0.001	36 (92.3)	76 (83.5)	119 (86.2)	0.416
Age, median, years	51.5 [39.8, 59.0]	66.0 [62.0, 73.0]	69.0 [64.0, 74.0]	< 0.001	67.0 [55.0, 74.0]	66.0 [62.0, 72.0]	68.0 [63.0, 74.0]	0.219
Smoker, *n*(%)	16 (33.3)	123 (58.3)	29 (50.9)	0.005	28 (71.8)	53 (58.2)	71 (51.4)	0.064
Pulmonary function								
FVC%, median		67.0 [56.7, 76.5]	50.8 [44.4, 63.2]	< 0.001	76.9 [64.9, 89.7]	67.7 [58.8, 75.3]	58.8 [47.6, 70.4]	< 0.001
DLCO%, median		48.2 [37.4, 67.0]	31.1 [22.8, 42.8]	< 0.001	71.4 [61.4, 81.6]	50.3 [42.0, 63.5]	34.4 [25.3, 44.1]	< 0.001
OI, median, mmHg		344.0 [395.0, 400.0]	175.0 [141.5, 232.5]	< 0.001	364.0 [338.0, 422.5]	357.0 [316.0, 426.8]	251.5 [161.8, 328.0]	< 0.001
Laboratory test								
WBC, median, 10^9/l		7.2 [6.0, 8.7]	8.8 [7.3, 11.3]	< 0.001	6.6 [5.4, 7.9]	7.0 [5.7, 8.6]	8.1 [6.7, 10.0]	< 0.001
CRP, median, mg/l		5.4 [3.5, 15.4]	17.5 [6.4, 46.7]	0.002	4.4 [3.3, 6.9]	5.1 [3.5, 9.5]	9.9 [4.5, 36.1]	< 0.001
LDH, median, U/l		231.0 [203.0, 282.0]	348.0 [256.5, 435.5]	< 0.001	212.0 [183.3, 243.0]	223.0 [202.0, 274.0]	278.0 [234.0, 370.3]	< 0.001
CC16, median, ng/ml	27.8 [21.8, 37.1]	79.7 [55.8, 117.2]	122.8 [85.2, 154.0]	< 0.001	43.6 [33.1, 61.1]	79.9 [61.1, 123.7]	100.8 [74.1, 140.3]	< 0.001
KL-6, median, ng/ml	198.5 [165.3, 298.0]	969.2 [575.0, 1370.5]	1945.0 [1184.5, 2601.5]	< 0.001	610.5 [503.3, 954.3]	938.2 [588.8, 1210.8]	1387.0 [863.0, 2285.0]	< 0.001
Outcome								
1 year mortality	0 (0)	38 (18.0)	42 (73.7)	< 0.001	0 (0)	5 (5.5)	75 (54.3)	< 0.001
2 years mortality	0 (0)	77 (36.5)	51 (89.5)	< 0.001	1 (2.6)	16 (17.6)	111 (80.4)	< 0.001
3 years mortality	0 (0)	96 (45.5)	53 (93.0)	< 0.001	2 (5.1)	29 (31.9)	118 (85.5)	< 0.001

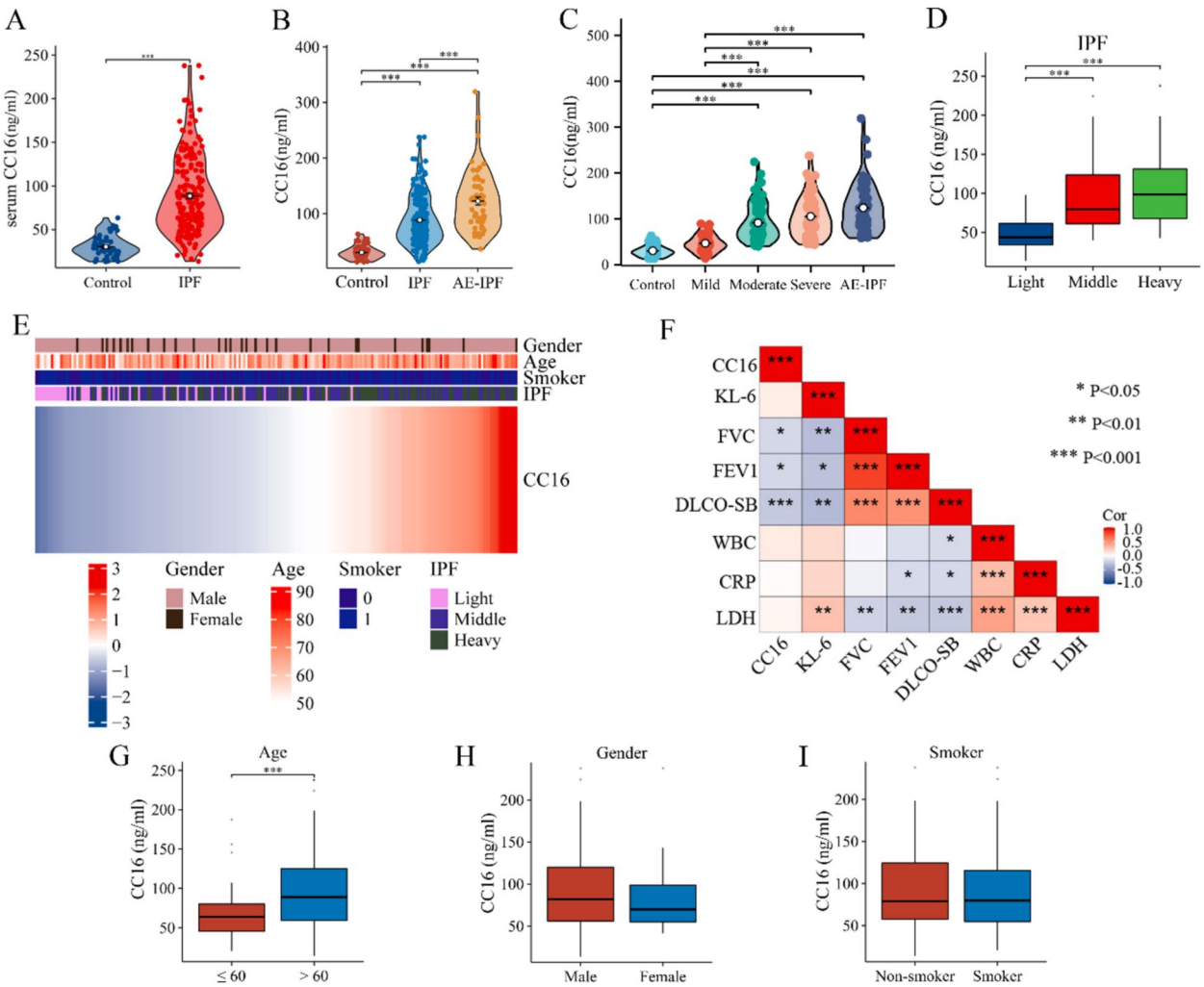

Fig. 1 Correlation analysis between CC16 and clinical characteristics. **A** Comparison of serum CC16 between control and IPF group. **B** Serum CC16 comparison between control, IPF and AE-IPF group. **C**, **D** Serum CC16 comparison between different severity group. **E** Heatmap visualized the relationship of CC16 expression and clinical variables. **F** Correlation analysis between CC16 and clinical characteristics, the color indicated the Pearson's correlation. **G** CC16 was significantly elevated in patients with age > 60. **H** CC16 showed similar level between male and female patients. **I** CC16 showed similar level between non-smokers and smokers. **P* < 0.05, ***P* < 0.01, ****P* < 0.001

except for gender and PaO_2 (Fig. 3A). The 11 factors were enrolled to conduct LASSO-penalized Cox regression analyses. By setting the penalty parameter (λ) to 1 standard error above the minimum (1se), a model containing CC16 and DLCO-SB was generated (Fig. 3B, C). The risk score was calculated as following: risk score = CC16 * 0.00554228382563167 + DLCO-SB (%) * (− 0.0160421088126014). Kaplan–Meier analysis revealed that patients' OS times in low-risk group were significantly longer than in high-risk group ($p < 0.001$, Fig. 3D). The specificity and sensitivity of the prognostic signature were also evaluated using time-dependent ROC analysis. For 1-year and 2-year survival, the AUC from ROC curve was 0.866 and 0.916, respectively (Fig. 3E). All the AUC were above 0.8 from 0.5 to 2.5 years, indicating excellent predictive efficacy (Fig. 3F). The predictive model for 1-year, 2-year and 3-year survival probability was visualized via a nomogram (Fig. 3G).

Model evaluation

The calibration curve of the model in Fig. 3H showed high agreement between the predicted survival probability and observed probability. DCA was performed to measure the clinical utility of the nomogram and compare it with GAP index, a widely-used scoring system predicting the mortality risk of IPF patients. Fig. 3I–K showed that the net benefits of the nomogram were superior to GAP index especially in predicting 2-year

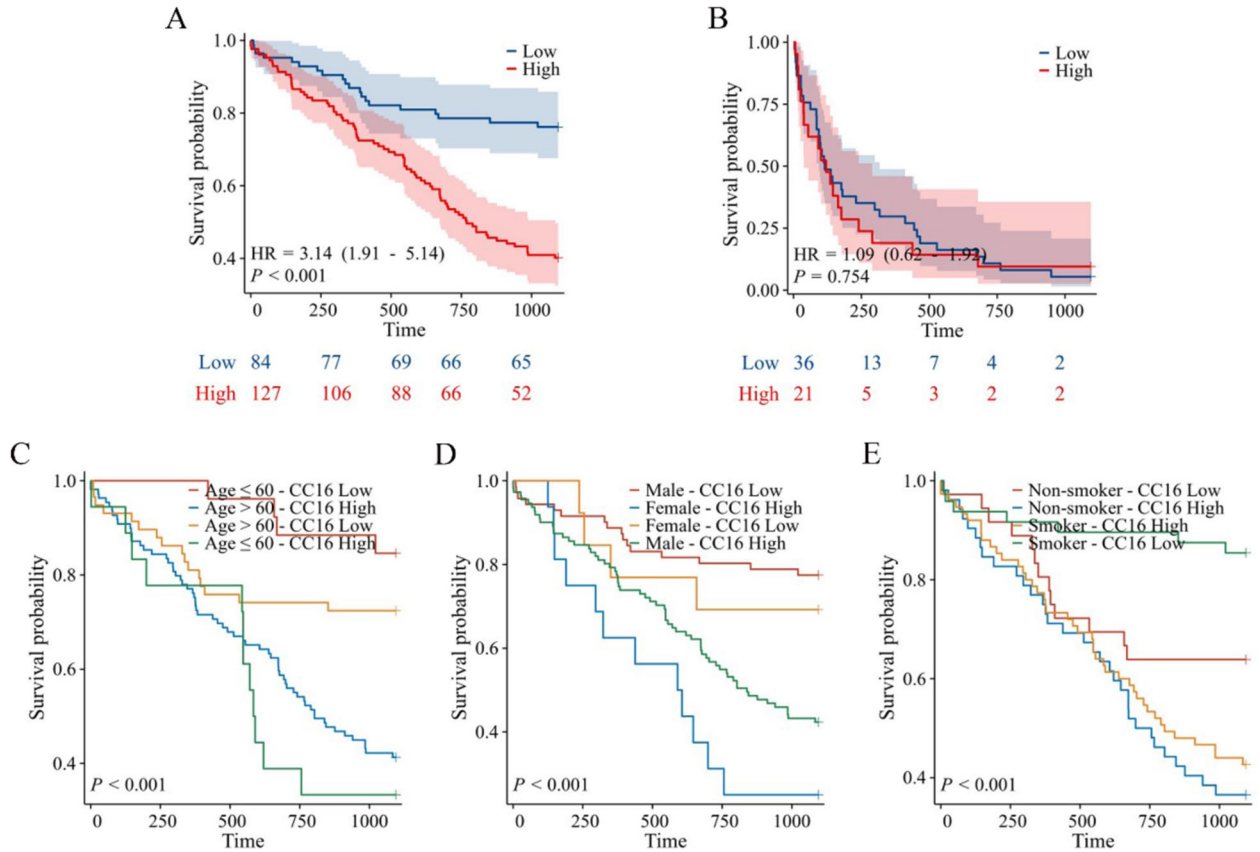

Fig. 2 Kaplan–Meier survival curves for CC16 in IPF and AE-IPF patients. **A** Patients with IPF were divided into CC16 low group and CC16 high group according to the minimum *P*-value between the two groups, the optimal cut-off value was 67.4175 ng/ml, then Kaplan–Meier survival analysis was used to analyze the survival difference between the two groups. **B** Kaplan–Meier survival analysis was conducted between low CC16 and high CC16 group in patients with AE-IPF, the optimal cut-off value was 129.655 ng/ml. **C** The CC16 low group and high group were grouped again according to age, and the survival difference among the groups was analyzed by Kaplan–Meier analysis. **D** The survival differences were compared between different CC16 level and different gender. **E** Kaplan–Meier survival analysis between different CC16 level and different smoking status

and 3-year prognosis. The performance of this CC16-based model was also examined in the testing set. IPF patients were also categorized into low- and high-risk groups based on the risk score calculated by the model. Kaplan–Meier curve revealed that the OS rate of the high-risk group was significantly lower than that of the low-risk group ($p = 0.002$, Fig. 4A). The AUC from ROC curve for 1-year and 2-year prognosis were 0.754 and 0.759, respectively (Fig. 4B). The AUC of time-dependent ROC curves were between 0.7 and 0.8 (Fig. 4C). DCA analysis also showed superior clinical utility than GAP index in predicting 2-year and 3-year prognosis in testing set (Fig. 4D–F). These results suggested that the model performed well in predicting the prognosis of patients with IPF and possessed potential clinical utility.

Predictive model construction for acute exacerbation of IPF

KL-6 has been reported to be a good diagnostic and prognostic predictor for IPF patients [24]. In present study, ROC curves were applied to estimate the predictive value of CC16 and KL-6 for IPF. The AUCs of CC16 and KL-6 were 0.941 and 0.976, respectively (Fig. 5A). As DLCO-SB can reflect the severity of pulmonary fibrosis, it was enrolled to predict acute exacerbation of IPF patients. The AUCs of CC16, KL-6 and DLCO-SB were 0.698, 0.760 and 0.750 in AE-IPF patients, respectively (Fig. 5B). Thus, we tried to combine three factors in a logistic model in order to improve the model's efficacy. The combined model was plotted with a nomogram (Fig. 5C), and the predictive value of the model were calculated by ROC curve, the AUC of this model was 0.815 (Fig. 5D).

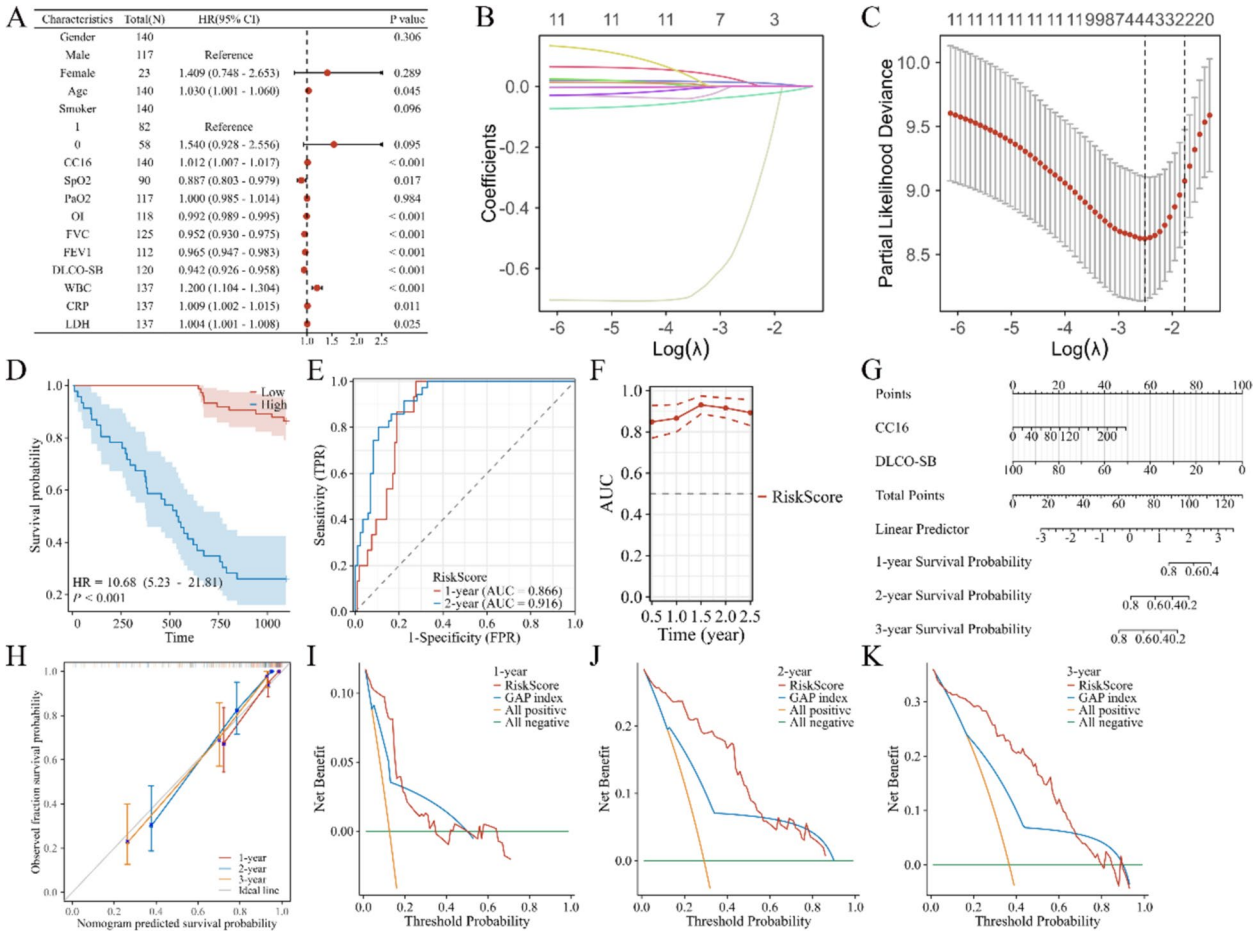

Fig. 3 Construction of the CC16-based prognostic signature. **A** Univariate Cox regression analysis of CC16 expression and clinical characteristics in the training set. **B** LASSO regression analysis, an upper abscissa indicated how many variables in this model have non-zero coefficients, with each curve representing a change in the coefficient of each variable. **C** Ten-fold cross-validation for parameter selection in the LASSO model. **D** In the training set, patients at high-risk have shorter OS than those at low-risk, based on a Kaplan–Meier curve ($P < 0.001$). **E** Time-dependent ROC curves of 1-year and 2-year. **F** Based on the risk score, AUCs for 0.5- to 2.5-year ROC curves were calculated. **G** A nomogram for prediction of one-, two-, and three-year survival probability in the training set. **H** Graphs showing the calibration curves for the nomogram prediction of survival rates at 1, 2, and 3 years. **I** 1-year DCA curve comparison between this nomogram model and GAP index. **J** 2-year DCA curve comparison. **K** 3-year DCA curve comparison

Calibration curve for the model showed high agreement between the predicted probability and the actual probability (Fig. 5E). The clinical utility of the nomogram was examined by DCA, the model showed a satisfactory clinical net benefit (Fig. 5F). These results indicated that the model combing serum CC16, KL-6 and DLCO-SB could help to identify the risk of acute exacerbation.

Increased CC16 expression in fibrotic lung tissues, BALF and serum

To further verify the relationship between CC16 and pulmonary fibrosis. Compared with that in normal lung tissues, CC16 protein expression was upregulated in IPF lung tissues, as detected by Western blot (Fig. 6A–C). CC16 in human BALF was significantly increased in IPF group compared with control group (Fig. 6D). In addition, Serum CC16 in BLM treated mouse also increased at 14th day (Fig. 6E). The protein expression of CC16 increased in fibrotic mouse lung tissues on day 7(Fig. 6F–H), day 14 (Fig. 6I–K) and day 21 (Fig. 6L–N). These results demonstrated that expression of CC16 was elevated in fibrotic lung tissues, BALF and serum and may be involved in pathogenesis of IPF.

Discussion

In current study, a prognostic prediction model for IPF was constructed based on serum CC16, and the model showed good performance to predict prognosis of IPF

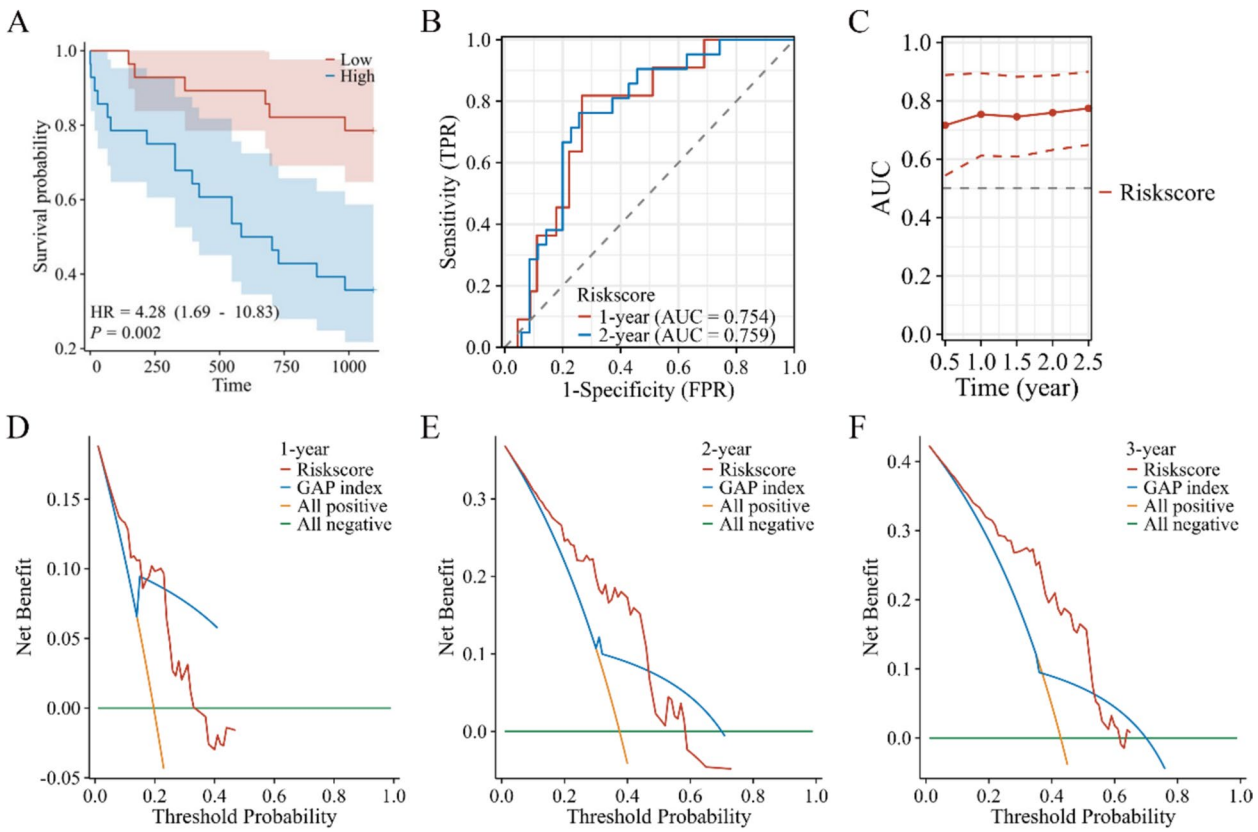

Fig. 4 Validation of CC16-based prognostic signature in the testing set. **A** Kaplan–Meier survival analysis in the training set indicated that patients at high-risk have shorter OS than those at low-risk ($P = 0.002$). **B** Time-dependent ROC curves were used to evaluate the performance of the model in predicting 1-year and 2-year survival probability. **C** Time-dependent AUCs from 0.5- to 2.5-years were calculated. **D**–**F** 1-, 2-, and 3-year DCA curves between this model and GAP index were conducted to evaluate the probability of clinical utility

and possessed potential clinical utility. The predictive model based on serum CC16, serum KL-6 and DLCO-SB also performed well at predicting the risk of acute exacerbation for patients with IPF. To our knowledge, this study may be the first to build predictive models for prognosis and acute exacerbation of IPF based on serum CC16. Moreover, level of serum CC16 was closely related to severity of pulmonary fibrosis in IPF patients, serum CC16 gradually increased with severity of lung fibrosis and it was especially higher in AE-IPF. Expression of CC16 significantly up-regulated in human fibrotic lung tissues and BALF, it also increased in BLM-induced mouse fibrotic lung tissues and serum. The increased CC16 level may be involved in pathogenesis of IPF.

In recent years, many studies have reported that ILD-GAP model was a simple and reliable clinical index for prognosis prediction of ILD patients, and it was usually selected as a comparison in some studies [25, 26]. The results of DCA suggested that our model performed better than GAP index to predict 2-year and 3-year mortality risk for IPF patients. And serum KL-6 was a recognized biomarker for prediction of severity, acute exacerbation, poor outcomes for ILD patients, and serum KL-6 can already be routinely tested in some hospitals [24]. Serum CC16, KL-6 and DLCO-SB were used to develop a predictive model for occurrence of acute exacerbation. Results of ROC, calibration curve and DCA indicated that this model also performed well in predicting the risk of acute exacerbation.

Elevated CC16 has been detected in lung tissues of IPF and BLM induced mice pulmonary fibrosis, and it had been regarded as an indicator of lung injury in previous researches. IPF is recognized as a disease characterized by lung epithelial injury, but the relationship between CC16 and IPF has rarely been reported. Previous research reported that epithelial cells respond to injury by secreting innate immunity proteins such as secretory leukocyte protease inhibitor (SLPI), elafin, CC16 and β-defensin-2, and among these proteins only CC16 was up-regulated in IPF [16]. In basis experiment, increased CC16 was detected not only in Club cells but also in AEC of patients with IPF [32]. Three studies have reported increased CC16 level in serum, BALF and sputum in patients with IPF, similar with our results [16, 27, 28].

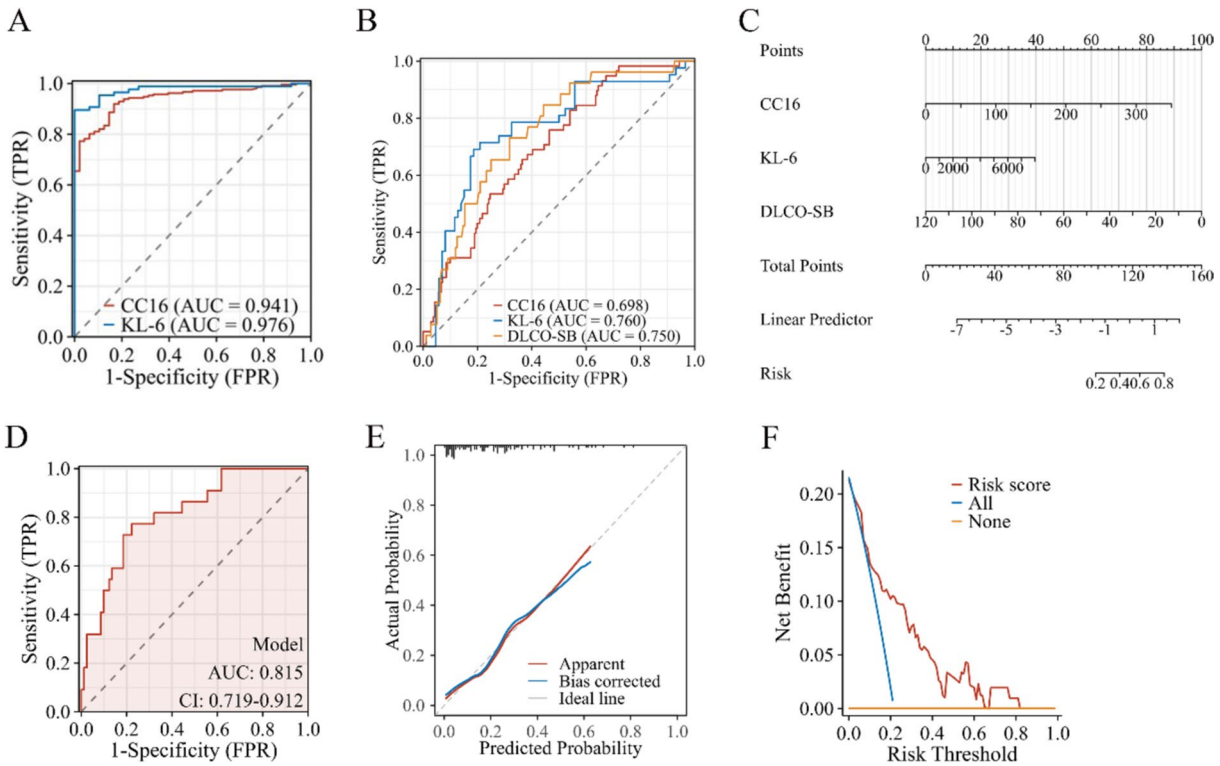

Fig. 5 Construction of CC16-based identification model for acute exacerbation. **A** ROC curve was applied to calculate the diagnostic value of CC16 and KL-6 for patients with IPF. **B** The diagnostic value of CC16, KL-6 and DLCO-SB to distinguish AE-IPF and IPF was calculated by ROC curves. **C** A nomogram using CC16, KL-6 and DLCO-SB for the risk of AE-IPF occurrence was constructed. **D** ROC curve and AUC of the model to verify the diagnostic value. **E** Calibration curves of the nomogram was generated for the cohort. **F** DCA for the nomogram was applied to evaluate the probability of clinical utility

Serum CC16 was significantly increased after acute exposure to smoke, whereas it returned to baseline level after 10 days of smoke exposure, which indicated that serum CC16 may be a potential marker for acute airway injury [29]. Increased CC16 was detected in peripheral blood after silica exposure, and it is sensitive than respiratory symptoms, chest computerized tomography scan and lung function test, which indicated that serum CC16 is a sensitive marker in early lung injury [30]. Recent research revealed that serum CC16 was obviously higher in ARDS patients than non-ARDS patients, and increased serum CC16 has been considered as a biomarker for ARDS diagnosis and prognosis [11, 31]. Up-regulated CC16 also has been regarded as a potential biomarker for COVID-19 associated lung injury [12] and elevated CC16 level may be useful to predict outcomes in patients with COVID-19 [32]. These studies suggested that CC16 is closely related to lung injury and IPF, and CC16 can reflect the level of lung injury earlier than clinical symptoms, lung function test or imaging.

The mechanisms of CC16 involved in pulmonary fibrosis are still unknown. Previous research indicated that CC16 knockout mice will sporadically develop focal pulmonary fibrosis and these mice exhibited high morbidity and mortality. And CC16 knockout mice were extraordinarily sensitive to BLM and an extremely low dose of BLM will lead pulmonary fibrosis. And supplementation with CC16 can prevent BLM induced pulmonary fibrosis by suppressing pro-fibrotic inflammatory T-helper 2 cytokines and TGF-β [33]. Recent research discovered that knockout of CC16 in mice led to an accelerated lung aging phenotype with exaggerated pulmonary inflammation with activated NF-κB, and CC16-/- mice developed more small airway fibrosis than wild type mice, the increased inflammatory factor including IL-10, CCL-2 and CCL5 [34]. Administration of Antiflammin-1 (AF-1), a derivative of CC16 and a synthetic nonapeptide, has been shown to have a protective effect against BLM-induced mouse pulmonary fibrosis by reducing the level of inflammatory factors such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) [35]. From these previous studies, CC16 may regulate inflammation to involve in pulmonary fibrosis, but more accurate studies are needed.

Acute exacerbation is a threatening disease status for IPF patients, and patients with acute exacerbation have

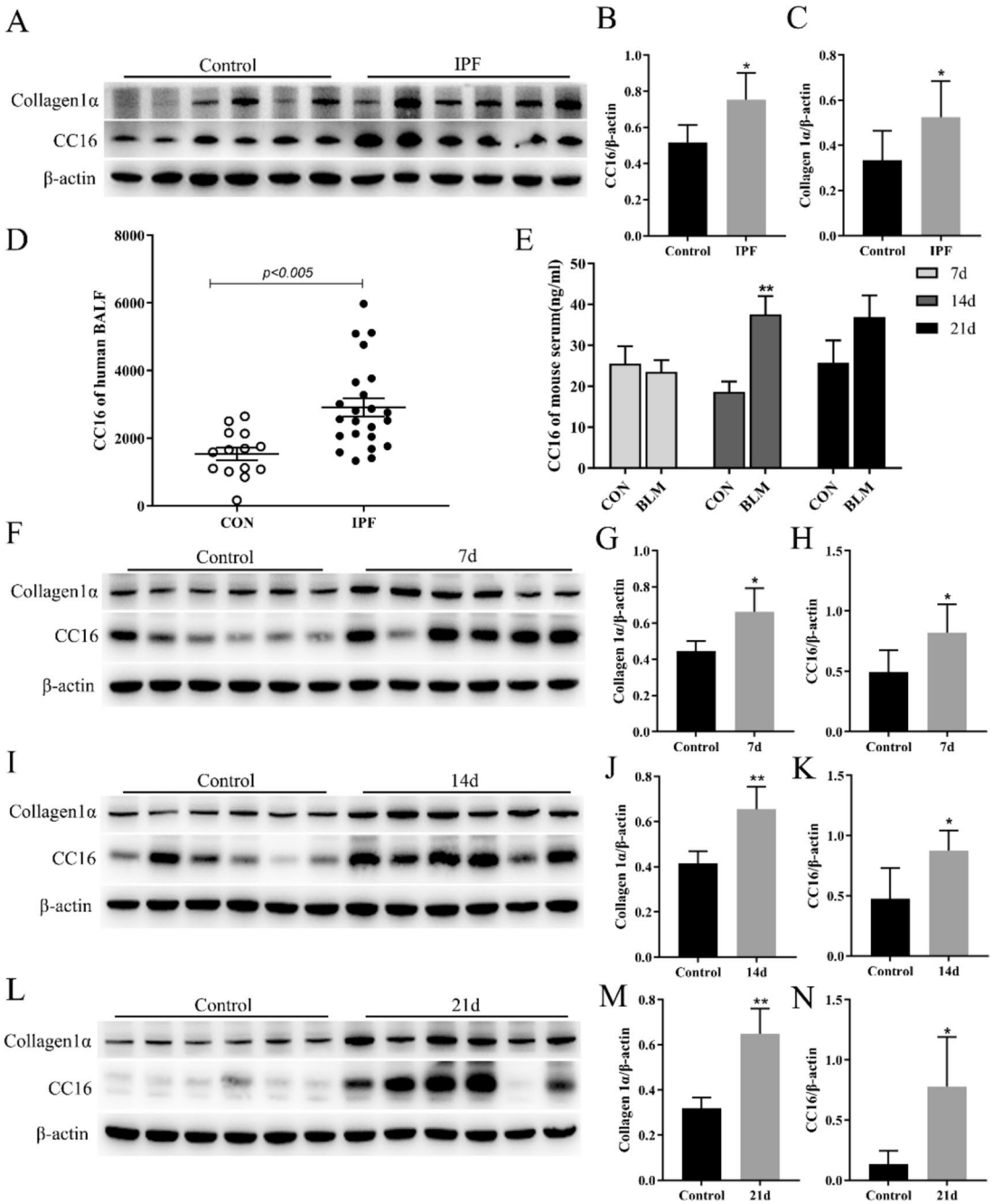

Fig. 6 Increased expression of CC16 in fibrotic lung tissues, BALF and serum. **A**–**C** Protein expression of CC16 and Collagen 1α in human lung tissues were detected by Western blot and greyscale values were calculated via Image J. **D** CC16 of human BALF were detected by ELISA in IPF and control group. **E** Serum CC16 of BLM treated mice were detected by ELISA at day 7, 14, 21. **F**–**H** Expression of CC16 and Collagen 1α were detected in BLM treated lung tissues on day 7. **I**–**K** CC16 and Collagen 1α were detected on day 14. **L**–**N** CC16 and Collagen 1α were detected on day 21 (*$P < 0.05$; **$P < 0.005$)

a high risk of death within short term. Recently, several studies have reported some scoring systems based on patient clinical characteristics to predict prognosis of patients with AE-IPF and risk of acute exacerbation. Sakamoto S et al. constructed a model named "PCR" by using PaO2/FiO2 ratio (P), CRP (C), and diffuse HRCT pattern (radiological) (R) to predict 3-month mortality in patients with AE-IPF [36]. Another predictive score model using radiographic honeycombing (H), age (A) and serum LDH level (L) was developed to discriminate

the risk of acute exacerbation in patients with idiopathic interstitial pneumonias, the discriminated C-index of HAL score system was 0.62 in exploratory cohort, and the C-index was 0.67 in a validation cohort [37]. The indexes were significantly lower than the AUC in our model (0.851). Compared with the above studies, our model may perform better in predicting the risk of acute exacerbation.

However, CC16 is not always up-regulated in respiratory diseases. In lung cancer, decreased serum CC16 at baseline associated with elevated mortality risk [38]. In COPD, low circulating CC16 levels in childhood predicted accelerated lung function decline in adults and may be associated with the development of COPD [39] and decreased serum CC16 have been used as a biomarker for COPD diagnosis and assessment [8]. Many evidences suggested that CC16 may play an anti-inflammatory and anti-oxidant role to protect lung from obstructive lung diseases [40]. Decreased CC16 in bronchial epithelial cells was proved to be associated with smoking-related lung function decline [41]. Under these circumstances, normal level of CC16 may play an important role in maintaining normal lung function. And we hypothesized that CC16 is up-regulated when lung parenchymal injury occurs, whereas CC16 secretion will decrease when Club cells and epithelial cells are damaged during airway injury.

This study has obvious limitations, the lack of external validation cohort makes the external extension performance of the model undetermined. And more clinical laboratory variables and symptoms should be included in the study for variable screening. We have also conducted some basic researches to explore the mechanisms of CC16 involved in pathogenesis of IPF. However, the underlying mechanisms are still unclear, and more basic researches are needed in the future.

Conclusions

This study constructed clinical model based on serum CC16 to predict the prognosis of patients with IPF and the risk of acute exacerbation. Current nomogram models could help clinicians identify patients with high risk of death and acute exacerbation in time, and guide clinicians to carry out effective treatments as early as possible to improve patients' prognosis and reduce mortality.

Abbreviations

IPF	Idiopathic pulmonary fibrosis
CC16	Club cell secretory protein 16
AE-IPF	Acute exacerbation of IPF
HRCT	High-resolution computed tomography
ROC	Receiver-operating characteristic curve
DCA	Decision curve analysis
KL-6	Krebs von den Lungen-6
DLCO-SB	Diffusion capacity for carbon monoxide in single breath
FVC	Forced vital capacity
GAP	Gender, age and physiology
COPD	Chronic obstructive pulmonary diseases
OSA	Obstructive sleep apnea
ARDS	Acute respiratory distress syndrome
BALF	Bronchoalveolar lavage fluid
CHP	Chronic hypersensitivity pneumonitis
CTD	Connective tissue diseases

Acknowledgements
Not applicable.

Author contributions
Yaqiong Tian, Xuan Zhou and Mi Tian were in charge of manuscript writing and data analysis, Lijun Ren, Ruyi Zou and Miaomiao Xie were primarily responsible for sample collection, data analysis and figure creation. Hanyi Jiang, Mei Huang and Jingjing Ding gathered clinical data of all patients. Yin Liu contributed to verifying and checking the diagnosis of these patients. Hourong Cai and Min Cao took charge of designing, making experiment plans and revising manuscript.

Funding
This work was supported by National Natural Science Foundation of China (Grant No.: 81970063 and 81900061) and Jiangsu Provincial Key Research and Development (Grant No.: BE2016611).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Nanjing Drum Tower Hospital of Medical School of Nanjing University (No. 31/93, 84/93, 29/01). Written informed consent was obtained from the participants, and this study did not involve any personal information.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 17 August 2024 Accepted: 22 December 2024
Published online: 10 January 2025

References

1. Hutchinson JP, McKeever TM, Fogarty AW, Navaratnam V, Hubbard RB. Increasing global mortality from idiopathic pulmonary fibrosis in the twenty-first century. Ann Am Thorac Soc. 2014;11(8):1176–85.
2. Henderson NC, Rieder F, Wynn TA. Fibrosis: from mechanisms to medicines. Nature. 2020;587(7835):555–66.
3. Raghu G, Remy-Jardin M, Myers J, Richeldi L, Ryerson C, Lederer D, et al. Diagnosis of idiopathic pulmonary fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med. 2018;198(5):e44–68.

4. Lakind JS, Holgate ST, Ownby DR, Mansur AH, Helms PJ, Pyatt D, et al. A critical review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or chronic pulmonary effects. Biomarkers. 2007;12(5):445–67.
5. Boers JE, Ambergen AW, Thunnissen FB. Number and proliferation of clara cells in normal human airway epithelium. Am J Respir Crit Care Med. 1999;159(5 Pt 1):1585–91.
6. Evans CM, Williams OW, Tuvim MJ, Nigam R, Mixides GP, Blackburn MR, et al. Mucin is produced by clara cells in the proximal airways of antigen-challenged mice. Am J Respir Cell Mol Biol. 2004;31(4):382–94.
7. Broeckaert F, Clippe A, Knoops B, Hermans C, Bernard A. Clara cell secretory protein (CC16): features as a peripheral lung biomarker. Ann N Y Acad Sci. 2000;923:68–77.
8. Rong B, Fu T, Gao W, Li M, Rong C, Liu W, et al. Reduced serum concentration of CC16 is associated with severity of chronic obstructive pulmonary disease and contributes to the diagnosis and assessment of the disease. Int J Chron Obstruct Pulmon Dis. 2020;15:461–70.
9. Palczynski C, Walusiak J, Krakowiak A, Halatek T, Ruta U, Krawczyk-Adamus P, et al. Glutaraldehyde-induced occupational asthma: BALF components and BALF and serum Clara cell protein (CC16) changes due to specific in halatory provocation test. Occupat Med (Oxford, England). 2005;55(7):572–4.
10. Gulec Balbay E, Ataoglu O, Ercelik M, Sahin IE, Admis O, Arbak PM. Serum Clara cell secretory protein (CC-16) in non-smoking patients with obstructive sleep apnea. Sleep Breathing. 2020;24(4):1607–12.
11. Lin J, Zhang W. Diagnostic and prognostic values of Club cell protein 16 (CC16) in critical care patients with acute respiratory distress syndrome. Clin Lab Anal. 2018;32(2): e22262.
12. Almuntashiri S, James C, Wang X. The potential of lung epithelium specific proteins as biomarkers for COVID-19-associated lung injury. Diagnostics. 2021;11(9):1643.
13. Zhang S, Jia Q, Song J, Tan Q, Yu G, Guo X, et al. Clinical significance of CC16 and IL-12 in bronchoalveolar lavage fluid of various stages of silicosis. Ann Palliat Med. 2020;9(6):3848–56.
14. Naha N, Muhamed JCJ, Pagdhune A, Sarkar B, Sarkar K. Club cell protein 16 as a biomarker for early detection of silicosis. Indian J Med Res. 2020;151(4):319–25.
15. Tian Y, Li H, Gao Y, Liu C, Qiu T, Wu H, et al. Quantitative proteomic characterization of lung tissue in idiopathic pulmonary fibrosis. Clin Proteomics. 2019;16:6.
16. Tsoumakidou M, Bouloukaki I, Thimaki K, Tzanakis N, Siafakas NM. Innate immunity proteins in chronic obstructive pulmonary disease and idiopathic pulmonary fibrosis. Exp Lung Res. 2010;36(6):373–80.
17. Buendia-Roldan I, Ruiz V, Sierra P, Montes E, Ramirez R, Vega A, et al. Increased expression of CC16 in patients with idiopathic pulmonary fibrosis. PLoS ONE. 2016;11(12): e0168552.
18. Kam MLW, Li HH, Tan YH, Low SY. Validation of the ILD-GAP model and a local nomogram in a Singaporean cohort. Respiration. 2019;98(5):383–90.
19. Wu Q, Xu Y. A clinical model for the prediction of acute exacerbation risk in patients with idiopathic pulmonary fibrosis. BioMed Res Int. 2020;2020:8848919.
20. Fu X, Lin J, Seery S, Ye J, Zeng S, Luo Y et al. A retrospective, nested case-control study to develop a biomarker-based model for ARDS diagnostics. Clin Lab. 2023; 69(1).
21. Lynch DA, Sverzellati N, Travis WD, Brown KK, Colby TV, Galvin JR, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. Lancet Respir Med. 2018;6(2):138–53.
22. Ganesh Raghu MRJ, Richeldi L, Thomson CC, Inoue Y, Johkoh T, et al. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med. 2022;205(9):e18–47.
23. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183(6):788–824.
24. Zhang T, Shen P, Duan C, Gao L. KL-6 as an immunological biomarker predicts the severity, progression, acute exacerbation, and poor outcomes of interstitial lung disease: a systematic review and meta-analysis. Front Immunol. 2021;12: 745233.
25. Ryerson CJ, Vittinghoff E, Ley B, Lee JS, Mooney JJ, Jones KD, et al. Predicting survival across chronic interstitial lung disease: the ILD-GAP model. Chest. 2014;145(4):723–8.
26. Hirata M, Hara Y, Fujii H, Murohashi K, Saigusa Y, Zhao S, et al. ILD-GAP combined with the monocyte ratio could be a better prognostic prediction model than ILD-GAP in patients with interstitial lung diseases. BMC Pulm Med. 2024;24(1):16.
27. Kokuho N, Ishii T, Kamio K, Hayashi H, Kurahara M, Hattori K, et al. Diagnostic values for club cell secretory protein (CC16) in serum of patients of combined pulmonary fibrosis and emphysema. COPD. 2015;12(4):347–54.
28. Ye Q, Fujita M, Ouchi H, Inoshima I, Maeyama T, Kuwano K, et al. Serum CC-10 in inflammatory lung diseases. Respiration. 2004;71(5):505–10.
29. Bernard A, Hermans C, Van Houte G. Transient increase of serum Clara cell protein (CC16) after exposure to smoke. Occup Environ Med. 1997;54(1):63–5.
30. Bernard AM, Gonzalez-Lorenzo JM, Siles E, Trujillano G, Lauwerys R. Early decrease of serum Clara cell protein in silica-exposed workers. Eur Respir J. 1994;7(11):1932–7.
31. Lin J, Tao W, Wei J, Wu J, Zhang W. Renal dysfunction reduces the diagnostic and prognostic value of serum CC16 for acute respiratory distress syndrome in intensive care patients. BMC Pulm Med. 2020;20(1):212.
32. Tiezzi M, Morra S, Seminerio J, Van Muylem A, Godefroid A, Law-Weng-Sam N, et al. SP-D and CC-16 pneumoproteins' kinetics and their predictive role during SARS-CoV-2 infection. Front Med. 2021;8: 761299.
33. Lee YC, Zhang Z, Mukherjee AB. Mice lacking uteroglobin are highly susceptible to developing pulmonary fibrosis. FEBS Lett. 2006;580(18):4515–20.
34. Laucho-Contreras ME, Polverino F, Rojas-Quintero J, Wang X, Owen CA. Club cell protein 16 (Cc16) deficiency increases inflamm-aging in the lungs of mice. Physiol Rep. 2018;6(15): e13797.
35. Liu W, Wan J, Han JZ, Li C, Feng DD, Yue SJ, et al. Antiflammin-1 attenuates bleomycin-induced pulmonary fibrosis in mice. Respir Res. 2013;14(1):101.
36. Sakamoto S, Shimizu H, Isshiki T, Nakamura Y, Usui Y, Kurosaki A, et al. New risk scoring system for predicting 3-month mortality after acute exacerbation of idiopathic pulmonary fibrosis. Sci Rep. 2022;12(1):1134.
37. Karayama M, Aoshima Y, Suzuki T, Mori K, Yoshizawa N, Ichikawa S, et al. A predictive model for acute exacerbation of idiopathic interstitial pneumonias. Eur Respir J. 2023;61(5):2201634.
38. Guerra S, Vasquez MM, Spangenberg A, Halonen M, Martinez FD. Serum concentrations of club cell secretory protein (Clara) and cancer mortality in adults: a population-based, prospective cohort study. Lancet Respir Med. 2013;1(10):779–85.
39. Guerra S, Halonen M, Vasquez MM, Spangenberg A, Stern DA, Morgan WJ, et al. Relation between circulating CC16 concentrations, lung function, and development of chronic obstructive pulmonary disease across the lifespan: a prospective study. Lancet Respir Med. 2015;3(8):613–20.
40. Lomas DA, Silverman EK, Edwards LD, Miller BE, Coxson HO, Tal-Singer R. Evaluation of serum CC-16 as a biomarker for COPD in the ECLIPSE cohort. Thorax. 2008;63(12):1058–63.
41. Lam DC, Kwok HH, Yu WC, Ko FW, Tam CY, Lau AC, et al. CC16 levels correlate with cigarette smoke exposure in bronchial epithelial cells and with lung function decline in smokers. BMC Pulm Med. 2018;18(1):47.

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