Elevated serum human epididymis protein 4 is associated with disease severity and worse survival in idiopathic pulmonary fibrosis: a cohort study

Mi Tian[1#], Kaifang Meng[1#], Yujuan Gao[2#], Ji Zhang[3], Miaomiao Xie[2], Yaqiong Tian[2], Xiaoqin Liu[2], Miao Ma[2], Ying Cai[3], Hongyan Wu[4], Jingjing Ding[1,2], Jingyu Chen[3], Hourong Cai[1,2]

[1]Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, Nanjing, China; [2]Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, China; [3]Jiangsu Key Laboratory of Organ Transplantation, Wuxi People's Hospital, Nanjing Medical University, Wuxi, China; [4]Department of Pathology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China

Contributions: (I) Conception and design: M Tian, K Meng, Y Gao, J Ding, H Cai; (II) Administrative support: H Cai, J Ding, J Chen; (III) Provision of study materials or patients: J Zhang, Y Cai, J Chen; (IV) Collection and assembly of data: M Xie, Y Tian, X Liu, M Ma; (V) Data analysis and interpretation: M Tian, K Meng, H Wu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Hourong Cai. Department of Respiratory and Critical Care Medicine, Nanjing Drum Tower Hospital Clinical College of Nanjing Medical University, No. 321 Zhongshan Road, Nanjing 210008, China. Email: caihourong2013@163.com; Jingyu Chen. Jiangsu Key Laboratory of Organ Transplantation, Wuxi People's Hospital, Nanjing Medical University, No. 299 Qingyang Road, Wuxi 214023, China. Email: chenjy@wuxiph.com.

Background: Elevated expression of human epididymis protein 4 (HE4) was previously described in connective tissue disease-associated interstitial lung diseases (CTD-ILDs) and cystic fibrosis (CF), but the clinical significance of HE4 has remained unknown in idiopathic pulmonary fibrosis (IPF), which is a progressive fibrosing ILD with a heterogeneous course that is in urgent need of reliable biomarkers in its clinical practice.

Methods: A total of 27 IPF patients with acute exacerbation status (AE-IPF), 32 IPF patients with stable status (S-IPF), and 29 sex-age matched healthy controls were retrospectively included. The levels of serum HE4 and Krebs von den Lungen-6 (KL-6) of the 3 cohorts were measured. In addition, the pulmonary expression of HE4 was evaluated in lung transplant specimens of IPF using immunohistochemistry and Western blot, and noncancerous lung tissue resected from early-stage lung cancer patients as controls. The endpoint of follow-up was March 1st, 2022, and the Cox regression model was used to analyze the prognostic value of HE4.

Results: The levels of HE4 and KL-6 were obviously elevated in AE-IPF patients compared to S-IPF (296.4 *vs.* 178.1 pmol/L for HE4, P<0.001; 2,007.0 *vs.* 990.5 IU/mL for KL-6, P<0.001) or healthy controls (296.4 *vs.* 51.8 pmol/L for HE4, P<0.001; 2,007.0 *vs.* 181.0 IU/mL for KL-6, P<0.001). Significant correlations were observed between serum HE4 levels and percent predicted diffusing capacity of the lung for carbon monoxide (DLCO%) (r=–0.526, P<0.001), percent predicted forced vital capacity (FVC%) (r=–0.344, P=0.024), gender-age-physiology (GAP) index (r=0.535, P<0.001), and oxygenation index (r=–0.550, P<0.001) in IPF patients. In histological analysis, overexpression of HE4 in mucosal epithelium of dilated bronchi was observed in IPF patients compared with controls. Multivariate cox regression revealed that serum levels of HE4 [hazard ratio (HR) =1.004, P=0.042] and GAP index (HR =1.374, P=0.010) were associated with worse survival in IPF patients.

Conclusions: The expression of serum HE4 was obviously elevated in IPF patients, especially in AE-IPF patients. In addition, serum HE4 could be utilized as a biomarker of disease severity and poor prognosis of IPF patients. These findings warrant further validation in larger, multi-center, and longitudinal cohorts.

Keywords: Idiopathic pulmonary fibrosis (IPF); gender-age-physiology index (GAP index); human epididymis protein 4 (HE4); acute exacerbation (AE)

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Introduction

Idiopathic pulmonary fibrosis (IPF), which is characterized by decreasing lung volumes and hypoxemic respiratory failure, has a median survival time of 2–5 years and a 5-year survival rate of 20–40% (1). As the primary cause of death in IPF patients, acute exacerbation (AE) is defined as an acute respiratory deterioration that presents clinically as sudden aggravation of dyspnea and new consolidation or bilateral ground-glass opacity (GGO) on chest imaging (2,3). The annual incidence of AE-IPF is as high as 20%, median survival after AE-IPF is generally no more than 3 months, and in-hospital mortality after AE-IPF usually exceeds 50% (4,5). Evidence suggests that AE of IPF is a typical alveolar inflammation involving diffuse alveolar damage and aggravated epithelial injury superimposed on the background of a chronic and fibrosing interstitial lung disease (6,7). However, the exact mechanism of AE-IPF remains unclear. Krebs von den Lungen-6 (KL-6), surfactant protein-A (SP-A), and SP-D are type II pneumocyte-derived molecules which have been well described for their usefulness as serum biomarkers of IPF (8-10). Elevated serum levels of these markers in patients with IPF were shown to be associated with poorer survival in IPF. Recently, alveolar macrophages-derived molecules such as matrix metalloproteinase-7 (MMP-7) and CC-chemokine ligand 18 (CCL18) are considered potential diagnostic and prognostic markers of IPF (9). Although there is increasing interest in exploring circulating serum biomarkers for evaluating the severity of disease and predicting IPF-related mortality, few biomarkers have been used in clinical practice.

Human epididymis protein 4 (HE4), also named WFDC2, has been found in the distal human epididymis and renal tubular epithelial, as well as in the respiratory tract (11-13), and is widely utilized as a biomarker in ovarian and endometrial cancer (14,15), with measurement of serum HE4 recently included in the tumor biomarker screening panel of females in clinical practice. Under normal physiological conditions, redundant deposition of extracellular matrix (ECM) components could be degraded by MMPs. Considered to be a crucial protein responsible for the deposition of ECM (16), HE4 inhibits the function of various proteases, such as MMPs and serine proteases, suppresses the degradation of type I collagen (13), and plays a crucial role in the pathogenesis of fibrosis. In a mice model of renal fibrosis, elevated expression of HE4 inhibited the activation of MMPs by upregulation of tissue inhibitor metalloproteinases (TIMPs), accelerating the process of fibrosis (16,17). Overexpression of HE4 has been reported in fibrotic diseases, such as cardiac fibrosis (18), renal fibrosis (13), cystic fibrosis (CF) (19), and progressive fibrosing interstitial lung diseases (PF-ILDs) (20). Recent studies further showed that serum HE4 was increased in connective tissue diseases (CTDs), including systemic sclerosis (SSc) (21), rheumatoid arthritis (RA) (22), and primary Sjögren's Syndrome (pSS) (23), and elevated HE4 levels were associated with the complication of ILD. Although the precise mechanism is uncertain, we speculated that the abnormal expression of HE4 was closely related to the formation of fibrosis and inflammatory response.

In this study, we investigated the expression of HE4 in IPF patients and evaluated its correlations with indicators of disease severity. We further compared HE4 levels between AE-IPF and stable IPF, and assessed whether serum HE4 could be utilized as a promising biomarker to predict poor prognosis of IPF. We present the following article in accordance with the STARD reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4042/rc).

Methods

Study subjects

A total of 27 AE-IPF and 32 S-IPF patients hospitalized in Nanjing Drum Tower Hospital between November 2017 and April 2018 were retrospectively included in this cohort study. In the Center of Physical Examination, 29 healthy subjects with no evidence of comorbidities were included as controls. The three cohorts were well matched for age, sex, and smoking history. The American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese

Respiratory Society (JRS)/Latin American Thoracic Society (ALAT) guidelines were used to diagnose IPF (24). The definition of AE was according to the updated criteria of AE-IPF in 2016 (3). Briefly, exacerbation of dyspnea typically occurred in the past month, there is new bilateral GGO and/or consolidation superimposed on the typical background of usual interstitial pneumonia (UIP) or possible UIP pattern on chest imaging, and deterioration cannot be explained entirely through fluid overload or heart failure. Medical records were reviewed to exclude participants with concurrent malignancy, kidney disease, and other known causes of ILD, such as CTD with autoimmune characteristics, drug toxicity, or occupational environmental exposure. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by ethics committee of Nanjing Drum Tower Hospital (No. 2016-138-01). Written informed consent to take part in this study and authorization to utilize their lung tissues and serum samples for research purposes were obtained from all participants.

Clinical data collection

Clinical features, demographics, and laboratory findings were retrospectively collected from medical records, and survival data were obtained through telephone interviews. The gender-age-physiology (GAP) index was introduced in our study on the basis of previous practice (25,26). Pulmonary function tests (PFTs) were conducted as recommended by ATS, and the reports were described as percentages of the predicted values according to the participants' height, age, and sex (27). The endpoint of follow-up was March 1st, 2022, and survival time was determined from the time of enrollment in our study to censoring or time of death.

Measurement of serum HE4 and KL-6

Blood samples were collected using conventional methods and immediately centrifuged, with the serum aliquoted and frozen at –70 ℃ until measurement. An enzyme-linked immunosorbent assay (Fujirebio Diagnostics, Gothenburg, Sweden) was used according to the manufacturer's instructions to quantitatively detect serum concentrations of HE4. Concentrations of KL-6 were measured using a KL-6 kit (Fujirebio, Inc., Tokyo, Japan) on an automatic LUMIPULSE G1200 immune analyzer (Fujirebio). Lactate dehydrogenase (LDH) and C-reactive protein (CRP) were measured by Beckman Coulter AU5800 automatic biochemical analyzer (Beckman, Germany) at Drum Tower Hospital.

Human lung tissues

Lung specimens from 6 IPF patients [2 females, 4 males; mean age ± standard deviation (SD): 58.7±7.9 years] undergoing lung transplantation surgery at the Key Laboratory of Organ Transplantation of Wuxi People's Hospital (Wuxi, China) were collected, and noncancerous lung specimens resected from 6 early-stage lung cancer patients (2 females, 4 males, mean age ± SD: 62.8±5.0 years) matched with sex and age at the department of thoracic surgery were included as controls.

Immunohistochemistry (IHC) staining

IHC staining was performed in lung specimens fixed with 10% formalin and embedded in paraffin before being cut into 5-μm-thick sections. After dewaxing and rehydration, the sections were incubated with 3% H_2O_2 for 5 minutes to block the activity of peroxidase. The sections were then incubated with rabbit monoclonal HE4 antibody (ab200828; Abcam, Tokyo, Japan) at 4 ℃ overnight, followed by secondary antibody (Vector Laboratories, Burlingame, CA, USA) at 25 ℃ for 60 minutes. Subsequently, the visualization of immunoreaction was conducted by 3,3-diaminobenzidine (DAB) chromogen solution (DAB substrate kit; Vector Laboratories) and then counterstained with hematoxylin.

Western blot

Total protein from the lung specimens was extracted using radioimmunoprecipitation assay (RIPA) buffer supplemented with protease inhibitor cocktail. Equal amounts (10–20 μg) protein of each sample were separated on a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, transferred onto polyvinylidene difluoride (PVDF) membranes, and incubated with the primary antibody at 4 ℃ overnight (anti-HE4, 1:100, Abcam), followed by horseradish peroxidase-conjugated anti-rabbit secondary antibody (diluted 1:5,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Western blot for glyceraldehyde 3-phosphate dehydrogenase (GAPDH; Bioworld Technology, St Louis Park, MN, USA) was performed to normalize the level of HE4.

Table 1 Demographic and clinical characteristics of idiopathic pulmonary fibrosis patients

Clinical characteristics	Controls (n=29)	AE-IPF patients (n=27)	S-IPF patients (n=32)	P value AE-IPF *vs.* S-IPF
Age, years	64.3±5.9	69.0±8.1	65.7±7.8	0.115
Male, n (%)	22 (75.9)	24 (88.9)	31(96.9)	0.486
Corticosteroids, n (%)	0 (0)	25 (92.6)	5 (15.6)	<0.001***
Smoking history, n (%)	14 (48.3)	17 (63.0)	14 (43.8)	0.141
LDH, U/L	NA	381.0 (277.0, 500.0)	222.0 (202.0, 253.0)	<0.001***
CRP, mg/L	NA	35.4 (13.6, 82.2)	4.1 (2.4, 11.0)	<0.001***
FVC% predicted	NA	49.2 (41.8, 63.4)	68.9 (59.1, 78.0)	0.001**
DLCO% predicted	NA	29.9 (22.7, 36.8)	49.0 (38.7, 69.9)	0.002**
TLC% predicted	NA	51.4 (43.8, 55.1)	65.2 (58.4, 77.5)	<0.001***
PaO_2, mmHg	NA	71.0 (56.0, 84.0)	76.5 (67.5, 83.0)	0.171
$PaCO_2$, mmHg	NA	39.9 (33.2, 43.4)	40.2 (38.9, 43.2)	0.563
Oxygenation index	NA	209.0 (141.0, 245.0)	362.0 (319.0, 395.0)	<0.001***
GAP index	NA	5.6±0.9	4.1±1.7	0.007**

Data are expressed as mean ± standard deviation or median (interquartile range), if not otherwise specified. **, P<0.01; ***, P<0.001. AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis; S-IPF, stable IPF; LDH, lactate dehydrogenase; CRP, C-reactive protein; FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; TLC, total lung capacity; PaO_2, arterial oxygen pressure; $PaCO_2$, arterial partial pressure of carbon dioxide; GAP index, gender-age-physiology index.

Statistical analysis

Continuous variables are expressed as the median [interquartile range (IQR)] or mean ± SD based on the normality test, and differences between the groups were compared by the Mann-Whitney U test or *t*-test. Spearman or Pearson correlations were performed to analyze the relationships between variables. Receiver operator characteristic (ROC) analyses were conducted to determine the area under the ROC curves (AUCs) of variables for discriminating AE-IPF from S-IPF. Kaplan-Meier curves were compared by log-rank test. Cox proportional hazard models were carried out to determine the significance of variables in the prognosis of IPF patients. Variables with P value less than 0.05 in univariate analysis were successfully included in multivariate regression analysis. Age, sex, and PFTs largely determined the GAP index. Therefore, these variables were not included in the multivariate regression model to avoid multicollinearity. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA, USA) was employed for statistical analysis and creating figures. A two-sided P<0.05 was defined as statistically significant.

Results

Clinical features of the enrolled patients

The demographic and clinical features of IPF patients, including 27 AE-IPF and 32 S-IPF, and 29 healthy controls, with no difference in the proportions of age, sex, as well as smoking history, are summarized in *Table 1*. Elevated levels of CRP, LDH, and GAP index along with decreased percent predicted diffusing capacity of the lung for carbon monoxide (DLCO%), percent predicted forced vital capacity (FVC%), percent predicted total lung capacity (TLC%), and oxygenation index were generally observed in AE-IPF patients compared with S-IPF. Almost all of the AE-IPF patients (92.6%) were treated with corticosteroids, whereas only 15.6% of S-IPF patients patients received corticosteroid treatment.

Serum levels of HE4 and KL-6 in the IPF patients

As shown in *Figure 1*, elevated median levels of both serum HE4 and KL-6 were observed in AE-IPF compared with S-IPF subjects [296.4 (231.1, 454.7) *vs.* 178.1 (126.9,

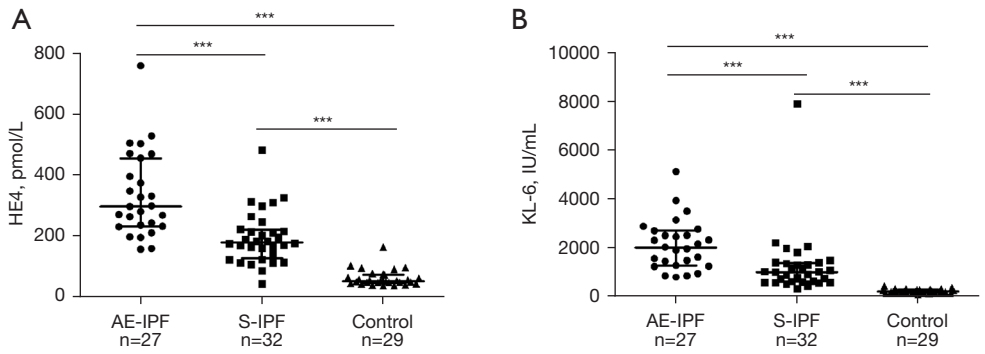

Figure 1 Comparison of serum HE4 and KL-6 levels in AE-IPF, stable IPF, and normal controls. (A) Serum HE4 levels were obviously higher in the AE-IPF compared with S-IPF or normal controls (both P<0.001). (B) Significantly elevated KL-6 levels were observed in AE-IPF compared with S-IPF or healthy controls (both P<0.001). ***, P<0.001. AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis; S-IPF, stable IPF; HE4, human epididymis protein 4; KL-6, Krebs von den Lungen-6.

220.0) pmol/L for HE4, P<0.001; 2,007.0 (1,254.0, 2,687.0) *vs.* 990.5 (584.3, 1,362.3) IU/mL for KL-6, P<0.001) or the healthy controls [296.4 (231.1, 454.7) *vs.* 51.8 (45.2, 73.5) pmol/L for HE4, P<0.001; 2,007.0 (1,254.0, 2,687.0) *vs.* 181.0 (129.0, 261.5) IU/mL for KL-6, P<0.001).

Comparison of HE4 expression in lung specimens from IPF patients and controls

The pulmonary expression of HE4 in IPF patients and controls was detected by IHC and Western blot. As illustrated in *Figure 2A,2B*, the bronchial mucosa and corresponding adjacent alveolar tissues were observed in controls, and the expression of HE4 was only scattered in the bronchial epithelium with focal distribution. In contrast, IPF lung tissues showed strong and relatively continuous HE4 expression in the mucosal epithelium of dilated bronchi, which formed the structure of honeycombing on the fibrous interstitial background (*Figure 2C,2D*). The expression of HE4 was further quantitatively analyzed in explanted lung specimens by Western blot. As shown in *Figure 2E,2F*, the expression of HE4 in IPF was significantly elevated compared with the controls (P=0.02).

ROC analysis for discriminating AE-IPF from S-IPF based on serum biomarkers

To determine the probable value of serum HE4 levels for discriminating AE-IPF from S-IPF, ROC curves were analyzed. The AUCs of serum HE4, KL-6, LDH, and CRP were 0.830 [95% confidence interval (CI): 0.725–0.935; P<0.001], 0.822 (95% CI: 0.715–0.929; P<0.001), 0.935 (95% CI: 0.876–0.993; P<0.001), and 0.843 (95% CI: 0.739–0.948; P<0.001), respectively, with serum LDH showing the greatest area (*Figure 3*).

Correlations between serum HE4 levels and the severity of IPF

Correlations between HE4 and indicators of severity, including PFTs (DLCO%, FVC%), oxygenation index, and GAP index, were evaluated in IPF patients. Serum HE4 levels inversely correlated with DLCO% (r=–0.526, P<0.001), FVC% (r=–0.344, P=0.024), and oxygenation index (r=–0.550, P<0.001). In addition, significant correlations between HE4 and GAP index (r=0.535, P<0.001), CRP (r=0.558, P<0.001), and KL-6 were also observed in IPF patients (*Figure 4*).

Survival analysis based on serum HE4 and KL-6

The median follow-up period in our study was 382 [35, 1,355] days, during which 52 (88.1%) patients with IPF died. To determine the discriminating ability of HE4 levels for distinguishing decedents from survivors, ROC analysis was conducted, and the AUC of HE4 was 0.764 (95% CI: 0.622–0.906) (*Figure 5A*). The optimal cutoff levels for serum HE4 and KL-6 were 191.9 pmol/L and 772 IU/mL, respectively, which were confirmed by the ROC curve with the highest Youden index. The Kaplan-Meier survival curves indicated worse survival in patients with AE-IPF compared with S-IPF (P<0.001; *Figure 5B*). The serum levels of HE4/KL-6

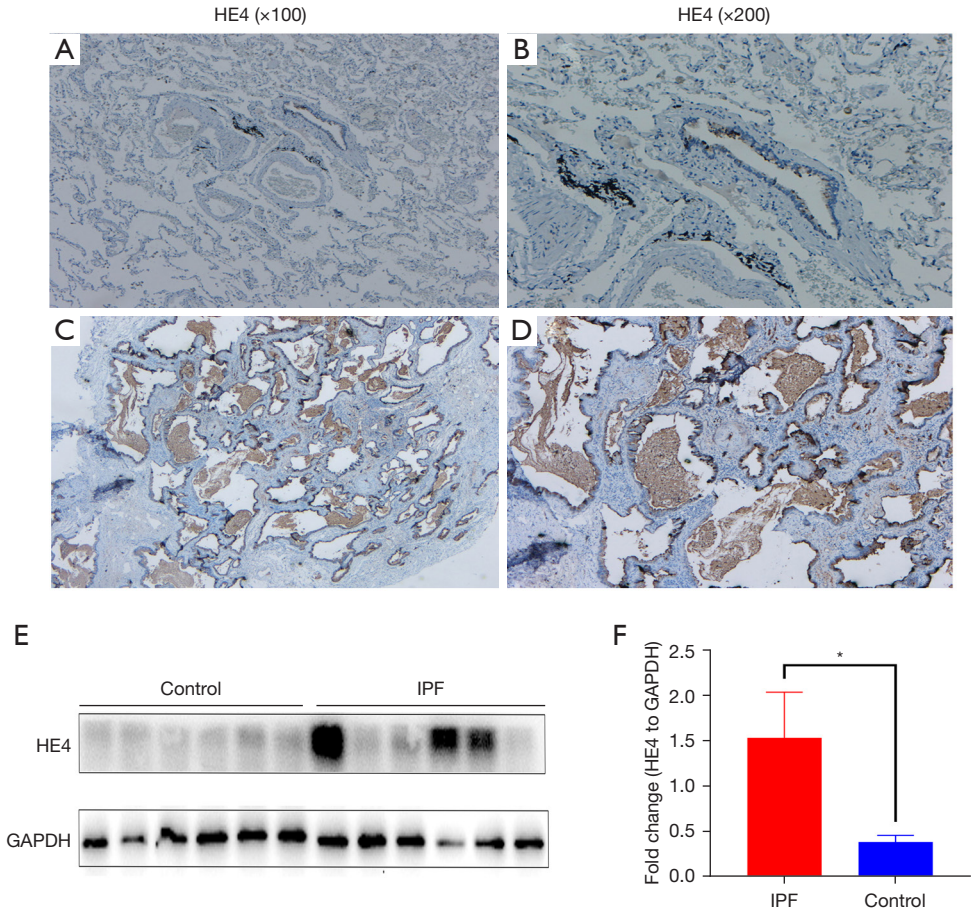

Figure 2 The expression of HE4 in lung tissues of IPF patients and controls. (A,B) The expression of HE4 from the controls' lungs was only scattered in the focal distribution in the bronchial epithelium (IHC staining, original magnification: ×100/×200). (C,D) IPF lung sections showed strong and relatively continuous expression of HE4 in the mucosal epithelium of dilated bronchi (IHC staining, original magnification: ×100/×200). (E,F) Relative expression of HE4 protein was quantified using Western blot, and the expression of HE4 in IPF was obviously elevated compared with the controls (P=0.02). *, P<0.05. IPF, idiopathic pulmonary fibrosis; HE4, human epididymis protein 4; IHC, immunohistochemistry.

were classified into low/high levels according to respective cutoff values, and IPF patients with higher serum HE4 (≥191.9 pmol/L) levels had worse survival compared with patients whose HE4 levels were <191.9 pmol/L (P=0.003; *Figure 5C*). Similarly, worse survival was observed in patients with higher KL-6 (≥772 IU/mL) levels compared with lower levels (P=0.001; *Figure 5D*).

Serum HE4 was an independent predictor of worse survival in IPF patients

To further determine the prognostic value of HE4 in all IPF patients, univariate and multivariate Cox regression analyses were performed (*Table 2*). We found that elevated age [hazard ratio (HR) =1.038, 95% CI: 1.002–1.075, P=0.040], LDH (HR =1.007, 95% CI: 1.005–1.010, P<0.001), CRP (HR =1.016, 95% CI: 1.008–1.025, P<0.001), HE4 (HR =1.004, 95% CI: 1.002–1.006, P<0.001), GAP index (HR =1.445, 95% CI: 1.164–1.793, P=0.001), along with decreased FVC% (HR =0.973, 95% CI: 0.950–0.998, P=0.030 and DLCO% (HR =0.972, 95% CI: 0.953–0.992, P=0.005) were related to poor outcomes in univariate analysis. Multivariate regression analysis revealed that elevated levels of HE4 and GAP index remained indicators associated with worse prognosis (HR =1.004, 95% CI: 1.000–1.007, P=0.042; HR =1.374, 95% CI: 1.080–1.747, P=0.010).

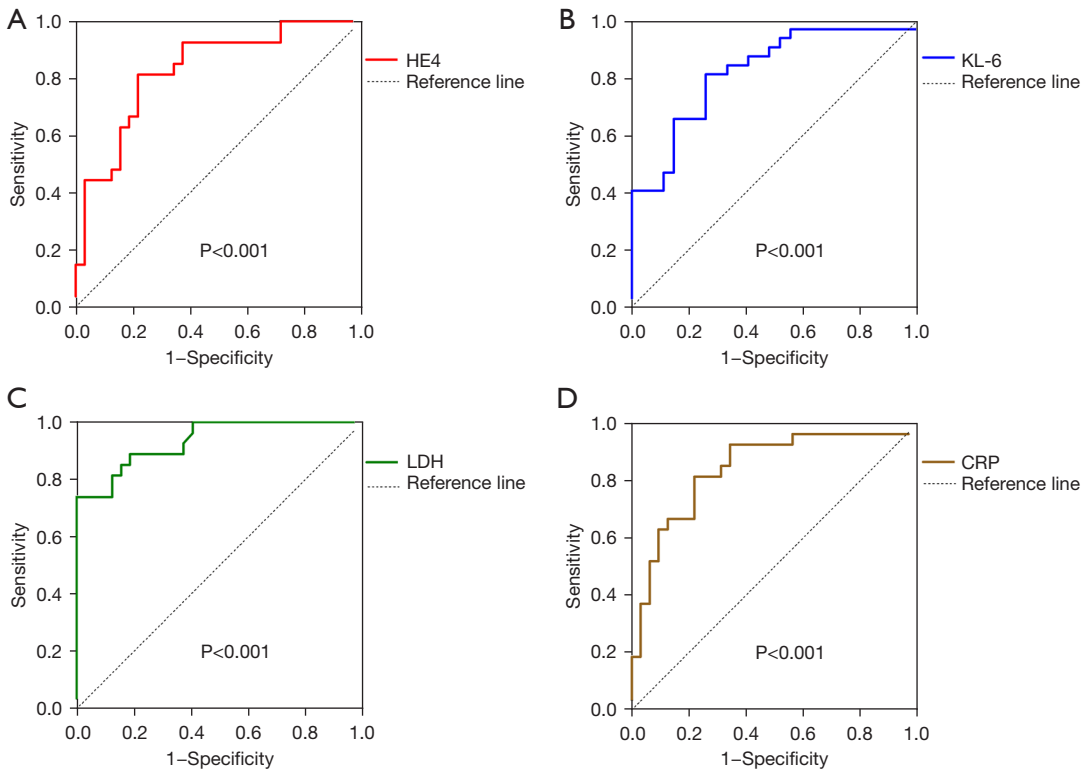

Figure 3 ROC analysis for discriminating AE-IPF from S-IPF based on serum biomarkers. The AUCs of HE4 (A), KL-6 (B), LDH (C), and CRP (D) were statistically significant in discriminating AE-IPF from S-IPF (P<0.001, AUC =0.830, 95% CI: 0.725–0.935; P<0.001, AUC =0.822, 95% CI: 0.715–0.929; P<0.001, AUC =0.935, 95% CI: 0.876–0.993; and P<0.001, AUC =0.843, 95% CI: 0.739–0.948, respectively). HE4, human epididymis protein 4; KL-6, Krebs von den Lungen-6; LDH, lactate dehydrogenase; CRP, C-reactive protein; AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis; S-IPF, stable IPF; AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

Discussion

Aberrant expression of HE4 in serum or lung sections has been well documented in CTD-ILD (17,21-23), and elevated HE4 has been associated with disease activity and systemic involvement (22,23), as well as disease severity and poor prognosis (17,21). However, the clinical value of HE4 in IPF, a progressive fibrotic pulmonary disease of unknown cause, has not been described. Our study found that the expression of HE4 in both lung tissues and serum was elevated in IPF patients. IPF patients with AE status had significantly higher levels of HE4 compared with stable subjects, which was also associated with a higher risk of mortality. In addition, serum HE4 was correlated with indicators of disease severity, and elevated HE4 levels, as well as GAP index, were associated with a worse prognosis.

HE4 is a secreted protein initially described as epididymis-specific protein and subsequently identified in multiple tissues including renal tubular epithelial cells, the oral cavity, and the respiratory tract (11,13). There is evidence that MMPs can degrade redundant deposition of ECM components under normal conditions, while HE4 inhibits the activity of several proteases, including MMP2 and MMP9, which play pivotal roles in the progression of fibrosis (13,16). The above evidence indicated that HE4 likely plays pathogenic roles in the progression of fibrosis. Although recent studies on HE4 have predominantly concentrated on its clinical value as a tumor biomarker, cumulative studies have described the important clinical significance of HE4 in other clinical settings, including CF (19), lung adenocarcinomas (11), pulmonary tuberculosis (28), as well as CTD-ILDs (17,21-23). Lin *et al.* and Zhang *et al.* reported that serum HE4 levels were obviously elevated in RA and SSc patients complicated with ILD compared with those without ILD (21,22). Chen

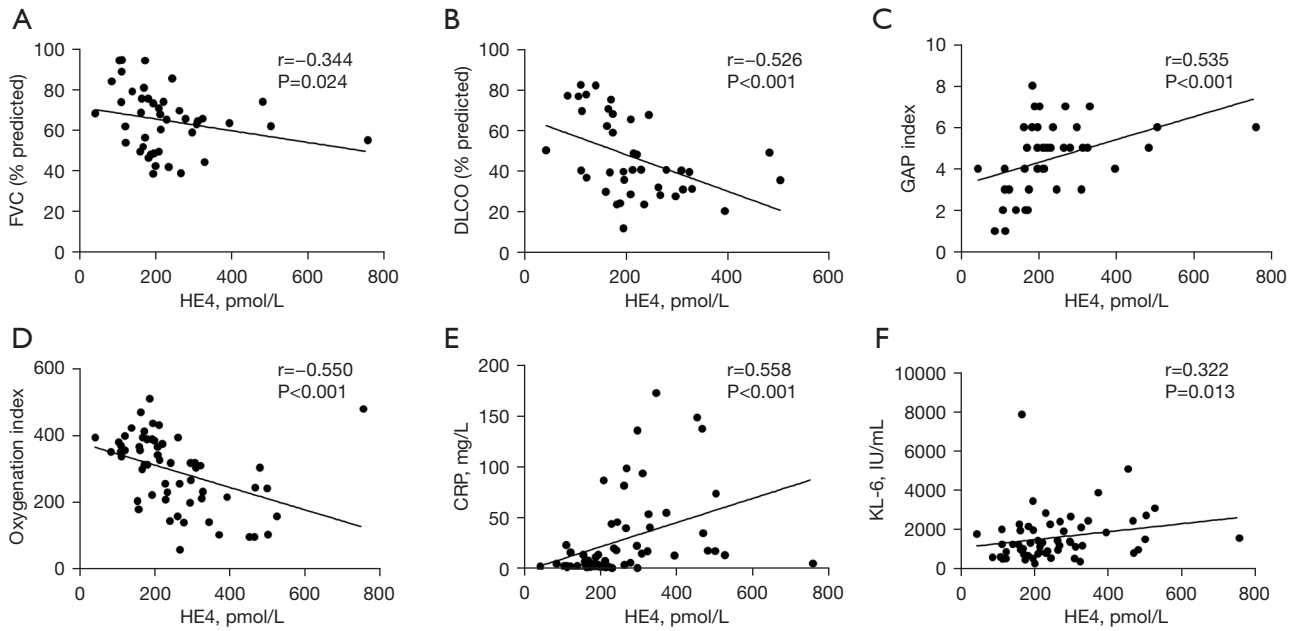

Figure 4 Correlations between serum HE4 levels and clinical parameters in IPF patients. (A,B,D) Serum HE4 inversely correlated with FVC% predicted (r=–0.344, P=0.024), DLCO% predicted (r=–0.526, P<0.001), and oxygenation index (r=–0.550, P<0.001). (C,E,F) Serum HE4 positively correlated with GAP index (r=0.535, P<0.001), CRP (r=0.558, P<0.001), and KL-6 (r=0.322, P=0.013). FVC, forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide; GAP index, gender-age-physiology index; CRP, C-reactive protein; KL-6, Krebs von den Lungen-6; IPF, idiopathic pulmonary fibrosis.

et al. subsequently validated that elevated serum HE4 was associated with disease activity and pulmonary/renal involvement in pSS, with the optimal cutoff values of 104 and 128.05 pmol/L (23). Recently, elevated serum HE4 level in IPF was reported in a prospective, case-controlled study (29), which prompted us to explore the clinical value of this biomarker in IPF patients.

Previous studies have shown that HE4 can be used as a severity indicator of CTD-ILD (17,21). As expected, our study showed that serum HE4 was elevated in AE-IPF compared with S-IPF, and elevated HE4 inversely correlated with PFTs (e.g., DLCO%, FVC%) and oxygenation index in IPF, indicating that HE4 may be involved in the progression of fibrosis and the occurrence of AE in IPF patients. Consistent with previous observations in PF-ILD (20) and CTD-ILD (17), our study demonstrated that serum HE4 could be considered an independent indicator to predict the prognosis of IPF. As a biomarker of lung epithelium-specific proteins, KL-6 can reflect well the degree of injury and regeneration of type II pneumocytes (17,30) and has been considered a promising predictor of the occurrence of AE-IPF and worse survival (31,32). In contrast, our study found that KL-6 could differentiate AE-IPF from S-IPF to some extent but was not an independent factor for the prognosis of IPF, which was consistent with previous research in other ILDs (17,20).

Previously described biomarkers reflecting the severity and prognosis of fibrosing lung diseases, including SP-A and SP-D, napsin A, as well as KL-6 (8-10), are mainly secreted by type II pneumocytes, which play pivotal roles in host defense, fibro-proliferative response, and repair/regeneration (10,33). In normal lung specimens, however, the expression of HE4 has been identified in the bronchial epithelium of the airway instead of the alveolar epithelium (11). Subsequently, overexpression of HE4 in bronchiolar epithelium from nonspecific interstitial pneumonia (NSIP) patients was detected by Nishiyama *et al.* (20). Our previous immunohistochemical analysis of RA-associated UIP (UIP-RA) patients indicated that HE4 was also primarily expressed in bronchiole epithelium instead of peripheral lung (17). In the present study, strong and relatively continuous expression of HE4 was only observed in dilated bronchi, still expressed in the mucosal epithelium, indicating the common pathological roles that

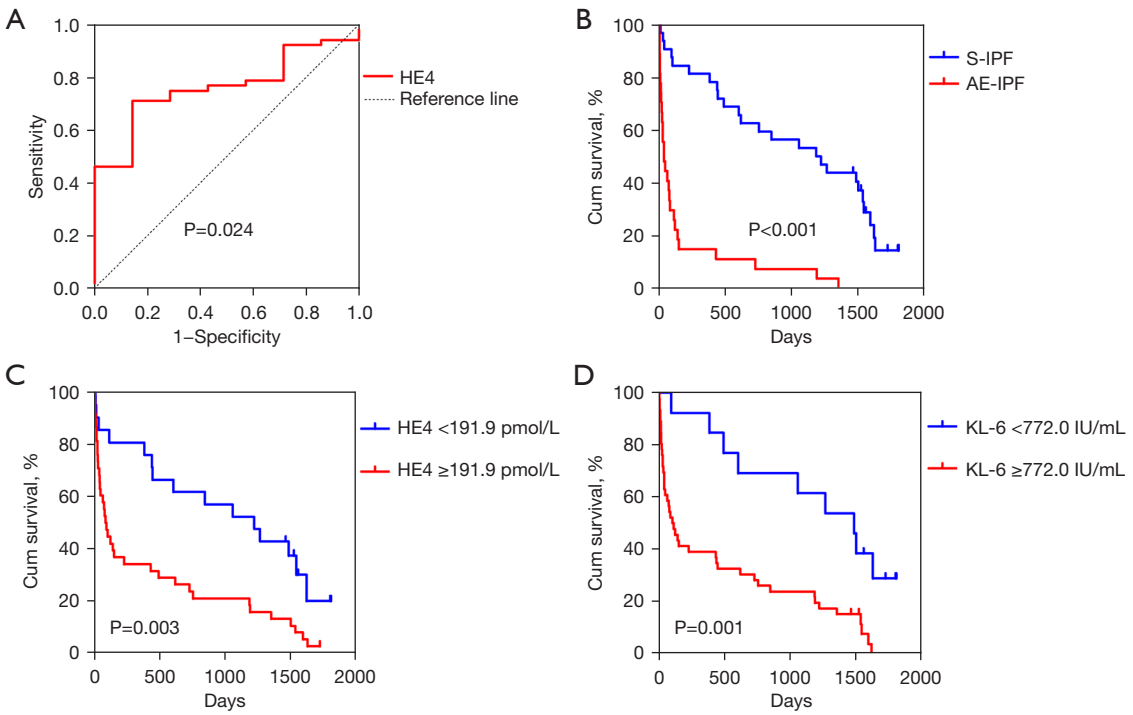

Figure 5 Prognostic value of HE4 in IPF patients and survival analysis. (A) Receiver operating characteristic curve analysis was conducted to differentiate the decedents from the survivors (AUC =0.764, 95% CI: 0.622–0.906, P=0.024). (B) Kaplan-Meier curves showed worse survival in AE-IPF compared with S-IPF (P<0.001). (C) Patients with higher serum HE4 (≥191.9 pmol/L) levels had worse survival compared with patients whose HE4 levels were <191.9 pmol/L (P=0.003). (D) Worse survival was observed in patients with higher KL-6 (≥772.0 IU/mL) levels compared with lower levels (P=0.001). HE4, human epididymis protein 4; AE, acute exacerbation; IPF, idiopathic pulmonary fibrosis; S-IPF, stable IPF; KL-6, Krebs von den Lungen-6; AUC, area under the curve; CI, confidence interval.

Table 2 Prediction of mortality in idiopathic pulmonary fibrosis patients by Cox regression analysis

Parameters	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age, years	1.038	1.002–1.075	0.040*	–	–	–
Male patient	0.463	0.165–1.299	0.143	–	–	–
Smokers	0.752	0.430–1.315	0.317	–	–	–
FVC%	0.973	0.950–0.998	0.030*	–	–	–
DLCO%	0.972	0.953–0.992	0.005**	–	–	–
LDH	1.007	1.005–1.010	<0.001***	0.999	0.992–1.008	0.902
CRP	1.016	1.008–1.025	<0.001***	0.997	0.979–1.015	0.731
HE4 (continuous)	1.004	1.002–1.006	<0.001***	1.004	1.000–1.007	0.042*
KL-6 (continuous)	1.000	1.000–1.001	0.170	1.000	1.000–1.000	0.964
GAP index	1.445	1.164–1.793	0.001**	1.374	1.080–1.747	0.010*

*, P<0.05; **, P<0.01; ***, P<0.001. DLCO, diffusing capacity of the lung for carbon monoxide; FVC, forced vital capacity; LDH, lactate dehydrogenase; CRP, C-reactive protein; HE4, human epididymis protein 4; KL-6, Krebs von den Lungen-6; GAP index, gender-age-physiology index; HR, hazard ratio; CI, confidence interval.

HE4 may play in the pathogenesis of ILD with different diagnoses.

The GAP index and GAP staging system is a multidimensional prediction model of mortality initially established by Ryerson *et al.* based on the North American population and is composed of sex, age, DLCO%, and FVC% (26). Our previous study showed that serum HE4 levels were significantly proportional to GAP stage, and shorter survival was observed in UIP-CTD patients with an advanced GAP stage compared to a lower stage (17). In the present study, HE4 level was positively correlated with the GAP index, and worse survival in IPF patients with higher GAP index were also observed. Notably, age and PFTs, including DLCO% and FVC%, largely determined the GAP index. Therefore, these variables were not included in the multivariate regression model to avoid multicollinearity.

Previous observations have described the important roles of CRP and LDH in the detection of AE status and prediction of mortality in IPF patients (31,32). As a cytoplasmatic enzyme that well reflects cell destruction or death, LDH has also been a biomarker of disease activity in various subtypes of fibrosing lung diseases, with high sensitivity but low specificity (34). The present study demonstrated that LDH was the most significant factor in identifying AE-IPF from S-IPF, with an AUC of 0.935. Consistent with Nagy *et al.* in CF (19), our study showed that serum HE4 was positively correlated with CRP and LDH, and elevated HE4 levels could also be used to detect the occurrence of AE-IPF. A growing number of studies have demonstrated that acute alveolar epithelial damage and unknown infection may play essential roles in AE of lung fibrosis (6,32), which may explain the evidently increased levels of CRP and LDH in AE-IPF. In addition, these results suggest that HE4 may be involved in the inflammatory response, except for the formation of fibrosis.

Several limitations should be considered in our study. First, this study was performed retrospectively in a small population from a single center, thus selection bias was introduced. Second, patients with inconsistent treatment regimens, such as whether they were treated with corticosteroids during hospitalization or subsequent anti-fibrosis drugs, may have affected the prognosis of disease. Third, we did not perform a longitudinal analysis of HE4 before and after treatment. Finally, overexpression of HE4 has been described in other types of ILDs, and further studies are needed to clarify whether serum HE4 can distinguish IPF from other ILDs.

In summary, our observations showed that HE4 was obviously elevated in serum and lung specimens of IPF patients and significantly correlated with indicators of disease severity. Moreover, our findings also suggested that measurement of serum HE4 was valuable for pulmonologists to evaluate the risk of AE-IPF occurrence and predict the prognosis of IPF. These findings are worthy of further validation in larger, multi-center, and longitudinal cohorts.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4042/rc

Data Sharing Statement: Available at https://atm.amegroups.com/article/view/10.21037/atm-22-4042/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-22-4042/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013) and was approved by ethics committee of Nanjing Drum Tower Hospital (No. 2016-138-01). Written informed consent to take part in this study and authorization to utilize their lung tissues and serum samples for research purposes were obtained from all participants.

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