Full title

Serum Level of Periostin Can Predict Long-term Outcome of Idiopathic Pulmonary Fibrosis

Short title

Periostin is a Biomarker of IPF

Authors

Morihiro Tajiri[a], Masaki Okamoto[a], Kiminori Fujimoto[b], Takeshi Johkoh[c], Junya Ono[d], Masaki Tominaga[a], Koichi Azuma[a], Tomotaka Kawayama[a], Shoichiro Ohta[e], Kenji Izuhara[f], and Tomoaki Hoshino[a]

Affiliations

[a]Division of Respirology, Neurology, and Rheumatology, Department of Internal Medicine, [b]Department of Radiology and Center for Diagnostic Imaging, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

[c]Department of Radiology, Kinki Central Hospital of Mutual Aid Association of Public Teachers, 3-1 Kurumazuka, Itami, Hyougo 664-8533, Japan

[d]Shino-Test Corporation, 1-56 Kanda Jinbocho, Chiyoda-ku, Tokyo 101-8410, Japan

[e]Department of Laboratory Medicine, [f]Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, 5-1-1 Nabeshima, Saga 849-8501, Japan

E-mail Addresses of Authors

Morihiro Tajiri: motajiri@med.kurume-u.ac.jp

Masaki Okamoto: okamoto_masaki@med.kurume-u.ac.jp

Kiminori Fujimoto: kimichan@med.kurume-u.ac.jp

Takeshi Johkoh: johkoht@aol.com

Junya Ono: junya.ono@shino-test.co.jp

Masaki Tominaga: tominaga_masaki@med.kurume-u.ac.jp

Koichi Azuma: azuma@med.kurume-u.ac.jp

Tomotaka Kawayama: kawayama_tomotaka@med.kurume-u.ac.jp

Shoichiro Ohta: ohtasho@cc.saga-u.ac.jp

Kenji Izuhara: kizuhara@cc.saga-u.ac.jp

Tomoaki Hoshino: hoshino@med.kurume-u.ac.jp

Corresponding author: Masaki Okamoto

Division of Respirology, Neurology, and Rheumatology, Department of Internal Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

Tel 81-942-31-7560; Fax 81-942-31-7703; Email: okamoto_masaki@med.kurume-u.ac.jp

Abstract

Background: KL-6 and surfactant proteins A and D are the only established serum biomarkers of idiopathic pulmonary fibrosis (IPF). We have previously shown that serum levels of periostin, a unique matricellular protein, are elevated and correlated with pulmonary function in patients with IPF.

We sought to determine whether the serum periostin levels correlate with overall survival (OS) and time-to-event (TTE), as a parameter reflecting long-term outcome, and with the extent of abnormality on chest high-resolution computed tomography (HRCT) scores in patients with IPF.

Methods: Twenty-nine patients with IPF were analyzed retrospectively. The mean observation period was 1035.2 ± 663.1 days (range, 112–1800 days). High-resolution computed tomography (HRCT) scores were calculated based on the extent of abnormality evidenced by HRCT. We evaluated if there were any correlations between the serum periostin levels and clinical parameters, including HRCT score, using Spearman's rank correlation coefficients and analyzed predictors of OS and TTE using the log-rank tests.

Results: We showed that the serum periostin levels significantly correlated with the increase of honeycombing score on HRCT during a 6-month period. Log-rank tests showed

that a higher serum periostin level was a predictor of a shortened OS and TTE. Greater extents of fibrotic lesions on HRCT scan were predictors of shortened OS and TTE.

Conclusions: In IPF patients, the serum periostin level may be a good predictive biomarker for an increase in the radiological fibrotic area and long-term outcome.

Key words: periostin, idiopathic pulmonary fibrosis, biomarker, high-resolution computed tomography

1.1 Introduction

Idiopathic pulmonary fibrosis (IPF), pathologically usual interstitial pneumonia (UIP), is the most common idiopathic interstitial pneumonia (IIP) of unknown etiology [1]. Previous studies have indicated that pulmonary function, 6-minute walk distance, and the serum levels of KL-6 and surfactant proteins A and D (SP-A and SP-D) can be biomarkers for predicting survival and/or disease activity in IPF patients [2–9]. High-resolution computed tomography (HRCT) is used clinically for differentiating IPF from other IIPs [10, 11]. Several studies have attempted to correlate abnormal HRCT findings with disease activity in IPF/UIP [12–15]. For example, Sumikawa et al. have reported that the prognosis of IPF is influenced by the extent of the fibrotic area on HRCT [12].

Periostin, an extracellular matrix (ECM) and matricellular protein, is composed of an EMI domain in its N-terminal portion, four tandemly lined fasciclin-1 (FAS1) domains in the middle, and an alternative splicing domain in the C-terminal portion [16, 17]. Periostin interacts with several integrin molecules (i.e., $\alpha_v\beta_1/\beta_3/\beta_5$) on the cell surface, playing an important role in the maintenance and development of bones and teeth and contributing to the progression of several tumor types [17, 18]. Many studies have shown that periostin is involved in the pathophysiology of fibrosis during the healing of myocardial infarction, bone marrow fibrosis, systemic sclerosis, allergic rhinitis, chronic rhinosinusitis, and atopic

dermatitis [16, 19–22]. We have previously reported that periostin is secreted from lung fibroblasts stimulated with interleukin (IL)-4 or IL-13, and contributes to subepithelial fibrosis in patients with bronchial asthma [23, 24]. Following treatment with the anticancer drug bleomycin, the expression of periostin has been shown to be up-regulated in the lungs of mice [25] and its expression has also been shown to be increased in the lungs and serum of patients with IIPs [26]. It has been reported that serum periostin levels in IPF patients (n = 37; 107.1 ± 11.9 ng/mL) are significantly higher than those in control subjects (n = 66; 39.1 ± 3.0 ng/mL) and that the serum periostin level correlates significantly with declines of vital capacity (VC) and diffusing capacity for carbon monoxide (D_{LCO}) during a 6-month period [26]. It is also well known that declines of VC or D_{LCO} are indicative of poor prognosis in IPF patients [2, 3]. Taken together, these findings suggest that the serum periostin level may be a prognostic predictor in IPF patients. Moreover, Naik, et al. have reported that the serum periostin level was a predictor of a decline in pulmonary function at 48 weeks in IPF [27]. However, it is still unclear whether the serum periostin level is correlated with long-term survival and/or HRCT abnormality in IPF patients. In the present study, we analyzed IPF patients for whom long-term observation was possible. We defined time-to-event (TTE) as a parameter reflecting the long-term outcome of IPF, as has been reported previously [28]. We analyzed the serum periostin levels, overall survival (OS),

TTE, and the extent of abnormal findings on HRCT (HRCT scores) in patients with IPF.

1.2 Materials and Methods

1.2.1 Study subjects

The patient characteristics are shown in Table 1. We included 29 patients (27 males, aged 66.8 ± 9.0 years) for whom long-term observations were possible. These patients had been reported previously [26] and had been under observation between 2004 and 2014 at our hospital. All patients were diagnosed with IPF in accordance with the recent official consensus statement on the diagnosis [1]. Other diseases such as connective tissue diseases, infections, and malignant diseases were excluded. All patients had been clinically stable with no disease exacerbations for more than 3 months prior to the first observation day. Diagnosis of acute exacerbation (AE) of IPF was defined in accordance with the criteria detailed in a previous report [29].

We defined the first observation date as the day of measurement of the serum periostin level. We observed the study subjects for at least 2 years, and for up to 5 years, after the first observation day. OS was defined as the period from the first to the final observation day (March 31, 2014). TTE was defined as the period from the first to the final observation day or the first day indicative of the event of IPF. We defined the events of IPF as a complicating AE or a decline from the VC baseline equal to or more than 10%, as reported previously [28]. Any patients who died of cancer were counted as censored cases.

Although 24 of 29 cases received no drug therapy at the same time on the first observation day, 5 cases received prednisolone in combination with cyclosporine A. All doses of prednisolone were equal to or less than 20 mg/day. In this study, none of the IPF patients received anti-fibrotic drugs during the follow-up period.

We received specific approval for all procedures from the Institutional Review Board (IRB) of Kurume University School of Medicine in accordance with the ethical standards of the Helsinki Declaration of 2008 (Approval date: August 2, 2008 and March 3, 2011; Approved #: 08067 and 10289, respectively) . Written informed consent was obtained from all patients.

1.2.2 HRCT image and score interpretation

HRCT examinations without contrast medium were performed at the time of the first observation day using a variety of scanners. The protocol consisted of end-inspiration in the supine position, and 0.5- to 1.5-mm collimation sections reconstructed with a high-spatial-frequency algorithm at 1-cm or 2-cm intervals. Images were interpreted at a window setting appropriate for viewing the lung parenchyma (window level, -600 to -700 Housfield units [HU]; window width, 1200 to 1500 HU).

We diagnosed IPF patients by HRCT, as reported previously [1]. Briefly, the HRCT

findings were classified as showing a definite UIP pattern when honeycombing with a predominantly peripheral and basal distribution was evident. The HRCT findings were classified as possible UIP pattern when a reticular pattern with a predominantly peripheral and basal distribution was evident, but with no honeycombing.

Two board-certificated diagnostic radiologists, who specialize in diffuse lung diseases with 24 and 22 years of experience in chest CT interpretation, evaluated the HRCT findings independently. The radiologists were blinded to the clinical information.

The two radiologists evaluated the presence, extent, and distribution of the CT features, which included the presence of ground-glass attenuation (GGA), airspace consolidation, reticulation, honeycombing, and emphysema. The lungs were divided into six zones (upper, middle, and lower on both sides), as reported previously [11–15]. The extents of all radiologic abnormalities were expressed as the percentage of lung parenchyma affected in each of the six zones, to the nearest 5% and were averaged. We defined total, fibrosis and inflammation score consisting of each of the radiologic abnormalities. We counted the scores for honeycombing, reticulation, GGA and airspace consolidation with traction bronchiectasis (TBE) into a fibrosis score. Similarly, we counted the scores of GGA and airspace consolidation without TBE into an inflammation score. The fibrosis and inflammation scores were then counted into a total score. The grade of TBE was quantified

by assessing the levels of the most proximal bronchial branches that were involved. TBE was scored as follows: 0 = none, 1 = bronchial dilatation involving bronchi distal to the fifth generation, 2 = bronchial dilatation involving fourth-generation bronchi, and 3 = bronchial dilatation involving bronchi proximal to the third-generation bronchi [12]. These TBE scores were assessed in each of the six lung zones and were averaged. HRCT images of subjects are shown in Figure 1. Disagreements with respect to the extent of the HRCT findings and the score of the TBE grade between the two radiologists were resolved by consensus after assessing the inter-observer agreement.

1.2.3 Measurement of periostin by ELISA

Serum samples were obtained from subjects and then stored at -80^{o} C for human periostin ELISA assay, which we had established previously [20–26]. Duplicated samples were assayed.

1.2.4 Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Correlations between the two parameters were evaluated using Spearman's rank correlation coefficient. Survival curves were obtained by the Kaplan-Meier method and differences in OS and TTE between

subgroups were analyzed using the log-rank test. The cut-off values were defined as the values with the highest Youden index (i.e., sensitivity + specificity – 1) on a receiver-operating characteristic (ROC) curve for distinguishing survivors from non-survivors [14]. P <0.05 was taken to represent statistical significance. All statistical analyses were performed using the JMP 10.0 (SAS Institute Japan, Tokyo, Japan).

1.3 **Results**

1.3.1 Inter-Observer agreement

Inter-observer agreement of radiologists is shown in Table 2. The agreement of the presence of HRCT abnormalities between the two observers was moderate to perfect (kappa = 0.56–1.0, all $P < 0.001$). The correlations between the two observers, in respect to the extent of the various radiologic abnormalities, were statistically significant (Spearman rank correlation coefficient, $r = 0.79–0.97$, all $P < 0.001$).

1.3.2 Characteristics and outcome of patients with IPF

The clinical data and histories of the 29 IPF patients are shown in Table 1. As we previously reported [26], the mean serum level of periostin was 98.0 ± 41.5 ng/mL.

Sixteen of the 29 subjects died during the follow-up period due to causes other than malignant disease. Two patients who died of cancers (bladder cancer and lung cancer) were counted as censored cases. Six patients were complicated AE and this was the cause of death in 3 of them. Causes of death included AE of ILD [n = 3], gradual deterioration of ILD [n = 4], infection [n = 2], heart disease [n = 3], and unknown [n = 4]. The mean observation period was 1035.2 ± 663.1 days (range 112–1800).

The observation period for the high serum periostin (≧106 ng/mL) group was shorter than that for the low serum periostin (<106 ng/mL) group (P = 0.048*). There was no difference in other variables between the high and low serum periostin groups.

1.3.3 Correlations among clinical parameters and histories in IPF patients

We analyzed whether the serum periostin level was correlated with clinical parameters and histories (Table 3). The serum periostin level was significantly correlated with the change in the honeycombing score (*P* = 0.045) on HRCT during the 6 months from the baseline but not the other HRCT scores.

Analyses of inter-group differences using the Fisher's exact test showed that the serum level of periostin was not correlated with gender, smoking status, or history of AE. Also, the serum periostin level was not correlated with the Brinkman index (data not shown). The baseline serum periostin level was not correlated with the baseline levels of KL-6 and LDH. Analysis of 12 patients whose serum periostin levels were measured at 6 months after the first observation day showed that the change in the serum periostin level (25.3 ± 32.9 ng/mL) was also not correlated with that of KL-6 (29.6 ± 571.8 IU/mL, r = 0.18, *P* = 0.59), and LDH (28.3 ± 87.6 IU/mL, r = 0.61, P = 0.060).

Our present results indicated that the serum periostin level was associated with an

increase in the area of fibrosis on HRCT scans in IPF patients.

1.3.4 Log-rank test for overall survival and time-to-event

The results of log-rank tests are shown in Table 4. Predictors of shortened OS identified as significant in log-rank test were a higher serum periostin level (P = 0.0072), a higher total (P = 0.0081), fibrosis (P = 0.014) and inflammation scores (P = 0.045), and TBE grade (P = 0.018) on HRCT. Similarly, significant predictors of shortened TTE were a higher serum periostin level (P = 0.0011) and a higher fibrosis score (P = 0.043) and TBE grade (P = 0.044) on HRCT scan (Table 4, Fig. 2). KL-6 was not a significant predictor if the cut-off level was set at 500 or 1000 IU/mL, based on the previous reports (data not shown) [5, 6]. Interestingly, honeycombing and reticulation scores were not significantly correlated with OS or TTE (data not shown). On the basis of analyses of other variables by log-rank test, a decline in VC and D_{LCO} of more than 10% was each a predictor of shortened OS and age, gender, smoking status, and emphysema score on HRCT were not associated with OS and TTE (data not shown). We did not analyze the change in HRCT score during 6 months as a predictor of OS and TTE because the number of subjects who underwent HRCT scans, at 6 months after the first observation day, was insufficient for a log-rank test. These results suggested that the serum periostin level, pulmonary function,

and the extents of the fibrotic areas on HRCT scans could be used to predict OS and TTE in IPF patients.

1.4 Discussion

In IPF patients, the need for biomarkers obtained by non-invasive and simple examinations has been highlighted. KL-6, SP-A, and SP-D are clinically used as serum biomarkers for IPF. However, there is little evidence to support their association with survival and response to therapies [6, 9]. In the present study, we show for the first time that the serum periostin level can be a predictor of shortened OS and TTE in IPF patients. The serum periostin level can be a predictor of long-term outcome in IPF patients. In contrast, serum KL-6 and LDH levels had no prognostic value for IPF. In addition, the serum periostin level might reflect a different mechanism of KL-6 release in IPF patients because there was no significant correlation between the two parameters. IPF patients with emphysema were thought to have different survivals from those without emphysema [30]. However, the extent of emphysema might not influence the results of the present study because the emphysema score on HRCT (mean value; 5.8 ± 5.6%) was not correlated with serum periostin level, OS, and TTE.

In addition, the extents of fibrotic areas and TBE grade on HRCT were significant predictors of shortened OS and TTE in the present study. These results are thought to be consistent with the previous study [12]. Although the honeycombing score was not a predictor of OS and TTE in the present study, this result might have been influenced by the

small sample size. These results suggested that the serum periostin level and the extent of the fibrotic lesion on HRCT were predictors of long-term outcome in IPF patients.

The honeycombing on HRCT scan may reflect the presence of histopathological fibrotic lesions in IPF [11–15]. Fibroblastic foci (FF) consisting of myofibroblasts and ECM proteins are thought to be key factors in the fibrotic process of IPF [31, 32]. King, et al. reported that FF represented the earliest manifestation of ongoing lung fibrosis and was correlated with survival in IPF patients [33]. In our previous study, we had shown that periostin was strongly expressed in FF but not in regenerative alveolar epithelium or macrophages, small airways, areas showing established fibrosis, or inflammatory cells in lung tissues obtained from IPF patients [26]. Moreover, serum periostin level significantly correlated with the 6-month changes in VC ($n = 26$, Spearman $r = -0.498$, $P < 0.01$) and D_{LCO} ($n = 21$, Spearman $r = -0.467$, $p < 0.05$), previously [26]. Moreover, our present study showed that the serum level of periostin was associated with an increased extent of honeycombing, reflecting marked fibrosis in IPF patients. Taken together, these findings suggest that periostin may play an important role in ongoing fibrosis and disease progression in IPF.

Tenascin-C is one of the ECM proteins produced mainly by myofibroblasts. The expression level of tenascin-C in the lungs is correlated with survival in IPF patients [34, 35]. No

previous report has indicated that the serum level of ECM protein can be a biomarker of IPF. Periostin has unique functions in promoting the epithelial-mesenchymal transition (EMT) and development of various cancers via the integrin / phosphatidylinositol 3-kinase/Akt pathway [36]. As the EMT is one origin of lung fibroblasts and is associated with the fibrotic process in IPF [31], periostin may contribute to the fibrotic process via the EMT in IPF.

The first limitation of this study is the small sample size because of a study in a single center. We will analyze the significance of this biomarker in a further study of larger populations. The second limitation is that drug therapy on the first observation day might have influenced the serum periostin levels. However, we believe that any such influence would likely have been small because the doses of prednisolone were relatively low.

In conclusion, the serum periostin level can be a good biomarker for predicting an increase in the radiological fibrotic area and long-term outcome in patients with IPF.

1.5 Acknowledgment

We thank Dr. Howard A. Young (NCI-Frederick, Frederick, MD) for editing and critically reviewing this manuscript.

1.6 Conflict of interest

Masaki Okamoto, corresponding author was supported by grants from Japan Foundation for Aging and Health, Ishibashi's Fund for the Promotion of Science, and Japan Society for the Promotion of Science.

Kenji Izuhara was supported by grants from Shino-test Co. Ltd and Chugai Pharmaceutical Co. Ltd.

Tomotaka Kawayama received lecture fees from Novartis Pharmaceuticals Japan, Boehringer Ingelheim Japan, and GSK Japan and received a grant from GSK Japan.

Takeshi Johkoh received lecture fees from Daiichi-Sankyo Co. Ltd, Takeda Co. Ltd, Kyorin Co. Ltd, Eizai Co. Ltd, and Shionogi Co. Ltd.

Tomoaki Hoshino received a grant from Novartis Pharmaceuticals Japan, Chugai Pharmaceutical Co. Ltd., and GSK Japan.

Other co-authors have no potential conflict of interest.

1.7 References

[1] Raghu G, Collard HR, Egan JJ, 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**: 788-824.

[2] Flaherty KR, Mumford JA, Murray S, et al. Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J Respir Crit Care Med. 2003; **168**: 543-8.

[3] Latsi PI, du Bois RM, Nicholson AG, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med. 2003; **168**: 531-7.

[4] du Bois RM, Albera C, Bradford WZ, et al. 6-minute walk test distance is an independent predictor of mortality in patients with idiopathic pulmonary fibrosis Eur Respir J. 2013, in press.

[5] Kohno N, Awaya Y, Oyama T, et al. KL-6, a mucin-like glycoprotein, in bronchoalveolar lavage fluid from patients with interstitial lung disease. Am Rev Respir Dis. 1993; **148**: 637-42.

[6] Yokoyama A, Kondo K, Nakajima M, et al. Prognostic value of circulating KL-6 in idiopathic pulmonary fibrosis. Respirology. 2006; **11**: 164-8.

[7] Ishikawa N, Hattori N, Yokoyama A, et al. Utility of KL-6/MUC1 in the clinical management of interstitial lung diseases. Respir Investig. 2012; 50: 3-13.

[8] Honda Y, Kuroki Y, Matsuura E, et al. Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. Am J Respir Crit Care. 1995; **152** (6 Pt 1): 1860-6.

[9] Greene KE, King TE Jr, Kuroki Y, et al. Serum surfactant proteins-A and -D as biomarkers in idiopathic pulmonary fibrosis. Eur Respir J. 2002; **19**: 439-46.

[10] Hunninghake GW, Zimmerman MB, Schwartz DA, et al. Utility of a lung biopsy for the diagnosis of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2001; **164**: 193-6.

[11] Sumikawa H, Johkoh T, Fujimoto K, et al. Usual interstitial pneumonia and nonspecific interstitial pneumonia: Correlation between CT findings at the site of biopsy with pathological diagnoses. Eur J Radiol. 2012; **81**: 2919-24.

[12] Sumikawa H, Johkoh T, Colby TV, et al. Computed tomography findings in pathological usual interstitial pneumonia: relationship to survival. Am J Respir Crit Care Med. 2008; **177**: 433-9.

[13] Gay SE, Kazerooni EA, Toews GB, et al. Idiopathic pulmonary fibrosis: predicting response to therapy and survival. Am J Respir Crit Care Med. 1998; **157**: 1063-72.

[14] Fujimoto K, Taniguchi H, Johkoh T, et al. Acute exacerbation of idiopathic pulmonary

fibrosis: high-resolution CT scores predict mortality. Eur Radiol. 2012; **22**: 83-92.

[15] Ichikado K, Suga M, Müller NL, et al. Acute interstitial pneumonia: comparison of high-resolution computed tomography findings between survivors and nonsurvivors. Am J Respir Crit Care Med. 2002; **165**: 1551-6.

[16] Oka T, Xu J, Kaiser RA, et al. Genetic manipulation of periostin expression reveals a role in cardiac hypertrophy and ventricular remodeling. Circ Res. 2007; **101**: 313-21.

[17] Ruan K, Bao S, Ouyang G. The multifaceted role of periostin in tumorigenesis. Cell Mol Life Sci. 2009; **66**: 2219-30.

[18] Fujimoto K, Kawaguchi T, Nakashima O, et al. Periostin, a cellular matrix protein, has potential as a novel serodiagnostic marker for cholangiocarcinoma. Oncol Rep. 2011; **25**: 1211-6.

[19] Oku E, Kanaji T, Takata Y, et al. Periostin and bone marrow fibrosis. Int J Hematol. 2008; **88**: 57-63.

[20] Yamaguchi Y, Ono J, Masuoka M, et al. Serum periostin levels are correlated with progressive skin sclerosis in patients with systemic sclerosis. Br J Dermatol. 2013; **168**: 717-25.

[21] Ishida A, Ohta N, Suzuki Y, et al. Expression of pendrin and periostin in allergic rhinitis and chronic rhinosinusitis. Allergol Int. 2012; **61**: 589-95.

[22] Shiraishi H, Masuoka M, Ohta S, et al. Periostin contributes to the pathogenesis of atopic dermatitis by inducing TSLP production from keratinocytes. Allergol Int. 2012; **61**: 563-72.

[23] Takayama G, Arima K, Kanaji T, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. J Allergy Clin Immunol. 2006; **118**: 98-104.

[24] Hayashi N, Yoshimoto T, Izuhara K, et al. T helper 1 cells stimulated with ovalbumin and IL-18 induce airway hyperresponsiveness and lung fibrosis by IFN-gamma and IL-13 production. Proc Natl Acad Sci USA. 2007; **104**: 14765-70.

[25] Uchida M, Shiraishi H, Ohta S, et al. Periostin, a matricellular protein, plays a role in the induction of chemokines in pulmonary fibrosis. Am J Respir Cell Mol Biol. 2012; **46**: 677–86.

[26] Okamoto M, Hoshino T, Kitasato Y, et al. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. Eur Respir J. 2011; **37**: 1119-27.

[27] Naik PK, Bozyk PD, Bently JK, et al. Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2012; **303**: 1046-56.

[28] Taniguchi H, Ebina M, Kondoh Y, et al. Pirfenidone in idiopathic pulmonary fibrosis.

Eur Respir J. 2010; **35**: 821-9.

[29] Collard HR, Moore BB, Flaherty KR, Brown KK, Kaner RJ, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2007; **176**: 636-43.

[30] Jankowich MD, Rounds SI. Combined pulmonary fibrosis and emphysema syndrome: a review. Chest. 2012; 141:222-31.

[31] Strieter RM, Mehrad B. New mechanisms of pulmonary fibrosis. Chest. 2009; **136**: 1364-70.

[32] Horowitz JC, Thannickal VJ. Idiopathic pulmonary fibrosis: new concepts in pathogenesis and implications for drug therapy. Treat Respir Med. 2006; **5**: 325–42.

[33] King TE Jr, Schwarz MI, Brown K, et al. Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med. 2001; **164**: 1025–32.

[34] Kaarteenaho-Wiik R, Mertaniemi P, Sajanti E, et al. Tenascin is increased in epithelial lining fluid in fibrotic lung disorders. Lung. 1998; **176**: 371-80.

[35] Kaarteenaho-Wiik R, Tani T, Sormunen R, et al. Tenascin immunoreactivity as a prognostic marker in usual interstitial pneumonia. Am J Respir Crit Care Med. 1996; **154**: 511-8.a

[36] Ruan K, Bao S, Ouyang G. The multifaceted role of periostin in tumorigenesis. Cell

Mol Life Sci. 2009; **66**: 2219-30.

Figure legends

Figure 1. HRCT findings.

Images of HRCT scans of a 59-year-old woman show GGA (A, black arrow) and airspace consolidation (B, black arrows). Those of a 67-year-old man show reticulation (C, black arrow) and honeycombing (D, black arrow). Figures 1E and G show images of a 77-year-old man with grades 1 (E, white arrows) and 3 (G, black arrow) TBEs. Figure 1F shows an image of 59-year-old woman with grade 2 (F, white and black arrows) TBEs.

Figure 2. **Survival curves for overall survival and time-to-event using the Kaplan-Meier Method**

TBE; traction bronchiectasis