Progranulin And Activin A Concentrations Are Elevated In Serum From Patients With Acute Exacerbations of Idiopathic Pulmonary Fibrosis

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Research Article

Keywords: idiopathic pulmonary fibrosis, acute exacerbation, Progranulin, activin A

Posted Date: April 26th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-398721/v1

Abstract

Purpose To examine the serum concentrations of Progranulin (PGRN) and activin A in patients with acute exacerbations of idiopathic pulmonary fibrosis (AE-IPF) in a pilot study.

Methods Twenty-one patients with AE-IPF were compared with 23 patients with stable idiopathic pulmonary fibrosis (IPF) as a control group. Serum PGRN and activin A levels, arterial blood gas measurements and lung function were determined in these two groups. Students t-test was used to compare the differences in PGRN and activin A levels between the two groups, and Spearman correlation coefficient was used to examine the relationship between serum PGRN and activin A levels with carbon monoxide diffusion capacity in patients with IPF.

Results Peripheral blood PGRN and activin A levels in patients with AE-IPF were 83.7+10.0 and 14.2$\pm$1.7 ng/ml (mean$\pm$SD), respectively, higher than those in the control group 61.0+5.8, and 5.8$\pm$1.0 ($p<0.001$). PGRN and activin A levels were significantly negatively correlated with carbon monoxide diffusion capacity ($r=-0.857$ $p<0.001$) and $r=-0.757$ ($p<0.001$).

Conclusion: PGRN and activin A may be involved in the pathogenesis of AE-IPF. They may be possible markers of activity in AE-IPF.

Introduction

IPF is a chronic, progressive, fibrotic interstitial pneumonia, the pathogenesis of which is not fully understood. There are currently no very effective therapeutic agents other than lung transplantation, although pirfenidone and nintedanib are thought to slow deterioration of lung function or reduce the frequency of acute exacerbations to some extent. The overall prognosis remains poor[1]. Most patients experience progressive decline over time but the clinical course can be highly variable[2]. Acute exacerbations of IPF represent sudden deteriorations in lung function occurring due to sudden accelerations in lung disease or superimposed acute lung injury which may be idiopathic.

Progranulin(PGRN) is a 539-amino acid secreted multifunctional growth factor thought to be involved in a variety of physiological and disease processes including embryogenesis, wound healing, inflammation, tumorigenesis, and host defence. PGRN is widely expressed in immune cells such as epithelial cells, macrophages, dendritic cells and also inneurones. It plays a pro-inflammatory role in post-injury repair, diabetes in the presence of insulin resistance, obesity, and an anti-inflammatory role in lipopolysaccharide-induced acute pneumonia, acute cerebral ischemia, and various autoimmune diseases in mice[3]. There is evidence of its involvement in wound healing and diverse conditions such as psoriasis and colitis in humans and in mouse models[4]. In a recent study serum PGRN was significantly higher in dermatomyositis (DM) patients than in polymyositis or healthy controls. Concentrations were significantly raised in DM combined with interstitial lung disease, and may be associated with reduced 6 month survival in this group[5]. Activin A is a multipotent cytokine, a member of the transforming growth

factor beta (TGF-b) superfamily, that is involved in inflammatory, tumour and fibrotic processes in a variety of tissues and organs, and its expression is elevated in idiopathic pulmonary fibrosis[6].

We have found no studies on PGRN or activin A in acute exacerbations of idiopathic pulmonary fibrosis, and this study aimed to examine the concentrations of PGRN and activin A in the serum of patients with acute exacerbations and compare them with those in stable idiopathic pulmonary fibrosis to investigate any potential pathogenetic significance. The possibility that one of both proteins might have potential as clinical markers of AE-IPF was also considered.

Materials And Methods

General data

Patients were selected from those who were hospitalized in Hainan Genaral Hospital for AE-IPF between January 2017 and June 2020. All met the diagnostic criteria for AE-IPF[1]:①Previous or concurrent diagnosis of IPF.②Acute worsening or development of dyspnea typically≤1 month duration ③ Computed tomography with new bilateral ground-glass opacity and/or consolidation superimposed on a background pattern consistent with usual interstitial pneumonia pattern ④Deterioration not fully explained by cardiac failure or fluid overload. The control group included patients with stable IPF who regularly attended follow up in the Respiratory Medicine Outpatient clinic of Hainan General Hospital. We applied the following criteria of IPF[7]：(1)Exclusion of other known causes of interstitial lung disease (2)The presence of a UIP pattern on high-resolution computed tomography (HRCT): Subpleural, basal predominance, reticular abnormality, honeycombing with or without traction bronchiectasis, absence of features listed as inconsistent with UIP pattern. All the patients accepted multidisciplinary discussion among respiratory physicians, imaging physicians and pathologists with rich experience in the diagnosis of ILD. None of the patients underwent surgical lung biopsy. Exclusion criteria included IPF patients with concomitant: (1)chronic obstructive pulmonary disease (COPD), asthma, bronchiectasis, pulmonary infection, pulmonary embolism or other respiratory diseases; (2) malignancy; (3) stroke, Alzheimer's disease, acute coronary syndrome, type 2 diabetes mellitus, obesity (BMI ≥ 30 kg/m2); (4) renal or hepatic dysfunction, cirrhosis, fibrosis; (5) Autoimmune diseases such as psoriasis, dermatomyositis, or rheumatoid arthritis; (6) patients receiving or who had received immunosuppressant or glucocorticoid therapy within the previous three months. The experimental protocol was approved by the Human Ethics Committee of Hainan General Hospital. Written informed consent was obtained from individual participants. The study group included 21 patients: 19 males and 2 females, aged between 52 and 71 with a mean age of 65.2 ± 6.8 years. The control group included 23 patients; 20 males and 3 females, aged between 49 and 68, with a mean age of 62.4 ± 5.7 years.

Methods

5 ml of peripheral venous blood was drawn from both groups of fasting patients within 24 hours after enrolment. The supernatant was extracted by centrifugation at 3000 rpm for 10 minutes. Serum levels of

PGRN and activin A were measured using enzyme-linked immunoassays(ELISA) kits (R&D Systems, USA) according to the product's specification. According to the kit specification, we have diluted the samples before performed the test. Blood gas analysis (Blood gas analyzer Radiometer ABL80) was performed immediately after 2 ml of peripheral arterial blood was collected. Pulmonary function tests (Medi soft Micro 5000, Belgium) were performed within 48 hours after enrolment. The main variables recorded were the measured/expected ratio of Forced Vital Capacity (FVC) as percent predicted and diffusion capacity for carbon monoxide (DLCO). Spirometry was conducted by the same physician according to international guidelines by a PFT spirometer (Medi soft Micro 5000, Belgium)

Statistical analysis

Using SPSS17.0 statistical software to analyse the data, patients' age, PGRN and activin A concentration, blood gas measurements, FVC and DLCO were expressed as $\bar{x}\pm s$. The differences between groups were analysed by Students t-test. Discrete data such as patient gender ratio and antifibrotic treatment were analyzed by χ2 test. Correlations between PGRN and activin A levels and DLCO in all patients were analysed by Spearman correlation analysis. P-values less than 0.05 were considered significant. All tests were two-tailed. P<0.05 was taken as a statistically significant difference.

Results

There were no statistically significant differences in general characteristics such as age, gender, duration of IPF and basic anti-fibrotic treatment status between the two groups, see Table 1 for details (p>0.05). The serum levels of PGRN and activin A in the study group were significantly higher than those in the control group (p<0.05).The level of A-a gradient of blood gas analysis in the study group was significantly higher than that in the control group (p<0.05). The levels of DLco and FVC%pred in the study group were significantly lower than those in the control group (p<0.05)(see Table 2 for details). A significant negative correlation was demonstrated between PGRN and DLCO; Spearman Correlation coefficient r=--0.859,p<0.001 indicated that serum PGRN concentration decreased as DLCO increased (see Figure 1). A significant negative correlation was demonstrated between activin A and DLCO; Spearman Correlation coefficient r=-0.757,p<0.001 indicated that serumactivin A concentration decreased as DLCO increased (see Figure 2).

Table 1
General characteristics of the enrolled participants

	AE-IPF	control group	χ^2/t	p-value
Number of patients	21	23		
sex(male)	19(90.5%)	20(87.0%)	χ^2 = 0.012	0.914
Age,mean ± SD	65.2 ± 6.8	62.4 ± 5.7	t = 1.485	0.145
duration of IPF,mean ± SD	1.2 ± 0.3	1.0 ± 0.5	t = 1.589	0.119
basic anti-fibrotic therapy*	9(42.86%)	12(52.17%)	χ^2 = 0.100	0.752

* basic anti-fibrotic therapy include pirfenidone and nintedanib

Table 2 Comparison of serum levels of PGRN, activin A, A-a gradient, DLCO and forced vital capacity in the two groups.

	AE-IPF	control group	*t*	*p*-value
PGRN（ng/ml）,mean ± SD	83. 7±10.0	61.0±7.3	8.64	＜0.001
activin A(ng/ml), mean ± SD	14.2±1.7	5.8±1.0	19. 7	＜0.001
A-a gradient , mean ± SD	38.2±5.28	34.7±3.61	2.58	0.013
DLco, mean ± SD	33.8±5.5	50.6±5.4	-6.79	＜0.001
FVC%pred, mean ± SD	39.4±5.8	48±6.0	-4.566	＜0.001

A-a gradient: The difference in blood oxygen pressure between alveoli and arterial capillaries

DLco: Carbon monoxide dispersio

FVC: forced vital capacity %pred:percentage predicted

Discussion

Idiopathic pulmonary fibrosis is the commonest form of idiopathic interstitial pneumonia the pathogenesis is of which is not understood though epithelial cell injury is thought to underlie abnormal healing and subsequent fibrosis. The natural history is variable; a steady predictable decline is common but some patients experience acute deteriorations of unknown aetiology termed AE-IPF. This small, single centre study has shown significant elevations of PGRN and activin A in patients with AE-IPF compared with stable IPF. No a priori power calculation was possible to determine sample size as no previous

estimates of PGRN or activin A were available in this population but this was intended as a pilot study. The PGRN concentrations determined in this study were similar to those measured by Tanaka et al in DM with ILD [5]. The negative correlations of activin A and PGRN with DLCO is of interest, though preliminary, as it could represent only linear interpolation of data from 2 distinct populations. However, if real, it may imply some cause and effect relationship ie that whatever processes reflected by increase in these growth factors, bearing in mind their multiple possible cellular origins and their multiplicity of effects, may relate directly to acute deterioration in the diffuse alveolitis and disorganization of alveolar structures, ultimately leading to an acute exacerbation of the interstitial fibrosis. The mechanism may be that PGRN and activin A are involved in the inflammatory response to common triggers of acute exacerbations of idiopathic pulmonary fibrosis such as bacterial or viral infections, gastric contents aspiration, and air pollution. Even if not mechanistically important PGRN and activin A could potentially be useful clinically as markers of AE-IPF.

Sputum PGRN concentrations have been reported to be higher in acute exacerbations of COPD (AECOPD) than in stable COPD patients or controls and to be negatively correlated with FEV_1. It has been suggested that PGRN might be an independent predictor of AECOPD[8]. However, in asthma, including occupational asthma, serum levels were reduced compared to controls[9,10], Serum PGRN concentrations were reportedly raised in acute community acquired pneumonia and correlated with poor prognosis[11]. In mouse models of cigarette smoke induced epithelial injury[12], endotoxin shock[13] and an LPS-induced acute lung injury model[14], PGRN depletion (knockdown) increases inflammation while over-expression or pre-treatment reduces it.

Activin A, a member of the transforming growth factor β family, is importantly involved in angiotensin II-mediated atrial fibrosis and in promoting fibroblast differentiation of endometrial mesenchymal stem cells[15,16]. It participates in fibrosis in liver, pancreas, and kidneys [17]. Activin A can stimulate fibroblast differentiation into myofibroblasts inducing the proliferation of lung fibroblasts, airway smooth muscle cells, and bronchial epithelial cells[18]. Levels were raised in airways severe asthma[19] and in the serum of patients with COPD compared to healthy controls [20]. Activin A and activin B are expressed at increased levels in alveolar epithelial cells and inflammatory cells in patients with idiopathic pulmonary fibrosis[6], and the activin A antagonist follicle suppressor can also reduce bleomycin-induced pulmonary fibrosis[17].

Potential weaknesses of this study are the small sample size and the limited number of relevant parameters measured. Factors related to IPF but also possible co-morbidities need to be further refined to exclude bias. Measurements were made only at a single time point and there is no sequential information about evolution of the signals. Future, larger prospective, ideally longitudinal, studies will be needed to confirm these findings and to focus on trying to dissect possible pro-inflammatory and anti-inflammatory effects of PGRN and activin A, and their mechanisms, in IPF and particularly in AE-IPF. Comparison of PGRN and activin A with other possible markers of disease activity in IPF eg KL-6[21)] are urgently needed to assist diagnosis and treatment of acute exacerbations of idiopathic pulmonary fibrosis.

Declarations

Acknowledgements

This paper was supported by the Hainan Provincial Natural Science Foundation of China(819QN354).

Funding: This study was supported by the Hainan Provincial Natural Science

Foundation of China(819QN354).

Conflicts of interest: The authors confirm that there are no conflicts of interest.

Availability of data and material: All data and material generated or used during the study appear in the submitted article.

Code availability : Not applicable

Authors' contributions : Tian Xie designed the research and wrote the paper. Lizhen Han analyzed the data. Yongxing Chen collected the data. Haihong Wu designed the research.

Ethics approval: The experimental protocol was approved by the Human Ethics Committee of Hainan General Hospital. Consent to participate (include appropriate statements)

Consent to participate: Written informed consent was obtained from individual participants.

Consent for publication: Not applicable.

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Figures

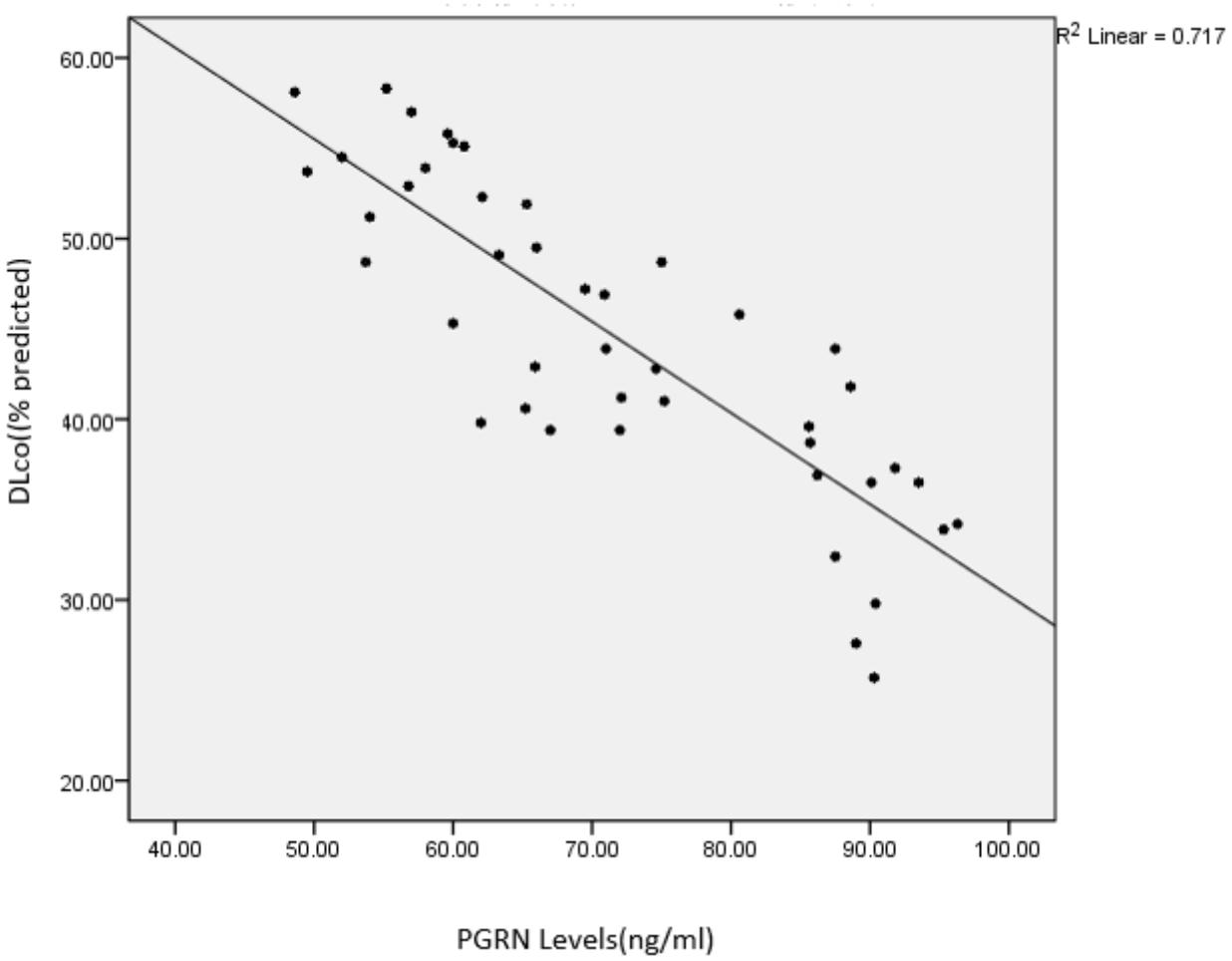

Figure 1

Correlation between PGRN levels and DLco. Negative correlation between PGRN and DLco in IPF patients.

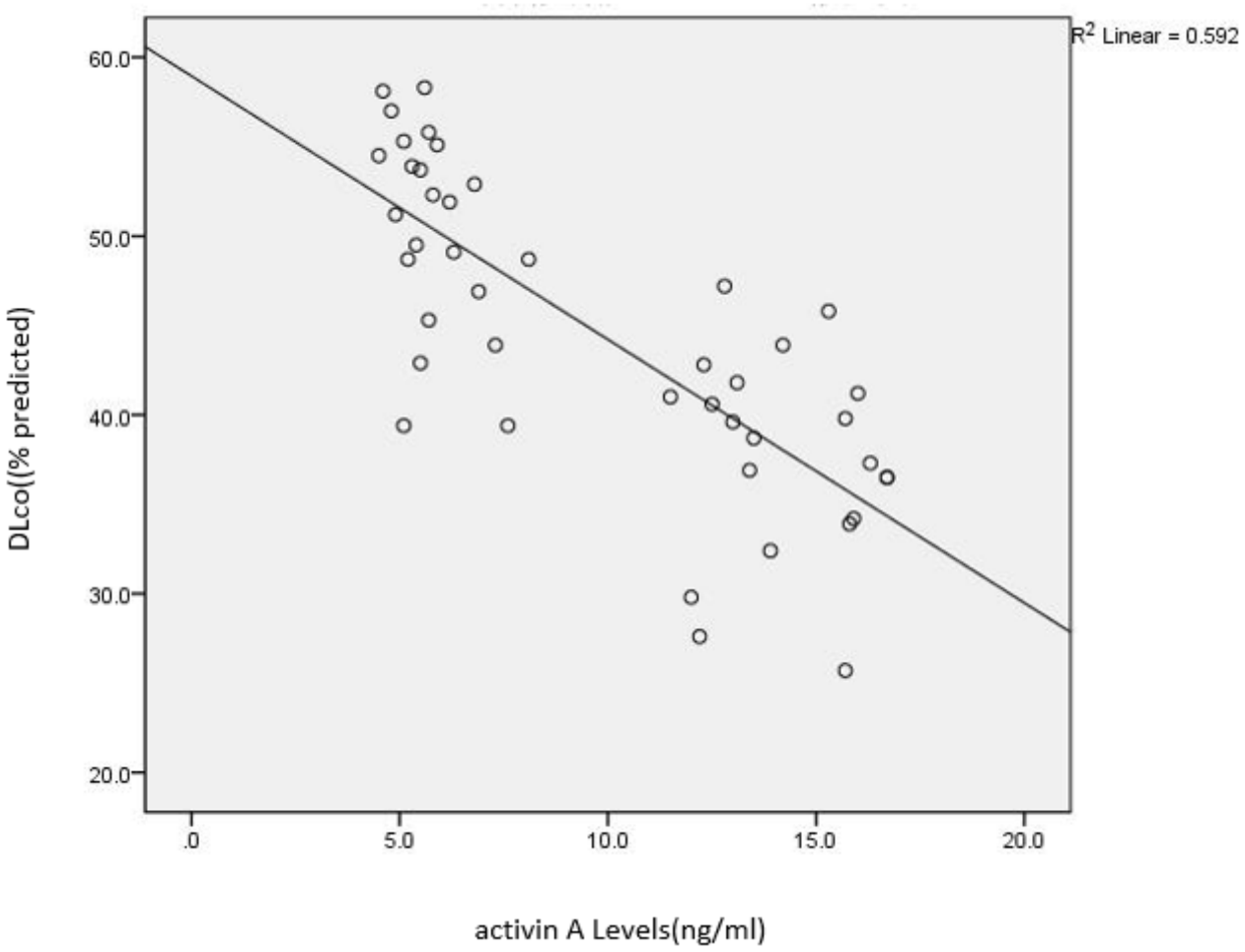

Figure 2

Correlation between activin A levels and DLco. Negative correlation between activin A and DLco in IPF patients.