ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original research article

# Serum autotaxin levels in chronic disease and acute exacerbation of fibrosing interstitial lung disease

Takuma Isshiki, Hiroshige Shimizu, Susumu Sakamoto, Akira Yamasaki, Shion Miyoshi, Yasuhiko Nakamura, Sakae Homma, Kazuma Kishi

Please cite this article as: Isshiki T, Shimizu H, Sakamoto S, *et al*. Serum autotaxin levels in chronic disease and acute exacerbation of fibrosing interstitial lung disease. *ERJ Open Res* 2022; in press (https://doi.org/10.1183/23120541.00683-2021).

Original research article

# Serum autotaxin levels in chronic disease and acute exacerbation of fibrosing interstitial lung disease

Takuma Isshiki[1], Hiroshige Shimizu[1], Susumu Sakamoto[1], Akira Yamasaki[1], Shion Miyoshi[1], Yasuhiko Nakamura[1], Sakae Homma[2], Kazuma Kishi[1]

1 Department of Respiratory Medicine, Toho University Omori Medical Center, Japan

2 Department of Advanced and Integrated Interstitial Lung Diseases Research, School of Medicine, Toho University, Japan

**Correspondence to:**

Takuma Isshiki

Respiratory Medicine

Toho University Omori Medical Centre

Ota-ku Omori nisi 6-11-1, Tokyo 143-8541, Japan.

Tel.: +81 3 3762 4151; Fax.: +81 3 3766 3551

E-mail: takuma.isshiki@med.toho-u.ac.jp

**Short title:** Serum autotaxin in fibrosing ILD

**Key words:** Autotaxin, PF-ILD, IPF, lysophosphatidic acid, acute exacerbation

**ABSTRACT**

***Background*:** Autotaxin (ATX) is an ecto-enzyme that catalyzes the hydrolysis of lysophospholipids to the lipid mediator lysophosphatidic acid (LPA). LPA/ATX signaling has emerged as a new therapeutic target for pulmonary fibrosis; however, the serum levels and dynamics of ATX during the clinical course of fibrosing interstitial lung disease (ILD) remain unknown.

***Objectives*:** This study sought to examine the serum ATX levels in fibrosing ILD in the chronic phase and in acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF). We aimed to elucidate the association between serum ATX level and clinical characteristics including disease progression and prognosis.

***Methods*:** In total, 119 patients with fibrosing ILD and 38 healthy volunteers as controls were enrolled in the study and their serum ATX activity was analyzed. We also included 6 male patients with AE-IPF in order to analyze the changes in serum ATX at the onset of AE-IPF.

***Results*:** Patients with fibrosing ILD showed significantly higher serum ATX levels compared with healthy controls in both genders. Percent change in forced vital capacity after 1 year correlated with serum ATX levels in female patients. High serum ATX levels (>0.721 mg/L) were associated with worse outcome in survival curve and multivariate analysis of male patients. Serum ATX activity decreased after the onset of AE-IPF.

***Conclusion*:** Serum ATX levels were significantly higher in patients with fibrosing ILD compared with healthy controls and this was associated with disease progression and outcome. This suggests the potential of serum ATX as a promising biomarker for the treatment of fibrosing ILD.

## Background

Fibrosing interstitial lung disease (ILD) is a heterogenous group of lung disorders characterized by fibrosis in the lung interstitium. Idiopathic pulmonary fibrosis (IPF), a subtype of fibrosing ILD, is a representative phenotype with radiological and/or histopathological usual interstitial pneumonia (UIP) pattern in which chronic and progressive pulmonary fibrosis of indeterminable cause develops in the lung and finally results in respiratory failure [1]. The clinical course of IPF is usually chronic, however, some patients develop an acute exacerbation of IPF (AE-IPF) with severe worsening of respiratory symptoms and grave prognosis after the onset [2]. Other subtypes of ILD, such as non-specific interstitial pneumonia (NSIP), connective tissue disease-associated ILD (CTD-ILD), unclassifiable ILD, and pleuroparenchymal fibroelastosis also have a progressive fibrotic phenotype[3].

Lysophosphatidic acid (LPA), a bioactive lipid mediator, has been reported to be associated with the development and progression of pulmonary fibrosis through binding to its receptors [4–7]. Autotaxin (ATX) is an extracellular enzyme that catalyzes the hydrolytic conversion of lysophospholipids, such as lysophatidylcholine (LPC), to LPA by its phospholipase D activity [8]. ATX is highly expressed in the human fibrotic lung and pharmacologic inhibition of ATX results in attenuation of bleomycin-induced pulmonary fibrosis [9]. The ATX/LPA axis has now emerged as a promising therapeutic target for pulmonary fibrosis and several clinical studies have been investigating the possible effectiveness of ATX and LPA inhibitors in patients with IPF and progressive fibrosing ILD [10–12]. However, to our knowledge, the association between LPA and ATX levels in blood and the attendant clinical characteristics have not been fully investigated in patients with IPF and other types of fibrosing ILD. Measuring serum ATX levels is more advantageous than LPA

measurements in the clinical setting. Primarily, ATX is stable without requiring strict temperature control after sample preparation while LPA is not stable at room temperature and is only measurable in plasma.

In this study, we aimed to elucidate ATX activity in the chronic phase of fibrosing ILD and after the onset of AE-IPF.

## Methods

*Patients*

We recruited 139 patients with fibrosing ILD in the chronic phase (n= 119) and after the onset of AE-IPF (n=20) seen at our institution during the period from November 2017 through October 2020. Since 4 patients had serum available from both the chronic and acute exacerbation phase, 135 patients were enrolled in the study. In addition, 38 volunteers were recruited to participate as healthy controls.

Patient characteristics extracted from the medical records included the following: age, gender, smoking history, laboratory data, pulmonary function test results, final diagnosis of fibrosing ILD, treatment for fibrosing ILD, and prognosis. The diagnosis of IPF and other type of fibrosing ILD was determined by using the guidelines of the American Thoracic Society (ATS)/European Respiratory Society (ERS)/Japanese Respiratory Society/Latin American Thoracic Association and the joint statement of the ATS and the ERS [13]. The diagnosis of AE-IPF was based on criteria proposed by the international working group on AE-IPF [2]. The database was locked in October 2020. The institutional review board of Toho University Graduate School of Medicine approved this study (approval number: A20100). All patients and healthy controls provided written informed consent to participate in the study.

*Blood collection and autotaxin quantification*

Peripheral blood was collected during the stable phase of fibrosing ILD. Blood collection in AE-IPF was performed at the onset before administered corticosteroid treatment. Peripheral blood samples were obtained from the patients in a tube and centrifuged at 3000 rpm for 10 min. The resulting supernatant after centrifugation was

aliquoted and stored at -80°C. ATX activity was measured by using fluorescence enzyme immunoassay in a commercial clinical laboratory (SRL, Inc., Tokyo, Japan).

*Data analysis*

Continuous variables comprised the unpaired t-test for both groups. Categorical variables were compared using the $\chi 2$ and Fisher's exact tests. Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cut-off value of serum ATX for prediction of outcome. Worse outcome was defined as death or inability to continue treatment for fibrosing ILD by transfer to either home health care or long-term hospital care. We used the Kaplan-Meier method to determine the prognosis. The log-rank test was used to compare the two groups. Hazard ratios along with 95% confidence intervals (95%CI) were calculated using Cox proportional hazards analysis to determine the independent predictor for onset of AE-IPF. All P values were two-sided and <0.05 was considered to indicate statistical significance. Statistical analysis was performed by using SPSS Statistics for Windows version 27.0 (IBM Corp., Armonk, NY) and GraphPad PRISM version 8.00 (MDF Co., Ltd., San Diego, CA).

## Results

*Baseline characteristics and gender differences*

Baseline characteristics of patients with chronic fibrosing ILD are shown in Table 1; 78 IPF patients and 41 non-IPF patients were included in the study. We compared serum ATX levels between male and female patients. Higher ATX levels were seen in females compared with male patients (0.724 ± 0.161 mg/L vs. 0.912 ± 0.224 mg/L; P < 0.0001). Thus, for subsequent investigations we divided the patients according to gender. Next, we examined the association of ATX activity and corticosteroid therapy. ATX levels were significantly lower in patients receiving corticosteroid therapy compared with those not receiving corticosteroids in both male and female patients (Males: 0.743 ± 0.160 mg/L vs. 0.580 ± 0.075 mg/L, P = 0.002; Females: 0.975 ± 0.236 mg/L vs. 0.789 ± 0.137 mg/L, P = 0.002). We thus excluded those patients receiving corticosteroid therapy for further analysis of chronic fibrosing ILD. No such difference was observed in the presence or absence of treatment with antifibrotic agents including pirfenidone and nintedanib (Males: 0.747 ± 0.153 mg/L vs. 0.736 ± 0.176 mg/L, P = 0.681; Females: 0.927 ± 0.218 mg/L vs. 0.873 ± 0.243 mg/L, P = 0.229).

*ATX activity in chronic fibrosing ILD*

We added a control arm comprising 38 healthy controls to analyze ATX activity in the chronic phase of fibrosing ILD (Table 1). Serum ATX levels were compared between patients with fibrosing ILD and healthy controls. Significantly higher ATX levels were seen in both genders among fibrosing ILD patients compared with healthy controls (Figure 1). There were no significant between-group differences in ATX levels among the subtypes of fibrosing ILD (Table 2). We then examined the

correlation of serum ATX activity with other clinical parameters including laboratory data and pulmonary function (Table 3). There was no significant correlation between ATX levels and other clinical parameters at baseline. The percent predicted change in forced vital capacity (%FVC) after 1 year was correlated with serum ATX levels in female patients.

*Association between ATX activity and outcome*

We identified the optimal cut-off value of ATX level for prediction of outcomes by using ROC curve analysis. The cut-off value was determined as 0.721 mg/L for male (n=61) and as 0.946 mg/L for female (n=33) patients for predicting death or discontinuation of treatment for fibrosing ILD within 3 years (Table 4). Survival curve analysis showed significantly worse prognosis in male patients with high serum ATX activity (>0.721mg/L) compared with those with low ATX activity (<0.721 mg/L) (Figure 2A). In addition, multivariate analysis using Cox proportional hazards model revealed that lower %FVC at baseline and high ATX level were independent predictors of worse outcome (Table 5). When we limited to IPF patients, high serum ATX activity and %FVC was still significant predictor of outcome (HR 8.288 [95%CI: 1.021, 67.256], P=0.048). However, there was no significant difference in survival between high and low serum ATX levels among female patients (Figure 2B).

*ATX activity in acute exacerbation of IPF*

Finally, we analyzed serum ATX levels in AE-IPF. Clinical characteristics at the onset of AE-IPF and treatment for AE-IPF are shown in Table 6. We compared ATX levels between stable male IPF patients without corticosteroid therapy (n=53) and male AE-IPF patients before receiving the first corticosteroid injection (n=6). As

shown in Figure 3, ATX levels were decreased after AE-IPF onset compared to stable state in male IPF patients (0.746 ± 0.163 mg/L vs. 0.564 ± 0.199 mg/L; P = 0.006).

## Discussion

This study demonstrated that serum ATX levels were elevated in fibrosing ILD patients compared with healthy controls. High ATX levels were associated with disease progression and worse outcome in fibrosing ILD. Low ATX levels were observed in AE-IPF compared with the stable state.

LPA is a bioactive lipid mediator that consists of a glycerol phosphate backbone with a single fatty acid. LPA binds to specific G protein-coupled receptors and mediates various inflammatory and fibrotic responses. $LPA_1$ is the most frequently reported LPA receptor associated with pulmonary fibrosis. LPA/$LPA_1$ signaling has a profibrotic role in epithelial cells, endothelial cells, and fibroblasts [4, 6]. Mice with genetic deficiency of $LPA_1$ and pharmacological inhibition of $LPA_1$ both demonstrated protection from fibrosis in a bleomycin mouse model of pulmonary fibrosis[4, 6]. Also, LPA induced fibroblast reorganization and proliferation thorough $LPA_2$ signaling. 5 LPA/$LPA_2$ signaling activates TGF-β expression in lung epithelial cells and fibroblasts, leading to myofibroblast differentiation [7].

ATX is a secreted lysophospholipase D that catalyzes the hydrolysis of LPC to LPA by phospholipase D activity. It is abundant in most biological fluids, including blood, bronchoalveolar lavage fluid, cerebrospinal fluid, and urine [14]. Immunohistochemical studies revealed that ATX is constitutively expressed in bronchial epithelial cells, alveolar epithelial cells, and alveolar macrophages in the lung [9]. Furthermore, ATX showed a higher staining intensity in IPF and F-NSIP lung than in control samples of healthy lung, suggesting that ATX is activated in the fibrotic lung [9]. Genetic deletion and pharmacological inhibition of ATX resulted in attenuation of bleomycin-induced pulmonary fibrosis in a mouse model [9]. Taken together, the LPA/ATX axis has now emerged as a potential therapeutic target in

pulmonary fibrosis. Several inhibitors targeting $LPA_1$ and ATX have now been entered into clinical trials of IPF and other types of fibrosing ILD [10–12]. The FLORA study, a phase 2a randomized placebo-controlled trial of ATX inhibitors in IPF, reported decreased plasma LPA C18:2 levels in the treatment group compared with the placebo group [10] . However, no study has analyzed serum ATX levels in fibrosing ILD to date.

In this study, we showed that serum ATX level is a potential biomarker to predict disease progression and worse outcome in fibrosing ILD. To our knowledge, this is the first report to examine the association of ATX levels with clinical characteristics in fibrosing ILD. Further studies are required to assess the relationship between serum ATX levels and response to LPA/ATX inhibitors if these drugs are proven to be effective.

In terms of utilizing blood levels in the clinical setting, serum ATX measurement is preferable to serum LPA. First, ATX is temperature stable after sample preparation [15]. Second, ATX is less affected by metabolic changes such as in diabetes mellitus, chronic kidney disease, and food intake [16]. In addition, there were no significant differences between age groups [17]. However, a gender difference in blood ATX levels has been reported [15]. The precise mechanism of this difference is yet to be determined, however, since female subjects and with pregnancy show high ATX levels in the blood, it might be associated with reproductive biology [15, 18]. In this study, ATX levels were significantly higher in female patients compared with male patients consistent with previous reports. Therefore, we analyzed the association of serum ATX level with clinical characteristics separately in both genders. Serum ATX level is elevated in pregnancy [18], malignant lymphoma [19], and liver fibrosis [20]. None of the patients in our study were pregnant or had malignant lymphoma or liver

fibrosis. Initiation of corticosteroid therapy was reported to decrease ATX levels in a dose-dependent manner [21]. Similarly, in this study, ATX levels were significantly lower in patients receiving corticosteroid therapy compared with those without corticosteroids in both male and female patients. Considering the effect of corticosteroids, patients who were receiving corticosteroid therapy at the time of serum ATX measurement in chronic fibrosing ILD were excluded from further analysis. No such difference was observed with antifibrotic therapy.

ATX levels were decreased after the onset of AE-IPF. Interpretating ATX level in AE-IPF is complicated since all patients treated corticosteroids in our institutional protocol. We excluded the patients had received the first dose of corticosteroids at the time of blood collection. The pathogenesis of AE-IPF characteristically is by increased type II alveolar epithelial cell injury and/or proliferation, coagulation disorders, and fibrotic deposition [2, 22]. ATX is mainly expressed in bronchial epithelial cells and alveolar macrophage in the lung [9]. Therefore, possible explanation for decreased ATX levels in AE-IPF is that severe epithelial damage of AE-IPF resulted in lower expression and production of ATX in bronchial epithelial cells.

This study has several limitations. First, this is a single-center analysis, and we could not observe longitudinal changes in serum ATX levels in patients with chronic fibrosing ILD. Changes in serum ATX activity would be useful to predict disease progression. Future study with validation cohort is needed to confirm our findings since we include a heterogenous fibrotic ILD phenotype in this study. Second, the healthy controls were younger than the patients and there were less subjects with smoking history. This could have added some potential bias. Gender differences have been reported in several studies, but a previous study showed similar levels

among different ages groups[17]. There was no significant difference between patients with or without smoking history in both genders (data not shown). Third, because of the small number of female patients who were not receiving corticosteroid therapy, we did not perform a multivariate analysis in female patients with fibrosing ILD. Similarly, we could not analyze ATX levels in AE-IPF among female patients.

## Conclusions

In this study, elevated serum ATX levels in fibrosing ILD patients compared with healthy controls and high ATX levels were associated with disease progression and worse outcome. Our findings indicated that serum ATX is a potential blood biomarker for diagnosing and predicting the outcome in fibrosing ILD. Inhibitors targeting LPA/ATX signaling have emerged as new treatment candidates for fibrosing ILD. The association of serum ATX with response to LPA and ATX inhibitors should be examined in the future if their utility is proven in clinical trials.

## Authorship and author contributions

T.I. had access to the data and takes responsibility for data accuracy. S.S., S.H., and K.K. contributed to the design of the study. T.I., H.S., A.Y., Y.N., and S.M. contributed to data collection. All authors were involved in drafting and revising the manuscript and gave their final approval of the version to be published.

## Conflicts of interest

This study received no specific funding from any company. S.H. received research grants from Nippon Boehringer Ingelheim Co., Ltd., Shionogi & Co., Ltd., and Chugai Pharmaceutical Co., Ltd. T.I., S.S., A.Y., H.S., S.M., Y.N., and K.K. have no conflicts of interest to declare.

## Funding

This study was supported by Project Research Grant No. 20-6 from Toho University School of Medicine with partial support by a grant from the Ministry of Health, Labour and Welfare of Japan, awarded to the Study Group on Diffuse Pulmonary Disorders.

## Acknowledgements

We thank Florence Ene (MBBS, PhD) for medical English editing.

## References


1. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, Behr J, Cottin V, Danoff SK, Morell F, Flaherty KR, Wells A, Martinez FJ, Azuma A, Bice TJ, Bouros D, Brown KK, Collard HR, Duggal A, Galvin L, Inoue Y, Gisli Jenkins R, Johkoh T, Kazerooni EA, Kitaichi M, Knight SL, Mansour G, Nicholson AG, Pipavath SNJ, Buendía-Roldán I, et al. Diagnosis of idiopathic pulmonary fibrosis An Official ATS/ERS/JRS/ALAT Clinical practice guideline. *American Journal of Respiratory and Critical Care Medicine* American Thoracic Society; 2018; 198: e44-e68.
2. Collard HR, Ryerson CJ, Corte TJ, Jenkins G, Kondoh Y, Lederer DJ, Lee JS, Maher TM, Wells AU, Antoniou KM, Behr J, Brown KK, Cottin V, Flaherty KR, Fukuoka J, Hansell DM, Johkoh T, Kaminski N, Kim DS, Kolb M, Lynch DA, Myers JL, Raghu G, Richeldi L, Taniguchi H, Martinez FJ. Acute exacerbation of idiopathic pulmonary fibrosis an international working group report. American Journal of Respiratory and Critical Care Medicine American Thoracic Society; 2016. p. 265-275.
3. Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, Ryerson CJ, Ryu JH, Selman M, Wells AU, Behr J, Bouros D, Brown KK, Colby T v., Collard HR, Cordeiro CR, Cottin V, Crestani B, Drent M, Dudden RF, Egan J, Flaherty K, Hogaboam C, Inoue Y, Johkoh T, Kim DS, Kitaichi M, Loyd J, Martinez FJ, Myers J, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *American Journal of Respiratory and Critical Care Medicine* 2013; 188: 733-748.
4. Tager AM, LaCamera P, Shea BS, Campanella GS, Selman M, Zhao Z, Polosukhin V, Wain J, Karimi-Shah BA, Kim ND, Hart WK, Pardo A, Blackwell TS, Xu Y, Chun J, Luster AD. The lysophosphatidic acid receptor LPA1 links pulmonary fibrosis to lung injury by mediating fibroblast recruitment and vascular leak. *Nature Medicine* 2008; 14: 45-54.
5. Huang LS, Fu P, Patel P, Harijith A, Sun T, Zhao Y, Garcia JGN, Chun J, Natarajan V. Lysophosphatidic acid receptor-2 deficiency confers protection against bleomycin-induced lung injury and fibrosis in mice. *American Journal of Respiratory Cell and Molecular Biology* 2013; 49: 912-922.
6. Funke M, Zhao Z, Xu Y, Chun J, Tager AM. The lysophosphatidic acid receptor LPA 1 promotes epithelial cell apoptosis after lung injury. *American Journal of Respiratory Cell and Molecular Biology* 2012; 46: 355-364.

7. Xu MY, Porte J, Knox AJ, Weinreb PH, Maher TM, Violette SM, McAnulty RJ, Sheppard D, Jenkins G. Lysophosphatidic acid induces αvβ36 integrin-mediated TGF-β activation via the LPA2 receptor and the small G protein Gαq. *American Journal of Pathology* Elsevier Inc.; 2009; 174: 1264–1279.
8. Tokumura A, Majima E, Kariya Y, Tominaga K, Kogure K, Yasuda K, Fukuzawa K. Identification of human plasma lysophospholipase D, a lysophosphatidic acid-producing enzyme, as autotaxin, a multifunctional phosphodiesterase. *Journal of Biological Chemistry* 2002; 277: 39436–39442.
9. Oikonomou N, Mouratis MA, Tzouvelekis A, Kaffe E, Valavanis C, Vilaras G, Karameris A, Prestwich GD, Bouros D, Aidinis V. Pulmonary autotaxin expression contributes to the pathogenesis of pulmonary fibrosis. *American Journal of Respiratory Cell and Molecular Biology* 2012; 47: 566–574.
10. Maher TM, van der Aar EM, van de Steen O, Allamassey L, Desrivot J, Dupont S, Fagard L, Ford P, Fieuw A, Wuyts W. Safety, tolerability, pharmacokinetics, and pharmacodynamics of GLPG1690, a novel autotaxin inhibitor, to treat idiopathic pulmonary fibrosis (FLORA): a phase 2a randomised placebo-controlled trial. *The Lancet Respiratory Medicine* Lancet Publishing Group; 2018; 6: 627–635.
11. Palmer SM, Snyder L, Todd JL, Soule B, Christian R, Anstrom K, Luo Y, Gagnon R, Rosen G. Randomized, Double-Blind, Placebo-Controlled, Phase 2 Trial of BMS-986020, a Lysophosphatidic Acid Receptor Antagonist for the Treatment of Idiopathic Pulmonary Fibrosis. *Chest* Elsevier Inc; 2018; 154: 1061–1069.
12. Lee G, Kang SU, Ryou J-H, Lim J-J, Lee Y-H. Late Breaking Abstract - BBT-877, a Potent Autotaxin Inhibitor in Clinical Development to Treat Idiopathic Pulmonary Fibrosis. *European Respiratory Journal* [Internet] 2019; 54: PA1293Available from: http://erj.ersjournals.com/content/54/suppl_63/PA1293.abstract.
13. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, Colby T v., Cordier JF, Flaherty KR, Lasky JA, Lynch DA, Ryu JH, Swigris JJ, Wells AU, Ancochea J, Bouros D, Carvalho C, Costabel U, Ebina M, Hansell DM, Johkoh T, Kim DS, King TE, Kondoh Y, Myers J, Müller NL, Nicholson AG, Richeldi L, Selman M, Dudden RF, et al. An Official ATS/ERS/JRS/ALAT Statement: Idiopathic pulmonary fibrosis: Evidence-based guidelines for diagnosis and management. *American Journal of Respiratory and Critical Care Medicine* 2011; 183: 788–824.
14. Barbayianni E, Kaffe E, Aidinis V, Kokotos G. Autotaxin, a secreted lysophospholipase D, as a promising therapeutic target in chronic inflammation and cancer. Progress in Lipid Research Elsevier Ltd; 2015. p. 76–96.

15. Nakamura K, Ohkawa R, Okubo S, Tozuka M, Okada M, Aoki S, Aoki J, Arai H, Ikeda H, Yatomi Y. Measurement of lysophospholipase D/autotaxin activity in human serum samples. *Clinical Biochemistry* 2007; 40: 274–277.
16. Ikeda H, Kobayashi M, Kumada H, Enooku K, Koike K, Kurano M, Sato M, Nojiri T, Kobayashi T, Ohkawa R, Shimamoto S, Igarashi K, Aoki J, Yatomi Y. Performance of autotaxin as a serum marker for liver fibrosis. *Annals of Clinical Biochemistry* SAGE Publications Ltd; 2018; 55: 469–477.
17. Usami Y, Ishimine N, Shiba A, Yamaura M, Kawasaki K, Sugano M, Uehara T, Honda T. Performance evaluation of a new liver fibrosis marker Autotaxin immunoassay. *Igakukensa* 2019; 68: 99–104.
18. Iwasawa Y, Fujii T, Nagamatsu T, Kawana K, Okudaira S, Miura S, Matsumoto J, Tomio A, Hyodo H, Yamashita T, Oda K, Kozuma S, Aoki J, Yatomi Y, Taketani Y. Expression of autotaxin, an ectoenzyme that produces lysophosphatidic acid, in human placenta. *American Journal of Reproductive Immunology* 2009; 62: 90–95.
19. Masuda A, Nakamura K, Izutsu K, Igarashi K, Ohkawa R, Jona M, Higashi K, Yokota H, Okudaira S, Kishimoto T, Watanabe T, Koike Y, Ikeda H, Kozai Y, Kurokawa M, Aoki J, Yatomi Y. Serum autotaxin measurement in haematological malignancies: A promising marker for follicular lymphoma. *British Journal of Haematology* 2008; 143: 60–70.
20. Nakagawa H, Ikeda H, Nakamura K, Ohkawa R, Masuzaki R, Tateishi R, Yoshida H, Watanabe N, Tejima K, Kume Y, Iwai T, Suzuki A, Tomiya T, Inoue Y, Nishikawa T, Ohtomo N, Tanoue Y, Omata M, Igarashi K, Aoki J, Koike K, Yatomi Y. Autotaxin as a novel serum marker of liver fibrosis. *Clinica Chimica Acta* 2011; 412: 1201–1206.
21. Sumida H, Nakamura K, Yanagida K, Ohkawa R, Asano Y, Kadono T, Tamaki K, Igarashi K, Aoki J, Sato S, Ishii S, Shimizu T, Yatomi Y. Decrease in circulating autotaxin by oral administration of prednisolone. *Clinica Chimica Acta* 2013; 415: 74–80.
22. Collard HR, Calfee CS, Wolters PJ, Woo Song J, Hong S-B, Brady S, Ishizaka A, Jones KD, King TE, Matthay MA, Soon Kim D. Plasma biomarker profiles in acute exacerbation of idiopathic pulmonary fibrosis. *Am J Physiol Lung Cell Mol Physiol* 2010; 299: L3–7.

Table 1. Baseline characteristics of patients with stable fibrosing ILD.

| | Patients | Healthy controls |
|---|---|---|
| Subjects, n | 119 | 38 |
| Age (years) | 70 ± 9 | 52 ± 11 |
| Sex, male, n (%) | 69 (58) | 16 (42) |
| Smoking history, yes, n (%) | 73 (61) | 11 (29) |
| Diagnosis of fibrosing ILD, n | | |
| IPF | 78 | - |
| NSIP | 11 | - |
| Unclassifiable ILD | 10 | - |
| PPFE | 8 | - |
| CTD-ILD | 12 | - |
| Treatment at the time of analysis, n | | |
| Corticosteroids | 25 | - |
| Pirfenidone | 19 | - |
| Nintedanib | 15 | - |
| Laboratory data | | |
| LDH, U/L | 252 ± 54 | - |
| KL-6, U/mL | 1029 ± 680 | - |
| SP-D, ng/mL | 215 ± 148 | - |
| Pulmonary function | | |
| FVC, mL | 2133 ± 743 | - |
| %FVC, % | 75.9 ± 20.4 | - |
| DLco, % | 59.8 ± 20.9 | - |

Data are presented as mean ± SD. ILD, interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; NSIP, non-specific interstitial pneumonia; CTD-ILD, connective tissue disease-associated interstitial lung disease; PPFE; pleuroparenchymal fibroelastosis; LDH, lactate dehydrogenase; SP-D, surfactant protein-D; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity; DLco, diffusing capacity for carbon monoxide.

Table 2. ATX activity by gender in fibrosing ILD patients without corticosteroids therapy at the time of blood sampling.

| Diagnosis of ILD | IPF | NSIP | Unclassifiable ILD | PPFE | CTD-ILD |
|---|---|---|---|---|---|
| Male | | | | | |
| Subjects, n | 53 | 3 | 1 | 4 | 0 |
| ATX activity, mg/L | 0.746 ± 0.163 | 0.793 ± 0.221 | 0.661 | 0.683 ± 0.988 | - |
| Female | | | | | |
| Subjects, n | 16 | 4 | 3 | 4 | 6 |
| ATX activity, mg/L | 1.003 ± 0.258 | 0.933 ± 0.105 | 1.217 ± 0.376 | 0.869 ± 0.195 | 0.876 ± 0.101 |

Data are presented as mean ± SD. ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis; NSIP, non-specific interstitial pneumonia; PPFE; pleuroparenchymal fibroelastosis; CTD-ILD, connective tissue disease-associated interstitial lung disease.

Table 3. Correlation between ATX levels and clinical parameters.

| | r | P value |
|---|---|---|
| Male (n=61) | | |
| KL-6 | -0.117 | 0.370 |
| SP-D | 0.040 | 0.757 |
| %FVC | -0.093 | 0.481 |
| DLco | -0.191 | 0.146 |
| Δ1 year %FVC (n = 28) | 0.142 | 0.472 |
| Female (n=33) | | |
| KL-6 | -0.009 | 0.957 |
| SP-D | 0.218 | 0.223 |
| %FVC | 0.051 | 0.784 |
| DLco, % | -0.135 | 0.471 |
| Δ1year %FVC (n = 13) | -0.676 | 0.014 |

KL-6, Krebs von den Lungen-6; SP-D, surfactant protein-D; FVC, forced vital capacity; DLco, diffusing capacity for carbon monoxide.

Table 4. ROC curve analysis to determine the optimal cut-off value of ATX for predicting the worse outcome of fibrosing ILD.

| | Cut-off value | AUC | Sensitivity | Specificity |
|---|---|---|---|---|
| Male (n=61) | 0.721 mg/dL | 0.766 | 92.9% | 61.7% |
| Female (n=33) | 0.946 mg/dL | 0.608 | 62.5% | 64.0% |

ROC, receiver operating characteristic; AUC, area under curve.

Table 5. Multivariate analysis using Cox proportional hazards model for predicting independent predictors of worse outcome in male patients with fibrosing ILD (n=61).

| | Hazard ratio | 95%CI | P value |
|---|---|---|---|
| Age (years) | 1.005 | 0.943-1.072 | 0.868 |
| Smoking history | 1.257 | 0.156-10.100 | 0.830 |
| KL-6 | 0.999 | 0.998-1.001 | 0.228 |
| %FVC | 0.964 | 0.930-0.999 | 0.043 |
| ATX > 0.721 mg/L | 8.295 | 1.049-65.558 | 0.045 |

CI, confidence intervals; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity.

Table 6. Baseline characteristics of male patients with AE-IPF

| | n=6 |
|---|---|
| Age (years) | 71 ± 6 |
| $PaO_2/FiO_2$ | 315 ± 52 |
| LDH, U/L | 332 ± 43 |
| KL-6, U/mL | 1808 ± 990 |
| SP-D, ng/mL | 318 ± 137 |
| CRP, mg/dL | 9.3 ± 10.9 |

Data are presented as mean ± SD. LDH, lactate dehydrogenase; SP-D, surfactant protein-D; KL-6, Krebs von den Lungen-6; AE-IPF, acute exacerbation of idiopathic pulmonary fibrosis

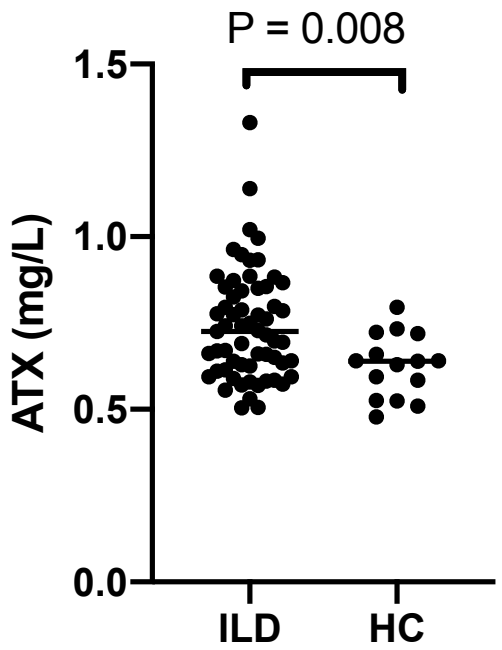
**Figure Legends**

Figure 1. Autotaxin (ATX) levels in patients with fibrosing interstitial lung disease (ILD) and healthy controls (HC). Fibrosing ILD patients showed significantly increased ATX levels in male patients (0.743 ± 0.160 mg/L vs. 0.626 ± 0.092 mg/L; P = 0.008) (A), and female patients (0.975 ± 0.236 mg/L vs. 0.786 ± 0.133 mg/L; P = 0.001) (B).

Figure 2. Survival curve of fibrosing interstitial lung disease (ILD) patients according to serum ATX levels. (A) Male patients with high ATX levels (>0.721mg/L) showed significantly better outcome than patients with low ATX levels (Log rank P = 0.003). (B) No such difference outcome was observed between high (>0.946mg/L) and low ATX levels in female patients (Log rank P = 0.478).

Figure 3. Autotaxin (ATX) levels in male patients with chronic idiopathic pulmonary fibrosis (IPF) and acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF). ATX levels were significantly decreased after the onset of AE-IPF compared with stable IPF (0.746 ± 0.163 mg/L vs. 0.564 ± 0.199 mg/L; P = 0.006).

Fig. 1. A


P = 0.008
1.5
1.0
0.5
0.0
ATX (mg/L)
ILD
HC

B

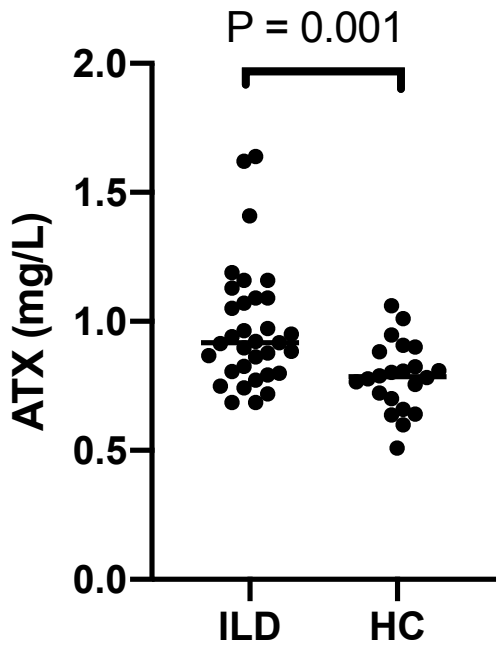
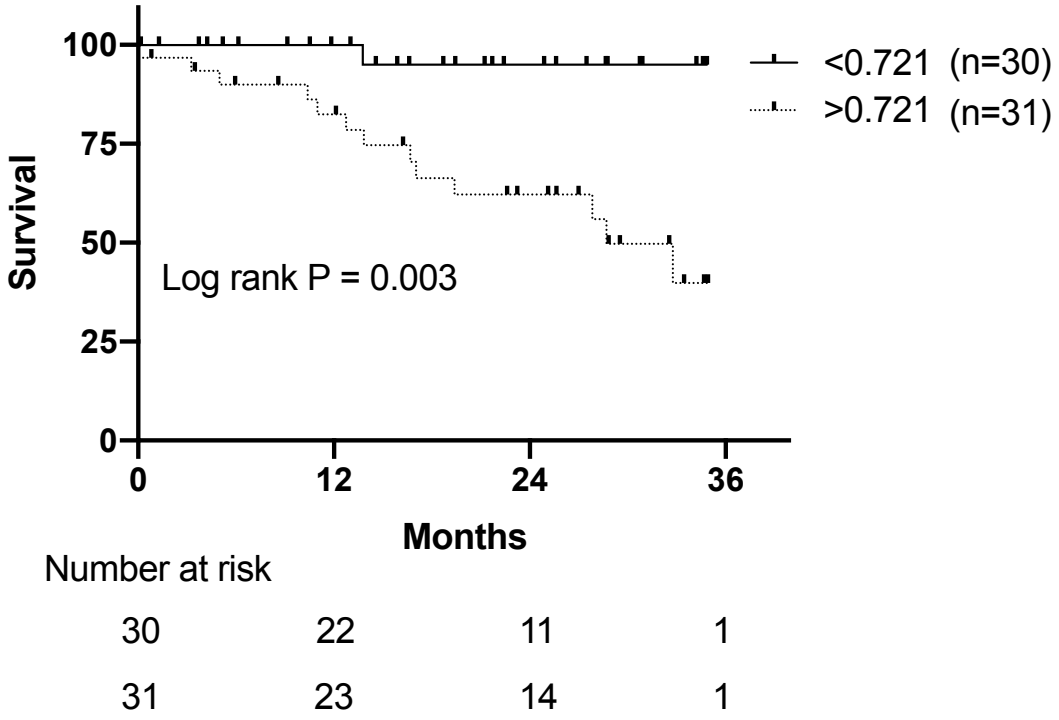

P = 0.001
2.0
1.5
1.0
0.5
0.0
ATX (mg/L)
ILD
HC

Fig. 2. A


100
75
50
25
0
Survival
Log rank P = 0.003
0
12
24
36
Months
<0.721 (n=30)
>0.721 (n=31)
Number at risk
30 22 11 1
31 23 14 1

B

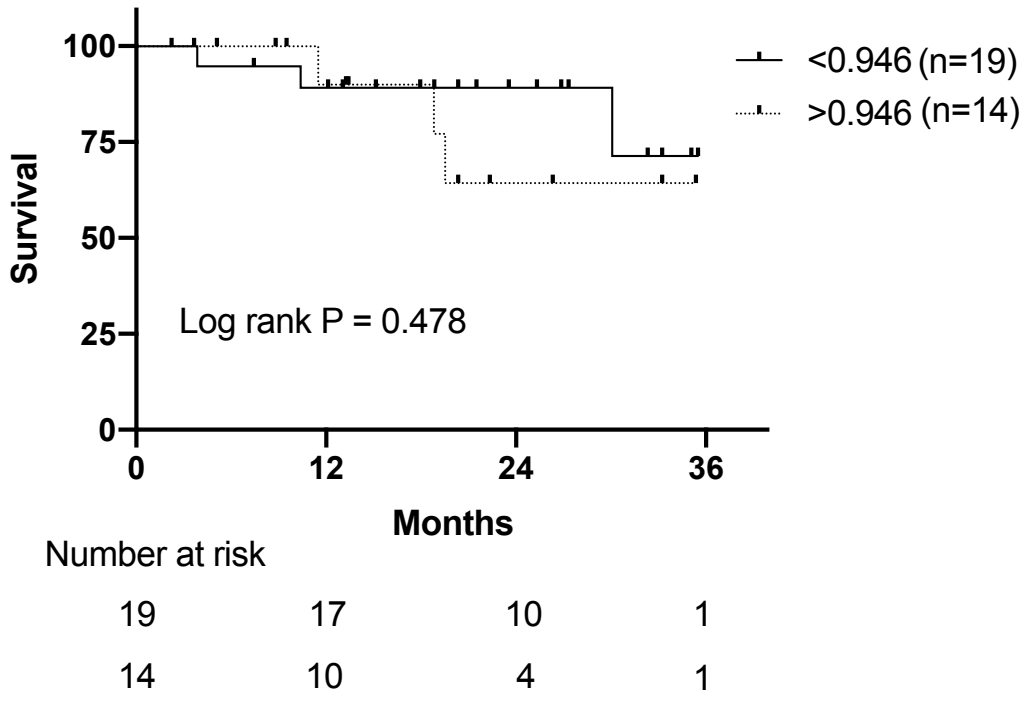
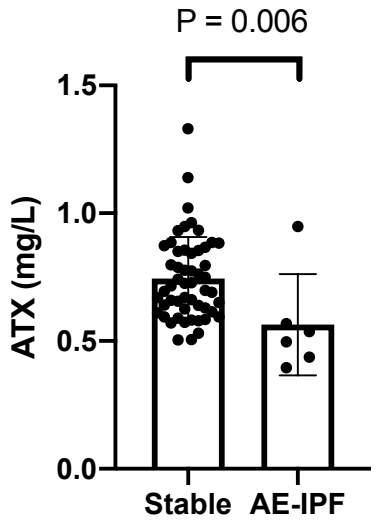

100
75
50
25
0
Survival
Log rank P = 0.478
0
12
24
36
Months
<0.946 (n=19)
>0.946 (n=14)
Number at risk
19 17 10 1
14 10 4 1

Fig. 3



Supplementary Table 1. Multivariate analysis using Cox proportional hazards model for predicting independent predictors of worse outcome in male patients with IPF (n=53).

| | Hazard ratio | 95%CI | P value |
|---|---|---|---|
| Age (years) | 0.971 | 0.891-1.060 | 0.514 |
| Smoking history | 1.098 | 0.134-9.002 | 0.931 |
| KL-6 | 0.999 | 0.998-1.001 | 0.296 |
| %FVC | 0.962 | 0.927-0.998 | 0.041 |
| ATX > 0.721 mg/L | 8.288 | 1.021-67.256 | 0.048 |

CI, confidence intervals; KL-6, Krebs von den Lungen-6; FVC, forced vital capacity.