Peer Review File
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Reviewer A

Thank you for your fruitful comments for our manuscript. We have carefully modified according to your comments.

1. Have the authors investigated the association between IL-11/%FVC and acute exacerbations? Since acute exacerbations account for the majority of deaths from IPF in Japanese patients, the paper would be more convincing if the association between IL-11/%FVC and acute exacerbations were also confirmed.

   (Reply 1-1) Thank you for your important suggestion. We have added the new investigation. We have added two tables (table 5 and 6). IL-11/%FVC tended to be associate with occurrence of acute exacerbation (AE) in IPF by multivariate Cox analysis with PDGF/%FVC. IL-11/%FVC was a significant predictive factor of AE in IPF were by Cox analysis using the significant clinical parameter (age) and PDGF/%FVC.
   (Change 1-1)
   Table 5 and 6.
   Abstract: L68-71, Line 72-73
   Introduction: Line 86-88, Line 90-91, Line 92
   Diagnosis of AE in IPF: Line 145-161
   Results: Line 254-268
   Discussion: Line 275-276, Line 336-346
   Conclusions: L360-362

2. The authors included IL-11/%FVC and PDGF/%FVC simultaneously in the multivariate models, but it is possible that the model would be inappropriate because of a significant correlation between the two. Did the authors check multicollinearity in these models?

   (Reply1-2) Thank you for your important comment. We have tested multicollinearity between IL-11/%FVC, PDGF/%FVC, and %FVC. Multivariate

Cox analysis showed that regression coefficient between IL-11/%FVC and PDGF/%FVC is 0.082.
(Change1-1)
Footnote of table 4, 6

3. Is IL-11 measurable in BALF? Have the authors investigated the correlation between serum IL-11 and IL-11 in BALF?

   (Reply1-3) Thank you for your good suggestion. I am sorry, but we have not measured the levels of IL-11 and PDGF and we cannot evaluate prognostic values of them.

4. In Table 1, %FVC for healthy controls should be included.

   (Reply1-4) Thank you for your important suggestion. However, we have %FVC data of healthy controls. Healthy controls were medical staffs in our hospital and their blood samples were obtained the medical check-up in our hospital. At the time, pulmonary function tests were not performed and we cannot use the data. Hence, we have hypothesized that %FVC of all healthy volunteers are 100% and calculated IL-11/%FVC and PDGF/%FVC as IL-11/100 and PDGF/100, respectively. This is shown in Line 207-208.

Reviewer B

Thank you for your fruitful comments for our manuscript. We have made reply to your comments.

1. Please show whether % FVC correlates with mMRC or %DLCO. If their markers have significant correlation, the results shown in table 2 may only show the above correlation and not the significance of IL-11 and PDGF.

   (Reply2-1) %FVC is generally correlated positively with %DLCO and negatively with mMRC in IPF. Then reviewer 1 have supposed it is natural that IL11/%FVC is significantly correlated with %DLCO and mMRC. However, cytokine

levels/%FVC were not always correlated with mMRC as we have already reported in our previous manuscript (JTD 2022; 14: 278-294). Fibrosis associated cytokines, including IL-7, IL-9, IL-13, basic FGF, and eotaxin and so on, per %FVC were significantly associated with mMRC in the manuscript. Hence, we suppose IL-11/%FVC are not always correlated with %DLco and mMRC, and we suppose it is important to confirm whether IL-11/%FVC is correlated with disease severity parameters including %DLCO and mMRC. We suppose IL-11/%FVC possibly reflect IL-11 levels per lung volume and significant correlation between IL-11/%FVC and mMRC might suggest importance of IL-11 in pathophysiology of IPF.

2. Cox proportional hazards analysis shows that IL-11/%FVC and PDGF/%FVC were associated with prognosis of IPF. However, this result shows the ability for predicting prognosis of %FVC, and may not show the ability of IL-11 and PDGF as prognostic biomarkers.
   Please explain that concerns.

   (Reply2-2) We have published our first paper showing prognostic value of PDGF in IPF (JTD2022; 14: 278-294). In the paper, we have shown that fibrosis-associated cytokines including PDGF, basic FGF, IL-17, IL-7, IL-9, eotaxin per %FVC is associated with prognosis of IPF; however, inflammatory cytokines/%FVC is not associated with its prognosis. If importance of PDGF/%FVC and IL11/%FVC for survival of IPF reflect only the influence of %FVC, most of cytokines/%FVC should be useful predictors of survival of IPF in our previous study (JTD 2022). We suppose cytokine/%FVC is a new parameter which is supposed to reflect cytokine production per lung volume. If serum IL-11 levels are same, %FVC can determine IL-11/%FVC as reviewer 2 say. However, IL-11 and %FVC changed simultaneously and we suppose relationship between IL-11/%FVC and %FVC is not so simple although significant correlation existed between these two parameters as shown in the footnote of Table 4. I understand what reviewer 2 suppose and then we have already included this problem in the limitation (Line 348-359) and values of cytokine levels/%FVC should be confirmed by further study.

Reviewer C

Thank you for your fruitful comments for our manuscript. We have carefully modified according to your comments.

1. How the samples of serum were stored? Since the patients were enrolled 2004 to 2009, the time from sapling to test should be at least 13 years. The authors should also describe how authors follow up patients, when and how healthy controls were enrolled?

   (Reply 3-1) We used Kinki-Chuo Chest Medical Center interstitial lung disease (ILD) biobank system and appropriately stored serum samples in a -30˚C freezer. The opening and closing of freezer door have been minimal.
   (Change 3-1)
   Method: Line 119-120

2. There were significant differences between healthy controls and the IPF patients for sex, age, and smoking status, since the enrollment of healthy control is relative easier than patients, the sample size of healthy controls seems too small in this study (n=20). Furthermore, the BMI data for the healthy controls were not presented.

   (Reply 3-2) Healthy samples are obtained from volunteers who offered their cooperation in the ILD research at the time of the medical check-up for our hospital staffs at our hospital as a clinical study approved by our institutional review board (approval number: No 586). The number of samples close to the patient population was small due to the fact that most of the staff were relatively young women. We could not use BMI because usage of BMI was not included in the plan of the research No. 586. As you suggested, the bias of healthy samples to patient's samples is a limitation of this study.

3. For multivariate analysis of IL-11/%FVC, in table 4, model 2 should include the variable "%FVC" as the author stated.
   (Reply 3-3) Thank you for your important opinion. Whether %FVC is included in the multivariate analysis depends on the correlation between %FVC and IL-11/%FVC. Spearman rank correlation revealed that rho between IL-11/%FVC

and %FVC is -0.777 ($p<0.001$). Hence, IL-11/%FVC and %FVC is highly correlated and %FVC had better be excluded from the multivariate analysis.
(Change 3-3)
Footnote in Table 4 and 6

4. In the abstract, summary of the result of this study should be more detailed. Such as the correlation value (ρ value and p value) between IL-11/%FVC and two clinical variables, the hazard ratio and 95%CI for the Cox regression analysis, and which clinical parameters used for adjustment.

   (Reply 3-4) According to your suggestions, we have added some figures in the abstract according to your suggestions.
   (Change 3-4)
   Abstract Line 61, 62-63, 66-71

5. Line 84, "because all of their serum sample had been used" should be corrected for grammar mistakes.

   (Reply 3-5) We have modified this sentence according to a native English Speaker of Editage. We have changed this part to "we could not measure the IL-11 in one patient because all of the patient's serum sample had been used and lost in previous experiments" (Method p.8, Line 172-174).

6. Line 91-92, "For example, if half of the lung becomes fibrotic, the volume of lung affected by fibrosis shrinks to one-fifth, and local cytokine production per lung volume increases by five-fold", why the volume of lung affected by fibrosis shrinks to one-fifth when half of the lung becomes fibrotic.

   (Reply 3-6) This is only an example. I would like to show you another example. If half of the lung becomes fibrotic and the volume of lung affected by fibrosis shrinks to one-fourth, and local cytokine production per lung volume increases by eight-fold, the total cytokine production in the lung is **1.5 times** as much as normal lung and serum levels of the cytokine is also **1.5 times** as much as normal subjects. Underlined parts above might be changed in each case and for each cytokine and bolded parts also can be changed accordingly. As a result, I would like to say "serum cytokine levels may not always be correlated with the severity of IPF". We

have slightly modified the sentences you pointed.
(Changes 3-6)
Method: L187-189

7. Line 116, “The median values for continuous variables in the two groups were compared” should be “The values” not “The median values”.

(Reply 3-7) Thank you for your suggestion. I have changed as you suggested.