Peer Review File

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Reply to Reviewer A:

Comment 1: Please use alternative to the unit Torr as more people shall understand this or use alternative as well.
Reply 1: We have changed Torr to Torr (mmHg) as more people shall understand well (p.4, Line 26).

Comment 2: Were the samples collected at the time of an exacerbation? Were there baseline blood samples for these patients too?
Reply 2: We have collected serum samples of each case at the diagnosis of IPF. We have no samples at the diagnosis of acute exacerbation.

Comment 3: I am not sure how you came up with the formula for measuring systemic production of cytokines? Has this been done before? Validated?
Reply 3: This is a very important point. The formula was used firstly in this manuscript. The formula was made under the hypothesis that the cytokines associated with pulmonary fibrosis was produced mainly from the cells of fibrotic area in IPF patients. It is difficult to evaluate actual systemic total production of each cytokine. If it is possible, we can use "systemic total cytokine production/FVC" as a parameter. However, it is impossible and we made the parameter "serum cytokine level/%FVC" in the place of "systemic cytokine production/FVC". How we have reached to "serum cytokine level/%FVC" was described in the method (p.6, Line 2).

Comment 4: I am unsure of what exactly this all means in terms of predictive power? Does the Cytokine level/%FVC mean in terms of odds ratios?
Reply 4: Cytokine level/%FVC do not mean odds ratio. We have calculated hazard ratio of "Cytokine level/%FVC" to evaluate risk for short survival time. The larger the Cytokine level/%FVC, the shorter survival time.

Comment 5: I would agree about BALF being difficult/dangerous to get from patients with IPF. Does this study generate enough power to risk that for prognosis?
Reply 5: I might have misunderstood what you would like to ask me; however, I would like to answer. This study is not performed to clarify the risk of BALF for IPF. Actually, all of the IPF patients in this study were diagnosed as IPF with the findings of BALF or TBLB; however, none of the cases experienced acute exacerbation directly after the diagnosis of IPF.

Comment 6: Where/what scenarios could this be applied? Transplant? Self management?
Reply 6: We suppose that the results we have shown in this manuscript suggest importance of PDGF in the pathophysiology of IPF, and that inhibition of PDGF effects is important for the management of IPF to improve survival of the disease. Effects of nintedanib on IPF might be predicted using PDGF/%FVC. We have modified the conclusions (p.12, Line 10).

Comment 7: Page 10 line 232 - "however this was not confirmed" - please explain?
Reply 7: As I have mentioned in Line 8 to Line 10 in page 10, the results of the studies (ref 40, 41, 42) have shown increase in PDGF in the lungs of IPF patients and PDGF could be a useful serum biomarker; however, significance of PDGF in the serum as a prognostic factor have not been confirmed by previous reports (p.11, Line 8).

Comment 8: The second limitation list page 10 is weak. What about the overall numbers?
Reply 8: All IPF cases diagnosed during the study period is 92 cases, and we could collect serum samples of 69 out of the 92 IPF cases. We have added the information in this section (p.11, Line 25).

Comment 9: Table 1. Please explain dead/alive

Reply 9: I am sorry that you could not understand what we would like to express. “dead/alive” means final state of each patient at the last follow up. I have modified the Table 1 (Table 1, p. 19).

Comment 10: Was mMRC compared to cytokine profile in the same way? Did this augment/strengthen the correlation?
Reply 10: I think you have suggested to examine each cytokine levels per mMRC as a predictor of survival and occurrence of acute exacerbation. We have used cytokine levels per %FVC because we suppose this parameter reflects cytokine production per lung volume, not because we adjust cytokine production with IPF severity. However, we have tried cytokine levels per mMRC score as you suggested. Modified MRC score 0,1,2,3,4 was converted to 1,2,3,4,5, respectively, because the scores were used as a denominator. Cox proportional hazard regression analysis with stepwise selection procedure was performed to predict survival. PDGF/mMRC (HR=1.002, p=0.026) and eotaxin/mMRC (HR=0.920, p<0.001) were significant predictors. Cox proportional hazard regression analysis to predict occurrence of acute exacerbation revealed PDGF/mMRC (HR=1.004, p=0.012) and IL-9/mMRC (HR=0.883, p=0.002) were significant predictors. These results were consistent with our data about “cytokine levels/%FVC” and suggested importance of PDGF in the clinical course of IPF. We have added the Table 11 (p.36) and some sentences (p.8, Line 25) to show the results about “cytokine levels/mMRC”.

Comment 11: I wonder whether table four if split into significance by value or not might be easier to follow?
Reply 11: I am grateful for your suggestion. I have modified the Table 4 as your suggestion (p.22).

Comment 12: The figures in table 9 could do with some explaining given the massive differences.
Reply 12: Hazard ratio is calculated for parameter change of 1. If range of each parameter is small, for example, less than 0.1, HR is large figure. Ranges of IL1β, IL2, IL4 were less than 0.05 and HRs of these parameters were large and shown using “e”.

Comment 13: Where could this practically fit into the clinical and/or research arena?
Reply 13: We suppose this comment is similar to the comment 5. Importance of PDGF is consistent with the effect of antifibrotic drugs on IPF. Whether serum levels of PDGF or PDGF/%FVC can predict effects of nintedanib on IPF is important problem to be solved in the future study (p.12, Line 10).

Reply to Reviewer B:
Thank you for your fruitful comments for our manuscript. We have carefully modified according to your comments. We have made reply to some of your comments.

Comment 1: The proposal to divide the levels of cytokines measured by body size and by FVC seems risky and highly questionable. In order for this method to be effectively effective, it would be necessary that the production of each cytokine under consideration should be proportional to the amount of healthy (or diseased) lung tissue at a given moment in a patient with IPF, and that this proportionality should be maintained within the group of patients at different degrees of severity.
Reply 1: Thank you for your important comments. We could not completely understand what you mean; however, I would like to answer to your comment. We suppose each cytokine production in IPF is heterogeneous in the lung because fibrotic lesions in the IPF lung is heterogeneously distributed. Although it is very difficult to evaluate volume of healthy lung, severe fibrotic lung and mild fibrotic lung, IPF with high proportion of healthy lung might show lower cytokine levels per %FVC, and IPF with high proportion of severe fibrotic lung might show higher cytokine levels per %FVC. To clarify this association is right, we examined the association between mMRC score suggesting severity of IPF, and each cytokine per %FVC in each IPF case. To show this point, we have added the Table 10 (p.34). We have added one paragraph (p.8, Line 18 to 23).

Comment 2: The proposed method also does not take into account the many factors intrinsic and extrinsic to the patient that could influence the levels.

Reply 2: As you mentioned, many factors are associated with the serum levels of cytokines. When we use serum levels of cytokine as a biomarker, we usually use it simply; however, some modification might make the biomarker more useful. We have made a new parameter, serum cytokine levels/%FVC. We have shown this parameter can predict mortality and AE occurrence and this parameter correlated with mMRC, another severity marker of IPF. As we have stated in the conclusions, this parameter need validation by future studies (p.12, Line 10).

Comment 3: The absence of significant correlations between the levels of the tested cytokines and the measured FVC% is a further confirmation of the weakness of this proposed index.

Reply 3: This is a very important point. I have shown the absence of significant correlations between serum cytokine levels and %FVC; however, in the previous reports, such correlation has been rarely reported. In my manuscript, we have misunderstood and stated that "Prasse et al. showed significant correlation between CCL18 and %FVC", however, Prasse, et al reported the change of %FVC and %TLC in 6 months was correlated with serum CCL18, and %FVC was not associated with CCL18. Tsoutsou et al. reported the correlation of %FVC with IL-8, not with IL-12, IL-2, IL4, IL-10, and Interferon-γ. Hence serum cytokine levels do not often correlate with %FVC. Our data that none of the measured cytokine levels were not correlated with %FVC is not inconsistent with previous reports.

We have modified second paragraph of discussion (p.9, L14-L25).

Comment 4: If the authors want to explore the role of the proposed cytokines in the lung environment alone, excluding possible systemic production, they can dose them in the BAL of the enrolled patients, although it is not clear how many actually performed it.

Reply 4: Thank you for your important comments. As you say, role of the proposed cytokines in the lung environment alone, excluding possible systemic production, might be better to use cytokine levels in the BAL. However, as I have mentioned in the discussion(p.10, Line 1-13), not all the IPF cases underwent BAL. Fibrosis is usually heterogeneous in IPF lung and it is chiefly distributed in the lower lung field. However, BAL is often performed in the middle lobe bronchus or lingular bronchus. In addition, actual lung volume washed by saline is not unclear, and cytokine production in the lung volume can not be measured. Then BAL is not ideal method to evaluated cytokine production in the lung volume. As you say, our parameter includes various problems; however, we suppose we can use it as one of the parameters to predict survival or acute exacerbation of IPF.

Comment 5: In the methods section is reported how the BAL was performed. All experimental assays were performed on the serum. I therefore believe that the description of the BAL methodology is out of place.

Reply 5: Because you have recommended that we had better delete the BAL method, we have deleted it in the modified manuscript.(P.5, Line 11)

Comment 6: The percentage of neutrophils in BAL reported in table 1 are decidedly low for a cohort of patients with IPF.

Reply 6: Thank you for your important comment. In IPF guideline in 2018, mean neutrophil percentage in BAL is from 5.9% to 22.08%, and in BAL guideline of Myers in 2012, that is more than 5% in more than 90% IPF patients. Hence, neutrophil percentage in BAL of our IPF patients is a little bit lower than previous guideline; however, from Japan Kondoh et al reported neutrophil percentage in BAL of their IPF patients is 2.0+-3.5% (Sarcoidosis Vasculitis and Diffuse Lung Diseases 2010), which is similarly lower than previous guidelines. Hence, our figures do not suggest our IPF cases are not true IPF.

Comment 7: The study of the correlations between the levels of the cytokines tested and the clinical and biological variables collected, including the impact of therapy, was not performed.

Reply 7: Thank you for your important comments. Corticosteroid therapy are not usually recommended for IPF now and all the fifteen cases did not respond to the therapy. Usage of corticosteroid before acute exacerbation was not associated with serum cytokine levels. Modified MRC score, another severity marker of IPF, associated with only IP-10. Cell populations in BAL was associated with some cytokines associated with inflammation. We have modified Table 7(p.28).

Comment 8: No data are available on healthy volunteers and whether these can be considered adequate for demographic characteristics to be compared with a group of patients with IPF.

Reply 8: Thank you for your important comments. We have added some background data of healthy volunteers (HVs) in the results. Age of HVs were significantly younger and significantly more females and non-smokers were included in the HVs (p.6, Line35 to p.7, L3). This is an important limitation and we have added in the paragraph of limitation (p.12, L3-L7).