Peer Review File

Article Information: https://dx.doi.org/10.21037/jtd-21-1830

Reviewer 1

In this manuscript, the authors proponed a novel prognostic gene model for risk stratification and individualized survival prediction for patients with IPF, investigating the gene expression profiles of bronchoalveolar lavage fluid obtained from the Gene Expression Omnibus.

The manuscript is well written and well presented, however,

1. I have major concerns as the therapy information of IPF patients are very low, this could affect the survival prediction.

Reply 1: We firstly thank the reviewer for the comments. The reviewer is completely right. Treatment strategies could affect the survival prediction for patients with IPF. Unfortunately, the dataset we used provides no therapy information besides age, gender, GAP (gender, age, and two lung physiology variables), survival status, and survival time. Moreover, it is the only dataset linking the survival data to the transcriptome data in BALF of patients with IPF. We will prospectively recruit real-world patients to explore the impact of treatment information on the survival prediction and we acknowledge the limitation in the "Discussion" section (see Page 18, line 702).

2. The conclusion is supported by too few events. In future analyses, it is advisable to validate the regulation of the seven genes and the inflammation, or oxidative stress on real samples.

Reply 2: We thank the reviewer for the very valuable advices. One limitation of our study is that molecular biology experiments were not carried out to further support the conclusion. Therefore, we will design and perform molecular biology experiments to validate the regulation of the seven genes and the inflammation, or oxidative stress on real samples in our future work. We also acknowledge the limitation in the "Discussion" section (see Page 19, line 720-722).

Overall, this is an interesting and novel study with potential relevant clinical implications.

Other minor revisions:

1. Page 14, line 301-303: The discussion presents conceptual repetitions.

Reply 1: The reviewer is right. We deleted the conceptual repetitions in the discussion (see Page 15, line 452).

2. Page 14, line 308-309: The authors write: “while delaying those who may not need it”. This sentence could be written differently, because all IPF patients generally need to receive LTx. Perhaps the authors could highlight the urgency or the non-urgency to receive LTx.

Reply 2: We thank the reviewer for the proposal. We modified the sentence as advised (see Page 16, line 458).

3. Figure 1: This figure could be better presented. Perhaps the Venn diagram can be followed by a supplementary table listing the DEGs.

Reply 3: We agree with the reviewer's comments. The DEGs should be listed. However, a figure with diagrams and tables is visually unattractive. As an alternative, we used a correlation network

diagram as a panel of Figure 1 to list the DEGs and renumbered the four panels accordingly (see the revised Figure 1, actually new Figure 2 after renumbering).

4. Figure 7: It is recommended to show the dendrogram defining the samples clusters, which is usually on the top of heatmap. Moreover, I suggest to include HD profiles.

Reply 4: We thank the reviewer for the suggestions. We showed the dendrogram defining the samples clusters in the revised Figure 7 (actually new Figure 8 after renumbering). In addition, the intent of Figure 7 is to demonstrate the difference of clinical features and the seven gene expression profile between high-risk and low-risk patients. The healthy donors (HDs) in the dataset we used provide no more clinical features besides age and gender, so the HD profiles were not included in the revised Figure 7 (actually new Figure 8 after renumbering). Nevertheless, we still provided a supplementary figure including HD profiles (age, gender, and the seven gene expression profile) and showing the dendrogram (see the Figure 1 below).

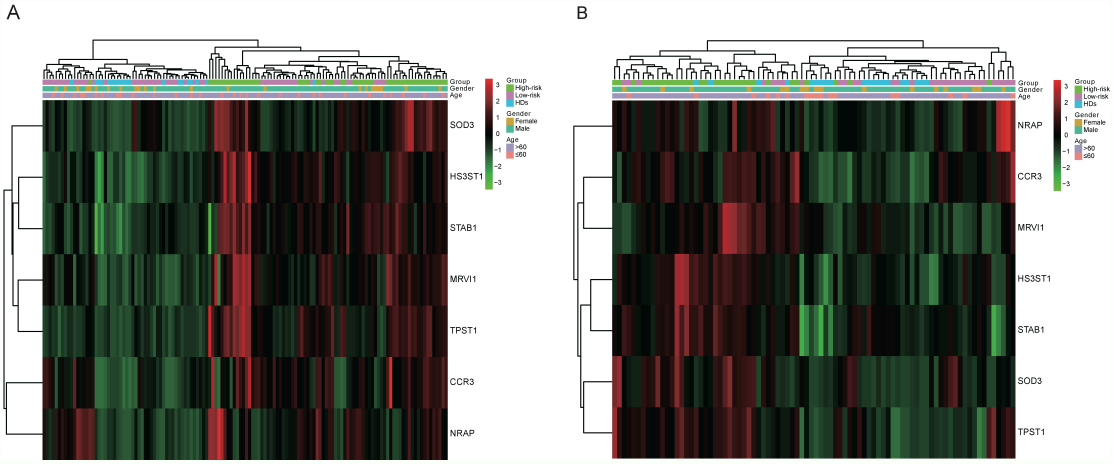

Figure 1 Clinical characteristics and seven gene expression profiles in different groups. Heatmaps of clinical data and gene expression in the derivation (A) and (B) validation cohorts.
Groups: high-risk group vs low-risk group vs healthy donors (HDs)
Clinical data: Age, Gender

Reviewer 2

The paper is well written and each step of the analyses well explained and supported by graphs and figures. Considering your main aim in setting up a strong stratification method based on gene expression profiles, your results are interesting. Here just some adjustments I might recommend:

1. line 109 : The OS acronym has never been mentioned before this line. I assume means overall survival.

Reply 1: The reviewer is right. OS is the acronym of overall survival. The full name of the acronym (OS) is given at first mention in the text (see Page 8, line 221).

2. line 247-255: there are some concepts being repeated in this introductive section of your discussion. In addition, no protein molecular biomarkers studies have never been cited in your work, despite several biomarker discovery studies on IPF are performed (for examples IPF studies by Landi C.)

Reply 2: We thank the reviewer for the comments. We deleted the repeated concepts in the introductive section of our discussion (see Page 16, line 459). In addition, we cited some relevant studies on IPF protein molecular biomarkers by Landi C (see Page 4, line 96, References 19-20).

3. line 330-332: The statement is quite strong as no validation on protein level of these gene products has been conducted and inflammatory state assessment of the patients has been evaluated neither.

Reply 3: We agree with the reviewer's point of view that the statement in line 330-332 is quite strong. We removed the statement in the absence of sufficient evidence (see Page 18, line 683-685).

Reviewer 3

In the present study the authors propose a novel BALF 7 gene model aiming to identify high- risk patients with IPF, in order to facilitate treatment modalities, such as the optimal timing for transplantation referral and antifibrotic treatment initiation.

This is an interesting work, with a good study design and presentation of results, accompanied by detailed statistical analysis; however, the potential value of the proposed model in clinical practice needs to be further investigated.

1. The authors should state more clearly the novelty and originality of their work in the introduction.

Reply 1: We thank the reviewer for the proposal. We further stated more clearly the novelty and originality of our work in the introduction section (see Page 5, line 124-136, and Page 6, line 148-153).

2. In the methods, to make the paper more easily readable, the authors could include some further details about the GLP platform (e.g. which IPF cohorts are included) and clarify the selection of samples.

Reply 2: We agree with the reviewer's suggestions. We included some further details about the GLP platform and clarified the selection of samples in the methods to make the paper more easily readable (see Page 6, line 160-167).

3. The flow chart of the study Fig S1, would better be moved to the methods session in the main body of the text and next figures should be renumbered accordingly. Figure 9 is of very bad analysis and quality.

Reply 3: We thank the reviewer for the very valuable advices. We moved the Fig S1 to the methods session in the main body of the text as Figure 1 (see Page 6, line 157) and next figures were renumbered accordingly. In addition, we revised the Figure 9 showing the two main enriched pathways (see the revised Figure 9, actually new Figure 10 after renumbering).

4. Is not apparent whether these genes have been already identified in other gene-profile studies.

Reply 4: At present, there exists five gene-profile studies based on the same dataset (GSE70866). However, the five gene models hold completely different gene signatures (as showed in the table below). Our model comprised seven genes, CCR3, SOD3, HS3ST1, MRVI1, NRAP, STAB1, and TPST1. Most of the seven genes have not been identified in the other four gene-profile studies except for STAB1 previously reported in Prasse A's study. Although the five models with distinct gene signature are derivated from the same dataset, our model perform best.

Table Comparison of five gene models.

Author Year	Gene model profile	Gene signature	C-index		AUCs	
			Derivation	Validation	Derivation	Validation
Prasse A et al 2019[1]	six-gene	ANKRD22, BMP6, IBSP, LOC284751, S100A14, STAB1	0.67		NA	NA
Li X et al 2021[2]	nine-gene	CCL8, HS3ST3B1, IL1R2, TPCN1, MARCKSL1, NALCN, PROK2, RAB15, S100A12	NA	NA	1-year 0.789 2-year 0.768 3-year 0.754	NA
Xia Y et al 2021[3]	four-gene	CCR2, HTRA1, SFN, TLR2	0.72	NA	1-year: 0.773 2-year: 0.772 3-year: 0.752	1-year: 0.760 2-year: 0.717 3-year: 0.748
Li M et al 2021[4]	five-gene	ACO1, ENPP2, MUC1, NRAS, ZFP36	NA	NA	1-year: 0.737 2-year: 0.772 3-year: 0.731	1-year: 0.891 2-year: 0.870 3-year: 0.678
Our study 2022	**seven-gene**	**CCR3, HS3ST1, MRVI1, NRAP, SOD3, STAB1, TPST1**	**0.815**	**0.812**	**1-year: 0.857 2-year: 0.918 3-year: 0.930**	**1-year: 0.850 2-year: 0.880 3-year: 0.925**

AUC, Area Under Curve; NA, not available.

References:

1) Prasse A, Binder H, Schupp JC, et al. BAL Cell Gene Expression Is Indicative of Outcome and Airway Basal Cell Involvement in Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med 2019;199:622-30.
2) Li X, Cai H, Cai Y, et al. Investigation of a Hypoxia-Immune-Related Microenvironment Gene Signature and Prediction Model for Idiopathic Pulmonary Fibrosis. Front Immunol 2021;12:629854.
3) Xia Y, Lei C, Yang D, et al. Construction and validation of a bronchoalveolar lavage cell-associated gene signature for prognosis prediction in idiopathic pulmonary fibrosis. Int Immunopharmacol 2021;92:107369.
4) Li M, Wang K, Zhang Y, et al. Ferroptosis-Related Genes in Bronchoalveolar Lavage Fluid

Serves as Prognostic Biomarkers for Idiopathic Pulmonary Fibrosis. Front Med (Lausanne) 2021;8:693959.

5. The discussion is rather long. In the first three paragraphs there is a repetition of already mentioned information that could be shortened and emphasize on the most outstanding findings of the present work. Concerning the structure of the discussion, the present study results and comparisons to previous literature should follow the same order in the whole discussion.

Reply 5: The reviewer is right. The discussion is lengthy and contains some repetitive information. We deleted the repeated information in the first three paragraphs of the discussion and emphasized on the most outstanding findings of our work (see the revised first three paragraphs of the discussion). In addition, we discussed our results and comparisons to previous literature following the same order in the whole discussion (see the revised discussion section).