**Peer Review File**
**Article information: http://dx.doi.org/10.21037/jtd-20-2596**

**Reviewer A**

***Comment:*** *The authors elegantly show that, using a standard diagnostic procedure, they are able to differentiate between patients at risk for developing ILD in SSc using fractionated BAL analysis, which is an important predictive finding to the field. Although the findings presented are clear, the authors should address the minor points below first.*
**Reply:** We thank the Reviewer for these insightful comments, which have helped us significantly improve the quality of our manuscript.

***Comment 1:*** *Are the proportions of cells obtained from the various fractions influenced by differences in treatment regimens between patients?*
**Reply 1:** A total of 18 patients (26.4%) were taking oral corticosteroids, 1 was taking prednisolone and cyclosporine, and 1 was taking methotrexate at the time of the BAL procedure. Steroid/immunosuppressant use reduced the number of eosinophils in FBAL-3 and FBAL-total. However, it did not affect the other cell types, including neutrophils. We have now added these data as Supplemental Table 1 and modified the text as advised (see Page 10, Line 194-197).

***Comment 2:*** *In table 1, authors show differences in steroid/immunosuppression use: please show/calculate whether these may influence the outcome of the study*
**Reply 2:** Steroid/immunosuppression use at baseline did not influence the outcome of the study, as described above. We have now added these data as Supplemental Table 1 and modified the text as advised (see Page 10, Line 194-197).

***Comment 3:*** *Same holds for the differences in background of auto-antibodies, please discuss whether there is any correlation there on number of cells in the different fractions*
**Reply 3:** The patients with anti-Scl-70 autoantibodies had more neutrophils in FBAL-3 compared to the patients without anti-Scl-70 autoantibodies. These antibodies have been associated with a poor prognosis, consistent with the poor outcome in neutrophilia in FBAL-3. We could not assess the other autoantibodies (RNP, centromere, RNA-polymerase) because of the small number of the cases (N<10). We have now added the data as a supplement Table 2 and modified the text as advised (see Page 10, Line 194-200).

***Comment 4:*** *BAL fractions also contain other cell-types (e.g. epithelial cells): could the authors provide information on the presence of other cell types in the different BAL fractions. Do the first fractions for example contain more ciliated cells, compared to fractions 3?*
**Reply 4:** As you pointed out, there were more ciliated/epithelial cells in FBAL-1 than in FBAL-2 and FBAL-3. However, we did not count the exact number of epithelial cells, as this is not generally done clinically. We have now added this information to the manuscript (see Page 10, Line 192-193).

***Comment 5:*** *The median range of fluid recovered was 47% (line 122, analysis of BAL): did the authors check whether the recovery of fluid was similar between groups, to make the comparison shown in figure 1 (survival curve)?*
**Reply 5:** The recovery rate of BAL was similar between the groups in Figure 1 (median 45% vs. 47%, p=0.955). We have now added the information to the manuscript (see Page 11, Line 222-223).

***Comment 6:*** *Bal fractions were send to microbiology and cytopathology: was BAL fluid also stored for cytokine analysis? In line 175-180 the authors discuss that neutrophils may contribute to endothelial injury. Therefore, cytokines levels in the different fractions, may also be used as predictive biomarkers of ILD development. If BAL fluid was stored, please add information on relevant cytokine levels of endothelial injury and profibrotic markers to support the findings.*
**Reply 6:** Unfortunately, we did not store the BAL fluid for cytokine analyses. This is a good idea to implement in future experiments. We have now mentioned this point in the limitations section (see Page 14, Line 275-278).

***Comment 7:*** *Please discuss the relevance of the correlation between FBAL and pulmonary function variables, shown in Table 3, for ILD patients: can this be used as a predictive marker, or are these variables relevant for early diagnosis and monitoring of patients?*
**Reply 7:** In Table 3, we wanted to emphasize the fact that FBAL provides more precise information on immune cell populations at the levels of the small airway and alveolar compartments than other fractions. For example, the proportion of neutrophils in the pooled BAL fluid may be strongly affected by the degree of neutrophils at the airway level. In our study, the neutrophils in FBAL-2 and FBAL-3 (alveolar space) showed a significant inverse correlation with the FVC % predicted, but those in FBAL-1 (bronchiolar space) showed no such correlation (Table 3). As shown in Table 3, an FBAL analysis may aid in understanding the severity of ILD at baseline. We have now mentioned these points in the discussion section (see Page 13, Line 249-253).

**Reviewer B**

***Comment:*** *The authors of the paper entitled "Fractional analysis of bronchoalveolar lavage in systemic sclerosis-associated interstitial lung disease" described the role of BAL cellularity in SSC associated ILD, analyzed a promising tool to better evaluate the role of BAL in these patients.*
*Although the role of BAL in ILD is an "OLD" hot topic, the open questions regarding BAL cellularity are is till controversial. The paper results well written and clear, however some point can be improved:*
**Reply:** We thank the Reviewer for these insightful comments, which have helped us significantly improve the quality of our manuscript.

*Major point:*
***Comment 1:*** *I suggest to the author to improve the introduction by adding the importance of BAL cellularity and recent contribution about this topic.*
**Reply 1:** We have now added recent studies showing the importance of BAL cellularity in the diagnosis of hypersensitivity. We have modified the introduction as suggested (see Page 4, Line 78-85).

***Comment 2:*** *I suggest to add the new score investigated such as:*
*The value of bronchoalveolar lavage for discrimination between healthy and diseased individuals.*
*Frye BC, Schupp JC, Rothe ME, Köhler TC, Prasse A, Zissel G, Vach W, Müller-Quernheim J. J Intern Med. 2020 Jan;287(1):54-65. doi: 10.1111/joim.12973. Epub 2019 Oct 15. PMID: 31612575*
*Bronchoalveolar Lavage Fluid Reflects a TH1-CD21low B-Cell Interaction in CVID-Related Interstitial Lung Disease. Friedmann D, Unger S, Keller B, Rakhmanov M, Goldacker S, Zissel G, Frye BC, Schupp JC, Prasse A, Warnatz K. Front Immunol. 2021 Feb 5;11:616832. doi: 10.3389/fimmu.2020.616832. eCollection 2020. PMID: 33613543*
**Reply 2:** We appreciate the Reviewer sharing these papers describing recent advances in BAL analyses. We have now added them to the introduction section (see Page 4, Line 86-87).

***Comment 3:*** *Results about Neutrophils resulted interesting. However it is well know that cellularity resulted affected by smoking habits. Can b the authors verify if effectively the increase of neutrophils resulted associated with smoke?*
**Reply 3:** While I agree that smoking generally affects BAL cellularity, especially neutrophils, there were no marked differences in the BAL cellularity between smokers (n=25) and non-smokers (n=43). Smoking status may have little effect on BAL cellularity because 22 of our patients (88%) were former smokers, and only 3 (12%) were current smokers in our study. We have now added Supplemental Figure 3 and revised the manuscript (see Page 10, Line 194-200).

***Comment 4:*** *In every case I also suggest to discuss the intrinsic bias of BAL and the change of cellularity due to amoking habits. I suggest some paper:*
*The effect of cigarette smoking on bronchoalveolar lavage protein profiles from patients with different interstitial lung diseases. Bargagli E, Cameli P, Carleo A, Refini RM, Bergantini L, D'alessandro M, Vietri L, Perillo F, Volterrani L, Rottoli P, Bini L, Landi C. Panminerva Med. 2020 Jun;62(2):109-115. doi: 10.23736/S0031-0808.19.03754-6. Epub 2019 Sep 24. PMID: 31577091*
*Interestingly new score has been analyzing such as neutrophil to lymphocytes ratio (Neutrophil-to-lymphocyte ratio in bronchoalveolar lavage from IPF patients: a novel prognostic biomarker? D'alessandro M, Bergantini L, Carleo A, Cameli P, Perrone A, Fossi A, Sestini P, Bargagli E. Minerva Med. 2020 May 14. doi: 10.23736/S0026-4806.20.06614-8. Online ahead of print. PMID: 32407050 ). I suggest to the author to evaluate this score in the present cohort.*
**Reply 4:** Thank you for the suggestions. I agree that a smoking habit can affect the BAL cellularity. We have now revised the text as advised (see Page 12, Line 237-240).

We evaluated NL-ratio in this cohort. The NL-ratio had a significant inverse correlation with the FVC % predicted in all fractions and the DLco % predicted in the FBAL-3 and FBAL-total. Furthermore, a higher NL-ratio in FBAL-3 was associated with the progression of ILD and end-stage ILD. Although these results are interesting, we have several questions, including which fractions of BAL are used to calculate the NL-ratio, and what are the meaning of and mechanisms underlying the elevated NL-ratio in SSc-ILD. Another concern is that readers might be confused, since the paper focuses on the fractional analysis of BAL. We hope to focus more on the NL-ratio in our next project. Thank you for the advice.

*Minor points:*
***Comment 5:*** *..”but decisions regarding the timing of initiation and optimal patient selection remain unclear” Please add a regerence/s*
**Reply 5:** We have now added a reference to the text (see Page 4, Line 74).

***Comment 6:*** *3,000 rpm substituted with ×g*
**Reply 6:** RPM has now been converted to g-force. We also noticed that the rotation speed was wrong: it is not 3000 rpm but 1500 rpm (280 ×g). We have now changed the text as advised (see Page 7, Line 141 and 143).

**Reviewer C**

***Comment:*** *The manuscript entitled "Fractional analysis of bronchoalveolar lavage in systemic sclerosis-associated interstitial lung disease" evaluates the utility of FBAL cellular composition in SSc-ILD characterisation. The study is essential, easy to follow and interpret the presented results. The authors shoved the possible practical approach of use FBAL-3 in SSc-ILD clinical investigation.*

**Reply:** We wish to express our appreciation to the Reviewer for their very insightful suggestions.

***Comment 1.*** *The manuscript is well written, however I miss more detailed discussion of biological role of neutrophils in SSc-ILD pathobiology and broad evaluation of neutrophil originated mediators of SSc-ILD course.*

**Reply 1:** Thank you for your suggestions. Recent studies have shown that HMGB1 and NETs contribute to the pathogenesis of endothelial damage and tissue fibrosis in SSc-ILD. We have now discussed the biological role of neutrophils and modified the discussion section (see Page 13, Line 262-267).