

## Peer Review File

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### List of Replies

#### Reviewer #1:

Comment 1: *The introduction is short. Some background on the impact of SARS-CoV2 lung injury would be a welcome addition. This helps to put some of the findings into context.*

Reply 1: As reviewer suggested, we have rewritten the Introduction to make it more reasonable and meaningful (Page 3-4, line: 63-88).

Comment 2: *The criteria used to grade the severity of COVID-19 should be included*

Reply 2: The criteria was added to Methods as requested (Page 4, line 105-112).

Comment 3: *More information on lymphocyte data acquisition and analysis should be included. The information provided is not sufficient.*

Reply 3: The details of lymphocyte data acquisition and analysis was added to Methods as requested (Page 5, line 117-124).

Comment 4: *What is the rationale for using twice the value of signal from 1 healthy control? Is data from 1 donor sufficient?*

Reply 4: We realized that 1 healthy control is not sufficient for analysis, so we added more data on this. Sera of another 9 healthy controls were obtained, and twice of mean OD450nm value of all 10 healthy control were used as cut-off value. Data were reanalyzed and paragraph related to this has also been rewritten (Page 8-9, 217-229), and this reanalysis didn't affect our main result in this part that S-RBD IgG antibody decline more dramatically than that of NP during our follow-up. Methods of ELISA has also been rewritten (Page 5, 128-137).

Comment 5: *How were the CT images assessed? Was this conducted by one observer?*

Reply 5: The CT images were assessed by one observer in the first place, and we realized that this is inappropriate, so all the chest CT scan were reassessed by two radiologists independently as described

in the revised version of Methods (Page 5,6; line:138-146). Also, the chest CT data at different time points were included this time to make a comprehensive and dynamic analysis. The paragraph related to radiological results and descriptions of images have been rewritten, two tables and four figures were added (Page 7, line: 176-193; Table 3-4, Figure 2-5).

*Comment 6: Arrows on the CT images would be useful to orientate the reader to the abnormalities and subsequent resolution of these abnormalities on follow-up.*

Reply 6: Arrows have been added to the chest CT images in the revised version as requested.

*Comment 7: A breakdown of the lymphocyte subpopulations according to disease severity should be completed. Does the severity of disease impact lymphopenia and subsequent recovery?*

Reply 7: Considering the Reviewer's suggestion, we've conducted a breakdown of the lymphocyte subpopulations according to disease severity and age at different time points, paragraph has been rewritten, two figures and 1 table were added (Page 8, line: 211-215; Figure 9, Figure 10, Table S1).

*Comment 8: Similar to above, does age have an impact on this outcome?*

Reply 8: As said above, analysis of the lymphocyte subpopulations according to disease severity and age at different time points was conducted as requested, paragraph has been rewritten, two figures and 1 table were added (Page 8, line: 211-215; Figure 9, Figure 10, Table S1).

*Comment 9: Are there any comorbidities that are important to report?*

Reply 9: Data of comorbidities has been showed in the table and described as requested (Table 1; Page 6, line: 164-165).

*Comment 10: Can you be sure the difference between antibody results is real? Is it plausible this is caused by variability in the assay?*

Reply 10: We fully understand reviewer's concern, due to the small size nature of our study, sampling error may be inevitable compared with the large-scale investigations, and this was mentioned in the limitation of our study in Discussion. However, we tried to improve the reliability of our results through different ways. Firstly, each sample has 3 duplications and mean value was used. Secondly,

blank controls were used, and value of blank controls between different ELISA plates were compared to exclude the potential batch effects, the data of blank controls were shown in Supplementary data. Thirdly, the data of antibody were derived from same group of patients in different time points, this could reflect the variation over time more precisely. A more detailed process of antibody data acquisition and analysis was written in revised version of Methods. (Figure S1; Page 5, line: 125-137)

*Comment 11: Results from other convalescent studies should be included - there are others published.*

Reply 11: Related studies were included, and majority of the Discussion has been rewritten (Page 9-11, line: 235-288).

*Comment 12: Can the authors speculate on the long term impact of the lymphopenia?*

Reply 12: There is still no such data on Covid-19, so we speculate on the long-term impact of the lymphopenia based on the past publications related to the role of CD4+ T cell in immunity and long-term follow-up study of SARS-CoV-1 infections in the Discussion (Page 10, line: 266-276).

*Comment 13: Carefully check manuscript for spelling and grammatical errors.*

Reply 13: We've checked the manuscript and made careful corrections to grammar and spelling mistakes. Revised proportions were marked in red.

Thanks again for your good comments! Hope this can meet your requests.

**Reviewer #2:**

*Comment 1: Lines 58-60: please revise English.*

Reply 1: We rewritten this paragraph to make it more readable as requested (Page4, line: 98-102).

*Comment 2: Results (line 91): It's not necessary to mention again the location of the hospital.*

Reply 2: The location of the hospital was deleted as requested (Page 9, line: 232-234).

*Comment 3: HRCT were performed 8 weeks later, as reported in the results. It*

*could be useful to specify lesions found at the onset of disease. CORADS should be specified, summarizing radiological patterns found on imaging. In this part of the manuscript, please add a table including these CT features.*

Reply 3: As requested, we added the chest CT data at three other time points: First chest CT scan after symptom onset, second chest CT scan after symptom onset and last chest CT scan before discharge, and all the data were analysis dynamically compared with data at 8 weeks after discharged, including CO-RADS score and patterns of abnormalities, the impacts of age and disease severity on the chest CT data were also analyzed. Paragraph was rewritten, two tables and four figures were added (Page 7, line: 176-193; Table 3-4, Figure 2-5).

*Comment 4: With regard to the results, no lesions or fibrotic changes were reported also in patients with severe disease: it could be useful to specify number of patients with severe disease.*

Reply 4: In imaging analysis part, all the chest CT scan were reassessed by two radiologists independently as described in the revised version of Methods (Page 5,6; line:138-146). The paragraph related to radiological results and descriptions of images have been rewritten. A table include the distribution of chest CT scan abnormalities in patients with different age and disease severity was also added as requested (Table 4)

*Comment 5: Figure 2c: CT pattern at 8 weeks seem to be positive for ground-glass attenuation, even if less evident.*

Reply 5: As described above, all the chest CT data were reassessed, and descriptions of images have been rewritten (Fig 3, 4)

*Comment 6: There are 2 figures "2" in the manuscript. Please re-order figures in the manuscript.*

Reply 6: Figures have been re-ordered as requested.

*Comment 7: Lines 123-126: It could be useful to rephrase, indicating results at first follow-up and results of the patients included in the second evaluation.*

Reply 7: The paragraph has been rewritten to make it more readable (Page 8, line: 195-204), and two figures were added to better present the lymphocyte data (Figure 6, 7).

Thanks again for your good comments! Hope this can meet your requests.

**Reviewer #3:**

*Comment 1: Although this study suggested with an interesting aspect on the immune status of convalescent patients, the overall manuscript is poorly completed. It is necessary to correct English, improve the quality of figure, and use accurate terms.*

Reply 1: We've checked the manuscript and made careful corrections to grammar and spelling mistakes. Accurate terms were used, and all figures were either redrawn or replaced by a high-resolution version.

*Comment 2: Line 2: Since RT-PCR exists in various ways for various bacteria, it should be written as RT-PCR for SARS-corona. In later paragraphs, I just kept writing it as RT-PCR, but all of them need to be corrected.*

Reply 2: All “RT-PCR” have been revised as “RT-PCR for SARS-CoV-2” and marked in red.

*Comment 3: Line 39: 'More importantly, level of 39 S-RBD specific IgG antibody decreased faster than expected and NP specific IgG.' The word 'expected' is vague and has no standards. Please describe it based on scientific standards.*

Reply 3: We realized that the word “expected” is subjective, and the sentence has been rewritten more accurately. (Page 3-4, line 56-58)

*Comment 4: Line 47: The Introduction was written very poorly. Rather than simply stating that it is not well known, it is necessary to introduce related studies and to present the reasons why the authors wanted to see images, lymphocytes, and IgG in this study.*

Reply 4: The Introduction has been extensively rewritten as requested, the reason we conducted this research as well as why we focused on images, lymphocytes, and level of IgG were described (Page 3-4, line 67-88)

*Comment 5: Line 60: If you just write CT, there may be several places in the brain and abdomen, so you should write it as chest CT. Also write chest CT for the description that follows.*

Reply 5: The term “chest CT” was used across the whole manuscript as requested.

*Comment 6: Line 74: Describe Elisa as ELISA.*

Reply 6: The term “ELISA” was used across the whole manuscript as requested.

*Comment 7: In the methods, describe in detail by what criteria the clinical grade written in lines 93, 94 is divided.*

Reply 7: The criteria of grading was added to Methods (Page 4, line 105-112)

*Comment 8: In the imaging part, statistics on CT itself have no clinical significance. This is because some people will be normal from the beginning. Therefore, it is necessary to analyze the degree of recovery by looking at serial. The inflammatory nodule is clinically meaningful whether it is a new one that did not exist at the beginning or which CT pattern was initially changed. Analyze again and write.*

Reply 8: We added the chest CT data at three other time points: First chest CT scan after symptom onset, second chest CT scan after symptom onset and last chest CT scan before discharge, and all the data were analysis dynamically compared with the chest CT data at 8 weeks after discharged, including CO-RADS score and patterns of abnormalities, the impacts of age and disease severity on the chest CT data were also analyzed. Paragraph was rewritten, two tables and four figures were added (Page 7, line: 176-193; Table 3-4, Figure 2-5).

*Comment 9: Also, it does not matter which symptoms and how many patients have clinical symptoms. This is because some people have no symptoms from the beginning. There were a few patients who initially had cough, and some of them still complain of cough. And how many patients complained of the ache of joints, and how many of them still complain.*

Reply 9: We add a table to show the symptoms from the beginning to convalescence phase, and this part of manuscript has been rewritten to describe more detailed (Table 2; Page 7, line 172-175)

*Comment 10: In my opinion, Table 1 itself is not that interesting data. Rather, make a table about the presence or severity of symptoms that appear at the first, 8 weeks, and 16 weeks for 26 people. Except for the image that had nothing from the beginning, it is necessary to write down which lesions were completely recovered, and which lesions left with lesions.*

Reply 10: As previously described, we've added data of chest CT scan and symptoms at different time points so that we can analysis dynamically as requested (Table 2-4; Figure 2-5; Page 7, line 172-175; Page 7, line: 176-193).

*Comment 11: There are two figures. Renumber the figure.*

Reply 11: The figures are renumbered properly as requested.

*Comment 12: And are there any people whose lymphocyte is low and high? The overall change of patients is more important than counting how many are normal at 8 weeks and how many are abnormal.*

Reply 12: In this part, we've conducted more detailed analysis on lymphocyte data, the data at different time point were presented, the overall change over time were analyzed, age and disease severity were also taken into consideration, and the paragraph has been rewritten (Page 8, line: 195-215; Figure 6,7,9,10; Table S1)

*Comment 13: Are there any IgG levels during hospitalization?*

Reply 13: We are very sorry for we didn't have IgG level data during hospitalization and no available sera sample. We included this in the limitation of our study, and other research regard to this question was briefly discussed in Discussion (Page11, line: 280-281, 299-300).

*Comment 14: It was said that this analysis of patients in the recovery phase would be helpful for vaccine research. Please provide a hypothesis based on specific evidence that it will be helpful for vaccine research by citing other journals.*

Reply 14: Considering your suggestion, we've added a new paragraph regarding to this question (Page 11, 289-298), and several more references were also cited (References 27-31).

Thanks again for your good comments! Hope this can meet your requests.

Other revisions need to be stated: 1. In this version of manuscript, we exclude the asymptomatic patient (n=1) due to lack of serial radiological and antibody data, it is reported that asymptomatic patients have different clinical and immunological characteristics <sup>[1]</sup> with symptomatic patients which may introduces bias, and it's hard to do further subgroup analysis with only one asymptomatic patient. 2. Majority of the manuscript has been revised whether mentioned by reviewers or not to make the manuscript more reasonable and readable, some of the changes may not be listed here but marked in red in the revised paper.

We tried our best to improve the manuscript, and we appreciate for Editors and Reviewers' work earnestly and hope that the correction will meet with approval.

Once again, thanks very much for your time, comments and suggestions.

#### **References**

1. Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020; 26: 1200–04.