Author Response 1

Reviewer: 1

Comments to the Author

Data missing about the following:

• Complete cell counts and the absolute count for platelet count, neutrophils, monocytes, lymphocytes were retrieved separately for the calculation of inflammatory index calculation of neutrophil-to-lymphocyte ratio (NLR)

• Few details about all reagents used, the company providing the kit and country method in short or add a reference

• Few details about flow cytometer reagent used, lymphocyte markers for T and B cells. The company providing the kit and country method in short or add a reference

Answer:

1. Thank you for the kind advice! In the original version of manuscript, the count for platelets was listed in Table 2, and the count of neutrophils and lymphocytes at admission were listed in Table 3, and the count of neutrophils and lymphocytes at discharge were listed in Table 4. TLC represents total lymphocyte count. We have added the data of the absolute count of monocytes in Table 2 in the revised manuscript.

2. In the Methods part, we added the details of reagents and protocols of the flow cytometry in the revised manuscript.

Reviewer: 2

Comments to the Author

The work is a retrospective description of white blood cells and specifically lymphocytes involved in adaptive immunity in a series of 373 patients with COVID-19 treated in two Shanghai hospitals. The numbers of each cell subtype are compared between patients classified as either severe or non-severe. The work is interesting because of the newness of this virus, and although previous works have offered similar descriptions before, the current research includes some new parameters (LDH, CRP, C3 and C4) and their correlations with lymphocytes subsets not previously reported. However, including the analysis of B lymphocytes would give very useful information about adaptive immunity.

There are some comments to make on a number of aspects.

1. The research includes 373 patients. Were they consecutively admitted to the guard or was there any criteria to select patients or any reason preventing any case inclusion (except consent not provided). Could there be any potential bias?

Answer:

In China, once a patient was diagnosed with COVID-19, he must be admitted to the local designated hospital and receive professional care of doctors and nurses. As a result, all the patients with confirmed COVID-19 were admitted to COVID-19 pneumonia-designated hospitals. So, there would be no potential bias for the inclusion criteria.

2. A brief or general description of the criteria used to classify patients as mild, moderate, severe and critical would be welcomed to allow the reader to figure out how those signs and symptoms relate to the laboratory findings and especially to the characteristics in Table Answer:

Thank you for the advice! We have added the criteria for classification in Methods in the revised manuscript.

3. Page 8 line 7: perhaps the authors would want to consider specifying that although the mean values of LDH and CRP declined to normal at discharge there were still patients in the severe group with CRP values above normal (at least that seems to be the situation at the sight of the standard deviation of the variable in severe patients at discharge)

Answer:

The discharge criteria of COVID-19 in China are listed below

1) Body temperature is back to normal for more than three days;

2) Respiratory symptoms improve obviously;

3) Pulmonary imaging shows obvious absorption of inflammation,

4) Nuclei acid tests negative twice consecutively on respiratory tract samples such as sputum and nasopharyngeal swabs (sampling interval being at least 24 hours).

Those who meet the above criteria can be discharged. As a result, it is possible that the patients were discharged with CRP values above normal. But these patients must go back to the clinic for follow-up examination.

4. Table 5: CD4/CD8 ratio needs revision since 1.92 corresponding to severe patients at admission does not fit with the CD4 and CD8 counts reported in the table (something like 1.17 is more likely), which means that some corrections are also necessary in the results section.

Answer:

We checked the data again and the result of CD4/CD8 ratio is correct. Although mean of CD8 of the severe group is 258.26, but the Standard Deviation of CD8 is 564.68, which is large compared with Standard Deviation of CD8 of non-severe group (185.65). As a result, CD8 of the severe group could vary considerably, leading to the great changes of CD4/CD8 ratio. Hence it makes sense that although CD4+CD3+ Lymphocytes count of severe group is much smaller than the non-severe group, CD4/CD8 ratio of the severe and non-severe groups seems to be similar.

5. Did the authors not measure B lymphocyte subset by flow cytometry? This subset is involved in adaptive immunity and might also provide important clues about severity. According to the representative histogram in Figure 2 there is a very low percentage of CD3+ cells among total lymphocytes in the severe patient group at admission, which implies that perhaps the percentage of B cells is much increased, even if B counts are not high.

Answer:

Thank you very much for the suggestion. It is very meaningful to study the impact of COVID-19 infection on B lymphocytes. However, in this study, we put our emphasis on the T lymphocyte subset, so we did not measure B lymphocyte subset by flow cytometry. We will take this advice into consideration and investigate the influence of COVID-19 infection on B lymphocytes in the further study.

6. Figure 1. This figure is not necessary since the same data are presented in tables 3 to 5. In addition, describing in the figure foot the same results as in the text is also not necessary.

Answer:

Thank you for the advice. We have deleted the complete Figure 1 and the figure foot, also we deleted the relevant information of Figure 1 in the revised manuscript.

7. Figure 2: Mean and Standard deviations of lymphocyte percentages should be presented in a table additionally to the representative histograms, even if only as a supplementary table. Are percentages indicated in each histogram those of the representative blood sample? Or are they the mean value of the group? This should be stated at the figure foot.

Answer:

The new table of lymphocyte percentages at admission and at discharge has been presented in supplement material as S-Table 1. The percentages indicated in each histogram represent one single patient whose flow cytometry result was shown in the figure of flow cytometry. We changed the group name from "moderate" to "non-severe", and "severe+critical" to "severe" in the new Figure 1, consistent with the figure foot. Nothing changes in the images and percentages of the flow cytometry in Figure 1. We also add the explanation of the percentages in the figure foot in the revised manuscript.

Minor corrections.

8. Page 5: immunologic in lines 46 and 58 should be "immunological".

9. Page 6, line 39, commonly should be "common"

10. Page 7, line 22: non-severe should be "severe"

11. Page 8, line 46: "LDH and CRP significantly increased upon admission" should be "LDH and CRP were significantly increased upon admission"

12. Page 8, line 50: "…and subtypes decreased at admission" should be "… and subtypes were decreased…"

13. Page 8 line 55: "NLR decreased" should be "NLR was decreased"

14. Page 9 line 11: T cells functions should be "T cells function"

15. Page 9 line 18: Consider substitution of "number of TLC" instead of "degree of TLC"

Answer:

Thank you so much for the clear and detailed advices of comment 8-15. We have revised them in the new version of manuscript.

16. Page 9 line 30: In "lymphocytes, lymphocyte subsets and TLC…" Are lymphocytes and TLC in this sentence different variables? Should one of both be deleted?

Answer:

Thank you for the correction. We have deleted "TLC" in this sentence in the revised manuscript.

17. Page 9, line 45-46: "Upon discharge… but not significant", does not seem to be true, since the only trends is for NLR and the rest of correlations are not even trends.

Answer:

Thank you for the correction! We checked the data in Table 6-7, in the Results part, we mentioned there was no correlation between lymphocyte with LDH, CRP in severe group at discharge, but in the Discussion part, we misread the information and this sentence was a mistake. We are truly sorry for that, and we have revised it in the new version of manuscript.

18. Table 3: LDH and CRP are in the title but are missing in the table.

Answer:

Thank you for reminding us. We have added the data in Table 3.

19. Table 3: The units of Neutrophil count and TLC are wrong since superscript of 10 should be 9 and not 6. The same is true for Table 4 "neutrophil count"

Answer:

Thank you for the correction. We have revised it in the new Table 3.

20. Figure 2; figure foot could be shortened; there is no need to repeat "representative plot….CD8+CD3+CD45+" if "(A,B,C,D)" is located after "…from severe COVID-19 patients at admission" and so on…

Answer:

Thank you for the suggestion. We have shortened the figure foot in the new version of manuscript.

21. Figure 3 title has a mistake. In addition, the plots have very bad visual quality, and the R or P values included in the graph are not visible. I would eliminate this figure since the same is already presented in tables 6 and 7.

Answer:

Thank you for the suggestion! We have deleted the entire Figure 3 in the revised manuscript.