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Blood molecular markers associated with COVID-19 immunopathology and multi-organ damage

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1st Editorial Decision 6th Aug 2020

Dear Yong-Zhen,

Thank you for submitting your manuscript to The EMBO Journal. I am sorry for the delay in getting back to you with a decision, but I have now received the three reports on your manuscript.

As you can see from the comments, the referees appreciate the findings reported and are overall supportive of publication here pending adequate revisions. While the referees raise many concerns, many of them can also be addresses with text changes, better description of how some of the work was done and more careful discussion addressing potential limitations as well. I think it would be helpful to discuss the raised points further and I am happy to do so. We can do so either via email or video whatever you prefer.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website:
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We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. Should you foresee a problem in meeting this three-month deadline, please let me know and I can extend an extension.

Thank you for the opportunity to consider your work for publication. Looking forward to discussing the revisions further

with best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

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- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
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Referee #1:

Review of Chen et al.

In the paper entitled "COVID-19 severity is associated with immunopathology and multi-organ damage", Chen and co-authors study the host response to COVID-19 pathophysiology by a multi-omics approach applied to samples from peripheral blood and plasma. Based on an extensive panel of expressed genes, proteins, metabolites and extracellular RNAs in a fairly comprehensive set of COVID-19 patients they characterize the immune response characterized by activation of IFN-I signalling and neutrophils, and high levels of inflammatory cytokines, in severe disease patient, which was contrasted by a robust T cell response in mild COVID-19. They use these set of biomarkers to point towards organ-specific damage and also to predict clinical outcome of disease.

While the authors should be applauded for bringing together an extensive and heterogeneous data set for a fairly large number of patients, the paper comes with significant shortcomings that must be addressed before considering this paper for publication. First, in many aspects the conclusions drawn are not supported by data. This is particularly true for their speculation on organ-specific damage. While it is interesting to see that organ specific proteins are identified to be upregulated in the severe patients, this reviewer considers it highly questionable to derive any conclusions from this observation obtained in blood w.r.t. organ-specific response in the absence of any data from

these organs. Such data could be particularly interesting for those six fatal patients by providing post mortem data (imaging, biomarker based etc.) from autopsies. While it may be difficult or even impossible to provide such data for their present patient cohort, at the very least they should try to link their findings to recent studies on COVID-19 autopsies. In any case, it is mandatory that the authors down tone the conclusions drawn from blood samples for damage of other organs. Second, the authors do not cite the relevant body of literature, which is of particular concern in this rapidly evolving field on COVID-19 research. Third, may methodological details are missing rendering the assessment of their results difficuklt and impossible in some places. Unfortunately, all of this reduces the value of their paper. For further details see major and minor points below.

Major points:

1) The title of the paper must be changed. Their data does not sufficiently support their claim that COVID-19 severity is associated with multi-organ damage. Further, the title should be precise in stating that this study is restricted to blood samples.
2) It is puzzling why the authors decided to put a focus on testis and not on other organs that have been shown in multiple studies to be primarily effected in COVID-19 (e.g., the heart, intestine, pancreas etc.). Here, again the authors fail to cite the relevant literature on autopsy based COVID-19 studies.
3) It is unclear how COVID-19 severity was defined here. According to WHO classification many of the patients (at least those that died) are presumably critical rather than severe. The description of the patient cohort is insufficient here. This is particularly important since others have shown that immune host response critically depends on severity of disease (see e.g. Chua et al. Nature Biotech 2020, https://doi.org/10.1038/s41587-020-0602-4)
4) Correlation of multi-omics data with biochemical parameters is very interesting. However, it does not justify the statement "suggesting that that the molecular changes identified directly impact the pathophysiology of COVID-19". Along the same line, the statement "Combined, these data reveal an association between specific molecular variations and COVID-19 pathophysiology" is by no means supported by their data.
5) "Compared to healthy controls, intensive alteration of tissue-enhanced proteins were observed in all COVID-19 patients, suggestive of multiple organ dysfunction including the lung, liver, brain, testis and intestine." This statement is very misleading. First of all, the term tissue enhanced protein should be explained and linked with a proper citation to the HPA. Note that a third of all genes are tissue enhanced so the conclusions drawn from their finding of an enrichment of those genes in blood samples w.r.t to organ damage must be interpreted with this in mind. This part of the manuscript is the weakest and the most over-interpreted part, which should be entirely reworked.
6) Higher neutrophil counts were observed in severe patients but not in mild patients during hospitalization (Fig. S3C). Examination of the neutrophil transcriptomic signatures revealed that excessive neutrophil activation was associated with severe rather than mild disease. This is not all novel and has been shown in multiple studies before. Citations are missing.
7) "Severe patients lost ~59.1% of their total T cell population, 62.3% of their CD4 T cells and 52.8% of their CD8 T cells. Importantly, the CD4 T cell population gradually recovered in the severe-survivors compared to the severe-fatality group". This data is impossible to interpret properly at presented here. Rather than showing relative values the authors must provide absolute standardized values (cells per ml or equivalent other absolute measures). The depletion of T cells may e.g. be a simple consequence of massive expansion of neutrophils in severe COVID-19.
8) "Additionally, T cells in the survivors were primed by dendritic cells and expressed high levels of IFNG and GZMB (Fig. 4C)." This finding is not new at all and the proper literature must be cited.
9) "Although viral load declined during the period of hospitalization in both survival and fatal cases, it remained elevated in fatal cases compared to survivors." This plot of viral load is surprising

because sustained high viral load in nasal swabs 40 days after hospital admission even in fatal COVID-19 has not been described in the literature. Typically, 4-6 weeks after hospitalization, even critical patients have little if any sign of viral RNA in nasal swabs with few exceptions. Insufficient information about viral load assessment are found in the methods section ("Quantitative viral load tests were performed using the BioDigital General dPCR kit (Jiangsu Saint Genomics..."). Given the variation in viral load estimates and the difficulty of obtaining standardized, quantitatively comparable viral load measurements it is essential to give all details on how the authors computed viral load. Further, individual trajectories of viral load per patient should be show in addition in particular for this six fatal cases.

Minor points:

1) In the introduction, the sentence "It is believed that SARS-COV-2 is able to use angiotensin-converting enzyme 2 (ACE 2) as a receptor for cell entry (Zheng et al., 2020; Zhou et al., 2020b)." should be corrected. It has actually been shown that this is the case (Hoffmann et al. 2020, CELL, https://doi.org/10.1016/j.cell.2020.02.052), and as such this paper should be cited first place here.
2) In the introduction the authors should properly cover the relevant literature on autopsy based COVID-19 studies organs that show the primarily effected organs in COVID-19 (e.g., the heart, intestine, pancreas etc.).
3) The coverage of publications studying expression of ACE2 and its co-factors is quite biased and incomplete.
4) The authors fail to cite recent papers studying the host immune response in the respiratory system in COVID-19 patients. (e.g., Liao, et al. (2020) Nature Medicine; Chua et al., Nature Biotech 2020, https://doi.org/10.1038/s41587-020-0602-4)
5) "Immune responses can cause severe damage to the cells or tissues that defend hosts against viral infection (Baseler et al., 2017; Cicchese et al., 2018; Newton et al., 2016)." Citations should be amended by those specifically studying SARS-CoV-2 infection.
6) Fig. 2C: circos plots: The authors should explain how these plots were generated, e.g., by the circlize package. In that case, they should refer to that paper.
7) Proper legend for Fig. S5c is missing. What do different colors, and line styles (dashed/connected) mean?

Referee #2:

The manuscript " COVID-19 severity is associated with immunopathology and multi-organ damage" by Chen et al investigates how host responses contribute to COVID-19 pathophysiology using a multi-omics approach to identify molecular markers in peripheral blood and plasma samples that distinguish COVID-19 patients experiencing a range of disease severities. Although the data is a monumental set of transcriptomic, proteomic, and metabolomic analysis there are several limitations that are not discussed or addressed by the authors that may limit the data interpretation and its applicability to COVID-19 patients. Of most significant concern is that the changes seen between mild and severe COVID-19 cases and more specifically those that survived

or died may be reflective of severe illness and not specific to COVID-19 itself. Said another way is not clear if the pathways and notable findings by the authors would not be seen in sepsis or other severe illnesses. The authors have no controls from other severe illnesses (e.g. bacterial pneumonia and sepsis). Without these controls it is difficult to interpret whether these findings are unique to COVID-19. With this said these findings are important even if not unique to COVID-19 and therefore the manuscript should be accepted pending major revision.
Comments for the author:
1. As stated above controls leveraging other mild to severe illnesses (another example flu and death from flu). Without showing that these differences it is not clear if these changes are unique to COVID or just to severe illness. Addition of these controls would greatly strengthen this manuscript.
2. The authors make the argument that the differences they see are characteristics of mild versus severe patients and "that distinguish COVID-19 patients experiencing a range of disease severities." Are these markers present early on in the disease process? Can they be used prospectively to partition patients who will go onto develop mild versus severe disease?
3. The authors claim that significant changes in the proteome were seen in mild and severe COVID-19 patients. For example markers attributed to the lung, liver, kidney, testis etc were altered and noted to be downregulated (e.g. ALB). This is hard to attribute specifically to an organ or to COVID-19 disease. In almost all disease states particularly when people are severely ill and not eating you see significant changes that are associated with illness and the fasting state. Albumin as are several markers are often diminished due to inflammatory or injury states and in themselves are not unique to a specific illness. Moreover the authors make the claim Fig.3C with a figure denoting brain injury and neuronal death. Current data that this occurs is quite limited and if the author wants to make claims of end-organ injury tissue sections etc for direct proof is needed.
4. Several cytokines, chemokines and interferons are altered (up/down) in their proteomic or transcriptomic analysis. ELISAs (preferable) or Western Blots is needed to confirm these findings and claims.
5. Further discussion on how these changes seen with COVID-19 compare and contrast that with other severe illnesses and particularly viral illnesses such as influenza would greatly enhance the impact of this work.

Referee #3:

In the manuscript "COVID-19 severity is associated with immunopathology and multi-organ damage," the authors utilize proteomics, metabolomics, and RNAseq to perform a systems-level analysis of physiological changes accompanying COVID-19 disease. The authors analyze samples from severe and mild COVID-19 patients, and compare them to healthy control individuals. The study is exhaustive and informative regarding the patterns and correlations observed between and among molecular alternations and clinical alterations accompanying COVID-19. Many interesting observations are reported, notably in the context of metabolism, tissue-related macromolecules, and the immune response. One critique of this paper is that the figures and legends require revision to better communicate the study design and the analysis performed. Another critique is that the authors do not sufficiently explain their analyses to the reader in the results text -- considering the choice of EMBO (a general interest journal), it would be helpful to explain their methodology a bit more, space permitting. This is an issue throughout the results section, and I suggest the authors revise the text accordingly.

The manuscript is organized largely based on results paragraphs and corresponding figures, so I

have grouped suggested revisions according to this same pattern. Specific comments below.

Line 35 - The authors may want to soften the language around prognostic indicators, for example one change might be "...exRNAs may be used as biomarkers to predict the clinical outcomes of SARS-CoV-2 infection.". A central question I have is whether the size of the cohort used, particularly considering the small number of fatal cases (n=6), is sufficient for a high degree of prognostic certainty. My impression is that more validation work would need to be done to validate biomarkers, particularly as they relate to mortality, and I suggest the authors address this where appropriate. Please correct me if I'm wrong.

Page 3 - I suggest streamlining the discussion of multiple organ infection by SARS-CoV-2 infection, it seems slightly repetitive in paragraphs 1 and 2 on this page.

Line 85 - Patient cohort and clinical characteristics (not "characters")

Figure 1:
-Please revise Figure 1A to better reflect the study design. The main text includes mention of throat swabs, but these are not included in Fig. 1A. Please indicate these in figure along with assay run with those samples.
-It is not clear what analysis is run on the sampling timepoints from the blood based on Fig. 1A - if all -omics analyses were run on every timepoint, please revise to illustrate this.
-The methods indicates that 1-5 timepoints were collected per patient, suggesting that for some patients time-resolved data was available and for some patients it was not. It may be helpful to mention for how many patients time-resolved data was available and analyzed.
-Figure 1B: please clarify y-axis labels
-Figure 1C: the authors may want to clarify these y-axis labels - the mention of both 'count' and '%' is somewhat confusing.

Figure 2:
-The labels in Figure 2B are too small to read.
-The left panel of Figure 2C would benefit from more explanation
-For Figure 2D, the abbreviation WGCNA is not defined. Also, the main text references 14 modules of proteins, however it appears that 'Modules' depicts a number of colors in a row at the bottom of this heatmap, and 'Modules' also relates to the right hand panel. It would be helpful if these designations were clarified.
-Figure 2E highlights some noteworthy coagulation-related proteins, I found this interesting.
-The heatmap colors in Figure 2E does not appear to be defined. Also, it is unclear how each of the three columns in 'Mild' and four columns in 'Severe' are defined.
-The heatmap colors in Figure 2F do not appear to be fully labeled. Also, it is unclear how each of the three columns in 'Mild' and four columns in 'Severe' are defined.

Tissue damaged caused by SARS-CoV-2, and Figure 3:

-The text in Figure 3A is too small to read. Fig. 3A is also somewhat challenging to interpret at first, it seems like the authors are trying to depict the abundance of the dysregulated proteins in relation to the relative abundance of non-dysregulated proteins (please correct me if I'm wrong). There may

be a clearer way to present or explain this data.
-The X-axis labels in Figure 3F can be shortened.
-Fig. 3G. The "X-Cell" acronym is "xCell" in other parts of the manuscript, please harmonize. Also, the use of colors to depict both the heatmap and the cellular populations is somewhat confusing, the authors might consider an alternative way to designate cellular subtypes in this heatmap.
-Fig 3H. It is unclear what the four columns in 'Control' and four columns in 'Mild', and six columns in 'Severe' are comprised of. Are these referring to specific individuals in the cohort? If so, how were they chosen?
-Figure 3I - Please correct me if I'm wrong, I don't see how age or gender are depicted in this subfigure (these are indicated in the color legend).

Line 150: The authors may want to specify the type of -omics under analysis early in this paragraph, e.g. exRNA.
Line 152 refers to Figure 3F, however this may mean Figure 3G.

The manuscript would benefit from a definition of "tissue-enhanced protein", as well as "proteins related to organ function", as these terms may not be commonly understood.

Immunopathological changes in COVID-19 patients, Figure 4 and Figure S3:

Line 185 - "The CD4 T cell population gradually recovered in the severe-survivors compared to the severe-fatality group (Fig. S3E)." The heatmap suggests that the T-cell numbers are higher in severe survivors, but I don't see an obvious upward trend over time.

Figure S3E - heatmap colorbar requires labeling.

Biomarkers predictive of clinical outcomes of COVID-19 patients:

It may be helpful if the authors could mention the size of each category of patient utilized to train their prognostic model, comment on the certainty of their findings in the context of their cohort size, and mention what further work would be required to validate biomarkers for COVID-19 prognostic use.

Discussion:

Line 315 - "The data generated here revealed that the number of AT1 and AT2 cells reduced significantly in severe patients..." Regarding the molecular profiling of blood plasma to verify tissue health, I wonder if the authors might address the shortcomings of this approach to measure the health of specific cell types in the body. I think these observations are complemented very well by citing autopsy studies, for example, but I wonder if alone they are sufficient to make such claims. Please correct me if I'm wrong.

Line 334 - "a role for T cells in SARS-CoV-2 infection not yet has been determined, likely reflecting "lymphopenia". I suggest rephrasing for clarity.

Response to reviewers' comments:

reviewer #1:

In the paper entitled "COVID-19 severity is associated with immunopathology and multi-organ damage", Chen and co-authors study the host response to COVID-19 pathophysiology by a multi-omics approach applied to samples from peripheral blood and plasma. Based on an extensive panel of expressed genes, proteins, metabolites and extracellular RNAs in a fairly comprehensive set of COVID-19 patients they characterize the immune response characterized by activation of IFN-I signalling and neutrophils, and high levels of inflammatory cytokines, in severe disease patient, which was contrasted by a robust T cell response in mild COVID-19. They use these set of biomarkers to point towards organ-specific damage and also to predict clinical outcome of disease.

Response: We thank the reviewer for his/her careful review and the constructive comments.

While the authors should be applauded for bringing together an extensive and heterogeneous data set for a fairly large number of patients, the paper comes with significant shortcomings that must be addressed before considering this paper for publication. First, in many aspects the conclusions drawn are not supported by data. This is particularly true for their speculation on organ-specific damage. While it is interesting to see that organ specific proteins are identified to be upregulated in the severe patients, this reviewer considers it highly questionable to derive any conclusions from this observation obtained in blood w.r.t. organ-specific response in the absence of any data from these organs. Such data could be particularly interesting for those six fatal patients by providing post mortem data (imaging, biomarker based etc.) from autopsies. While it may be difficult or even impossible to provide such data for their present patient cohort, at the very least they should try to link their findings to recent studies on COVID-19 autopsies. In any case, it is mandatory that the authors down tone the conclusions drawn from blood samples for damage of other organs. Second, the authors do not cite the relevant body of literature, which is of particular concern in this rapidly evolving field on COVID-19 research. Third, may methodological details are missing

rendering the assessment of their results difficult and impossible in some places. Unfortunately, all of this reduces the value of their paper. For further details see major and minor points below.

Response: As suggested, we have added citations concerning recent autopsy and other relevant COVID-19 studies, toned down the descriptions of multi-organ damage, and provided more methodological details in the revised manuscript.

Major points:

1) The title of the paper must be changed. Their data does not sufficiently support their claim that COVID-19 severity is associated with multi-organ damage. Further, the title should be precise in stating that this study is restricted to blood samples.

Response: As suggested, we changed the title into "Immunopathology and multi-organ damage in COVID-19".

2) It is puzzling why the authors decided to put a focus on testis and not on other organs that have been shown in multiple studies to be primarily effected in COVID-19 (e.g., the heart, intestine, pancreas etc.). Here, again the authors fail to cite the relevant literature on autopsy based COVID-19 studies.

Response: As shown in Fig. 3 and Fig. EV2, we analysed all organs that were likely to be affected by COVID-19, including lung, liver, brain, heart, intestine, testis, pancreas etc. Among these, the lung, brain and liver exhibited the most marked variation in tissue-enhanced proteins and so were described in more detail. These organs have also received considerable attention in multiple studies.

Although it has been reported that the SARS-CoV-2 receptor ACE2 can be highly expressed in testes (Verma et al., 2020; Wang et al., 2020; Wang and Xu, 2020), the effect of the virus on this organ remains unclear. Herein, we noted the significant variation in testis-enhanced protein expression in COVID-19 patients in our data. In addition, relevant citations have been added as suggested. See lines 68-71 and 315-322.

3) It is unclear how COVID-19 severity was defined here. According to WHO classification many of the patients (at least those that died) are presumably critical rather than severe. The description of the patient cohort is insufficient here. This is particularly important since others have shown that immune host response critically depends on severity of disease (see e.g. Chua et al. Nature Biotech 2020, https://doi.org/10.1038/s41587-020-0602-4)

Response: As described in the Methods section, patients in this study were divided into two groups based on their clinical signs and the need for oxygen: (i) mild – with clinical signs of pneumonia but without oxygen support, and (ii) severe – with oxygen support using non-invasive ventilation, tracheal tube, tracheotomy assist ventilation or extracorporeal membrane oxygenation (ECMO). Accordingly, the mild patient group included both mild (4 patients) and moderate (46 patients) patients under the WHO classification, while the severe group comprised severe (1 patient) and critical (15 patients) patients. We combined patients in this matter to obtain sufficiently large and balanced sample sizes. Importantly, this grouping did not influence, particularly the importance of the host immune response in shaping COVID-19 severity. We have modified the text to better describe the grouping structure (lines 414-421).

4) Correlation of multi-omics data with biochemical parameters is very interesting. However, it does not justify the statement "suggesting that that the molecular changes identified directly impact the pathophysiology of COVID-19". Along the same line, the statement "Combined, these data reveal an association between specific molecular variations and COVID-19 pathophysiology" is by no means supported by their data.

Response: We have revised and toned down this section. Please see lines 96-127.

5) "Compared to healthy controls, intensive alteration of tissue-enhanced proteins were observed in all COVID-19 patients, suggestive of multiple organ dysfunction including the lung, liver, brain, testis and intestine. " This statement is very misleading. First of all, the term tissue enhanced protein should be explained and linked with a proper citation to the HPA. Note that a third of all genes are tissue enhanced so the conclusions drawn from their finding of an enrichment of those genes in blood samples w.r.t to organ damage must be interpreted

with this in mind. This part of the manuscript is the weakest and the most over-interpreted part, which should be entirely reworked.

Response: The reviewer is correct and we apologize for our misleading statements. The tissue-enhanced proteins were defined by HPA (Uhlén et al., 2015), identifying proteins encoded by genes with an elevated expression (i.e. at least four-fold higher mRNA levels) in the specific tissue compared to the average level in all other tissues. We have added the definition/citation of HPA in the revised manuscript (lines 582-589).

Plasma proteins are generally synthesized in the liver, but also originate from a variety of other tissues and cells as a result of secretion (Anderson and Anderson, 2002; Guipaud, 2013). Plasma protein levels reflect human physiological biochemical states and have been used for the diagnosis and prognosis of many diseases (Geyer et al., 2017).

To better understand the impact of COVID-19 on patients, we mapped our plasma proteome data with HPA database. In total, 544 tissue-enhanced proteins were detected, among which 335 tissue-enhanced proteins were expressed significantly differently in COVID-19 patients' blood samples compared with healthy controls. Specifically, we found that proteins such as GLUL, GLUD2, GLUD1 that participate in neurotransmitters transport, and HTRA3, GRIK3, and GRIA3 that act as neurotransmitters receptors, were changed in plasma of patients. Although the alternation of these tissue-enhanced proteins in blood suggests the tissue were influenced by COVID-19, in accord with the reviewer's comments have we toned down the conclusions in this section. Please see lines 128-168.

6) Higher neutrophil counts were observed in severe patients but not in mild patients during hospitalization (Fig. S3C). Examination of the neutrophil transcriptomic signatures revealed that excessive neutrophil activation was associated with severe rather than mild disease. This is not all novel and has been shown in multiple studies before. Citations are missing.

Response: Citations have been supplemented as suggested (lines 184-185).

7) "Severe patients lost ~59.1% of their total T cell population, 62.3% of their CD4 T cells and 52.8% of their CD8 T cells. Importantly, the CD4 T cell population gradually recovered in the severe-survivors compared to the severe-fatality group". This data is impossible to interpret properly at presented here. Rather than showing relative values the authors must provide absolute standardized values (cells per ml or equivalent other absolute measures). The depletion of T cells may e.g. be a simple consequence of massive expansion of neutrophils in severe COVID-19.

Response: Sorry for the confusion. As shown below, these relative values were calculated by the absolute standardized values (cells per ml) of T cells detected clinically. We have added the absolute values and modified the text to make it clearer (lines 195-201).

In addition, we decided to utilize a widely-used deconvolution algorithm, i.e. xCell (Aran et al., 2017), to decipher the relative composition for many more immune cell types from the bulk RNA-seq data. For each RNA-seq library from patients of different levels of disease severity, we performed a global per library normalization before subjecting the data set to xCell, making the between-sample comparison of expression level of a given cell type meaningful.

Exam_name	Mild_mean (cells per ml)	Severe_mean (cells per ml)	Perc.
CD3#	937.2400	383.375000	59.1%
CD4#	554.0800	208.625000	62.3%
CD8#	344.9600	162.812500	52.8%

8) "Additionally, T cells in the survivors were primed by dendritic cells and expressed high levels of IFNG and GZMB (Fig. 4C)." This finding is not new at all and the proper literature must be cited.

Response: Citations have been added as suggested (lines 202-203).

9) "Although viral load declined during the period of hospitalization in both survival and fatal cases, it remained elevated in fatal cases compared to survivors." This plot of viral load is surprising because sustained high viral load in nasal swabs 40 days after hospital admission even in fatal COVID-19 has not been described in the literature. Typically, 4-6 weeks after hospitalization, even critical patients have little if any sign of viral RNA in nasal swabs with few exceptions. Insufficient information about viral load assessment are found in the methods section ("Quantitative viral load tests were performed using the BioDigital General dPCR kit (Jiangsu Saint Genomics..."). Given the variation in viral load estimates and the difficulty of obtaining standardized, quantitatively comparable viral load measurements it is essential to give all details on how the authors computed viral load. Further, individual trajectories of viral load per patient should be show in addition in particular for this six fatal cases.

Response: The reviewer gives us a good suggestion. The method for viral load measurement has been described in more detail as suggested (lines 437-444). In addition, viral load of each patient is now provided in Table S8.

Minor points:

1) In the introduction, the sentence "It is believed that SARS-COV-2 is able to use angiotensin-converting enzyme 2 (ACE 2) as a receptor for cell entry (Zheng et al., 2020; Zhou et al., 2020b)." should be corrected. It has actually been shown that this is the case (Hoffmann et al. 2020, CELL, https://doi.org/10.1016/j.cell.2020.02.052), and as such this paper should be cited first place here.

Response: Modified as suggested, please see lines 62-63.

2) In the introduction the authors should properly cover the relevant literature on autopsy based COVID-19 studies organs that show the primarily effected organs in COVID-19 (e.g., the heart, intestine, pancreas etc.).

Response: Added as suggested (lines 68-71).

3) The coverage of publications studying expression of ACE2 and its co-factors is quite biased and incomplete.

Response: As suggested, we have now cited more of the relevant literature (lines 63-68).

4) The authors fail to cite recent papers studying the host immune response in the respiratory system in COVID-9 patients. (e.g., Liao, et al. (2020) Nature Medicine; Chua et al., Nature Biotech 2020, https://doi.org/10.1038/s41587-020-0602-4)

Response: Modified as suggested, please see lines 75-76.

5) "Immune responses can cause severe damage to the cells or tissues that defend hosts against viral infection (Baseler et al., 2017; Cicchese et al., 2018; Newton et al., 2016)." Citations should be amended by those specifically studying SARS-CoV-2 infection.

Response: Modified as suggested, please see lines 171-172.

6) Fig. 2C: circos plots: The authors should explain how these plots were generated, e.g., by the circlize package. In that case, they should refer to that paper.

Response: We appreciate the reviewer's reminder. Yes, we used the circlize package to draw circos plots. We have added a description on the generation of circos plots in the "Methods" section and cited the paper in the revised manuscript. Please line 668.

7) Proper legend for Fig. S5c is missing. What do different colors, and line styles (dashed/connected) mean?

Response: We appreciate for reviewer's advice. We have added the following sentences to describe the meaning of the different colors and line styles in figure Fig. EV5c (Fig. S5c in previous version):

"Each color represents a model. A solid line represents the average for each model, whereas a dashed line represents one random iteration for each model."

reviewer #2:

The manuscript " COVID-19 severity is associated with immunopathology and multi-organ damage" by Chen et al investigates how host responses contribute to COVID-19 pathophysiology using a multi-omics approach to identify molecular markers in peripheral blood and plasma samples that distinguish COVID-19 patients experiencing a range of disease severities. Although the data is a monumental set of transcriptomic, proteomic, and metabolomic analysis there are several limitations that are not discussed or addressed by the authors that may limit the data interpretation and its applicability to COVID-19 patients. Of most significant concern is that the changes seen between mild and severe COVID-19 cases and more specifically those that survived or died may be reflective of severe illness and not specific to COVID-19 itself. Said another way is not clear if the pathways and notable findings by the authors would not be seen in sepsis or other severe illnesses. The authors have no controls from other severe illnesses (e.g. bacterial pneumonia and sepsis). Without these controls it is difficult to interpret whether these findings are unique to COVID-19. With this said these findings are important even if not unique to COVID-19 and therefore the manuscript should be accepted pending major revision.

Response: We thank the reviewer for this careful review and the comments.

Comments for the author:

1. As stated above controls leveraging other mild to severe illnesses (another example flu and death from flu). Without showing that these differences it is not clear if these changes are unique to COVID or just to severe illness. Addition of these controls would greatly strengthen this manuscript.

Response: Of course, we agree with the reviewer that data from another illness such as influenza would improve this manuscript. While it is very difficult to add such controls in this study, differences of tropism, replication and innate immune response between SARS-CoV-2 and other respiratory pathogens have been reported recently and we now describe some of these results.

SARS-CoV-2 was found to replicate better than SARS-CoV, but not as well as the 2009 pandemic influenza H1N1 virus, in bronchial epithelium. In addition, a less potent induced effect on proinflammatory cytokines was found in SARS-CoV-2 compared to H5N1, H1N1, and MERS-CoV (Hui et al., 2020). Examination of lungs from patients died from COVID-19 and H1N1 infection found that, vascular angiogenesis could distinguish pulmonary pathobiology of COVID-19 from that of severe H1N1 infection. Moreover, significant differences of CD4 T cell, CD8 T cell and neutrophil counts, as well as the expression of inflammation-related genes were observed between patients with COVID-19 and those with influenza (Ackermann et al., 2020). Hence, we believe that at least some of changes we observed between mild and severe COVID-19 patients are likely to be specific to COVID-19. As noted above, we have modified the text and added discussion comparing COVID-19 and other viral respiratory illnesses as suggested. Please lines 369-384.

2. The authors make the argument that the differences they see are characteristics of mild versus severe patients and "that distinguish COVID-19 patients experiencing a range of disease severities." Are these markers present early on in the disease process? Can they be used prospectively to partition patients who will go onto develop mild versus severe disease?

Response: Yes. As we showed in the principal component analysis (Fig. 7A), patients with good (mild and severe survivor) and poor (severe in-hospital and severe fatality) prognosis could be prospectively partitioned based on exRNA, transcriptome, proteome, and clinical covariate data from samples collected at the first timepoint. Accordingly, we further identified prognostic biomarkers from each of the four types of data. However, because of the relatively small patient sample size utilized in this study, additional work is needed to confirm the reliability and practicality of these biomarkers. This is clearly stated in the Discussion section of the manuscript.

3. The authors claim that significant changes in the proteome were seen in mild and severe COVID-19 patients. For example markers attributed to the lung, liver, kidney, testis etc were altered and noted to be downregulated (e.g. ALB). This is hard to attribute specifically to an organ or to COVID-19 disease. In almost all disease states particularly when people are

severely ill and not eating you see significant changes that are associated with illness and the fasting state. Albumin as are several markers are often diminished due to inflammatory or injury states and in themselves are not unique to a specific illness. Moreover the authors make the claim Fig.3C with a figure denoting brain injury and neuronal death. Current data that this occurs is quite limited and if the author wants to make claims of end-organ injury tissue sections etc for direct proof is needed.

Response: In fact, plasma proteome contains proteins from a variety of tissues and cells as a result of secretion (Anderson and Anderson, 2002; Guipaud, 2013). Plasma protein levels could reflect human physiological biochemical states and have been used for the diagnosis and prognosis for many diseases (Geyer et al., 2017).

To determine the impact of COVID-19 on patients, we mapped our plasma proteome data with the Human Protein Atlas (HPA) database and determined different expressions of tissue-enhanced proteins. Tissue-enhanced protein was defined as those encoded by genes that have an elevated expression (at least four-fold higher mRNA level) in the specific type of tissue compared to the average level in all other tissues (Uhlén et al., 2015). In total, we identified 544 tissue-enhanced proteins, among which 335 were expressed significantly differently in COVID-19 patients compared with healthy controls. Moreover, most of these altered tissue-enhance proteins that are related to fundamental functions of specific tissue were significantly downregulated in COVID-19 patients. Although it is hard to determine whether all of these changes are specific to COVID-19, our findings still provide important basic data for molecular changes in COVID-19 patients.

As shown in Fig. 3 and Fig. EV2 (Fig. S2 in previous version), we analysed and summarized all organs that were likely to be affected in COVID-19 infection, including lung, liver, brain, heart, intestine, testis, pancreas etc. Among these, lung, brain and liver, that showed the most significant variations in tissue-enhanced proteins, were described in detail. Brain injury and/or neurologic manifestations have also been observed in COVID-19 patients in several studies (Kandemirli et al., 2020; Mao et al., 2020), receiving increasing attention (Langfelder and Horvath, 2008). In addition,

multiorgan SARS-CoV-2 tropism including brain has been reported recently (Puelles et al., 2020). However, as the reviewer suggests, we have modified and toned down the statement on the section of "Tissue damage", please see lines in 128-168.

4. Several cytokines, chemokines and interferons are altered (up/down) in their proteomic or transcriptomic analysis. ELISAs (preferable) or Western Blots is needed to confirm these findings and claims.

Response: In fact, the absolute values of many cytokines, chemokines and interferons were obtained clinically using Cytometric Bead Array (CBA) and are shown in Table S2. Similar alterations between severe and mild COVID-19 patients: e.g. a high level of inflammatory cytokines (IL-6, IL-8, IL-10), neutrophil and monocytes in severe patients, were observed in both the clinical biochemical parameters and the omics data. We have modified the main text to make this clearer. Please see lines 172-180.

In addition, elevated levels of cytokines, chemokines and interferons in severe COVID-19 patients, especially in fatal COVID-19 cases, has also been confirmed in other studies. More relevant literatures have been added to support these findings.

5. Further discussion on how these changes seen with COVID-19 compare and contrast that with other severe illnesses and particularly viral illnesses such as influenza would greatly enhance the impact of this work.

Response: Many thanks for this good suggestion. We have modified the text and added discussion comparing COVID-19 and other viral respiratory illnesses as suggested. Please see lines 369-384.

reviewer #3:

In the manuscript "COVID-19 severity is associated with immunopathology and multi-organ damage," the authors utilize proteomics, metabolomics, and RNAseq to perform a systems-level analysis of physiological changes accompanying COVID-19 disease. The authors analyze samples from severe and mild COVID-19 patients, and compare them to healthy control individuals. The study is exhaustive and informative regarding the patterns and correlations observed between and among molecular alternations and clinical alterations accompanying COVID-19. Many interesting observations are reported, notably in the context of metabolism, tissue-related macromolecules, and the immune response. One critique of this paper is that the figures and legends require revision to better communicate the study design and the analysis performed. Another critique is that the authors do not sufficiently explain their analyses to the reader in the results text -- considering the choice of EMBO (a general interest journal), it would be helpful to explain their methodology a bit more, space permitting. This is an issue throughout the results section, and I suggest the authors revise the text accordingly.

Response: We thank the reviewer for these helpful comments. We have tried to clarify the figures and legends, and explained the methodology as much as possible.

The manuscript is organized largely based on results paragraphs and corresponding figures, so I have grouped suggested revisions according to this same pattern. Specific comments below.

Line 35 - The authors may want to soften the language around prognostic indicators, for example one change might be "...exRNAs may be used as biomarkers to predict the clinical outcomes of SARS-CoV-2 infection.". A central question I have is whether the size of the cohort used, particularly considering the small number of fatal cases (n=6), is sufficient for a high degree of prognostic certainty. My impression is that more validation work would need to be done to validate biomarkers, particularly as they relate to mortality, and I suggest the authors address this where appropriate. Please correct me if I'm wrong.

Response: We appreciate for the reviewer's advice and agree that future work is clearly needed to definitely confirm the reliability and practicality of these biomarkers. The

sentence in Line 35 of the Abstract section has been toned down to say: "Finally, we identified some genes, proteins and exRNAs as potential biomarkers that might be useful in predicting prognosis of SARS-CoV-2 infection." We have clearly stated this caveat in the Discussion section of the manuscript. Finally, we have tried to soften the language throughout the manuscript wherever appropriate.

Page 3 - I suggest streamlining the discussion of multiple organ infection by SARS-CoV-2 infection, it seems slightly repetitive in paragraphs 1 and 2 on this page.

Response: Thanks for the suggestion. We have revised this section, introducing the current state and clinical features of the COVID-19 pandemic in paragraph 2, while discussed two factors that might impact the COVID-19 severity: (i) multiple organ infection by SARS-CoV-2, and (ii) host immune responses in paragraph 3.

Line 85 - Patient cohort and clinical characteristics (not "characters")

Response: Modified as suggested.

Figure 1:

-Please revise Figure 1A to better reflect the study design. The main text includes mention of throat swabs, but these are not included in Fig. 1A. Please indicate these in figure along with assay run with those samples.

Response: Modified as suggested.

-It is not clear what analysis is run on the sampling timepoints from the blood based on Fig. 1A - if all -omics analyses were run on every timepoint, please revise to illustrate this.

Response: As suggested, both Figure 1 and its legend have been modified to reflect the study design more clearly. In addition, samples with specific sampling time used in each omics analyses are now listed in Table S1.

-The methods indicates that 1-5 timepoints were collected per patient, suggesting that for some patients time-resolved data was available and for some patients it was not. It may be helpful to mention for how many patients time-resolved data was available and analyzed.

Response: Yes, time-resolved data was available for some, but not all, patients. Related information has been supplemented in the text (see lines 426-428) and also listed in Table S1 in detail.

-Figure 1B: please clarify y-axis labels.

Response: Modified as suggested.

-Figure 1C: the authors may want to clarify these y-axis labels - the mention of both 'count' and '%' is somewhat confusing.

Response: Modified as suggested.

Figure 2:

-The labels in Figure 2B are too small to read.

Response: Thanks for the comments. We carefully revised all the figures including Figure 2B to make them clear to read.

-The left panel of Figure 2C would benefit from more explanation

Response: We apologize for the unclear explanation of Figure 2C. This shows different expression of proteins in samples collected at the first timepoint. The heatmap showed on left panel indicated the dynamic expression patterns of proteins overrepresented in healthy control (1,656 proteins), mild patients (1,547 proteins) and severe patients (2,362 proteins). The top categories enriched for clusters are shown. Values for each protein in each samples (columns) are color-coded based on expression level, low (blue) and high (red) z-scored FOT. Please see details in the modified legend of Figure 2C.

-For Figure 2D, the abbreviation WGCNA is not defined. Also, the main text references 14 modules of proteins, however it appears that 'Modules' depicts a number of colors in a row at the bottom of this heatmap, and 'Modules' also relates to the right hand panel. It would be helpful if these designations were clarified.

Response: Thanks for the comments. We apologize for the unclear explanation. WGCNA is the abbreviation of "weighted gene co-expression network analysis"

(Langfelder and Horvath, 2008). In our research, we applied weighted gene co-expression network analysis (WGCNA) to separate the proteomic profiles into 33 co-expression modules (ME0-ME32), in which ME0 refers to Module Grey, ME1 refers to Module turquoise, etc. as shown at the bottom of the heatmap.

Among the 33 modules, 6 showed significant correlation with clinical parameters (asterisk in heatmap): ME 14 (Module cyan) was strongly associated with TBIL: total bilirubin (Pearson r: 0.72), DBIL: Direct Bilirubin (Pearson r: 0.76), cTnl: cardiac troponin I (Pearson r: 0.62), and MYO: myoglobin (Pearson r: 0.67). ME 15 (Module midnightblue) was strongly associated with IL-6 (Pearson r: 0.99) and IL-10 (Pearson r: 0.98). ME2 (Module blue) was positively correlated with HS-CRP (Pearson r: 0.54). ME18 (Module lightgreen) was positively correlated with BR-PCT: platelet hematocrit (Pearson r: 0.95). ME1(Module turquoise) was positively associated with APTT: activated partial thromboplastin time (Pearson r: 0.77). ME17 (Module grey) was negatively associated with O2SAT: oxygen saturation (Pearson r: -0.96).

We further performed GO enrichment analysis on these six clinical associated modules as shown on the right-side of Figure 2D. The enriched pathways were labeled with their corresponding modules. To avoid misunderstanding, we added module names on the GO enrichment analysis panel of Figure 2D, and revised the manuscript, please see lines 115-118 and the modified Figure 2D for details.

-Figure 2E highlights some noteworthy coagulation-related proteins, I found this interesting.

-The heatmap colors in Figure 2E does not appear to be defined. Also, it is unclear how each of the three columns in 'Mild' and four columns in 'Severe' are defined.

-The heatmap colors in Figure 2F do not appear to be fully labeled. Also, it is unclear how each of the three columns in 'Mild' and four columns in 'Severe' are defined.

Response: Thanks for the positive comments. We apologize for the unclear label. For the heatmap on the previous version of the paper, we grouped the mild patients randomly into three groups and severe patients randomly into four groups, and calculated the average protein abundance of each group. The color of each cell represents average

protein abundance, low (blue) and high (red) z-scored FOT. Same method was used to generate heatmaps in Figure 3H (changed to boxplots now). To make the figure easier to read, we have revised Figure 2E and 2F to boxplots and shown the different expression of proteins in Module 1 (Fig. 2E) and 2 (Fig. 2F) between mild and severe patients. Please see the modified Figure 2E, F for details.

Tissue damaged caused by SARS-CoV-2, and Figure 3:

-The text in Figure 3A is too small to read. Fig. 3A is also somewhat challenging to interpret at first, it seems like the authors are trying to depict the abundance of the dysregulated proteins in relation to the relative abundance of non-dysregulated proteins (please correct me if I'm wrong). There may be a clearer way to present or explain this data.

Response: We apologize for the unclear presentation of Figure 3A. The goal of Figure 3A is to show the number of alternated tissue-enhanced proteins compared to the number of total tissue-enhanced proteins detected in our data. As suggested, we revised Figure 3A into rose plots, please see the modified Figure 3A in the revised manuscript.

-The X-axis labels in Figure 3F can be shortened.

Response: Modified as suggested.

-Fig. 3G. The "X-Cell" acronym is "xCell" in other parts of the manuscript, please harmonize. Also, the use of colors to depict both the heatmap and the cellular populations is somewhat confusing, the authors might consider an alternative way to designate cellular subtypes in this heatmap.

Response: We have harmonized the term "X-Cell" in the manuscript. Following the reviewer's suggestion, we revised Figure 3G into bar plots, please see the modified Figure 3G in the revised manuscript.

-Fig 3H. It is unclear what the four columns in 'Control' and four columns in 'Mild', and six columns in 'Severe' are comprised of. Are these referring to specific individuals in the cohort? If so, how were they chosen?

Response: Sorry for the unclear label of Figure 3H. Please see our response above to Figure 2E for the heatmap in the previous version of the paper. To make the figure easier to read, we have revised Figure 3H to boxplots which showed different expression of cell type specific proteins among health control, mild and severe patients. Please see the modified Figure 3H for details.

-Figure 3I - Please correct me if I'm wrong, I don't see how age or gender are depicted in this subfigure (these are indicated in the color legend).

Response: Sorry for the unclear label. The age and gender of individuals were depicted on the top panel of each heatmap. The figure legend has been modified for a clearer description.

Line 150: The authors may want to specify the type of -omics under analysis early in this paragraph, e.g. exRNA.

Response: Modified as suggested, please see lines 152-153.

Line 152 refers to Figure 3F, however this may mean Figure 3G.

Response: Yes, it should be Figure 3G and 3H. Thank you for the correction.

The manuscript would benefit from a definition of "tissue-enhanced protein", as well as "proteins related to organ function", as these terms may not be commonly understood.

Response: Thanks for the comments. We have added a definition as suggested and modified the statement to make it clearer. Pleases see lines 130-135 and 582-589.

Immunopathological changes in COVID-19 patients, Figure 4 and Figure S3:

Line 185 - "The CD4 T cell population gradually recovered in the severe-survivors compared to the severe-fatality group (Fig. S3E)." The heatmap suggests that the T-cell numbers are higher in severe survivors, but I don't see an obvious upward trend over time.

Response: Sorry for the inaccurate statement. In fact, Fig. S3E (Fig. EV3E in the revised version) aimed to show a certain level of T cell activation in the severe-survivor group

which could not be observed in the severe-fatality group. We have modified the statement in the revised version of the paper, please see lines 199-201.

Figure S3E - heatmap colorbar requires labeling.

Response: Thanks for the reminder. The color bar label has been added in Fig. EV3E (Figure S3E in the previous version). In the legend for Fig. EV3E, we also added the following sentences to describe the labeling of colorbars of the heatmap:

"The colors of the heatmap represent the Z-scaled values of fractions of each cell type from different stages and subgroups. The red color stands for a higher proportion whereas the blue color denotes a lower cell population."

Biomarkers predictive of clinical outcomes of COVID-19 patients:

It may be helpful if the authors could mention the size of each category of patient utilized to train their prognostic model, comment on the certainty of their findings in the context of their cohort size, and mention what further work would be required to validate biomarkers for COVID-19 prognostic use.

Response: We greatly appreciate for reviewer's advice. Accordingly, we added:

(1) A description of the size of each category of patients utilized to train the prognostic models in the "Methods" section (lines 616-623):

"The numbers of samples used in predictive modeling were as follows: for the exRNA-seq and the matched clinical testing data sets, a total of 37 patients were used, including 28 patients with good outcomes and 9 patients with poor outcomes. For the RNA-seq data set, 63 patients were used, including 55 patients with good outcomes and 8 patients with poor outcomes. For the proteomics data set, 31 patients were used, including 21 patients with good outcomes and 10 patients with poor outcomes. Samples from the first sampling timepoint from each patient were used in the analysis."

(2) Regarding the certainty of our findings in the context of cohort size, we would like to bring the attention of the reviewer to the section on the effect of sample size on model performance illustrated in Fig. EV5C (lines 648-656):

Specifically, we did try to estimate the effects of sample size on the accuracy and variability of the prognostic models. We performed learning curve model comparison

(LCMC) analysis and found that 15 samples for clinical variables, 23-30 samples for exRNA-seq data were sufficient for building prognostic models, when AUC reached 1 (Fig. S5C). The sample sizes for the proteomic and mRNA-seq data were between those observed for clinical variables and the exRNA data. Thus, we concluded that there is potential utility of the prognostic biomarkers for predicting the clinical outcomes of SARS-CoV-2 infection although the sample size used in this study is limited due to practical constraints.

(3) Regarding what further work would be required to validate biomarkers for COVID-19 prognostic use:

We clearly stated the caveat of limited sample size in the Discussion section of the manuscript (lines 399-402). We hope that after the publication of our work, larger scientific and clinical studies will be performed to test the ideas presented.

Discussion:

Line 315 - "The data generated here revealed that the number of AT1 and AT2 cells reduced significantly in severe patients..." Regarding the molecular profiling of blood plasma to verify tissue health, I wonder if the authors might address the shortcomings of this approach to measure the health of specific cell types in the body. I think these observations are complemented very well by citing autopsy studies, for example, but I wonder if alone they are sufficient to make such claims. Please correct me if I'm wrong.

Response: There is now more evidence from other groups that SARS-CoV-2 can cause multi-organ damage in severe COVID-19 patients. Citation of more related studies and autopsy findings have there been added. Please lines 315-318.

In addition, ground-glass opacity in computed tomography (CT) images revealed severe lung injury in these patients. A set of biochemical parameters are commonly used to indicate organ dysfunction and we observed significant variations of some of these biochemical parameters, such as higher levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), Creatine kinase (CK), B-type

natriuretic polypeptide (BNP), Urea in severe patients (Table S2), suggesting organ dysfunction including liver, heart, kidney in these patients.

Notably, while the plasma proteome is generally synthesized in liver, it contains proteins from a variety of other tissues and cells as a result of secretion (Anderson and Anderson, 2002; Guipaud, 2013). Plasma protein levels reflect human physiological biochemical states and have been used for diagnosis and prognosis for many diseases (Geyer et al., 2017). We have also softened the language in the section of "Tissue damage" to make it more accurate. Please see lines 128-168.

Line 334 - "a role for T cells in SARS-CoV-2 infection not yet has been determined, likely reflecting "lymphopenia". I suggest rephrasing for clarity.

Response: When we originally submitted our paper, little was known about the function of T cell response in SARS-CoV-2 infected individuals. Our work shows that the mild disease group showed robust T cell responses, and the ones who failed to activate T cell responses usually suffered severe COVID-19 disease. This effect has now been confirmed by other papers (Braun et al., 2020; Sekine et al., 2020). The relevant text has been modified for clarity and the recent literature has now been cited. Please see lines 349-354.

References

Ackermann, M., Verleden, S.E., Kuehnel, M., Haverich, A., Welte, T., Laenger, F., *et al.* (2020). Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Engl J Med 383: 120-128.

Anderson, N.L., and Anderson, N.G. (2002). The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics 1: 845-867.

Aran, D., Hu, Z., and Butte, A.J. (2017). xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol 18: 220.

Braun, J., Loyal, L., Frentsch, M., Wendisch, D., Georg, P., Kurth, F., *et al.* (2020). SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature.

Geyer, P.E., Holdt, L.M., Teupser, D., and Mann, M. (2017). Revisiting biomarker discovery by plasma proteomics. Mol Syst Biol 13: 942.

Guipaud, O. (2013). Serum and plasma proteomics and its possible use as detector and predictor of radiation diseases. Adv Exp Med Biol 990: 61-86.

Hui, K.P.Y., Cheung, M.-C., Perera, R.A.P.M., Ng, K.-C., Bui, C.H.T., Ho, J.C.W., *et al.* (2020). Tropism, replication competence, and innate immune responses of the coronavirus SARS-CoV-2 in human respiratory tract and conjunctiva: an analysis in ex-vivo and in-vitro cultures. Lancet Respir Med 8: 687-695.

Kandemirli, S.G., Dogan, L., Sarikaya, Z.T., Kara, S., Akinci, C., Kaya, D., *et al.* (2020). Brain MRI findings in patients in the intensive care unit with COVID-19 Infection. Radiology: 201697.

Langfelder, P., and Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. BMC bioinformatics 9: 559.

Mao, L., Wang, M., Chen, S., He, Q., Chang, J., Hong, C., *et al.* (2020). Neurologic manifestations of hospitalized patients with Coronavirus Disease 2019 in Wuhan, China. JAMA Neurol 77: 1-9.

Puelles, V.G., Lütgehetmann, M., Lindenmeyer, M.T., Sperhake, J.P., Wong, M.N., Allweiss, L., *et al.* (2020). Multiorgan and renal tropism of SARS-CoV-2. N Engl J Med 383: 590-592.

Sekine, T., Perez-Potti, A., Rivera-Ballesteros, O., Straling, K., Gorin, J.-B., Olsson, A., *et al.* (2020). Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell DOI: https://doiorg/101016/jcell202008017.

Uhlén, M., Fagerberg, L., Hallström, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., *et al.* (2015). Proteomics. Tissue-based map of the human proteome. Science 347: 1260419.

Verma, S., Saksena, S., and Sadri-Ardekani, H. (2020). ACE2 receptor expression in testes: implications in COVID-19 pathogenesis. Biol Reprod.

Wang, S., Zhou, X., Zhang, T., and Wang, Z. (2020). The need for urogenital tract monitoring in COVID-19. Nat Rev Urol 17: 314-315.

Wang, Z., and Xu, X. (2020). scRNA-seq profiling of human testes reveals the presence of the ACE2 receptor, A target for SARS-CoV-2 infection in spermatogonia, leydig and sertoli cells. Cells 9: 940.

1st Revision - Editorial Decision 16th Oct 2020

Dear Yong-Zhen,

Thank you for submitting your revised manuscript to the EMBO Journal. I have now had a chance to take a careful look at the revised version and the revision has also been seen by the original referee # 1 whose comments are provided below.

As you can see the referee appreciate the introduced changes and support publication here. The referee has a few remaining points that I would like to ask you to address in a revised version address with appropriate text changes and additional information in the method section.

Regarding the title - I agree with the comment made by the referee, but not so sure that I like the one proposed by the referee, maybe try to come up with some variation on this. Happy to give more input on this if needed.

When you submit your revised manuscript will you also please take care of the following points.

- Figures 2, 3, 5 and EV1 are very busy and I am wondering if you should split some of them into 2 figures. OK to have more main figures and 6 EV files. I will leave the decision up to you.

- Dataset EV1 contains Tables S1-S9. Please upload each Table separately and add a legend in a separate tab.

- We don't encourage data not shown (pg 9) - please take a look and rephrase or provide the data.

- For the reference list - for articles with more than 10 authors the author list should be cut after 10 authors followed by et al.

- The ORCID ID is missing for Chen Ding and Tong-Yu Zhu

- Please double check grant # 2019FY101500. It is listed as 2019FY101400 in the submission system

- The appendix is missing a ToC

- We include a synopsis of the paper (see http://emboj.embopress.org/). Please provide me with a general summary statement and 3-5 bullet points that capture the key findings of the paper.

- We also need a summary figure for the synopsis. The size should be 550 wide by [200-400] high (pixels). You can also use something from the figures if that is easier.

That should be all!

Let me know if we need to discuss something further

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

Instructions for preparing your revised manuscript:

Please check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
https://bit.ly/EMBOPressFigurePreparationGuideline

IMPORTANT: When you send the revision we will require
- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
- Expanded View files (replacing Supplementary Information)
Please see out instructions to authors
https://www.embopress.org/page/journal/14602075/authorguide#expandedview

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The revision must be submitted online within 90 days; please click on the link below to submit the revision online before 14th Jan 2021.

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

This reviewer appreciates the multiple efforts the authors have taken in improving their manuscript along the detailed comments of all reviewers. However, I still disagree with some of the statements of the authors:

1) The title is still misleading and does not properly reflect the results of this paper. Instead of "Immunopathology and multi-organ damage in COVID-19" I would suggest a title such as "Immunopathology in the blood suggests impact on multi-organ damage in COVID-19"
2) Most of the inappropriate claims have been properly rephrased and citations complemented. Still, there is a new claim in the revised paper that needs to be rephrased (Page 9, line 166-167): „Together, our data indicate that COVID-19 has a great impact on functions of multiple organs including lung damage." I would suggest again to downtone this statement, e.g., by replacing "great impact" by "potential impact".
3) I appreciate the additional details on methods and measurements of viral loads in the revised version (Table S8 and Methods section). Unfortunately, I was unable to retrieve Table S8 from the submission. Further, I still miss details in the methods section about how the authors normalized RT-PCR measurements. Given the unusually prolongued levels of relatively high viral load the quantification of high viral loads need to be explained.
4) Additional comment:
Page 16, line 319-320: In the discussion of "how SARS-CoV-2 is able to cross the blood-brain or the blood-testis barriers?" the authors may wish to discuss the recently described role of the olfactory mucosa for SARS-CoV-2 propagating into the CNS.
https://www.biorxiv.org/content/10.1101/2020.06.04.135012v1

Response to reviewers' comments:

Referee #1:

This reviewer appreciates the multiple efforts the authors have taken in improving their manuscript along the detailed comments of all reviewers. However, I still disagree with some of the statements of the authors:

Response: We sincerely thank the reviewer for this careful review and the comments provided.

1) The title is still misleading and does not properly reflect the results of this paper. Instead of "Immunopathology and multi-organ damage in COVID-19" I would suggest a title such as "Immunopathology in the blood suggests impact on multi-organ damage in COVID-19".

Response: Thank you for the suggestion. We have changed the title to "Molecular markers in the blood are associated with Immunopathology and multiorgan damage in -COVID-19" which we believe is a more accurate description of our paper.

2) Most of the inappropriate claims have been properly rephrased and citations complemented. Still, there is a new claim in the revised paper that needs to be rephrased (Page 9, line 166-167): „Together, our data indicate that COVID-19 has a great impact on functions of multiple organs including lung damage." I would suggest again to downtone this statement, e.g., by replacing "great impact" by "potential impact".

Response: Modified as suggested. Please see lines 167-168.

3) I appreciate the additional details on methods and measurements of viral loads in the revised version (Table S8 and Methods section). Unfortunately, I was unable to retrieve Table S8 from the submission. Further, I still miss details in the methods section about how the authors normalized RT-PCR measurements. Given the unusually prolonged levels of relatively high viral load the quantification of high viral loads need to be explained.

Response: We apologise for the confusion. We used digital RT-PCR to quantify viral loads in SARS-CoV-2 positive samples. Specifically, 15 µL RNA solution of each sample was mixed with 20 µL reaction buffer. This reaction buffer comprised 7 µL 5×RT-PCR buffer, 3 µL Taq polymerase and 10 µL SARS-CoV-2 detection mix provided by the kit (BioDigital General dPCR kit, Jiangsu Saint Genomics, Cat no. CSJ-3-0018). As suggested, we have modified the text to make this clearer. Please see lines 440-449.

Unlike Real-Time RT-PCR (qRT-PCR), digital RT-PCR does not require a standard curve to provide absolute quantification of viral loads. It measures the true number of molecules (template nucleic acid) as one droplet only contains one DNA molecule. Hence, digital PCR can provide absolute quantification, which refers to the number of droplets that are fluorescing due to proper amplification.

4) Additional comment:

Page 16, line 319-320: In the discussion of "how SARS-CoV-2 is able to cross the blood-brain or the blood-testis barriers?" the authors may wish to discuss the recently described role of the olfactory mucosa for SARS-CoV-2 propagating into the CNS. https://www.biorxiv.org/content/10.1101/2020.06.04.135012v1

Response: Added as suggested. Please see lines 326-329.

2nd Revision - Editorial Decision 27th Oct 2020

Dear Yong-Zhen,

Thank you for submitting your revised manuscript to the EMBO Journal. I have now had a chance to take a look at it and I appreciate the introduced changes. I am therefore very pleased to accept the manuscript for publication here.

Congratulations on an important study

With best wishes

Karin

Karin Dumstrei, PhD
Senior Editor
The EMBO Journal

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Corresponding Author Name: Chen Ding, Tong-Yu Zhu, Yong-Zhen Zhang
Journal Submitted to: The EMBO Journal
Manuscript Number: EMBOJ-2020-105896

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi 2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

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B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	This study included 66 clinically diagnosed and laboratory confirmed COVID-19 patients hospitalized at the Shanghai Public Health Clinical Center, Shanghai, China between January 31st and April 7th, 2020. These patients were selected based on the following criteria: 1) severe patients – with oxygen support using non-invasive ventilation, tracheal tube, tracheotomy assist ventilation or extracorporeal membrane oxygenation (ECMO) or 2) mild patients with serial blood and throat swab samples.
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Not available
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	1) For RNA-seq data, a QC analysis and library filtering were performed before downstream biological analysis. Libraries that passed the following criteria were retained: (i) more than five million reads; (ii) more than 90% of reads aligned to the human reference genome; (iii) over 10,000 genes were expressed (a gene with FPKM>0.5 was identified as an expressed gene). In addition, to monitor data quality across batches, libraries of some heathy control samples were constructed and sequenced 2-3 times. The average expression profile of the multiple libraries from each heathy control sample were calculated for follow-up analyses. 2) For exRNA data, A QC analysis was performed prior to biological analysis by removing (i) libraries with low sequencing depths (<1M raw reads); (ii) libraries with mapping ratio lower than 50%, and (iii) libraries with low transcript-genome ratios. To minimize the impact of noise due to low expression levels, only 769 miRNAs with at least 1 count per million in no less than 10% of the total number of samples were included in the final analysis.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	No
For animal studies, include a statement about randomization even if no randomization was used.	Not available
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	No
4.b. For animal studies, include a statement about blinding even if no blinding was done	Not available
5. For every figure, are statistical tests justified as appropriate?	Yes.

Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	Both Kolmogorov-Smirnov (K-S) test and Shapiro-Wilk test were used for normality test. For data that meet the assumption of normal distribution, a Student's t-test or parametric ANOVA test was used for a statistical test, otherwise Mann-Whitney-Wilcoxon tests or nonparametric ANOVA test was used.
Is there an estimate of variation within each group of data?	No
Is the variance similar between the groups that are being statistically compared?	No

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Not available.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Not available.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Not available.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Not available.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Not available.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	All human samples included in the present study were obtained after approval of the research by the Shanghai Public Health Clinical Center Ethics Committee (YJ-2020-S018-02)
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	A written informed consent was obtained from each patient. All experiments in this study conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	Not available.
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	All the data generated in this study could be obtained and/or reused non-commercially and under proper citation or appreciation of this study.
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	Not available.
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not available.
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not available.

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	The datasets produced in this study are available in the following databases: (1) RNA-Seq and exRNA-Seq Data: NODE OEP000868 (http://www.biosino.org/node/project/detail/OEP000868) (2) Raw mass spectrometry data: iProX IPX 0002186001 (https://www.iprox.org/page/subproject.html?id=IPX0002186001)
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	Access to the datasets produced in this study was provided in the manuscript under the "Data Availability" section.
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	Human clinical datasets was provided in Table S1 and S2. Other datasets produced in this study was provided in the manuscript under the "Data Availability" section.
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	Not available.

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	Not available.