

Peer Review File

Article Information: <https://dx.doi.org/10.21037/cdt-23-381>

Reviewer A

This study presents interesting findings with clinical value of showing that SARS-cov-2-induced inflammatory injury to the cardiac tissues even after the patient recovering from it. I have some specific comments for improvement:

Comment 1:

Abstract: fine.

Reply1 :

Thank you for your comments.

Comment 2:

Introduction: good literature review with research gap identified.

Reply2 :

Thank you for your comments.

Comment 3:

Materials and Methods: Patients who underwent open heart surgery are mainly due to MVR and LA volume reduction, so this should be listed as the first inclusion criterion. Authors mentioned that all patients underwent chest CT to exclude lung diseases. What about these CT scans? were they normal or abnormal? This should be briefly mentioned in the Results.

Reply3 :

Thank you for your valuable suggestions. We have revised the inclusion criterion and MVR and LA volume reduction was listed as the first inclusion criterion. Besides, chest CT results of all patients were added in the results.

Changes in the text:

We have modified our text as advised (page7-8, line109-1113) and (page12, line192-197).

Comment 4:

Is Mann-Whitney test correct for comparison of continuous variables? Please double check it.

Reply4 :

Thank you for your comments. In this study, the variables (such as age, BMI, etc.) exhibited a non-normal distribution, potentially attributed to the limited sample size. Therefore, Mann-Whitney U test was used to analyze the continuous variables in our study. Thus, the results were expressed using median and interquartile range (p25, p75). Additionally, the statistical analysis of this study has been reviewed and validated by a statistical professor from our institution. Sorry for make you confused, and we have updated the statistical methods and related content accordingly.

Changes in the text:

We have modified our text as advised (page 11, line185-188).

Comment 5:

Figure 1 the flow chart seems quite simple. More information should be added, in particular, the last box of 9 patients: Test team n=9: revise to: final study patients n=9, cardiac tissue samples analysed for pathological findings, etc. Something like that.

Reply5 :

Thank you for your valuable suggestions, and we have updated the flow chart in this manuscript. Additional details regarding the study design and methodology have been included in response to your comment.

Changes in the text:

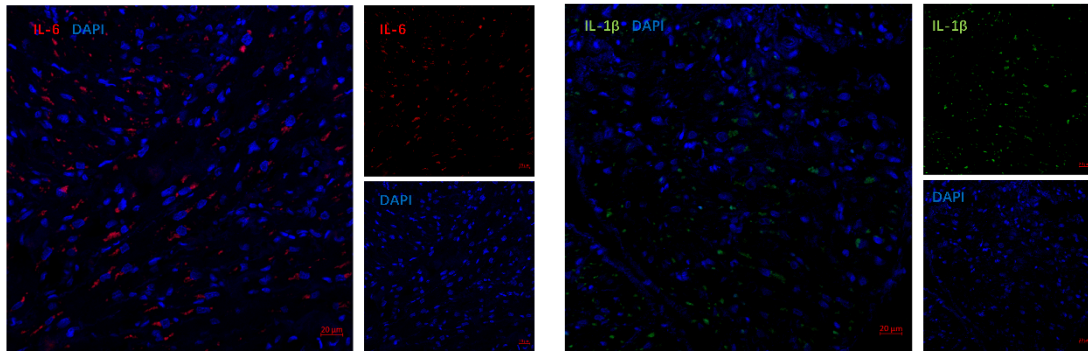
Thank you for your valuable suggestions. We have modified the flow chart as advised (page22).

Comment 6:

Results: I would suggest providing one or two figures showing pathological specimen of cardiac tissues with inflammatory changes.

Reply6 :

Thank you for your valuable suggestion. To present cardiac inflammatory changes more effectively, we have conducted the immunofluorescence detection of IL-6 and IL-1 β in cardiac tissue from one SARS-CoV-2(+) patient. Proinflammatory cytokines IL-6 and IL-1 β were observed in cardiac tissue (figure 3 and figure 4, also show below). Accordingly, we revised the main text.

**Changes in the text:**

We have modified our text as advised (page3, line39-40; page13, line235-237; page26, figure 3 and figure 4).

Comment 7:

Discussion: needs some improvement. Study limitations were not mentioned. Thus needs to be provided before the conclusion.

Reply7 :

Thank you for your comments. We have added the limitations of this article before the conclusion.

Changes in the text:

We have modified our text as advised (page18, line329-336).

Comment 8:

Overall, I feel like that the manuscript needs to be strengthened as it gives me an impression that some parts were simply presented such as Results.

Reply8 :

Thank you for your comments. We have added the limitation of our study, revised the manuscript including the results and discussion section, and update the references (ref 12, 15,17,19,26, 27).

Changes in the text:

We have modified our text as advised (pgae3, line45-46; page5, line52; page11, line171-181; page12, line191-197, line205-211; page13, line213-227; page14-15, line246-260; page16-17, line295-303).

Reviewer B

I am sincerely thankful for the opportunity of revising this original article. The following study was designed to investigate the persistent SARS-CoV-2 colonization and associated inflammatory markers presence in cardiac tissue in patients scheduled for open-heart surgery who tested negative upon admission. The authors investigate the presence of the virus in cardiac muscle as well as the expression of inflammatory markers up to 6 months after COVID-19 infection. This brings novelty since no other similar article (for whom I personally searched) has not related to such a long period after acute infection. This article (if published) might not only help in determining the maximum duration for persistent SARS-CoV-2 myocardial involvement, but also help clinicians make decisions depending on their patient's COVID status. Long COVID should be further studied and taken into special consideration in vast majority of patients who suffer from chronic diseases (including heart diseases). It could exacerbate these states, worsen established treatment outcomes and undermine clinicians' effort.

Acknowledging it might change the current routine in handling many chronic diseases including heart diseases. The paper itself is well written and concise. The tables and figures are clear for the most part. The authors are honest about their study limitations and present the current state of knowledge well.

Nevertheless, I have few minor comments for the authors:

Comment 1:

Why weren't patients older than 70 years old included in the study? If there weren't any maybe the inclusion criteria should be changed to "over 18 years old"?

Reply1 :

Thank you for your kind reminding. The patients in this study were not over 70 years old. We have adjusted the inclusion criteria accordingly.

Changes in the text:

We have modified our text as advised (page8, line110).

Comment 2:

Did tests used upon admission include PCR for SARS-CoV-2?

Reply2 :

Thank you for your comments. The patients underwent one PCR test (throat swabs) for SARS-CoV-2 before hospital admission (outpatient department), and the results of all patients were negative. This has been updated in the article.

Changes in the text:

We have modified our text as advised (page7, line100; page8, line110).

Comment 3:

I would change the inclusion criterion (4) to "mitral valve replacement (MVR) + left atrial (LA) volume reduction" and move the explanation about discarded material down to the line 94. Exemplary sentence could be "During MVR + LA procedures tissues of left atrium were used as samples for testing (they are normally discarded)." or "During

MVR + LA procedures routinely discarded tissues of left atrium were used as samples for testing.” or similar.

Reply 3 :

Thank you for your valuable suggestion. We have updated the inclusion criteria, and, in consideration of its significance, adjusted it to be the first item. The explanation of discarded materials has been moved to the subsequent section. Thank you for your valuable suggestion.

Changes in the text:

We have modified our text as advised (page8, line109-113; line117-118).

Comment 4:

In Figure 1. – maybe “Tested population” will be more appropriate than “Test team”? I leave decision up to the authors.

Reply4 :

Thank you for your valuable suggestion. The relevant sections have been updated according to the two reviewers’ comments.

Changes in the text:

We have modified Figure 1 (page22).

Comment 5:

In Table 1. – I don’t see explanation for end-diastolic volume abbreviation. I am also confused about mechanical ventilation expressed in minutes – to which specific time in the (peri-operatively) it relates (since ICU stay is expressed in days). Also, I believe there is a typo regarding post-operation hospital stay ("stays").

Reply 5 :

Thank you for your valuable suggestion. We have examined the description of our mechanical ventilation and corrected the error where “h” was mistakenly written as “min”. We have thoroughly checked this manuscript again, rectifying spelling errors, and improving the abbreviation section.

Changes in the text:

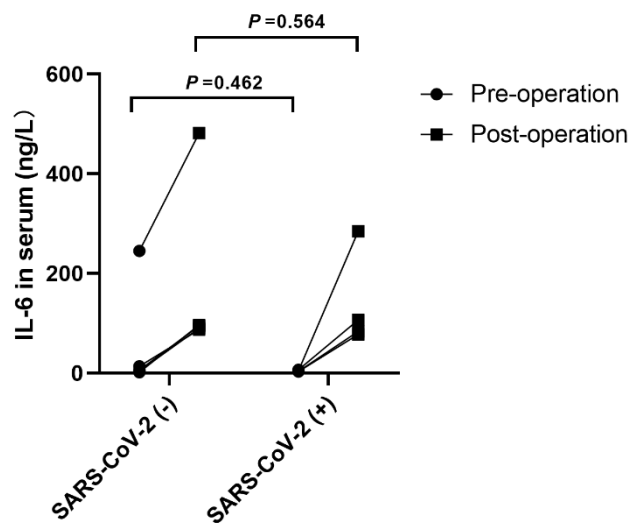
We have modified our text as advised (page23, Table1).

Comment 6:

The authors write that “while only one SARS-CoV-2 (-) patient displayed an abnormal elevation in IL-6 levels following surgery”. In Figure 3. I can see that IL-6 level in this patient was abnormal, also preoperatively?

Reply 6 :

Thank you for your valuable suggestion. We reexamined this patient and found that the preoperative IL-6 level was also abnormal. To present our research findings more effectively, we have redrawn the figure 5 (also show below). The relevant sections have been modified accordingly.



Changes in the text:

We have modified our text as advised (page13, line243-244; page26, Figure5).

Comment 7:

Table 2. - Please make sure all abbreviations from Table 2. are explained. These might not be clear for people who are not clinically oriented (i.e. abbreviations for creatinine or aminotransferases).

Reply 7 :

Thank you for your review. We have rechecked the abbreviations in Table 2 and provided explanations for abbreviations, including creatinine and transaminases. The relevant sections have been adjusted.

Changes in the text:

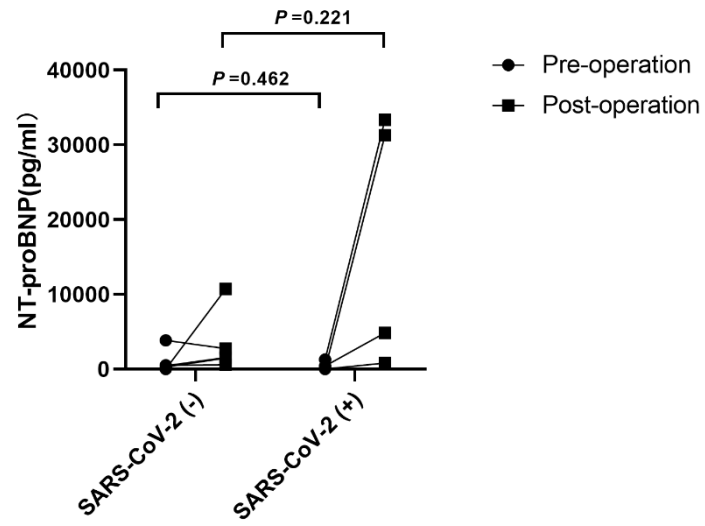
We have modified our text as advised (page28, Table2).

Comment 8:

I find it interesting that the postop increase in natriuretic peptide was expressed in SARS-CoV-2 (+) group (however statistically insignificant) since they undergone the same exact procedure as SARS-CoV-2 (-) group. Maybe it is just a coincidence (small study group) or maybe this is a consequence of persistent myocardial involvement (just wondering).

Reply 8 :

Thank you for your comments. We have analyzed the patients' preoperative and postoperative NT-proBNP. Indeed, the SARS-CoV-2 (+) patients exhibited higher levels of NT-proBNP compared to SARS-CoV-2 (-) patients, although the differences did not reach statistical significance. Due to the small sample size, this trend may be coincidental or may indicate sustained myocardial involvement. Thank you for your valuable advice. We have revised this section in the result (Figure 6, also show below) and revised the discussion section.



Changes in the text:

We have modified our text as advised (page14, line253-256; page30, Figure6).