Peer Review File

Article Information: http://dx.doi.org/10.21037/cdt-535

Detailed Responses to Reviewer A

General Comments:

Thank you for this manuscript which contribute to more understand cardiac involvement in COVID-19. The authors should make some modifications to increase the quality of the manuscript.

Reply:

We thank the Reviewer for the positive comments on our study. We have followed your suggestions and made every possible effort to address the concerns. Detailed responses are below.

Major Comments:

Comment 1:

Since "Cardiac injury" is a broad term change this by "myocardial injury" will be more specific.

Reply:

We thank the Reviewer for the constructive suggestion about our study. We accordingly have changed the "cardiac injury" to "myocardial injury".

Comment 2:

Precise all exclusion criteria in the manuscript.

Reply:

We thank the Reviewer for the constructive suggestion. We have added the exclusion criteria as follows (kindly see line 155-156): "Studies were excluded if they did not reported defined myocardial injury indexes or published in meta-analysis or case report."

Comment 3:

The authors should precise the specific type of studies that they have considered: cases series, case control, Cohort or RCT.

Reply:

We thank the Reviewer for the constructive suggestion. We have defined the included study types as follows (kindly see 151-155): "Studies of any types (case series study, cross-sectional study, case control study, cohort study, or randomized controlled trial) were eligible for inclusion if they included SARS-CoV-2 infected adult patients; reported the qualitative data of cardiac specific biomarkers (troponin or CKMB); or reported the data of myocardial injury with detailed definition."

Comment 4:

Knowing that MRI is a sensitive tool to assess myocardial injury, the authors should explain why they did not include such studies.

Reply:

We fully agree with the Review's opinion. MRI can assess myocardial injury including myocardial edema, intramyocardial hemorrhage, infarct size, microvascular obstruction, with high spatial resolution and excellent reproducibility. However, given the difficulty of performing echocardiography and MRI under strict isolation while wearing personal protective equipment, and the associated risk to staff, the exact prevalence and nature of cardiac dysfunction in COVID-19 may difficult to be fully illuminating. We according have added the related statement in the limitation section as follows (kindly see line 429-433): "Secondly, given the difficulty of performing echocardiography or cardiac magnetic resonance imaging under strict isolation, the exact prevalence and nature of myocardial injury in COVID-19 may difficult to be fully illuminating. Thus, in the present study, we used myocardial enzymology indexes as the definition of myocardial injury."

Comment 5:

It is important to precise in the methodology that all preexisting cardiovascular risk factors or established diseases will be taken into consideration in the meta-regression in order to rule out the specific effect of SARS Cov2.

Reply:

We thank the Reviewer for the constructive suggestion. We accordingly have revised the sentence as follows (kindly see 212-214): "To address the potential risk factors associated with myocardial injury, all preexisting cardiovascular risk factors or

established diseases will be taken into consideration in the meta-regression."

Comment 6:

It is also unclear if there were some autopsy studies which were included in the manuscript. This is important because authors mentioned cardiac injury in non survivors.

Reply:

We thank the Reviewer for pointing out this issue. Evidence from autopsies found that 35% of heart samples in SAR-CoV infected patients presented the viral genome, which raised the possibility of direct impair of cardiomyocytes by the virus. SAR-CoV-2 might share the same mechanism as the highly homologous with SAR-CoV. Nevertheless, no pathological studies have demonstrated the presence of SAR-CoV-2 within myocardial tissue. In addition, most of the autopsy studies were case report. Thus, autopsy studies are not suitable for the present study to assess the incidence of myocardial injury. We have stated the related sentence in the discussion section as follows (kindly see 372-378): "Evidence from autopsies found that 35% of heart samples in SAR-CoV infected patients presented the viral genome, which raised the possibility of direct impair of cardiomyocytes by the virus. SAR-CoV-2 might share the same mechanism as the highly homologous with SAR-CoV. Nevertheless, a recent pathological study failed to demonstrate the presence of SAR-CoV-2 within myocardial tissue. Therefore, the question of whether the SAR-CoV-2 could directly damage the heart requires further scientific verification."

Detailed Responses to Reviewer B

***General Comments*:**

My hearty congratulations to the authors for choosing to work on this highly contagious and fatal pandemic the world is currently facing. These diseases leads to several complications like cardiac injuries which can adversely affect the outcomes of COVID-19 patients leading to deaths in some cases. Again the theme is well chosen, the study is original, authentic, succinctly written. Kindly find my review comments below.

Reply:

We thank the Reviewer for the positive comments on our study. We have followed your suggestions and made every possible effort to address the concerns. Detailed responses are below.

Major Comments:

Comment 1:

Introduction:

---the word cardiac injury is vague and should be defined or more elaborations should be made on its meaning. Are the authors talking of acute coronary syndrome, ischemic heart disease, angina pectoralis, myocardial injury or what?

---It is important to briefly state the pathophysiology of cardiac injury in COVID-19 in the introduction.

---Apart from COVID-19 itself, there are other factors which lead to cardiac injury in these patients. These include cardiovascular risk factors (smoking, hypertension, obesity, physical inactivity, advanced age), severe forms of the disease and medications such as hydroxychloroquine or chloroquine.

---This should be stated in the introduction. Thank you for giving us statistics on the prevalence of cardiac injury in COVID-19 patients. But to better understand the burden of cardiac injury in this disease you have to equally state the case-fatality rate due to cardiac injury in COVID-19 patients. Please provide this lacking information.

Reply:

We thank the Reviewer for the suggestions about our introduction section. We accordingly have stated the definition of cardiac injury, the pathophysiology of cardiac injury, risk factors related to cardiac injury, and the case-fatality rate due to cardiac injury in the revised introduction: (kindly see line 97-100) “Myocardial injury,

defined as elevated levels of troponin or creatine kinase isoenzyme (CK-MB) regardless of new abnormalities in electrocardiography and echocardiography, have been reported with the rate of 7.2% in the initial COVID-19 study."; (kindly see line 102-106) "The pathophysiology of COVID-associated myocardial injury have not well established but likely involve the direct damage to cardiomyocytes, systemic inflammation, myocardial interstitial fibrosis, interferon mediated immune response, exaggerated cytokine response, in addition to coronary plaque destabilization, and hypoxia."; (kindly see line 106-110) "Apart from COVID-19 itself, there are other factors associated with myocardial injury in these patients, which include cardiovascular risk factors (smoking, hypertension, obesity, physical inactivity, advanced age), severe forms of the disease and medications such as hydroxychloroquine or chloroquine."; (kindly see line 110-113) "Currently published meta-analyses have reported that more myocardial injury happened in severe COVID-19 patients, which was subsequently associated with deteriorative outcomes (mortality and need for Intensive Care Unit-ICU care)"; (kindly see line 114-119) "The present study therefore summarized all available evidence for a comprehensive and rigorous systematic review focused on myocardial injury incidence in COVID-19. In addition, to state the case-fatality rate related to cardiac injury, variations of myocardial injury incidence were also examined by disease severity (non-survivors, severe patients, and non-severe patients)."

Comment 2:

Methods:

---why did your search term only include COVID-19 and nothing related to cardiac injury, this would have fine-tuned your search strategy.

---Congratulation for retrieving preprint articles.

---Why restrict your study population to adults only. What was the justification for not studying the pediatric population? Are they not at risk of cardiac injury if they have COVID-19?

---What was done to studies reporting cardiac injury in COVID-19 without a detailed definition? Were they excluded or you performed a sub-analysis on them?

---Its imperative to tell eligibility of including studies in this systematic review based on the diagnosis COVID-19. Was this diagnosis based on RT-PCR or based on clinical presentation? Was the a homogeneous definition for all included COVID-19 patients?

---You assess methodological quality of studies using the Newcastle-Ottawa Scale (NOS) meant for observational epidemiological studies. Hence, we agree you did not include experimental studies such as randomized control trials (RCT) which need a different tool for assessment. If RCT were included in this study look for the tool that best assess their methodological quality, mention it and revised the study accordingly.

Reply:

We thank the Reviewer for the suggestions about our method section. Regarding search strategy, to avoid the accidentally exclusion of studies, we firstly search the terms only related to COVID-19, and then manually screen studies if they reported

the qualitative data of cardiac specific biomarkers (troponin or CKMB) or reported the data of myocardial injury with detailed definition.

Regarding the exclusion of pediatric population, we found that the studies of pediatric population were limited before starting this study. In addition, the potential mechanism of cardiac injury in pediatric COVID-19 patients is fully unclear. Thus, to reduce the selective bias associated with population, we finally performed the present study based on adult patients.

Regarding the definition of cardiac injury, in the present study, we defined myocardial injury as serum levels of troponin or CK-MB above the 99th percentile upper reference limit, regardless of new abnormalities in electrocardiography and echocardiography. Finally, among 53 studies, 3 studies (62%) used troponin, 11 (21%) applied troponin or electrocardiography or echocardiography, and the remaining 9 (17%) employed CK-MB as cardiac injury definition. To strengthen the results, sensitivity analyses were conducted by only including studies that used troponin or electrocardiography or echocardiography as definition of myocardial injury. The result of sensitivity analysis (23%; 95%CI, 18%-27%) was in line with the primacy result (21%; 95% CI, 17%-25%). The related statements were presented as follows: (kindly see line 165-167) “Myocardial injury was defined as serum levels of troponin or CK-MB above the 99th percentile upper reference limit, regardless of new abnormalities in electrocardiography and echocardiography.”; (kindly see line 206-212) “To strengthen the robustness of the results, further serial sensitivity analyses were conducted by including studies that real time RT-PCR assay was performed

using a SARS-CoV-2 nucleic acid detection, or studies that used troponin or electrocardiography or echocardiography as definition of myocardial injury, or excluding studies that involved potentially repetitive patients in the same hospital with period within range of other studies; or excluding studies that sample size were <50."; (kindly see line 259-262) "Thirty-three studies (62%) used troponin, 11 (21%) applied troponin or electrocardiography or echocardiography, and the remaining 9 (17%) employed CK-MB as cardiac injury definition."; (kindly see line 281-284) "Sensitivity analyses by removing a single study at 1 time; or including studies that patients were detected with RT-PCR assay; or including studies that cases were defined as troponin; or excluding studies that involved potentially repetitive patients or sample size were <50 confirmed the robustness of primacy results."

Regarding the diagnosis standard of COVID-19, we only included studies that used real time RT-PCR assay for the diagnosis of COVID-19 or clinical diagnosis based on the Guidance for COVID-19 (7th edition) released by the National Health Commission of China. Among 53 studies, 91% of studies used RT-PCR method for confirming COVID-19. To strengthen the results, sensitivity analysis was conducted by including studies that was performed using a SARS-CoV-2 nucleic acid detection. The result of sensitivity analysis (20%; 95%CI, 17%-24%) was in line with the primacy result (21%; 95% CI, 17%-25%). The related statements were presented as follows: (kindly see line 163-165) "COVID-19 patients was the laboratory diagnosis using real time RT-PCR assay or clinical diagnosis based on the Guidance for COVID-19 (7th edition) released by the National Health Commission of China.";

(kindly see line 206-212) “To strengthen the robustness of the results, further serial sensitivity analyses were conducted by including studies that real time RT-PCR assay was performed using a SARS-CoV-2 nucleic acid detection, or studies that used troponin or electrocardiography or echocardiography as definition of myocardial injury, or excluding studies that involved potentially repetitive patients in the same hospital with period within range of other studies; or excluding studies that sample size were <50.”; (kindly see line 257-258) “The majority of studies (48/53, 91%) used RT-PCR method for confirming COVID-19.”; (kindly see line 281-284) “Sensitivity analyses by removing a single study at 1 time; or including studies that patients were detected with RT-PCR assay; or including studies that cases were defined as troponin; or excluding studies that involved potentially repetitive patients or sample size were <50 confirmed the robustness of primacy results.”

Regarding the methodological quality of included studies, we accordingly have added the method of RCT (kindly see line 179-180): “The methodological quality of included RCTs was evaluated according to Cochrane Collaboration Risk of Bias Tool.”

Comment 3:

Results:

---Your search strategy was not well formulated, hence, you retrieved so many useless studies as we can see in the result. See a librarian to help you adjust your search strategy and revised your methods and results accordingly. You will see you would

retrieve less studies while still maintain the 53 eligible studies.

---Precise whether the included 7,679 patients were from the general population and if some were healthcare providers. If some were health care providers what was their number and percentage, state those who died, had severe or non-severe forms of the disease and cardiac injuries.

---For the remaining 9 present of studies which did not used RT-PCT to confirm COVID-19, what methods did the authors use to confirmed COVID-19. And why include these studies as it remain uncertain if the patients really had COVID-19.

---They might have had other infections respiratory diseases like rhinitis, influenza, bronchitis, pneumonia, tuberculosis. There are suspected cases and bring bias into your study. Remove them from your study to have a homogeneous population. Unless you have a tangible argument.

---What were the percentages of the different types of cardiac injury you found in terms of angina pectoris, myocardial infection etc. This is what is important for clinical practice. Because, so far your review only speaks of the importance of requesting cardiac enzymes such as troponin and/or CPK MB for clinical follow-up of COVID-19 patient in view of preventing cardiac injury. But we do not know which type of cardiac injury to be more careful of or search for in COVID-19 patients.

---Rectify this: The mean age ranged from 37 to 72 years to The patients' age ranged from 37 to 72 years. 37 to 72 years is not the mean age please consult a statistician to calculate you the mean age as a unique value eg 65 years (with or without standard deviation).

---Its better to give us the overall percentage of males in the 7,679 COVID-19 patients included. The 36% to 81% does not help. Give a unique value eg 51%. See a statistician for help.

---Page 8, last paragraph : change ‘’indicating that severe patients was” to ‘’ indicating that severe patients were”.

---please “Risk factors associated with cardiac injury” can you assess if ethnicity (black African, black American, Asian, UK, European for instance), healthcare personnel (if present in your study population), heart failure, prior or current pulmonary embolism, treatment protocols with hydroxychloroquine or chloroquine and obesity were risk factors for cardiac injury in the in univariable meta-regression

---I suggest you analyze patients with RT-PCR diagnosis of COVID-19 separately from those with clinical diagnosis of COVID-19 to reduce bias/heterogeneity in the results if you insist on keeping patients with clinical diagnosis of COVID-19.

-In table S2 add a column of the different study designs (cross-sectional, case-control, cohort, RCT) of the all the studies in this stable. In the introductory paragraph of your result section, tell us the number and percentages of cross-sectional, case-control, cohort, RCT.

Reply:

We thank the Reviewer for the suggestions about our result section. Regarding search strategy, to avoid the accidentally exclusion of studies, we firstly search the terms only related to COVID-19, and then manually screen studies if they reported the qualitative data of cardiac specific biomarkers (troponin or CKMB) or reported the

data of myocardial injury with detailed definition.

Regarding the healthcare providers, all the included studies did not provide the information related to health care providers. Thus, 7,679 patients were from the general population.

Regarding the diagnosis standard of COVID-19, we only included studies that used real time RT-PCR assay for the diagnosis of COVID-19 or clinical diagnosis based on the Guidance for COVID-19 (7th edition) released by the National Health Commission of China. Among 53 studies, 91% of studies used RT-PCR method for confirming COVID-19. The remaining 5 studies used RT-PCR method or clinical diagnosis definition for confirming COVID-19. The related statement were presented as follows: (kindly see line 163-165) "COVID-19 patients was the laboratory diagnosis using real time RT-PCR assay or clinical diagnosis based on the Guidance for COVID-19 (7th edition) released by the National Health Commission of China."; (kindly see line 257-259) "The majority of studies (48/53, 91%) used RT-PCR method for confirming COVID-19. The remaining 5 studies used RT-PCR method or clinical diagnosis definition for confirming COVID-19."

Regarding the suspected cases of COVID-19, we only included studies that used real time RT-PCR assay for the diagnosis of COVID-19 or clinical diagnosis based on the Guidance for COVID-19 (7th edition) released by the National Health Commission of China. It is hard to stratify the patients of laboratory diagnosis and clinical diagnosis, thus we performed sensitivity analysis by only including studies that used real time RT-PCR assay for the diagnosis of COVID-19. The result (20%;

95%CI, 17%-24%) was in line with the primacy result (21%; 95% CI, 17%-25%). The related statements were presented as follows: (kindly see line 206-212) “To strengthen the robustness of the results, further serial sensitivity analyses were conducted by including studies that real time RT-PCR assay was performed using a SARS-CoV-2 nucleic acid detection, or studies that used troponin or electrocardiography or echocardiography as definition of myocardial injury, or excluding studies that involved potentially repetitive patients in the same hospital with period within range of other studies; or excluding studies that sample size were <50.”; (kindly see line 281-284) “Sensitivity analyses by removing a single study at 1 time; or including studies that patients were detected with RT-PCR assay; or including studies that cases were defined as troponin; or excluding studies that involved potentially repetitive patients or sample size were <50 confirmed the robustness of primacy results.”

Indeed, the present study did not obtain the detailed cardiac injury types such as angina pectoris and myocardial infarction. Thus, we added the statement in the limitation section (kindly see line 439-441): “Finally, we did not assess the clinical diagnosis (angina, myocardial infarction, etc.) associated with elevated myocardial enzymes as well as the dynamic change of troponin and the association between myocardial injury and mortality.”

As stated, we have revised the sentence as follows (kindly see line 263): “The mean age was 54 years and the percentage of male was 54.1%.”

As stated, we have revised the sentence as follows (kindly see line 290):

"indicating that severe patients were associated with significantly higher risk of myocardial injury."

Regarding the detection of risk factors. Among 53 studies, only one study was performed in USA and the others were all conducted in China. In addition, no healthcare personnel was available in the included studies. Other factors (heart failure, prior or current pulmonary embolism, hydroxychloroquine treatment, obesity, et al.) stated by reviewer were less than 25% of data points (at least 13 studies reported certain risk factor), to satisfy sufficient statistical power, thus these variables finally did not involve in the model of meta-regression. Related statement was presented as follows: (kindly see line 214-215) "As a rule, at least 25% data points should be available for each variable in univariable meta-regression."; (kindly see line 299-303) "Eleven variables with more than 25% data points (mean age, gender, smoking, hypertension, diabetes, cardiovascular disease, cerebrovascular disease, chronic pulmonary disease, chronic kidney disease, liver disease, and cancer) were assessed in univariable meta-regression. The results suggested that the incidence of myocardial injury were not associated with any of the above comorbidities."

Regarding the diagnosis of COVID-19, we only included studies that used real time RT-PCR assay for the diagnosis of COVID-19 or clinical diagnosis based on the Guidance for COVID-19 (7^{th} edition) released by the National Health Commission of China. Among 53 studies, 5 studies involved the patients with laboratory diagnosis and clinical diagnosis. However, no separate data was available with two types of diagnosis. To reduce bias/heterogeneity in the results, we performed sensitivity

analysis by only including studies that used real time RT-PCR assay for the diagnosis of COVID-19. The result (20%; 95%CI, 17%-24%) was in line with the primacy result (21%; 95% CI, 17%-25%). The related statements were presented as follows: (kindly see line 206-212) "To strengthen the robustness of the results, further serial sensitivity analyses were conducted by including studies that real time RT-PCR assay was performed using a SARS-CoV-2 nucleic acid detection, or studies that used troponin or electrocardiography or echocardiography as definition of myocardial injury, or excluding studies that involved potentially repetitive patients in the same hospital with period within range of other studies; or excluding studies that sample size were <50."; (kindly see line 281-284) "Sensitivity analyses by removing a single study at 1 time; or including studies that patients were detected with RT-PCR assay; or including studies that cases were defined as troponin; or excluding studies that involved potentially repetitive patients or sample size were <50 confirmed the robustness of primacy results."

As stated, we have add a column of the different study designs (cross-sectional, case-control, cohort) in table S2. Accordingly, the related statement was presented as follows (kindly see line 253-255): "Among them, 21 studies (39%) were cross-sectional studies, 16 (30%) were case-series studies, 14 (26%) were case-control studies, and 2 (5%) were cohort studies."

Detailed Responses to Reviewer C

***General Comments*:**

The authors addressed an urgent question regarding the global pandemic, and they

did a comprehensive meta-analysis on the incidence of cardiac injury in coronavirus disease 2019 (COVID-19). The manuscript can be further improved by following comments below.

Reply:

We thank the Reviewer for the positive comments on our study. We have followed your suggestions and made every possible effort to address the concerns. Detailed responses are below.

Major Comments:

Comment 1:

The authors used some interaction analysis to evaluate the risk difference of different illness severity. More statistical details should be provided for such a method.

Reply:

We thanks the suggestion raised by Reviewer. We accordingly have provided more details as follows (kindly see line 200-204): "The interaction analysis (*P* for interaction) using Cochran's Q test were applied to evaluate the risk difference of different illness severity. Interaction is referred to as effect modification, which investigates whether the effect of intervention in the primacy outcome varied between the subgroup such as disease severity."

Comment 2:

On Page 6 Line 1, "clinical characteristics (mean age, gender ratio, smoking

ratio,...": terms of "mean" and "ratio" should be removed.

Reply:

We thank the Reviewer for the suggestion about our study. We have revised the sentence as follows (kindly see line 175-179): "clinical characteristics (age, gender, smoking, and the comorbidities of hypertension, diabetes, cardiovascular disease, cerebrovascular disease, chronic pulmonary disease, chronic kidney disease, liver disease, and cancer), and data on cardiac injury (occurrence number and total number)."

Comment 3:

Tables S6 and S7 are busy. The authors may consider using forest plots.

Reply:

We thanks the suggestion raised by Reviewer. Table S6 presented the leave-1-out sensitivity analysis for 4 groups (overall, non-severe patients, severe patients, and non-survivors). Thus, four corresponding forest plots will be conducted, which may lead to the confusion for the reviewer and reader. We finally have revised the table S7 as a forest plot.

Comment 4:

The heterogeneity is substantial for all meta-analyses. Also, baseline characteristics vary across all studies. If feasible, the authors could try meta-analysis based on adjusted relative risks from each study.

Reply:

We thanks the suggestion raised by Reviewer. Regrettably, all the included studies did not report the adjusted relative risks related to cardiac injury, thus the pooled relative risks from crude data may introduce certain bias. We have added the statement in the limitation section (kindly see line 435-437): "Also, all the included studies did not report the adjusted relative risks related to cardiac injury, thus the pooled relative risks from crude data may introduce certain bias."

Comment 5:

What is the so-called "trim and fill method to deal with the publication bias"?

Reply:

We thanks the suggestion raised by Reviewer. We have added the related statement as follows (kindly see line 218-221): "The trim and fill method requires no assumptions about the mechanism that lead to publication bias, provides an estimate of the number of missing studies, and also provides an estimated intervention effect to adjust the publication bias."

