Point-by-point reply to the Reviewers comments

Reviewer(s)' Comments to Author:

Reviewer: 1

Comments to the Author
First of all congratulations to the authors to this important work. Although this cohort is significantly smaller compared to other studies, the overall message is in line with the finding of larger oberservations (women are more frequently affected by Long-COVID-19) and adds to the overall evidence. Nevertheless, there are a few suggestions below, which should be implemented into the revised manuscript.

Response:
Thank you for your comments to the author, which has been noted and appreciated. We carefully revised the manuscript according to your suggestions, as well as those from the other reviewer.

1. Spelling/typos:

Page 2, line 9: Would make two sentences and not us a semicolon. Per WHO definition it is either Covid-19 or SARS-CoV-2 infection (not Covid-19 infection). Please remove "infection".
Response:
We added two sentences on page 2, line 9. We removed the word "infection" .

Page 2, line 17: long-term
Response:
Correct as suggested

Page 2, line 19: SARS-CoV-2
Response:
Correct as suggested

The abstract in general: Please use one consitant form of Long-COVID-19 syndrome (Long-Covid-19 syndrome) - mimum in the abstract and preferably also in the rest of the text. The same is true for SARS-CoV-2 and COVID-19.
Response:
Correct as suggested

Page 4, line 24/25: chest high-resolution computed tomography
Response:
Correct as suggested

Page 12, line 31: Post-COVID infection is a non-excisting term to my knowledge. Would suggest to change it to "post-COVID sequelae" or another term of your choise.
Response:
Correct as suggested

2. Scientific comments:

Introduction: Would agree to the statement that we currently know little about the Long-COVID-19 syndrome, but a lot of the available literature is missing. Would recommend to include:

https://pubmed.ncbi.nlm.nih.gov/34027514/
https://pubmed.ncbi.nlm.nih.gov/34454673/
https://www.medrxiv.org/content/10.1101/2021.06.30.21259757v1 (multicentric, with > 2.000 patients)
https://pubmed.ncbi.nlm.nih.gov/34163090/

Response:
The suggested references were included in the paper. Two recent publications from our group were also added.

Methods: The manuscript describes exclusion, but no detailed inclusion criteria. It is obviously difficult to assess certain symptomes with a severe pre-excisting co-morbidity, but this patients are of great interest as well (higher risk a severe COVID-19) and headache, anosmia and other symptoms would not be affected. Excluding this patients is a weakness and should be discussed!

Response:
In this study, inclusion criteria were all patients aged ≥18 years with laboratory confirmed case of COVID-19, referred for a follow-up visit at the University-Hospital of Parma between May 2020 and March 2021, at least 3 months post-infection, and after having consented to participate.
Only three patients were excluded for different reasons: One patient affected by Marfan syndrome showed, at the time of echocardiographic imaging, a severe mitral insufficiency which was not previously diagnosed with an important cardiac remodeling; therefore, it would not have been possible to distinguish the sequelae of COVID-19 of this patient from those produced by the valve defect. This patient underwent cardiac surgery three month later.
We also excluded two patients affected by systemic sarcoidosis that involved numerous apparatus and organs; they both had many symptoms lasting for many years, and it would have been impossible to distinguish Long-COVID-19 syndrome from the pre-existing disease.
Comorbidities such as stable coronary artery disease and chronic respiratory disease were not considered exclusion criteria.

3. Results & Figures: In my opinion the p-values should be included into the figures. It is not so appealing, if one has to search in the figure legends. A good figure should deliver the message at first glance.
Response:
We thank the Reviewer for the astute suggestion. We included the p-values into the Figures, when statistically significant.

Figure 1: About this figure I am a bit puzzled. Guess the median values did not show any differences. If so, I would rather show the median number (being higher in women).
Response:
We thank the Reviewer for the comment. We added the median value of each marker in all groups of Figure 1.

Figure 2: If you would use to color schemes for males and females the figure would definitely benefit from this adaptation.
Response:

We modified the colors of Figures 2 according to the Reviewer's suggestion.

Tables: Very nice and informative!
Response:
We thank the reviewer for the comment.

4. Statistics: It is solid. There are no further suggestions.
Response:
Thank you for the comments.

5. Discussion: Autoimmune mechanisms as a very likely cause (see B007 trials, University Hospital of Erlangen) should be discussed - also in the context of vaccination (is probably removing auto-reactive, non-switched IgD- CD27- T cells). Here is a probable link between higher rate of autoimmunity in women compared to men (e.g. SLE, MS, RA, etc.).

https://www.medrxiv.org/content/10.1101/2021.04.11.21255153v1.full

Response:
We agree with the reviewer that autoimmune mechanisms could be involved in SARS-CoV-2 infection as well as in the sex-related humoral response and B cell responses to vaccination. A higher rate of autoimmunity helps to explain the more favorable course of the COVID-19 in women compared to men but is also responsible for the higher incidence of chronic autoimmune disorders, as systemic lupus erythematosus, Rheumatoid arthritis, and Multiple sclerosis, conditions that are more prevalent in females.
We included one more reference: "Pradhan A, Olsson P-E. Sex differences in severity and mortality from COVID-19: are males more vulnerable? Biology of sex differences 2020;11:53".
However, we must emphasize that neither the assessment of blood antibodies (COVID-19) nor gender related differences in immune response was purpose of our study. None of the enrolled patients in the present study had previously received the COVID vaccine (the study was completed before COVID vaccine marketed authorization).

As mentioned above the exclusion of patients the a severe pre-existing condition is a weakness of the trial. At one point we also have to find out, how the disease course of severe co-morbidities is influenced

Response:

Please refer to our previous response.

Reviewer: 2

Comments to the Author
notes on review

Summary
Long Covid is a very relevant topic and I applaud your group's effort for the significant amount of work required to create this study. Although I think that it is clearly publishable, I have some concerns about the paper's methodology, and I would suggest some major revisions.
Response:

Thanks to the Reviewer for her/his appreciation of our manuscript. We carefully revised the manuscript according to your suggestions, as well as those from the other reviewer.

Although I have included specific comments below, mu greatest concern is that there appears to be an overly heavy emphasis on acute symptomology and change of symptomology between acute and the long haul time period. If I understand correctly how the data was collected, I believe that you asked pts to recall their acute symptoms on average 5 months after their illness, if so, I think this information is very vulnerable to recall bias. I think the paper would be stronger if you stick with the strength of your data- sex differences in the real time symptoms that patients presented with to your long haul covid clinic, and objective data surrounding the acute covid visits. If you wanted to include the retrospective subjective data, I would downplay these results and comment upon them in the limitation section of the paper.

Response:

The reporting of acute symptomology was carefully collected through many sources: the clinical history of patients, medical records, and the hospital discharge letters. We agree with you that this information could contain some inaccuracies, and this point has been added as a limitation in the discussion of the paper. However, the main aim of this study was not to study the persistence of symptoms. In fact, acute symptoms are only some of the covariates present in the multivariate models of having long term symptoms as an output variable. Although sometimes significant, acute, long term symptomatology is not always correlated. As the reviewer states, this relationship can be influenced by recall bias. The main aim of this study was to test the gender differences in symptom perception in a longer phase, and such a relationship is not necessarily linked to acute symptomology and to changes in symptomology between acute and the long haul timeframes. On the contrary, our article demonstrates that for several symptoms the gender-symptom relationship in long-term COVID-19 was completely different from that of the acute phase.

Other minor suggestions-

- I'd consider including a paragraph that summarizes current theories as to potential causes of long haul covid- residual virus/virus fragments/autoantibodies/reawakening of other dormant viruses etc.

I think the paper would also be stronger if you included a more generous discussion about how sex based differences in the innate and adapative immune system may contribute to sex based differences in long haul covid (Sabra Klein has written a lot about sex based differences in immunology and there are also some review papers on sex based differences in myalgic encephalomyelitis.

Response:

The exact cause of Long-COVID-19 is currently unknown. Either a residual inflammation or an autoimmune reaction could lead to a persistent infection, possibly triggered by residual virus/virus fragments or reawakening of other dormant viruses (Medical Hypotheses 2021;146:110469).

It is well known that women mount stronger innate and adaptive immune responses than men, resulting in faster clearance of pathogens and greater vaccine efficacy in females than in males, but this also contributes to their increased susceptibility to inflammatory and autoimmune diseases, such as systemic lupus erythematosus , Rheumatoid arthritis , and Multiple sclerosis .

We have expanded the discussion of sex-based differences in the innate and adaptive immune system as you suggested. An additional reference by Sabra Klein was included in the paper (see reference n. 4 and 25).

- Finally, I think the paper is too long. As there is a fair amount of discussion on the change of symptoms between acute and long haul presentations, I suspect if you eliminate some of these comparisons, it will be more concise.

Response:-

We emphasized the retrospective data and underlined the different course of symptoms in males and females. The assessment of risk factors for long-term consequences requires a longitudinal study linked to retrospective sex-disaggregated data. The aim of this study was to develop personalized strategies to prevent Long-COVID-19 syndrome.

Specific comments
-Although I love this quote, I honestly think that your paper is looking to evaluate physiological differences based on biological sex (with possible some gender influence as to how symptoms are described) As such, it seems like a more appropriate opening paragraph would emphasize biology or at least biology and sociocultural influences, as such it only highlights gender
Response:
We expanded this topic in the Discussion.

page 4 Line 43- would include info as to what population Carfi's paper evaluated, pt's sick enough with Sars Cov2 to be hospitalized?
Response:
We included more details about the population of Carfi's paper. Carfi A et al. evaluated 143 patients who had recovered from COVID-19, 12.6% of them admitted to an Intensive Care Unit and 4.9% requiring mechanical ventilation. 72.7% of the participants had evidence of interstitial pneumonia.

page 5 line 4- would include percentage of long haul women versus men in this sentence from Sigrid's study
Response:
Sigrid's study is an ongoing open access, and it did not report the percentage of Long-COVID-19 in men and in women. This study was removed and replaced with the more relevant, multi-centric cohort study, as suggested by the first reviewer (Gebhard CE et al "Sex- and Gender-specific Risk Factors of Post-COVID-19 Syndrome: A Population-based Cohort Study in Switzerland ", reference n.16).

Need to clarify exclusion criteria- you suggest pts with significant "heart and respiratory disease" as pts who would be excluded, but then in result section you also suggest that many patients in follow up actually had coronary artery disease and chronic respiratory disease.
Response:
As was included in the response to Reviewer 1, only three patients were excluded for different reasons: The patient affected by Marfan syndrome showed, at the time of echocardiographic imaging, a severe mitral insufficiency, and he underwent cardiac surgery three month later.
We also excluded two patients affected by generalized sarcoidosis that involved numerous apparatus and organs; they both had many symptoms lasting for many years, and it would been impossible to distinguish the long COVID-19 syndrome from the pre-existing disease.
Comorbidities as stable coronary artery disease and chronic respiratory disease were not considered exclusion criteria.

page 7 line 57- reword- confusing as by definition anyone with BMI > 30 is "obese"- perhaps you meant "participants" where you placed word obese?
Response:
Corrected as suggested.

It is a little unclear how study participants who were outpts got referred to clinic- it suggests that this was a consecutive group, did ALL sars cov2 pos pts get referral regardless of symptomology or was there further selection.

Response:

The sample included patients with: (a) a complicated acute COVID-19 infection phase; (b) a positive TC score, regardless of the presence of symptoms; (c) the persistence of symptoms some weeks after recovery. In May 2020, a dedicated office was opened at the Hospital of Parma that referred patients for a standard diagnostic pathway after receiving a report from a hospital doctor or a general practitioner.

in results section-

It is still a little confusing as to collection points of data were all subjective acute and chronic symptoms collected at initial follow up appt? Or were initial symptoms collected at initial appt and then chronic symptoms followed out to 23 weeks (meaning that there were several follow up appt). If the former, at that time frame of 5 ½ months, I worry that the acute phase symptomology data is very vulnerable to recall bias.

Response:

The report of acute symptoms were collected at the time of the follow-up visit from a variety of sources: a check of medical records, discharge letters, and patients' medical histories. We respectfully maintain that retrospective data from medical records can be considered robust. During the same period, chronic symptoms were also collected.

page 13 line 46-

I would add a sentence here reminding audience who your patient population was- no sex difference in CPAP use in pts who survived their illness, I suspect if you were to look at all comers for who got hospitalized and got cpap -including those who died- that you would find a sex difference.

Response:

The literature reports that men, in the acute phase, receive more intensive care than women due to more severe illness. However, we did not consider this point.

page 16- I am a bit surprised you started off with pain perception as I suspect that many sex and gender researchers believe that sex differences in both acute and long covid are heavily rooted in significant differences in the innate and adaptive immune system (albeit as you suggested, immunological function and pain perception are likely interconnected.) Perhaps, you might consider starting off hypothesis more broadly with sex-based differences in physiology and then move on to discuss sex-based differences in immune system and then, the possible interconnection with pain perception.

Response:

We agree with you, and we revised this paragraph. First, we discussed sex-based differences in the immune system, and this was followed by a discussion of a possible interconnection with pain perception.

Page 17

sentence 12, would suggest eliminating "thirdly" and just segue into discussion about precedence of other diseases also showing sex/gender differences in symptom reporting sentence 23- would eliminate rather than angina, or substitute rather than classically taught symptoms of angina

Response:

We eliminated "thirdly," and we substituted angina with "typical angina".

54- again, details as to how pts were referred to follow up and how many people who were referred actually followed up is not entirely clear to me based on methods and results.

Response:

The sample included patients with: (1) a complicated acute COVID-19 infection phase; (2) a positive TC score, regardless of the presence of symptoms; and (3) the persistence of symptoms some weeks after recovery. In May 2020, a dedicated office was opened at the Hospital of Parma that referred patients for a standard diagnostic pathway after receiving a report from a hospital doctor or a general practitioner.

Considerations for limitation section- see above comments on retrospective subjective data collection.

Response:

The comments on retrospective data collections were added in the Limitation section, at the end of the Discussion.

As there was a signal that steroid use was different between males and females and that steroid use is the only known pharm agent that has been shown to definitively improve mortality in all comer hypoxic covid patients, does your sex difference in who received steroids need a sentence or two in limitation section? May also want to comment that future studies may want to consider if therapeutic medications given to active covid pts changes their risks for developing long haul symptoms or the types of symptoms that they develop- especially immunomodulators (or even vaccines.)

Response:

We agree with the Reviewer's comment. We found a tendency to a greater use of steroid in females compared to males, as showed in Table 3 and in the Result. These results further confirm a more severe Long-COVID-19 syndrome in women than in men.