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Peer Review File

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Review Comments A:

Comment 1: This is a very timely clinical research paper for the world's Covid-19 pandemic. Despite the small number of cases, a retrospective nature without proper control, this is a paper that would offer help for clinicians at the frontline daily worldwide in their effort to save their Covid-19 patients. This is particularly important considering Covid-19, in a short 7+ months, has caused > 700,000 deaths and >$USD 86 trillion in economic losses worldwide and yet there are no specific drugs approved for Covid-19 treatment.

Reply1：We appreciate for your kind comments.

Review Comments B:

"Application of high dose intravenous vitamin C on patients with COVID-19 pneumonia in severe condition: a retrospective case series study" by Zhang et col. is an interesting report that collects data about the use of high doses of intravenous vitamin C for the treatment of COVID-19 patients. This topic is relevant due the need for new therapies in COVID-19, the cheap price of the drug and its wide availability also to low-income countries. The article is well written and the level of English is adequate. The results are discussed within the limitations of the study (retrospective case series) and an appropriate reminder is done regarding the need for randomized clinical studies. The paper has a further value because while early administration of vitamin C has proven beneficial in critically ill patients, here some preliminary data about the "early" intervention (<24h) after disease exacerbation is investigated. Authors should however revise different parts of the manuscript to improve reader's understanding:

- **Comment 1** The introduction should include the dose of vitamin C utilized in the literature for either sepsis/ARDS. Then, the dose applied in this study should be mentioned in the abstract,

introduction and contextualized within the discussion. In addition, it is not clear if all the patients have been treated with HDIVC routinely (as described in Figure 1) and a rescue increased dosage is applied to the described case series, or HDIVC is applied ONLY when disease worsening is observed. Authors should describe more clearly the study protocol, avoid contracting sentences and underline it in the abstract, introduction and methods. Finally, a discussion about the rationale of such increment in dosage related to the severity of cases is needed.

Reply 1： We appreciate for your kind suggestion.

The dose of vitamin C utilized in the literature for sepsis/ARDS is 50 mg/kg actual body weight every 6 hours for 96 hours, and this information has been added in the revised manuscript. (line 100-101)

The dose applied (median [IQR], mg/kg [body weight]/day) in this study were (162.7 [71.1-328.6]) for severe and (178.6 [133.3-350.6]) for critical patients. This has been added in the abstract, introduction and discussion part of the revised manuscript. (line 54-56; line109-111; line308-310).

HDIVC was applied only after disease aggravation and this was described in the first paragraph of method parts. By the way, the HDIVC routine usage we mentioned in supplemental figure 1 was a part of the HDIVC protocol we testified in a prospective controlled study but not this manuscript. We are sorry for the misunderstanding caused by our unclear description.

In the revised manuscript, we described the study protocol more clearly as you requested (line128-132).

In this retrospective case serious study, the vitamin C treatment initiation as well as its dosage was judged according to the common opinion of Shanghai expert panel. The vitamin C dosage showed no difference between severe and critical patients (table 1, 162.7 [71.1-328.6] mg/kg/d vs 178.6 [133.3-350.6] mg/kg/d, P=0.667). Furthermore, we testified if HDIVC protocol (as supplementary figure 1 showed) exerted beneficial effect to COVID-19 in the following RCT study (not this study). Therefore, the rationale for dosage increment of Vitamin C will discussed in the following RCT study. Fowler, et al reported (doi:10.1186/1479-5876-12-32) that severe sepsis patient receiving high dose intravenous vitamin C (200mg/kg/d), but not low dose (50mg/kg/d), showed higher serum level of vitamin C (3000 um vs 300 um) and significantly faster decline in the regression slopes of SOFA score. This may explain the rationale of dosage

increment with disease severity.

-Comment 2 The title should be revised with particular attention of mentioning disease worsening and increment of dose, especially if the patients are already under HDIVC.

-Reply 2：thanks for your kind suggestion. We revised the title to ""Beneficial aspects of high dose intravenous vitamin C on patients with COVID-19 pneumonia in severe condition: a retrospective case series study" as you suggested (line1-3). As mentioned before, this study does not investigate the dosage increment and its effect as the retrospective study property. The patients included in this study were not treated with HDIVC until disease aggravation.

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-Comment 3 Table 1: Authors should address "time from admission to HDIVC administration". Also, if all the patients are routinely under HDIVC authors should consider present some statistics of % and time of disease worsening in this population from their database.

-Reply 3: thanks for your useful suggestion. We revised Table 1 according to your suggestion. The patient enrolled in this study were not treated with HDIVC before disease aggravation.

- Comment 4 Authors should consider plotting results of the inflammatory markers, immune response or organ function as bar/line graphs.

-Reply 4: Thanks for your great suggestion. We added the bar graphs to show the trend of biomarkers of inflammatory response, immune response and organ function as Figure 1.

- **Comment 5** Authors should consider including in the Bibliography relevant papers that addressed the HDIVC in sepsis for the first time (Line 80: DOI: 10.1016/j.chest.2016.11.036) and its use as immunomodulatory agent against viruses (https://doi.org/10.1080/14787210.2020.1706483).

-Reply 5: thanks for your kind suggestion. The Bibliographies (DOI: 10.1016/j.chest.2016.11.036) has been cited in this manuscript as reference 6. However, we did not find your suggested Bibliographies ((https://doi.org/10.1080/14787210.2020.1706483), and we added another Bibliographies which support the anti-virus role of HDIVC as reference 11 (line 131).

-**Comment 6** The discussion should be enriched with a consideration about vitamin C safety

profile in healthy patients and in special populations (kidney failure/renal replacement therapy).

-Reply 6: Thanks for your kind suggestion. The discussion for vitamin C safety profile has been added in the revised manuscript (line 325-330).

-Comment 7 A figure with the summarized beneficial effects of HDIVC in COVID-19 would be appreciated.

-Reply 7: Thanks for your kind suggestion. We summarized the beneficial effect of HDIVC in COVID-19 in supplementary Figure 2

Review Comments C:

The topic is interesting and innovative.

The abstract is clear and also the aim of the work.

-Comment 1 I suggest to insert in keyword also C-reactive protein.

-Reply 1 : Thanks for your kind suggestion. We added C-reactive protein as keyword in the revised manuscript (line 73).

-Comment 2 In the material and methods is not clear intravenous vitamin C administration. Please clarify it.

-Reply 2 The dosage of vitamin C was determined according to the common opinion of expert group based on the previous clinical study and research work. For severe patients, the dosage (median (IQR)) is 162.7 (71.1-328.6) mg/kg (body weight)/days and for critical patients, was 178.6 (133.3-350.6) (line176-178).

-Comment 3 It would have been interesting to evaluate the levels of IL -6. why did the authors not evaluate this aspect? In this regard I suggest you consult this work “Functional role of dietary intervention to improve the outcome of COVID-19: A hypothesis of work”

-Reply 3 Thanks for your kind suggestion. The data of IL-6 missed in a huge mount as the property of retrospective study. We will evaluate the effect of HDIVC on IL-1 in the following RCT study.

-Comment 4 Does vitamin C correlate negatively or positively with protein C and lymphocyte levels? Please clarify this aspect.

-Reply 4: we showed in this study the lymphocyte counts as well as CD4+ T cell significantly increased by GEE model. We are not quite sure if vitamin C was correlated with protein C and lymphocyte level as we did not measure the level of vitamin C.

-Comment 5 I suggest to report the molecular role of vitamin C in the lung.

-Reply 5: we showed that after HDIVC, the PF indicator was significantly improved. The specific mechanisms might include:1) the regulatory of NETosis (*Nutrients 2013, 5, 3131-3150*.); 2) reducing inflammation via attenuation of NF-ϰB activation (Mediators of inflammation. 2014;2014:426740.); 3) enhancing lung epithelial barrier function by promoting epigenetic and transcriptional expression of protein channels at the alveolar capillary membrane that regulate alveolar fluid clearance (*Inflammation 2019, 42,1585–1594*); this part has been added into the 4th para of discussion part (line298-302).

-Comment 6 Can there be any negative effects from taking high doses of vitamin C ?

-Reply 6: thanks for your question. The potential adverse effect of vitamin C is the formation of renal stone which, however, are not reported for temporary HDIVC till now. We added a discussion on negative effects of vitamin C in the revised manuscript (line325-330).

Review Comments D:

-Comment 1 There are 14 authors to the manuscript.

It seems that not a single one of them has taken any look at the reference list.

Ref 1 author is E M.

Ref 2 authors are A S, G P. and there are no volume or page information

Etc.

Etc.

That kind of manuscript writing is unacceptable

All 14 authors should take responsibility of the manuscript. No one has.

-Reply1: We are sorry for the mistake of reference which has been corrected in the revised manuscript. The reference followed the form of “Vancouver” which automatically generated using Endnote software.

-Comment 2 As to the science of this paper, there is no consideration that people are cured of self-limiting acute infections over time. When a person has virus infection and fever, it is highly likely that the person does not have fever any more after a week. Whatever we are doing when there is fever, we can "explain" that it was that doing which "took off" the fever. Very unsound reasoning.

Therefore, we need controlled trials.

In acute infections we can compare if there is any difference between the treatment and control groups in fever or CRP or in anything else.

-Reply2: We agree with your opinion about the limitation of this study. The case serious study design restrained the availability of control group. This study mainly focused on the outcomes change before and after HDIVC in the situation of disease aggravation. This might partly support the role of HDIVC as a rescue therapy.

-Comment 3 Sometimes it is reasonable to carry out time series analysis such that measurements are carried out before an intervention and the trajectory before intervention can be compared with that after the intervention.

If there is a stable level of outcome (say blood pressure) and a drug is started and then the outcome changes to a new level that is stable for a long term, it is reasonable to consider that the intervention explains the change at the level.

Here the authors do not have any control group.

Also they do not have any information of the endpoints before vitamin C administration.

They could have, since the patients were hospitalized for several days before the intervention.

-Reply 3: Thanks for careful consideration. The data on Day0 (Table 2) is the information on aggravation and before HDIVC treatment.

-Comment 4 The authors do not seem to understand what P-value means.

"no statistically significant differences were found regarding age (P=0.255), body weight (P=0.469)"

When P-value is between some 0.01 and 0.2, it is reasonable to give 2 digits. However, three digits is never useful.

When P = 0.469, the 3 digits do not give any useful information. It should be presented as P = 0.5.

The same problem is in the accuracy of the estimates

"59.01±37.9 mg/L)"

When the 37.9 describes how very inaccurate the "59.01" is, it is obvious that the ".01" has no information.

Thus, such an estimate should be written as 59+/- 38

-Reply 4: Thanks for your suggestion. We change all the P value and estimate into two digits in the revised manuscript.

-Comment 5 "The ameliorating effect of HDIVC on CRP in severe patients was statistically better than the one in critical patients (P=0.0125)."

"improving effect of HDIVC on the outcomes of severe patients was better than the one of critical patients."

That is false.

Such an association is explained by regression to the mean.

One or some of the 14 authors should have such basic courses in statistics that he or she would be familiar with that concept.

-Reply 5: thanks for your suggestion. The regression to the mean is an unavoidable affair, which might lead to overstatement of the therapeutic effect of HDIVC. We have added this into the limitation of the revised manuscript (line340-341).

Introduction:

-Comment 6"COVID-19 ... and the following cytokine storm,"

However, there is no relevant evidence for that "cytokine storm"

https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2767939

-Reply 6: thanks for your careful consideration. We carefully read the paper you provided and the term of "cytokine storm" are widely debated in COVID-19. We added reference 9 which support this viewpoint of "cytokine storm" in the revised manuscript (line 99). Besides, the use of dexamethasone has been shown to resulted in lower 28-day mortality among those who were receiving either invasive mechanical ventilation (N Engl J Med. 2020;NEJMoa2021436.). This might partly support the role of cytokine storm in severely ill patients of COVID-19.

-Comment 7 The authors do not discuss AT ALL the fact that people are cured from acute infections over time, and therefore we cannot draw any firm conclusions about the effects of vitamin C.

The authors give a strong impression in their text that it is vitamin C that caused the observed changes, without mentioning that COVID is usually always a self-limiting disease, which makes it very challenging to show effects of vitamin C in this kind of case series.

I do not consider that it is absolutely impossible to carry out a meaningful case series report about vitamin C for COVID patients. However, this manuscript is fully unsatisfactory.

Reply 7: thanks for your comments. We can not agree with your opinion as the patients enrolled in this study were either severe or critical type who not self-limiting. We actually observed the benefit from HDIVC in the situation of disease aggravation.

Review Comments E:

-Comment 1 The title should be more precise. For example:

"Beneficial aspects of high dose intravenous vitamin C on patients with COVID-19 pneumonia 2 in severe condition: a retrospective case series study"

-Reply 1: Thanks for your kind suggestion. We changed the title into "Beneficial aspects of high dose intravenous vitamin C on patients with COVID-19 pneumonia in disease aggravation: a retrospective case series study" in the revised manuscript following your request (line 1-3).

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