Author’s response to reviews

Title: Positive regulatory effects of perioperative probiotic treatment on postoperative liver complications after colorectal liver metastases surgery: a double-center and double-blind randomized clinical trial

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Author’s response to reviews: see over
Dear Maria Lourdes O.Catarroja,

Thank you for your encouraging letter and valuable advice. We also greatly appreciate the positive comments from the reviewers.

We would like to re-submit our manuscript, which has been revised according to the comments of the reviewers. We have addressed each comment raised by the reviewers and revised the paper accordingly. All amendments are highlighted in red font in the revised manuscript. Point-by-point replies are included below. Furthermore, this manuscript has been edited and proofread by a medical English teacher of Sun Yat-sen University. We hope that the revised manuscript is now acceptable for publication in your journal.

We look forward to hearing from you soon.

Sincerely yours,

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Response to Reviewers’ Comments:

Reviewer 1:

1. Abstract background: note, postoperative intestinal infection is still common
   Answer: We have revised the abstract according to your advice. Please check! Thank you!

2. Abstract aim: Zonulin needs a small introduction like: zonulin, a marker of intestinal permeability,...
   Answer: We have made the corresponding revision as your suggestion. Please check! Thank you!

2. Line 56: to evaluate the impact on the liver...
   Answer: We have modified the word as your advice. Please check! Thank you!

4. Line 58-61: grammatical review
   Answer: We have modified this sentence. Thank you!

5. Line 62: ? Paralyzed ??
   Answer: Thank you! We have modified this word.

6. Line 100: infectious complication need to be avoided, but you cannot say that antibiotics are not the best choice to treat them
   Answer: Thank you for your positive comments. We have amend the description.

7. Line 104: ... Bacteria may be expands?? Need grammatical correction
   Answer: We have changed this word into a better one. Thank you!
8. Line 126: our previous study...

Answer: We have added the word study. Thank you!

9. Line 127: transmucosal permeation or permeability?

Answer: Thank you for your remind. We have changed this word.

10. Line 332 to 334: this needs to be correlated with the amount of liver resection. Level of AST/ALT will vary considerably between massive reaction vs 1 single small border metastasis. Are the 2 groups comparable on this?

Answer: Thanks for your comment. We had used the local resection surgery which had little impact on the result of the experiment. This is a limitation here and we will overcome it in the further study.

11. Line 357-360: does the liver resection not increase by itself directly the zonula in lever (or indirectly)? You use the term "liver barrier". What do you mean exactly? You cannot assume that it is similar than the Intestinal TJ junction. This needs more clarification

Answer: Thanks for your comment. In fact, there are many papers which used the term “liver barrier” as we can search on the NCBI. We cannot exclud the interference of intestinal barrier in this study completely.

12. Line 359: We mean... Another verb would be more appropriate

Answer: Thank you for your comments. We have amend the verb.

13. Line 501: despite... Grammatical review

Answer: Thank you. We have revised this sentence.

14. Line 582: that could be the first limitation
**Answer:** We have revised this phrase. Thank you so much.

15: line 571: disable? Grammatical review

**Answer:** Thank you. We have modified this word.

Reviewer 2:

1. in the abstract, as well in the material section is not clear at all with type of surgery the patients received. It appears intuitive to think that they underwent synchronous colo-rectal and liver resection but there is no mentioning of the extent and type of hepatectomy.

**Answer:** Thank you so much for your comments. We used the local resection for metastatic tumor , while for large tumor, the segmental hepatectomy.

2. There are a multitude of endopoints. It is not clear which is the primary one and the secondary one.

**Answer:** Thank you for your comments. We have simplified the paper and emphasized the key point as your suggestion.

3. The introduction is too long and full of redundant information and sometimes also real commonplaces (such as epidemiology and role of surgery)

**Answer:** Thank you for your comments. We have shortened the introduction and checked the description.

4. statistical methods are unclear, especially the sample size calculation (12 pts/group) and 134 patinetns were enrolled (great confusion !!)

**Answer:** Thank you for your comments. In fact, it is 120 not 12. We made the mistake because of carelessness.
5. It may be that most of the finding are related to pringle manoeuvre !. There is not data on how may patients received pringle and the time of pringle.

Answer: We have not used the pringle maneuver in the surgery for there is no need to block the blood. Thank you so much.

6. A mean of three hours for a combined colorectal resection and hepatic resection seems not believable at all.

Answer: Thanks for your comment. We did the

7. The description of inclusion and exclusion criteria are repetitive

Answer: Thank you for your comments. We have shortened the introduction and deleted the repetition.

8. Which stain of probiotic bacteria were used ?? (BL-88, LA-11, LP ??). An international coding for probiotic should be used otherwise no one may reproduce the study design

Answer: Thank you for your comment. We had made the description and you can find it in line 159 of this paper.

9. Are these probiotics dried or alive ?

Answer: Thank you. The probiotics were alive.

10. Which is the definition of septicaemia? Positive blood cultures or simple SIRS ?

Answer: Thank you very much for your comment. We had made the definition of septicaemia through clinical diagnosis.

11. bowel preparation may affect deeply the survival of probiotic. Did the
authors test for viability

Answer: Thank you for your suggestion and we intend to discuss this problem in our further study.

12. I am always quite doubtful when I see zero surgical complications in such an extensive surgery. 134 simultaneous colorectal resection and liver resection with no surgical-related morbidity is very uncommon to find in the international literature. I have looked at the same group previous publications and it seems that in none of their trials there is one anastomotic leak, fistula, or bleeding in hundreds of cases.

Answer: Thank you for the comment. There were many complications as we can see in the table2 postoperatively, but no death occurred and the anastomotic fistulawere not included.

13. It is unclear why only 117/134 completed the study.

Answer: Thank you for the comment. We have described that 4 patients were lost to follow-up and 8 patients discontinued intervention on the fig. 1.

14. Are there patients receiving TPN or enteral feeding?

Answer: The patients received TPN for 3 days and no enteral feeding. Thank you for your comment.

15. The authors state that some infections such as central line and pneumonia are not correlated with intestinal infection. Which is the rational for this statement?

Answer: Thank you for the comment. We had compared the differences of
these indicators between the PRO group and the control group, and we feel very sorrow for stated unclear here.

16. diarrhea is not an infectious complication unless pathogens are proven in the stools.

Answer: Thank you so much, we have modified the postoperative complications.

17. there is overlapping period of patients enrollement with their previous publications about RCTs. How is it possible?

Answer: Thank you for your positive comment. In this study, we choose the patients who had hepatic metastases, while the patients had no hepatic metastases in the previous study.

18. in the present study they could not reproduce the effect of probiotic on intestinal permeability (as measured by L/M ratio) found their previous publications. Why?

Answer: Thank you. In the previous study, we underwent the gastrointestinal surgery which caused the changes of intestinal permeability, but there has no impact in the operation of liver of this study.

19. In the discussion is too long and there are multiple repetitions of the results and prosaic sentences (liver get a dual blood supply, etc)

Answer: Thank you, we have deleted the Superfluous information in the discussion.