Peer Review File
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Review Comments

The study proposes that preoperative dietary supplementation with inulin or 5-aminosalicylic acid (5-ASA), which modulates gut microbiota and activates the PPAR-γ pathway, could improve anastomotic healing, reduce local tumor spread, and mitigate systemic inflammation. The study is based on animal models, which may not fully replicate the complexity of human physiology and disease progression. Therefore, the translational potential to human patients requires further investigation. The study's findings may not be generalizable to all CRC patients due to variations in diet, genetics, and other environmental factors that influence gut microbiota. While the study suggests a link between gut microbiota and AL, the precise mechanisms by which inulin and 5-ASA exert their effects are not fully elucidated. The optimal dosage and duration of inulin or 5-ASA supplementation prior to surgery are not clearly defined, which is crucial for clinical application. The long-term safety and potential side effects of preoperative supplementation with inulin or 5-ASA are not addressed. Please also briefly review whether there are any planned or ongoing human clinical trials to validate these findings in a clinical setting. What specific changes in gut microbiota are associated with improved outcomes, and can these be targeted more directly? How does individual variability in gut microbiota composition affect the response to dietary supplementation? Further, What are the economic implications of implementing such dietary interventions in clinical practice, and what is the cost-benefit analysis?

We would like to thank the reviewer for his/her thoughtful and detailed feedback. Here we have separated the comments and answered one by one for clarity purposes.

Comment 1: The study is based on animal models, which may not fully replicate the complexity of human physiology and disease progression. Therefore, the translational potential to human patients requires further investigation.

Reply 1: *We agree that the translational potential of this work is limited, as the studies were performed using an animal model that does not fully replicate human physiology. However, we consider this research to be at a preliminary stage, and the use of animal models represents a first approach to test the hypothesis formulated by the authors. Nevertheless, these results require cautious interpretation and there is a need for designing and performing clinical trials to validate the effects of preoperative dietary supplementation with inulin on anastomotic leakage and systemic inflammation in CRC patients. This fact has been now discussed in the conclusion part of the manuscript (page 8, lines 174-181).*

Comment 2: The study's findings may not be generalizable to all CRC patients due to variations in diet, genetics, and other environmental factors that influence gut microbiota. While the study suggests a link between gut microbiota and AL, the precise mechanisms by which inulin and 5-ASA exert their effects are not fully elucidated.

Reply 2: *We are fully aware that the study discussed here has significant limitations. As the reviewer rightly highlights, the results may not be generalizable to all CRC patients due to the multifactorial nature of the disease. Therefore, despite these being novel findings that shed light on potential mechanisms involved in the relationship between microbiota and the occurrence of anastomotic leak in CRC patients, further studies in humans are necessary to elucidate the role of each one of the contributing factors.*

Additionally, the primary aim of the paper under discussion was, on the one hand, to determine the relationship between poor anastomotic healing and the increased risk of cancer recurrence, and on the other hand, to explore nutritional strategies aimed at modifying the microbiota to evaluate if these strategies may improve anastomotic healing. In this regard, the proposed the use of inulin, which is a prebiotic (dietary fiber) that has been shown to modify the microbiota by increasing species that produce short-chain fatty acids, particularly butyrate. In this article, the authors identified PPARγ as one of the potential targets for improving the course of anastomosis. Since PPARγ is modulated by butyrate, they proposed, as a preliminary study, the use of prebiotic to explore whether it could improve the clinical course in a mouse model of anastomosis, with 5-ASA employed simply as a positive control, though the latter was not the focus of the study.

Comment 3: The optimal dosage and duration of inulin or 5-ASA supplementation prior to surgery are not clearly defined, which is crucial for clinical application. The long-term safety and potential side effects of preoperative supplementation with inulin or 5-ASA are not addressed.

Reply 3: *We acknowledge the limitations of the study in this regard. As previously stated, one of the aims of the study was to explore potential nutritional strategies using a murine model to improve anastomotic healing. While we fully agree that the optimal dosage and duration as well as long-term safety and side effects of any supplementation (whether pharmaceutical or nutritional) should be addressed as is essential for clinical application, the authors of the original paper did not provide such information.*

Comment 4: Please also briefly review whether there are any planned or ongoing human clinical trials to validate these findings in a clinical setting.

Reply 4: *Thanks for your suggestion. We have performed a thoroughly search for clinical trials either planned or ongoing in humans using available tools of search. We have found one planned clinical trial on ClinicalTrials.gov with ID NCT05860322 which has been registered by the same authors. This information has been now added to the new version of the manuscript (page 8, lines 174-181).*

Comment 5: What specific changes in gut microbiota are associated with improved outcomes, and can these be targeted more directly?

Reply 5: *We agree with the reviewer that it is important to elucidate the underlying changes in microbiota associated with improved outcomes. The conclusion of the authors was based on the changes in the levels of short chain fatty acids, microbial-derived metabolites, after the treatment.*

Comment 6: How does individual variability in gut microbiota composition affect the response to dietary supplementation?

Reply 6: *We acknowledge that interindividual variability in gut microbiota profiles is a critical limitation in this type of studies. However, the results addressed by the authors are mainly based on preclinical studies using both cell and animal models. In this regard, the nutritional intervention was performed using inbred strains of mice (genetically identical) bred in the same conditions. Thus, although the interindividual variability is not relevant in this study, this should be taken in consideration when performing this study on human being.*

Comment 7: Further, what are the economic implications of implementing such dietary interventions in clinical practice, and what is the cost-benefit analysis?

Reply 7: *The economic implications of implementing any dietary interventions in clinical practice are indeed important to consider. While dietary supplementation with inulin and 5-ASA may offer a cost-effective strategy for improving surgical outcomes, a thorough cost-benefit analysis is needed. We believe that further studies, including clinical trials, are needed to establish the potential benefits, and that such studies should also explore the feasibility and scalability of these interventions.*