Author’s response to reviews

Title: Perioperative dynamics and significance of plasma-free amino acid profiles in colorectal cancer

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Author’s response to reviews:

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Dr. Guangde Tu
Academic Editor
BMC Surgery

Dear Dr. Tu,

Thank you very much for reviewing our paper entitled “Perioperative dynamics and significance of plasma-free amino acid profiles in colorectal cancer” (Manuscript ID BSUR-D-17-00249), and thank you very much for extending the submission deadline.

We have carefully considered the academic editor and reviewers’ comments and have implemented all appropriate changes. We have revised the manuscript according to the various comments (point-by-point responses are included below) and have indicated the revised sections with red font in the attached manuscript. We believe that these revisions have significantly improved the quality of the manuscript, and hope that it is now acceptable for publication in BMC Surgery.

Yours sincerely,
Responses to Reviewer 1

Thanks to the authors and editor for inviting this review. The authors present a small observational study examining the impact of surgical resection of CRC on concentrations of PFAAs which have been identified as being associated with the presence of CRC in the AICS scoring system. The authors report that resection of the primary tumour in patients without metastatic disease results in a reduction in AICS in the majority of patients, suggesting that these levels of PFAAs are driven by the tumour, rather than themselves being causally implicated in the disease process. Although the study is small, the analysis is appropriate, and the conclusions drawn reasonable. The authors identify the main limitations of small numbers and the significant variation in timing of postoperative blood sampling. I have a few comments;

Response:

Thank you for your kind comments.

Per your suggestions and those of the other reviewer, we have revised our manuscript.

Reviewer #1-1a

The authors suggest that levels of AICS reflect the presence of the tumour, explaining the reduction in the postoperative period. Is there any additional evidence to support this? In particular, have such bloods been drawn from any patients who went on to have disease recurrence? If AICS was found to rise again in this scenario then both the hypothesis, and the case for the use of AICS as a prognostic and then predictive measure would be strengthened.

Response:

Thank you for the above comment.

In this study, we could confirm only 5 recurrent patients. We have added this information to Additional file 3 and the following sentences.

p.16, Line 300—303 :
“In this study, only 5 cases showed recurrence during the follow-up. In the future, the number of cases will be increased prospective verification with predetermined blood collection time points and long-term studies with follow-up until recurrence are needed.”

The aim of this study was to show that PFAAs are driven by the tumor, rather than themselves being causally implicated in the disease process. We plan to study the application of this marker for predicting recurrence by using a different protocol in the near future. In fact, we are currently examining the results of this study and collecting patient cases using a new protocol. We are planning to conduct this hypothesis verification research based on this preliminary study and hope to verify the role of PFAAs as a recurrence or prognostic marker.

Reviewer #1-1b

If this has not yet been tested in CRC, is there any evidence from other solid tumours in the literature?

Response:

To our knowledge, there are 2 previous studies in other solid tumors. These have been included in the manuscript (references no. 23 and 24).


Reviewer #1-2

Following on from the first comment, both the preoperative AICS values, and fall after surgery were stage independent. If PFAAs are produced by the tumour or host-tumour interactions, is there any evidence of relationship with tumour burden outside of TNM stage?

Response:

Thank you for your thoughtful comment.

According to the results of a previous clinical research of 280 cases, there was no significant difference in AICS sensitivity among the stages (reference no. 14).

Recent advances in analytical techniques have enabled disease states to be analyzed through the comprehensive measurement of metabolites. However, we consider that problems remain in the
clinical setting with regard to the reproducibility, quantification, and cost of this type of analysis. The approach to such an analysis using “AminoIndex Technology” is based on the assessment of diseases and physical conditions using plasma amino acid concentrations obtained through the measurement of a particular subset of metabolites. Measurement of amino acid metabolites is particularly useful for predicting various conditions, because amino acid metabolism is closely related to many other metabolic pathways, such as glucose and lipid metabolism, and amino acids can therefore be considered as “hubs” to which many types of metabolites on the metabolic map are connected.

Reviewer #1-3

As the authors state in the discussion, the local and systemic immune response is increasingly recognised to drive many tumour-host interactions, being prognostic in most solid tumours. Furthermore, and of particular importance in the context of PFAAs, systemic inflammation is recognised to significantly perturb commonly measured micronutrients and plasma proteins. Were any measures of local or systemic inflammation measured in these patients before and after surgery, e.g. NLR, mGPS? This would be important as the host inflammatory response may both be a confounder of PFAAs and/or be a key underlying process relating them to the tumour.

Response:

We thank the reviewer for these pertinent suggestions.

According to your advice, we have now analyzed additional data on the CRP and albumin levels and have calculated the mGPS (please refer to Table 4 in the revised manuscript).

We consider that the levels of PFAAs in the tumor-bearing state vary not only by immunity and inflammation, but also by metabolism of the cancer itself and metabolic fluctuation of the host, such as that occurring in distant tissues.

However, in this study, the number of cases was small, and it was not possible to show a significant difference according to the patients’ inflammatory condition (Table 4.) In the next clinical trial, we are planning to proceed with the measurement of PFAAs, as well as measurements of CRP and albumin, while taking into account the postoperative blood collection date.

Based on this comment, we have revised the paper as follows:

P. 8, Line 149 – P. 9, Line 153:

“The modified Glasgow Prognostic Score (mGPS)

We calculated the mGPS value using the C-reactive protein (CRP) and albumin (Alb) values from biochemical tests performed pre- and postoperatively. Subsequently, the mGPS was
divided into 3 groups (I: Alb $\geq$ 3.5 g/dl and CRP $\leq$ 0.5 mg/dl, II: Alb < 3.5 g/dl or CRP > 0.5 mg/dl, III: Alb < 3.5 g/dl and CRP > 0.5 mg/dl) according to previous research [20].”

Changes in Alb, CRP, and the mGPS between the pre- and postoperative periods

Table 4 shows the preoperative and postoperative Alb (g/dl), CRP (mg/dl), and mGPS values. In the rank B+C group, the preoperative Alb level was approximately 4.0 g/dl, with no difference between the pre- and postoperative levels. The preoperative CRP level was high, at 0.4±0.9 mg/dl, and decreased to 0.2±0.2 mg/dl after surgery; however, a significant difference was not recognized. Regarding the mGPS, the percentage of patients classified as group I (Alb $\geq$3.5g/dl and CRP $\leq$0.5mg/dl) increased postoperatively, although no statistically significant difference was observed (Table 4).”

However, assessment of postoperative changes using the mGPS, a prognostic indicator, showed no significant difference in this study, although the proportion of patients classified as group I tended to increase.”

Responses to Reviewer 2

Reviewer #2: In this paper, the authors compared the value and rank of the AICS (AminoIndex Cancer Screening) before and after surgery. They have clarified that the AICS rank and value had declined postoperatively in the majority of the cases that had high preoperative level. They had concluded that AICS might be a good biomarker in the management of colorectal cancer. Although this paper provides us important information about the significance of AICS, I feel there remain some issues to be solved in this paper.

Response: We thank the reviewer for these pertinent suggestions. Per your suggestions and those of the other reviewer, we have revised our manuscript.

Reviewer #2-1.

I feel that when considering the efficacy of AICS for as biomarker, the data regarding the recurrent status of the tumor is necessary.

Response: Thank you for your appropriate comment.

In this study, we could confirm only five recurrent patients. We have added this information to Additional file 3 and the following sentences.

p.16, Line 300−303:

Additional file 3:
“In this study, only 5 cases showed recurrence during the follow-up. In the future, the number of cases will be increased prospective verification with predetermined blood collection time points and long-term studies with follow-up until recurrence are needed.”

The aim of this study was to show that PFAAs are driven by the tumor, rather than themselves being causally implicated in the disease process. We plan to study the application of this marker for predicting recurrence by using a different protocol in the near future. In fact, we are currently examining the results of this study and collecting patient cases using a new protocol. We are planning to conduct this hypothesis verification research based on this preliminary study and hope to verify the role of PFAAs as a recurrence or prognostic marker.

Reviewer #2-2

The authors examined the significance of AICS using subgroup analysis, but the number of each group is relatively small to draw any conclusion (e.g., sidedness, histology).

Response: Thank you for pointing this out. We agree that the number of cases certainly is small for subgroup analyses; however, we consider the numbers to be sufficient for the purpose of this research.

Reviewer #2-3. I'm interested in the data of the patients with preoperative rank A (excluded from this analysis). Could you show us the data of this group?

Response: Thank you for your interest. We have now included the data of patients with preoperative rank A in Additional file 4 (Table).

Reviewer #2-4

The discussion section seems to be redundant.

Response: We thank the reviewer for this pertinent suggestion. We have modified the Discussion section to be more concise.