

Dear Editor:

We wish to thank the editor and the reviewer for their comments and would like to resubmit the revised version of our manuscript entitled “Blood-based biomarkers for early detection of esophageal squamous cell carcinoma” (Manuscript NO: 53493). We have revised the manuscript according to the comments of the reviewers, and highlighted the changes in red in the revised version of the manuscript. A detailed description of the changes made is provided in the Response to the reviewers following this letter.

All authors have read and approved this revised version of the manuscript. No part of this paper has been published or submitted elsewhere, and no conflict of interest exists for any of the authors. We appreciate your reconsideration of our manuscript.

Sincerely,

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## Response to the reviewers

(Changes are highlighted in red in the revised version of the manuscript)

Reviewer #1: Authors describe clearly about the new biomarkers for ESCC. They have summarized most of the method for detecting the ESCC. However, I still have several minor concerns.

**Comment 1:** In the introduction, authors mentioned about the disadvantages (high cost and invasiveness) of endoscopy which is the present standard method for detecting the early ESCC. Therefore it would be easier for the readers to imagine the difference between endoscopy and new biomarkers, if there is a table comparing the advantages and disadvantage between these techniques.

**Response:** Thanks for your suggestion. The table of advantages and disadvantage between endoscopy and new biomarkers were added in the main text in the revised manuscript (Table 1).

Table 1. Technologies for detection of ESCC.

Technology	Quantitative	Qualitative	Advantages	Disadvantage	References
Endoscopy	-	√	1. Obvious observation of esophageal mucosal changes and lesion size and morphology 2. Low false negative rate and false positive rate	1. Invasiveness 2. Easy to cause complications such as sore throat after examination 3. High cost	15,16
Blood-based Biomarker	√	√	1. Non-invasive 2. Easy to operate 3. Low cost 4. Suitable as a screening tool 5. Identification of asymptomatic patients at risk	1. High false negative rate and false positive rate	16,17,18

**Comment 2:** This topics is about detecting the early ESCC; however, most of the data of the new biomakers are not from early ESCC patients. So it is difficult to say that these newly biomarkers can detect early ESCC.

**Response:** Thanks for your suggestion. In response to your question, this is indeed a point we have not considered, and this is the direction we need to improve in the future. The details were already added in the Discussion part, which now reads “Meanwhile, we note that some of the studies in this review did not include patients with early ESCC

[37,76,108], so in future studies, the early diagnostic value of these markers needs to be further evaluated.” (page 21 of the revised manuscript)

**Comment 3:** Since the authors wrote about NMR and MS, a non-invasive investigation, it might be better not to use the word "blood-based biomarkers" in the title or in the manuscript.

**Response:** Thanks for your suggestion. Both NMR and MS tests mentioned in this review are based on serum tests, but not tissue. More specifically, specific metabolites were identified in serum by NMR and MS, and then were analyzed by gas-chromatography/mass-spectrometry (GC/MS) and liquid chromatography-mass spectrometry (LC-MS). So, we believe that serum metabolites belong to the category of blood-based biomarkers.