Dear Editor,

Thank you for giving us an opportunity to revise our manuscript. We appreciate editor and reviewers very much for their positive and constructive comments and suggestions on our manuscript entitled “Biomarkers for Hepatocellular Carcinoma Based on Body Fluids and Feces” (Manuscript NO: 61866).
We have studied reviewers’ comments carefully and have made revision which marked in red in the paper. We have tried our best to revise our manuscript according to the comments.
The main corrections in the paper and the responses to the reviewers’ comments are as follows:

Responds to the reviewers’ comments:

Reviewer #1: The comments can be summarized as follows:
1. While the manuscript mention in the Conclusion that most of the molecular biomarkers cited may improve diagnosis and management of HCC in the future, it would be necessary also to include this comment in the Abstract. This is because despite promising, most of these biomarkers are not currently used on a daily basis.
2. Page 12, In the phrase "hepatitis C virus (HCV) information", the word "information" is likely to be misplaced. It seems the word "infection" is more appropriated.
3. Legends for Figure 1 could be expanded and include a slightly more detailed explanation about the figure.

Response: 1. We have made correction according to the Reviewer’s comments. The following sentences have been added in the Abstract (Page 3). most of which may improve diagnosis and management of HCC in the future
2. The word “information” in Page 12 has been replaced by “infection”.
3. Legends for Figure 1 have provided a slightly more detailed explanation about the figure. (Page 32). Multiple molecular biomarkers derived from blood, urine and feces, including proteins, metabolites, circulating nucleic acids, circulating tumor cells, extracellular vesicles, and gut microbiota, have great potential to diagnose early hepatocellular carcinoma, predict responses to specific therapies, evaluate prognoses before or after therapies, and be developed as new therapeutic targets for this tumor.

Reviewer #2: The comments can be summarized as follows:
1. This manuscript provides an enumeration of plenty of biomarkers and studies that leaves the reader quite unsatisfied: A more explicit critical appraisal from the clinical point of view would be expected; the final conclusion ("In conclusion, testing body fluids and feces has a role to play in serving as a minimally-invasive and effective tool in improving diagnosis and management of HCC in the future") is vague.
2. Figure 1 should be omitted; it does not yield substantial information.

3. Stylistic/linguistic improvement is required (e.g., "A multicenter retrospective study reported that a decrease in perioperative serum AFP to be an independent risk factor for prognosis in HCC patients after liver resection", "The detection of CTC with stem-like phenotypes can used for diagnosis, prognosis and therapeutic response evaluation in HBV-related HCC", "In addition, use of probiotics in murine HCC models inhibited HCC development, suggesting this strategy to have a potential to be used as a therapeutic option for HCC patients in the future, provided that further studies can show alterations of gut microbiota by certain probiotics can also be observed in human", etc.). 4. Page 12/35: What is meant by "patients with chronic hepatitis C virus (HCV) information"? Table 2 and Page 13/35: "Nacetylated" -> N-acetylated.

Response:

1. We thank Reviewer #2 for the favorable comments. The more detailed and definite conclusion has been added in the CONCLUSION (Page 16). As for blood-based biomarkers, despite wide utilization of AFP, AFP-L3, and DCP in most countries, the sensitivity of a single biomarker for the early diagnosis of HCC is relatively low. In turn, those diagnostic models (e.g., the GALAD model, the ASAP model, and HES algorithm) have satisfactory diagnostic performance, which not only facilitates early detection of HCC in high-risk populations, but also avoids undue healthcare costs. These models need to be further optimized and validated in different countries and different populations before widely applied in clinical practice. With the approval of miRNA7™ molecular diagnostic kit for HCC diagnosis and therapeutic monitoring in China, liquid biopsy, especially cfDNA methylation and miRNA test, has a good application prospect in the diagnosis of HCC. However, the maturity of related technology, cost reduction, and the establishment of internationally recognized reference standards are required to ensure early clinical application. At present, imaging examination is routinely used for prognosis evaluation and post-treatment monitoring around the world. But computed tomography (CT) or magnetic resonance imaging (MRI) combined with serum AFP or DCP test is recommended as a regular follow-up examination strategy after HCC treatment according to Chinese, Japanese, and other clinical practice guidelines. Besides, EVs with specif ic therapeutic agents to liver cancer cells are regarded as a novel therapeutic method for HCC. The mechanism of EVs arrival at and uptake by target cells needs to be further investigated, and these findings from in vitro experiments are urgent to be verified in animal models due to complicated internal environment. As to urinary biomarkers, multiple protein, metabolite, or nucleic acid changes in urine potentially allow for HCC diagnosis and prognosis evaluation. But in fact, no effective urinary biomarkers for HCC have been applied in clinical practice now, and most of the studies are still in the exploratory stage. For gut microbiota from feces, whether gut microbiota can be used for early detection of HCC or as a novel therapeutic remedy should be further studied and clarified. In conclusion, future well-designed multicenter prospective studies on biomarkers for HCC derived from body fluids and feces as well as transformation of the relevant basic research results into clinical application will optimize current strategies of HCC diagnosis and management.

2. Figure 1 and the corresponding legends have been optimized (Page 32). The revised figure is attached in "uploaded files" and figure legends are as follows: Multiple molecular biomarkers derived from blood, urine and feces, including proteins, metabolites, circulating nucleic acids, circulating tumor cells, extracellular vesicles, and gut microbiota, have great potential to diagnose early hepatocellular carcinoma, predict responses to specific therapies, evaluate prognoses before or after therapies, and be developed as new therapeutic targets for this tumor.
3. Some inappropriate statements have been corrected. Page 6: A multicenter retrospective study noted that a decrease in perioperative serum AFP might be an independent risk factor for prognosis in HCC patients after liver resection. Page 15: In addition, use of probiotics in murine HCC models inhibited HCC development, suggesting this strategy might have a potential to be used as a therapeutic option for HCC patients in the future, provided that alterations of gut microbiota by certain probiotics could also be observed in human. Page 34: A CTC panel including four putative stem cell biomarkers showed great potential in HCC diagnosis, outcome prediction as well as treatment response evaluation 4. The word “information” in Page 12 has been replaced by “infection”. The word “Nacetylated” in Table 2 and Page 13 has been replaced by “N-acetylated”.

Reviewer #3: The comments can be summarized as follows:
1. In the manuscript authors mostly summarize the literature without any critical opinion or discussion about limitations or advantages. In the manuscript there is numerous biomarkers – which are appropriate to use? Can they be used in the clinic or should they be evaluated in the future? The fact is, that they are not accepted as suitable in the diagnostic purposes. Thus, all above mentioned questions need to be properly and critically discussed in the manuscript.

Response: 1. Considering the Reviewer’s suggestion, the more detailed and definite summaries have been added in the CONCLUSION (Page 16). As for blood-based biomarkers, despite wide utilization of AFP, AFP-L3, and DCP in most countries, the sensitivity of a single biomarker for the early diagnosis of HCC is relatively low. In turn, those diagnostic models (e.g., the GALAD model, the ASAP model, and HES algorithm) have satisfactory diagnostic performance, which not only facilitates early detection of HCC in high-risk populations, but also avoids undue healthcare costs. These models need to be further optimized and validated in different countries and different populations before widely applied in clinical practice. With the approval of miRNA7™ molecular diagnostic kit for HCC diagnosis and therapeutic monitoring in China, liquid biopsy, especially cfDNA methylation and miRNA test, has a good application prospect in the diagnosis of HCC. However, the maturity of related technology, cost reduction, and the establishment of internationally recognized reference standards are required to ensure early clinical application. At present, imaging examination is routinely used for prognosis evaluation and post-treatment monitoring around the world. But computed tomography (CT) or magnetic resonance imaging (MRI) combined with serum AFP or

DCP test is recommended as a regular follow-up examination strategy after HCC treatment according to Chinese, Japanese, and other clinical practice guidelines. Besides, EVs with specific therapeutic agents to liver cancer cells are regarded as a novel therapeutic method for HCC. The mechanism of EVs arrival at and uptake by target cells needs to be further investigated, and these findings from in vitro experiments are urgent to be verified in animal models due to complicated internal environment. As to urinary biomarkers, multiple protein, metabolite, or nucleic acid changes in urine potentially allow for HCC diagnosis and prognosis evaluation. But in fact, no effective urinary biomarkers for HCC have been applied in clinical practice now, and most of the studies are still in the exploratory stage. For gut microbiota from feces, whether gut microbiota can be used for early detection of HCC or as a novel therapeutic remedy should be further studied and clarified. In conclusion, future well-designed multicenter prospective studies on biomarkers for HCC derived from body fluids and feces as well as transformation of the relevant basic research results into clinical application will optimize current strategies of HCC diagnosis and management.

Attached please find the revised version, which we would like to submit for your kind consideration. We would like to express our great appreciation to you and reviewers for comments on our paper.

Looking forward to hearing from you.

Sincerely yours,
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