

Reviewer #1:

Specific Comments to Authors: Nannini G et al attempted to review metabolomics profile in gastrointestinal cancers and found that NMR metabolomics has demonstrated to be an optimal approach for diseases' diagnosis. English writing is fair (no grammatical error) and this work is worth enough for possible publication in WJG.

Major comments: none.

Minor comments:

- 1) Provide page number in the manuscript.

Reply: *As rightly suggested, we have provided to insert page number*

- 2) Page 6, line 1. ... from Gln to -ketoglutarate ... is ... from Gln to α -ketoglutarate...

Reply: *Thanks for suggestion, we have corrected -ketoglutarate in α -ketoglutarate (pag 7 line 198)*

Reviewer #2:

Specific Comments to Authors: In the article entitled "Metabolomics profile in gastrointestinal cancers: update and future perspectives", Nannini G and colleagues critically reviewed the issue of metabolomic as a potential biomarker for gastrointestinal (GI) cancers diagnosis. They proposed the application of nuclear magnetic resonance (NMR) based metabolomics in bio-fluids to identify biomarkers on facts observed in studies for GI cancers, such as pancreatic cancer, gastric cancer and colorectal cancer. Considering the NMR-based metabolomics in GI cancers is still at its unfolding, authors made objective evaluations of related studies' objectivity and scientificity, and recommend that future related studies should design multicentric researches involving a high number of patients and multiple GI cancers. However, some points in this review need to be further considered.

Major comments: none.

Minor comments:

- 1) In my opinion, it will be better that introduce in the INTRODUCTION PART the pros and cons of NMR in metabolomics compared with GC and current hotspots in NMR metabolomics appliance in medicine.

Reply: *In agreement with the suggestion, we have added an introduction to the two main techniques and its principal differences (please see introduction section, 138-143 lines.*

- 2) Authors suggested that urinary metabolomics represents a good non-invasive alternative to determine tumor-associated perturbations; moreover, urine metabolomic analysis could be easily implemented to be used as wide scale population screening. However, all of studies mentioned in the URINE SAMPLES part seemed like retrospective studies, in my opinion, the conclusions draw by these might not be applied in population screening. And considering the heterogeneity among study objects, most objects in researches with valid conclusions were diagnosed cancer patients, non-invasive methods did little good to these patients since they probably had undergone surgeries already. Do author agree with this?

Reply: *Yes, authors totally agreed with the reviewer.*

The mentioned sentence has been corrected to be clearer and moved to the end of the paragraph. New prospective studies must be done based on these preliminary evidences to demonstrate the validity of the retrospective study done.

3) Some studies only included small sample size using metabolomics, what is the opinion of authors about their repeatability?

Reply: *Robustness of the results is strictly dependent on sample size. Studies on bigger and multicentric cohorts are needed to validate results obtained with a small sample size.*

4) The authors can properly discuss the mechanism of metabolites in GI, rather than just focusing on the studies about differential of metabolites in GI cancers. Also, the mechanism of which how metabolites in urine are effected by gastrointestinal tumors. Furthermore, authors not just illustrate the results of the study, but also have to intersperse the meaning of the study and their own perspective.

Reply: *As rightly suggested, we added, in the introduction section (93-103 lines), the role of metabolites in GI. Moreover, we have explained the major drawback of urine, concerning the high sample variability that should be taken into account (see lines 415-424). Identified metabolites' alterations should be weighted according to patient lifestyle, diet and comorbidities*

5) There are redundant tables listing the altered metabolite levels identified in blood samples, urine samples and fecal water samples, it would better to use the figure and legends to simplify the presentation. After all, a picture is worth a thousand words.

Reply: *In agreement with the reviewer, we have inserted a new figure (fig. 3) in order to summarize the most significant metabolites identified. However, we believe that it is hard to full substitute the table with a figure due to the large amount of data take into consideration, We think that the coexistence of both (table and figure) is the best solution for readers*

6) Since the authors regard fecal water sample as a priority in all bio-fluids for the metabolic NMR analysis, a summary table can be elaborated to clearly present this strength through comparing the advantages and disadvantages of various bio-fluids.

Reply: *Thanks for the suggestion, we added a summary outline (fig. 2) to clearly summarize the advantages and disadvantages of various bio-fluids*

7) In the abstract, the abbreviation "NMR" should be clearly presented as full name.

Reply: *As suggested, the abbreviation "NMR" in the abstract has been presented with its full name "nuclear magnetic resonance"*

8) "Warburg effect is a shift from ATP synthesis by oxidative phosphorylation to ATP generation through glycolysis, also in aerobic condition." This sentence should indicate the source: Warburg effect was firstly reported in the 1920s. (Warburg O, 1924, Biochemische Zeitschrift)

Reply: *In agreement with the reviewer, we added the suggested reference: O. Warburg, K. Posener, E. Negelein: Ueber den Stoffwechsel der Tumoren; Biochemische Zeitschrift, Vol. 152, pp. 319-344, 1924. (in German). Reprinted in English in the book On metabolism of tumors by O. Warburg, Publisher: Constable, London, 1930.*

9) Page 11. liver metastasis (LC). What does this abbreviation, LC means? liquid chromatography or liver cancer? 1

Reply: *We are sorry there was a mistake; as the reviewer suggests the abbreviation corresponds to "liver cancer (LC) metastasis".*

10. There were no conclusions summarized from Dykstra M.A. et al. (2017) study. Please check the completeness.

Reply: *Thank you for the advice. Conclusions for Dykstra M.A. et al. (2017) study have been summarized in the dedicated paragraph.*