April 15, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 10254-edited.doc).

Title: Circulating MACC1 (Metastasis Associated in Colon Cancer 1) transcripts in gastric cancer patient plasma as diagnostic and prognostic biomarker

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The manuscript has been improved according to the suggestions of reviewers:

Reviewer 1

The m/s presents an interesting research study concerning the quantitation of MACC1 transcript in blood of gastric cancer patients. Quantitative results were compared in terms of cancer metastases and overall survival of patients and also in conjugation with another proposed biomarker, S100A4. The m/s is well-written, however there are some points requiring clarification or correction, to be the m/s suitable for publication.

Major points

1. Table I: The stages I-IV(M0) and I-IV(M1) are not clearly indicated.

Answer:

We modified table I to make it more comprehensive and understandable.

2. The general question for gastric cancer is its early diagnosis, because of its aggressiveness and the m/s does not discuss on this subject, although Table I provide evidence that six of nine patients have cancer of stage I.

Answer:

In this paper we explored the potential benefit of circulating MACC1 levels as a prognostic and diagnostic biomarker. Significantly higher MACC1 levels were measured in the plasma of patients with primary diagnosis of gastric cancer compared to healthy volunteers.

Furthermore, we could show, that MACC1 can be used to identify individuals suffering of gastric cancer with a sensitivity of 0.68 (CI 0.45 – 0.85) and a specificity of 0.89 (CI 0.77 – 0.95).

However, only six patients out of the cohort of patients with present gastric cancer (consisting of patients with primary diagnosis with or without synchronous metastasis and patients with metachronous metastasis) had a stage I gastric cancer and the cohort was too small to determine the diagnostic value for this specific group.

In order to clarify this point and to underline the importance of early diagnosis we modified paragraph 1 of the Introduction section and additionally included reference 2.

3. Figure 1: According to point 2, the figure should be corrected to include the stage I(M0) patients.

Answer:

The figure includes all patients newly diagnosed without synchronous organ metastases (stage I-IV, M0), patients newly diagnosed with synchronous organ metastases stage IV, M1), patients with metachronous metastasis and patients in follow-up.

We hope that we could clarify this point with the modifications done in table 1.

4. Figure 3: The authors should explain why they have combined in the same group patients with none biochemical markers and patients having one of these markers in high levels. Otherwise, the figure should be removed, since it is rather confusing the reader.

Answer:

As previously shown S100A4 is also linked to tumor progression and development of metastasis in gastric cancer. Therefore, we wanted to investigate whether the combination of both the metastasis biomarkers, MACC1 and S100A4 will further improve the prognostic value of liquid biopsies.

Reviewer 2

The research article "circulating MACC1 transcripts in gastric cancer patient plasma as diagnostic and prognostic biomarker" by Burock et al 2014 provides a useful insight into the use of MACC1 transcripts to provide a biomarker for gastric cancer, and associated patient survival rates. The manuscript is interesting and draws upon sound clinical data sets. I would therefore recommend publication of this article if the authors address the following minor points:

1. Provide the full unabbreviated name of MACC1 in the manuscript title and define this and any other abbreviations/acronyms used prior to textual usage.

Answer:

We included the full name for MACC1 in the manuscript title and checked and explained the abbreviations throughout the manuscript.

2. Provide a brief explanation in the introduction section describing the function of MACC1 gene product, and hence outline why levels would likely correlate with gastric cancer diagnosis and prognosis.

Answer:

We included the requested paragraphs in the introduction section.

Finally, do the authors have any provisional evidence that suggests that MACC1 levels would be a useful biomarker following cancer treatment interventions/ any specific types of intervention? – as this is only briefly touched upon in the discussion.

Answer:

So far, there are no data available concerning MACC1 levels measured prior to and following cancer treatment interventions. Therefore, we just briefly touched upon in the discussion.

Reviewer 3

Overall it is a well-written manuscript which addresses a important research question. Only the statistical analysis for me seems to be a little bit old fashioned.

Some comments:

1) Why not using resampling approaches and respective confidence intervals for the cut-off value of MACC1?

Answer:

We completely agree that the analysis performed for this paper is performed in a traditional way. However, little is known by now about the distribution of MACC1 levels in gastric cancer patients' population. Therefore, we cannot be sure whether our population is representative and we are reluctant in using resampling approaches. Furthermore, resampling approaches are not commonly used in this field till now ad we wanted to be comparable to the analysis previously done by our own or other working groups.

2) For me it seems to be a not to complicated two-dimensional optimization problem (with objective function sensitivity + specificity or balanced accuracy or bookmarker informedness) to determine optimal cut-offs for the combination of MACC1 and S100A4. What about the sensitivity and specificity of this combination of biomarkers? Again resampling approaches could be used. Furthermore, one could also think applying statistical/machine learning methods to solve this problem.

Answer:

For the diagnostic value of MACC1 we used a very small patient cohort of patients with present gastric cancer consisting of patients with primary diagnosis with or without synchronous metastasis and patients with metachronous metastasis. As both biomarkers, S100A4 and MACC1 were not available neither for all healthy volunteers nor for all patients we did not consider to evaluate the diagnostic impact for a combination of both markers.

However, we used the optimal cut-off value for MACC1 as described and for S100A4 as previously reported (Stein et al, J Mol Diagn 2011) for all patients and healthy volunteers were both markers were available. We found a sensitivity of 58% and a specificity of 100% for patients with both markers elevated.

As little is known about the distribution of these markers in this patient cohort we are not in favor of using resampling methods in this case.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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