Author's response to reviews

Title: Circulating tumor cells and survival of patients with gastric cancer: a meta-analysis

Authors:

Zhen-yu Zhang (zhangsurgery@qq.com)
Zhen-ling Dai (circulating@qq.com)
Xiao-wei Yin (translational@qq.com)
Shu-heng Li (prilo@qq.com)
Shu-ping Li (microfluidic@qq.com)
Hai-yan Ge (gesurgery@qq.com)

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Dear editors and reviewers,

Thank you for your comments on our manuscript. Those comments are all valuable and very helpful for revising and promoting our research work. They are also of important guiding significance to our researches. We have studied the comments carefully and have made corrections which we hope to meet with approval. Revised portions are marked in red in the manuscript.

Below are our specific responses to the reviewers’ comments.

Comments from Reviewer Zhen-Ning Wang

1. There were no exclusion criteria on the redundant studies based on same patient population. The studies by Arigami[32], Arigami[36], Arigami[42], and Uenosono[48] (as well as studies by Wu[25] and study by Uen[26]) were conducted by same institution and may be based on same patients. Thus same patients are evaluated several times and the pooled results are doubtful and may be incorrect. Moreover, Statistical Approaches, third paragraph, “For studies with multiple arms (i.e., resectable and unresectable groups) or multiple markers (i.e., cytokeratin 18 and 19), each of the subgroups was considered an independent data set”, it contributed double/treble patients evaluation.

Response 1:

Thank you for your suggestions. We agree with the reviewer that duplicated assessments should be avoided in meta-analysis to the greatest extent. We have
rechecked the included studies by Arigami[32], Arigami[36], Arigami[42], and Uenosono[48], all of which are reported by the same institution in Japan. First of all, the research by Uenosono[48] with patients enrolled from February 2005 to December 2012 is unlikely to include subjects that reported in the other three researches by Arigami[32, 36, 42]. Because the periods for enrollment of the three works are from 2002 to 2005. Secondly, although a number of patients might be simultaneously enrolled in these reports, different markers are applied to define the positive event of CTCs in individual studies, i.e., B7-H4 by RT-PCR in Arigami[32], B7-H3 by RT-PCR in Arigami[36], STC2 by RT-PCR in Arigami[42] and CK8/18/19&EpCAM&CD45 by the CellSearch system in Uenosono[48]. Each of the markers independently indicates CTC status in different studies, (although some patients may be tested for all of the markers), resulting in different CTC detection rates (75.5% in ref [32], 50.5% in ref [36], 46.2% in ref [42] and 31.1% in ref [48]). Thus, even the same patient may be identified in opposite CTC statuses by detection of different markers. In fact, each of the four studies contributes different survival and exposure information to the analysis. In practice, the three researches [32, 36 and 42] by Arigami et al can be considered as three independent subgroups within a combined study. Thus, it is better to keep the four researches as independent units of the meta-analysis in order to gain the maximum information, in spite that they come from the same institution. Similar practices can also be found in some other meta-analyses and systematic reviews on prognosis of CTCs (i.e., PMID: 20389297, 21311967 and 24901848). To further validate this criterion, we have made sensitivity analyses by
removing one of the four studies and the results were demonstrated to be stable (please see Figure S1 and S2 in Additional file 5). In addition, when we only included the latest study by Arigami et al. (ref [42]), the overall effects changed very slightly. (OS: HR=1.83, 95%CI[1.50-2.24], n=28, \(I^2=35.48\%\))(data not shown in the manuscript). Therefore, no exclusion of any one of the four studies is also feasible.

As for the study by Wu[25] and the study by Uen[26], the situation is similar. Their patient enrollment periods are different from each other (January 2003 - March 2004 and April 2002 - September 2003, respectively) with different patient size (64 and 52, respectively). They use different methods (HTCAM and RT-PCR, respectively) to determine CTC status with different marker criteria. In addition, sensitivity analyses by removing any of the two studies did not change the total results significantly (please see Figure S1 and S2 in Additional file 5). Therefore, it is better to include both researches in order to keep the maximum information.

As for complex data structures such as studies with multiple arms (i.e., resectable and unresectable groups), patients in resectable group will never be included in the other group. Thus, both groups can be regarded as independent subgroups within the study. This does not lead to reassessments.

As for the statement in the section of statistical approaches: “studies with multiple markers”, these studies refer to studies that define CTC presence as the positivity of any one marker used. To keep intra-study independence and keep maximum information, we used each marker as independent subgroups. The reasons are similar as we have mentioned above. Such data processing method was also used in some
other meta-analyses on prognosis of CTCs (i.e., PMID: 2131196, 24901848). For example, in ref [29], both CK19 and CK20 were detected to independently predict the OS of gastric cancer patients. However, in ref [28], although multiple markers including CK8, CK18, CK19 and EpCAM were applied by the CellSearch system, no one of them can independently predict the survival because CTCs were regarded to be present only when all of the four markers are positive. In consideration of the reviewer’s comments, we have revised the manuscript to make the statement more rigorous.

2. Statistical Approaches, third paragraph “we used data from pre-therapy samples in prior to intra/post-therapy samples because those data were usually dependent”. In my opinion, clinical study and relevant meta-analysis (PubMed: 11176124, 20100481, and 22799295) indicated that the post-therapy sampling time might reflect the most relevant CTC status. And the authors also mentioned similar situation in Discussion and they were inconsistent. E.g., Discussion, fifth paragraph “Baseline detection had risks of failing to provide information about the actual burden of CTCs after therapies thus might be unable to accurately predict survival of patients post treatments.” and Subgroup Analyses and Meta-Regression, first paragraph “the prognostic role of CTCs for RFS was not observed in…”authors should clarify the difference on CTCs positivity level in pre-therapy and post-therapy and underlying reasons and mechanisms.

Response 2:
We agree with the reviewer. Because we did not know whether there were any significant changes on the total effects by using pre-therapy rather than intra/post-therapy data when we were designing the precedence. We thus further performed sensitivity analysis to test whether the results were reliable, as was stated in the statistical approaches section. Such sensitivity analysis could indirectly indicate whether the precedence was adequate. But the authors had pointed out in the manuscript that it was not clear whether pre-therapy sampling was superior to intra/post-therapy sampling because few studies compared multiple time points for CTC detection. Useful evidence was still limited. As a result, based on available data and current meta-analysis, we have added some detailed discussions on the potential reasons for the differences in positive rates of CTCs between pre-treatment and post-treatment detection (please refer to the fifth paragraph in the Discussion section). The manuscript has been revised accordingly.

3. Statistical Approaches, fourth paragraph “We did not assess the quality of included studies, because widely accepted standard was not available for prognostic studies [19]”, the reference 19 is a meta-analysis, thus whether the citation is correct.

Response 3:

Thank you. We are very sorry for the mistake. The “reference [19]” was incorrectly cited. In addition, I have discussed with my coauthors and we think that it is still necessary to make quality assessment on the included studies. Although there are no commonly accepted assessment tools, we have introduced the Newcastle-Ottawa
Scale (NOS) for cohort studies into our revised manuscript because it has been widely used and also been recommended by the Cochrane Collaboration. Our manuscript has been revised accordingly (please see Table 1 in the manuscript and Table S2 in Additional file 4). In addition, sensitivity analysis by removing low quality studies has also been performed (please refer to the Quality Assessment and Sensitivity Analyses section, page 10, line 213-217).

4. The subgroup analysis on detection methods was only based on cytological and molecular methods. For providing useful information to clinicians and investigators, the authors should also conducted subgroup analysis by PCR, ICC, CellSearch, and HTCMA.

Response 4:

We agree with the reviewer. We have further performed subgroup analyses by RT-PCR, CellSearch and other methods (including HTCMA [25], FACS-ICC[27] and ICC[41]). Subgroup analyses are performed only when there are two or more studies included in the subgroups. We have revised the manuscript as well (please see Table 2, the variable of Approach). We found that both RT-PCR and CellSearch systems were demonstrated to be valid approaches to detected CTCs in predicting patient survival of GC. However, as unbalanced number of studies was included in the subgroups on RFS and OS, limited information could be drawn to directly compare RT-PCR with the CellSearch system.
5. MicroRNA acts as a novel marker for detection of CTCs, authors should brief clarity the sensitivity and specificity.

**Response 5:**

We agree with the reviewer and have described the diagnostic accuracy of microRNA in the second paragraph in the Baseline Characteristics section, where the clarification has been marked in red (page 8, line 165-168). Thank you.

6. If the number of studies was available, the authors should conduct in-depth subgroup analyses considering the methods and sampling time simultaneously.

**Response 6:**

We agree with the reviewer. We have further conducted the subgroup analyses by method together with sampling time. When the studies were grouped by method and sampling time simultaneously, the heterogeneity became unobvious in the RT-PCR group of PFS and OS, indicating that sampling time was an important source of inconsistence across studies. The manuscript has been revised accordingly. Please refer to Table S3 in Additional file 4.

**Comments from reviewer : Nuh N Rahbari**

1. The authors should perform a quality assessment of all included studies. It is correct that there is no universal agreement as to which tool should be used for observational studies. However, this does not justify the fact not to do a quality
assessment at all.

**Response 1:**

Thank you for your suggestion. We agree with the reviewer and have made quality assessment of included studies with the Newcastle-Ottawa Scale (NOS) for cohort studies. The scale is recommended by the Cochrane Library. Please see Table S2 in Additional file 4 for details. In addition, sensitivity analysis by removing low quality studies was also performed (please refer to the Quality Assessment and Sensitivity Analyses section, page 10, line 213-217).

2. There are already several meta-analyses published on the same topic. The authors should clearly point out the novelty of their study.


**Response 2:**

We agree with the reviewer. The two meta-analyses listed above were published after the submission of our manuscript to the journal of *BMC cancer*. So, we think it is necessary to discuss the novelty of our work, although many of the contents and
statistical methods of our meta-analysis are different from them.

For example: Compared to another two meta-analyses on the similar topic of GC, we applied many advanced statistical methods in our meta-analysis, such as one study removed”, trim and fill method, meta-regression, fail-safe numbers as well as cumulative meta-analyses. These methods were helpful to get deeper and more comprehensive insights into the prognostic value of CTCs and potential heterogeneity of included studies. We also conducted subgroup analyses by publication year, country, country, patient size, detection rate besides detection method and time point.

We have revised the manuscript accordingly. Please refer to the second and third paragraphs in the Discussion section for details (page 11-12, line 236-259).

3. The authors need to clearly define their inclusion criteria for the studies to be included in the present meta-analysis.

Response 3:

We agree with reviewer and we have described the inclusion criteria in the manuscript. Please refer to the second paragraph of the Search Strategies and Study Selection section (page 4-5, line 83-91). The paper has been revised accordingly.

4. It is not clear, why the authors included studies that investigated the role of miRNAs as biomarkers, as these do not represent circulating tumor cells or specific tumor cell (epithelial) markers.

Response 4:
Thanks for the reviewer’s comments. Unlike classic tumor markers, miRNAs are expressed without restrictions to epithelial or tumor cells. However, many miRNAs have been demonstrated to be involved in tumor growth and metastasis. Altered expression of miRNAs can frequently be detected with many cancers including GC. Although miRNAs in blood may be derived from neoplastic (including CTCs) and non-neoplastic sources, the elevated levels of them (above cutoff value) can be used for diagnosis and prognosis of GC. We consider that the detection of miRNAs with increased concentrations indicate the presence of CTCs to a certain extent (although may be not as sensitive and specific as epithelial markers). Therefore, we included the type of marker in order to keep maximum information for us to make comprehensive assessments. In addition, we have added a brief introduction of the sensitivity and specificity of miRNAs (page 8, line 165-168). Thank you.