Author's response to reviews

Title: Prognostic significance of Epstein-Barr virus infection in gastric cancer: a meta-analysis

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Author's response to reviews: see over
Dear Editor,

Title: Prognostic Significance of Epstein-Barr Virus Infection in Gastric Cancer: A Meta-analysis

Author: Xuechao Liu, Jianjun Liu, Haibo Qiu, Pengfei Kong, Shangxiang Chen, Wei Li, Youqing Zhan, Yuanfang Li, Yingbo Chen, Zhiwei Zhou, Dazhi Xu, Xiaowei Sun

Name of Journal: BMC Cancer

We are very grateful to you for reviewing our manuscript and reconsidering it in your journal. We greatly value the comments from your reviewers and find them to be very helpful in improving our work and the overall manuscript. The manuscript has been updated based on your reviewer’s comments, and in doing so, the paper is improved. We have answered the comments in a point-by-point fashion as follows below, and resubmit our revised paper to your journal as a new manuscript.

Thanks for your kindly help and time in dealing with this and we look forward to hearing from you.

The manuscript has been improved according to the suggestions of reviewers:
1. Format has been updated
2. We have added page numbers to the PRISMA checklist and ensured our manuscript reporting adheres to PRISMA guidelines.
3. Revision has been made according to the suggestions of the reviewer:

   (1) Reviewer: Masanao Murakami

   Reviewer's report:

   In this manuscript, Xuechao Liu and co-workers collected 372 studies from PubMed and EMBASE and finally they used 24 studies (6.4%) in their analysis. These 24 studies were almost recent studies, because I guess old studies did not carry out statistical analysis clearly. Although authors checked studies by Funnel plot and it kept symmetry, I felt Funnel plot was a slight lean. Moreover, excluded old studies showed evidences for prognostic state such as important character information in EBVaGC which are low frequency of apoptosis, less p53 abnormal expression, and higher expression of IL-1b. Surely, these publications did not mention about prognostic significance. The authors also mentioned in the text; there are several similar statistical reports. I understand importance of statistical meta-analysis, but compared to their results, I could not find ‘what’s new’ except large scale analysis.

Comment for individual text.
1) P2 l60 and P3 l71; The relationship between EBV and gastric cancers were already reported by Dr. Selves J et al, Dr. Yuen ST et al, Dr. Shibata D et al, Dr. Tokunaga M et al, and Dr. Galetsky SA et al. Therefore “EBV has gradually been considered” “EBVaGC has been identified as a distinct disease” should be changed.
2) P4 l70; add space after references.
3) P4 l86; Abbreviations should be spelled out in full when first used in the text.
4) Author should unify description <95%CI or 95% CI>.
5) P6 l122; Abbreviations should be spelled out in full.
6) P7 l138; add space after references.
7) Author should uniformed style for EBV associated gastric cancers. Author used several forms such as EBVaGC, EBV-positive GC, EBV-associated GC.
8) P14 l290; add space after references.

Response:
Thanks very much for your important suggestions.
1. Of 24 included studies, only 2 studies were published before 2000.
2. We also found that Funnel plot was a slight lean, but the judgments were subjective in nature. The Begg’s test and Egger’s test were used to further examine asymmetry of the funnel plot. The P values of both tests were >0.05 respectively, which suggested publication bias was modest and not statistically significant (on line 219-224 at page 10).
3. Surely, we excluded some old studies which didn’t mention about prognostic significance, but they showed evidences for prognostic state such as important character information in EBVaGC which were low frequency of apoptosis, less p53 abnormal expression, and higher expression of IL-1b. In fact, we also realized that other possible prognostic factors were difficult to be examined with respect to their effect on the prognosis of EBVaGC (on line 350-351 at page 16).
4. Indeed, by now, the conclusion that the presence of EBV has a favorable impact on GC patient’s survival has been reported by Camargo MC et al in a pooled analysis, which included 4,599 GC patients from 13 studies in Asia (n=8), Europe (n=3), and Latin America (n=2). We performed an extensive search for articles that examined the association between EBV and the prognosis of GC. Of note, we first overcame limits of size and region to further validate the conclusion. More importantly, in subgroup analysis, we found that the protective role of EBV infection was only observed in Asian patients rather than European and American patients. Furtherly, we deeply and broadly discussed how come such a difference between Asian and Western countries. We postulated that there might be differences in genetic factors or the way of treatment between Asian and Western countries (on line 290-317 at page 14).

Comment for individual text.
1) “EBV has gradually been considered” “EBVaGC has been identified as a distinct disease” have been changed (on line 71-73,81-84 at page 4).
2) We have added space after references (on line 81 at page 4).
3) Abbreviations have been spelled out in full when first used in the text (on line 104 at page 5).
4) We have unified description <95% CI>.
5) Abbreviations have been spelled out in full (on line 143 at page 7).
6) We have added space after references (on line 160 at page 8).
7) We have uniformed style for EBV-associated gastric carcinoma (EBVaGC).
8) We have added space after references (on line 338 at page 16).

(2) Reviewer: Corey Casper
Reviewer's report:
This manuscript by Liu et. al. attempts to answer the question of whether Epstein Barr Virus (EBV) status impacts survival after a diagnosis of gastric cancer
through the conduct of a systematic review. Overall, the review is well-conducted according to established practices and identifies a sufficient number of cases to allow for a meaningful analysis and interpretable results.

Specific comments are listed below:

**Major Compulsory Revisions**

1. The authors include studies in which meet defined criteria for EBV determination (ISH for EBER), but it would be informative to know what studies failed to meet the authors' criteria for "sensitive and reliable" methods for EBV detection and were therefore discarded and what method these studies used. Did the exclusion of these studies influence the results of the systematic review?

2. The endpoint for the authors' analysis is "overall survival", but no time point for this endpoint is specified. Obviously studies with insufficient follow-up or heterogeneity in reported OS time periods could lead to bias or misclassification in this systematic review.

3. A limitation of this study is that it infers causality between EBV status and survival in patients with gastric cancer, but other factors can account for differences in survival between EBV+ patients and EBV-negative patients. It would be informative to see how the established prognostic factors for gastric cancer survival differed between EBV+ and EBV-negative cases across the studies (TNM, etc). Also, EBV+ gastric cancers are typically located in non-antral sites. A discussion of how differences in tumor location may impact survival is warranted.

**Response:**

Thank you so much for your valuable comments. There are three questions and our answers are:

1. Surely, one of our eligibility criteria was “to provide a sensitive and reliable method for detection of the existent status of EBV”. But, most of studies were excluded because of insufficient statistical data. Though a part of excluded studies used other methods for the detection of EBV, for example polymerase chain reaction-enzyme immunoassay (PCR-EIA), no studies were excluded just because of inappropriate detection method (on line 190-194 at page 9).

2. The time point for "overall survival" has been specified. We are also aware that studies with insufficient follow-up or heterogeneity in reported OS time periods could lead to bias or misclassification. Therefore, the estimated median follow-up time is provided (on line 115-118 at page 6 and on line 194-196 at page 9).

3. We were aware of the fact that other factors might account for differences in survival between EBV+ patients and EBV-negative patients, which had been discussed as the limitations of this study. In addition, according to your suggestion, a discussion of how differences in tumor location may impact survival has been added (on line 351-357 at page 16).

(3) **Reviewer: Hironori Yoshiyama**

Reviewer's report:

Comments (Major revision)

In this manuscript, Liu X et al. investigated the effect of EBV infection on survival
of patients who are suffered from gastric cancer. The manuscript is written clearly using understandable English. And the methods and results seem to be appropriate and draw convincing results for readers. However, the conclusion that the presence of EBV has a favorable impact on gastric cancer patient’s survival” has been reported by others. Thus, the reviewer awaits for novel information for treatment of patients with EBV-associated gastric cancer. Because of this, the reviewer rated it as requiring major revisions.

Major comment
The authors conducted a meta-analysis to assess the prognostic significance of EBV infection in gastric cancer and found that EBV presence has a favorable impact on Asian GC patient’s survival. However, the protective role of EBV infection European and American patients could not be observed (on line 253 and 254 at page 12). The authors had better discuss how come such a difference between Asian and Western countries. Probably, there must be difference in the way of diagnosis and treatment between Asian and Western countries.

Minor comment
On line 75, 76, and 77 at page 4, the author used words, such as “some”, “inconsistent”, and “no definitive” These words must substitute by more descriptive words. Please describe what is clearly understood for the prognosis of EBV-associated gastric cancer and what is still unclear and should be dissolved. By doing so, readers can delve deeper into the problems in the research on EBV-associated gastric cancer.

Response:
Thank you very much for you important comments.

1. We speculated that there might be difference in the way of diagnosis and treatment between Asian and Western countries, though we didn’t find sufficient information from included studies. According to your suggestion, we performed an extensive search for articles about treatment of patients with EBV-associated gastric cancer. We discussed the potential difference in the way of treatment between Asian and Western countries in detail in our revised manuscript (on line 297-3317 at page 14).

2. These words have been replaced by more descriptive words. By doing so, readers can delve deeper into the problems in the research on EBV-associated gastric cancer and the aim of our study (on line 86-95 at page 4).