Author's response to reviews

Title: The prognostic significance of vascular invasion in patients with resectable gastric cancer: a large retrospective study from southern china

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Author's response to reviews: see over
Mr. Ryan Relox and Dr. Wei Jiang,
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Title: The prognostic significance of lymphovascular invasion in patients with resectable gastric cancer: a large retrospective study from southern China

Dear Mr. Ryan Relox and Dr. Wei Jiang,
Thank you for reviewing the above-referenced manuscript submitted earlier to your office. We would like to take this chance to express our appreciation to you and the Reviewers.
In accord with the Reviewers’ comments, the manuscript has been revised accordingly, and the changes have been highlighted with red ink in the revised manuscript. We feel that the revised manuscript has been strengthened by the Reviewers’ suggestions and comments, and we are very appreciated of their time and effort. A point-by-point response to the reviewers’ comments and suggestions has been prepared and follows this cover letter.
The format for our revised manuscript has been properly prepared and conforms to BMC Cancer style.
If there are any questions or problems for our revised manuscript, please feel free to contact me.

Sincerely yours,

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Response to comments of the Reviewers

Reviewer #1: In several malignancies, including gastric cancer, lymphovascular invasion has been reported to be associated with a poor clinical outcome. Within a retrospective cohort from southern China, Li et al demonstrate similar findings and correlate lymphovascular invasion with other clinical and pathologic features. Although not novel, the authors’ study consists of a large cohort of 1148 patients undergoing gastrectomy with curative intent. However, there are a number of issues that should be addressed:

1. The authors should clearly define disease-free survival and disease-specific survival. Further, cancer-specific survival (CSS) should be corrected to disease-specific survival. Within the Materials and Methods it is unclear how the authors calculated disease-free survival. This should be clarified.

Reply: Thanks for the Reviewer's correction, the term “cancer-specific survival (CSS)” has been replaced by “disease-specific survival (DSS)”. In our study, the disease-free survival (DFS) was defined as the time from surgery to recurrence or death from gastric cancer (GC), whichever came first. The disease-specific survival (DSS) was the time from the date of surgery to the date of death from GC or to the last follow-up. DFS and DSS were calculated using the Kaplan-Meier method. These information have been clearly clarified in our revised manuscript (See the 154th line of page 7, the 155th to 157th lines of page 8 and the 191st to 192nd lines of page 9 in the revised manuscript).

2. Within the Materials and Methods, a number of variables should be clearly defined. For example, how was elevated CEA and CA19-9 defined.

Reply: The Reviewer is right that the cutoff values of elevated CEA and CA19-9 are not clearly defined. In the present study, serum concentrations of CEA and CA19-9 were measured using the immunoradiometric method. The cut-off values for CEA and CA19-9 were 5.0 ng/ml and 35.0 U/ml, respectively. The elevated levels of CEA and CA19-9 were defined as the serum concentrations above respective cut-off value. The description of the elevated CEA and CA19-9 has been added to the Materials and
Methods section. Additionally, the criteria for pathologic variables have been described in our revised “Materials and Methods” section (See the 144th to 148th lines of page 7, the 173rd to 176th lines of page 8 and the 177th to 180th lines of page 9 in the revised manuscript).

3. Within the Results and Table 2, the authors correlate various clinical and pathologic variables with disease-specific survival by univariate and multivariate analyses. As the authors have disease-free survival data, were similar results identified? The manuscript would benefit it similar analyses were performed with disease-specific survival. These should be added within the Results section and as an additional Table.

Reply: In accord with the reviewer’s suggestion, we analyzed the association of various clinicopathologic variables with DFS by univariate and multivariate analyses, and found that the presence of lymphovascular invasion (LVI) was an independent predictor of DFS in patients with GC after curative resection. This findings further supported LVI as a potential prognostic factor for GC. These data have been added within the "Results" section and Table 3 (See the 254th to 256th lines of page 12 in revised manuscript and Table 3).

4. In the Discussion, the authors are encouraged to relate their findings to prior reports. Were there any differences or similarities? If any differences, why?

Reply: We appreciated the Reviewer's good suggestions! We compared the incidence of LVI detected in our cohort with prior reports, found variations in the incidence of LVI reported in the literature. The different detection methods might be contributed to this discrepancy. Additionally, in line with previous observations in several small-scale studies, we confirmed the negative impact of the presence of LVI on DSS and DFS in a large cohort of 1148 patients with gastric adenocarcinoma undergoing gastrectomy. Furthermore, our data showed a worse prognostic impact of LVI stratified by TNM stage. More importantly, LVI was identified as an independent prognostic factor by multivariate analysis. In accordance with the reviewer’s
suggestion, we have clearly described these in our revised “Discussion” section (See the 275th to 281st lines of page 13, the 297th to 308th lines of page 14 and the 309th to 318th of page 15 in the revised manuscript).

5. Within the Materials and Methods, Pathologic Evaluation, the authors mention that particular attention was taken toward artifacts due to peritumoral edema and tissue shrinkage. The authors should further clarify how they dealt with these cases and how was vascular invasion determined.

Reply: Good suggestion! In this study, LVI was defined as the invasion of vessel walls by tumor cells and/or the existence of tumor emboli within an endothelial-lined space. Furthermore, the lumen of blood and/or lymph vessel were identified based on one or more of the following criteria: (i) lined by endothelium; (ii) with supporting smooth muscle or elastica; (iii) filled with lymphatic fluid or red blood cells (Gabbert et al., Int J Cancer 1991, 49(2):203-207). Any other circumstance was considered as artifacts due to peritumoral edema and tissue shrinkage. In light of the Reviewer’s comments, we described this clearly in our revised “Materials and Methods” section (See the 178th to 180th lines of page 9 and the 183rd to 187th lines of page 9 in the revised manuscript).

6. In the Discussion, the authors should provide a reference for "NCCN Guidelines for Gastric Cancer."

Reply: In light with the Referee’s suggestion, the reference for "NCCN Guidelines for Gastric Cancer." has been provided in the revised “Discussion” section (See the revised reference 21).

7. There are a number of terms within the manuscript that should be corrected. For example, lymph vessel invasion should be changed to lymphatic invasion.

Reply: The Referee is right that there are some grammatical errors in our manuscript. We have improved the language and presentation of the manuscript, and have removed as many spelling mistakes as we could. Thank you for the Reviewer's
correction, the word “lymph vessel invasion” has also been replaced by “lymphatic invasion” (See multiple places in the revised manuscript).

8. On page 6, the authors have used the term “et al” incorrectly. This should be corrected.

Reply: In accord with the reviewer’s suggestion, “et al” has been changed to “et al.”. Thank you for the correction (See the 113rd line of page 6 in the revised manuscript).

9. Within the Materials and Methods, Pathologic Evaluation, “all HE-stained” should be changed to “all H&E-stained”.

Reply: In accord with the reviewer’s suggestion, “all HE-stained” has been changed to “all H&E-stained” (See the 168th line of page 8 in the revised manuscript).

10. Page 10, the term endothelium-lined should be changed to endothelial-lined.

Reply: In light with the Referee’s suggestion, the term “endothelium-lined” has been replaced by “endothelial-lined” (See the 208th line of page 10 in the revised manuscript).

11. On Page 13, the sentence “Under the quality control of pathologic evaluation,” should be reworded.

Reply: Thank you for the Reviewer's correction, the sentence “Under the quality control of pathologic evaluation” has been replaced by “With quality control of pathologic evaluation” (See the 288th line of page 14 in the revised manuscript).

Reviewer #2: In this retrospective study, the authors analyzed prognostic impact of lymphovascular invasion (LVI) (focusing on cancer specific survival (CSS) and disease free survival (DFS)) in 1148 patients from Southern China with gastric adenocarcinoma, who received surgical resection and clinical follow up. LVI was detected by simple microscopic evaluation of H&E sections of the tumors and scored
as present or absent. Statistical analysis of the data showed that the presence of LVI in the tumor sections correlated with many known histological features of poor prognosis including large tumor size, poor differentiation, deep invasion, nodal involvement, and high TMN stage, etc. Interestingly, a multivariate analysis of the data also indicated that LVI was an independent adverse predictor of DFS and CSS.

1. An obvious strength of the study is its large number of patients with long clinical follow up data. There is a good opportunity to make new discoveries and draw important conclusions from this cohort. However, the presented data fail to show any exciting novelty. The only new finding is the authors’ claim of LVI as an independent adverse predictor of DFS and CSS. Yet, this result is less convincing, particularly in the context that the same analysis did not reveal nodal metastasis status as a statistically significant factor (Table 2). The authors should provide necessary explanation.

Reply: It is true that nodal metastasis status was not evaluated as a statistically significant prognostic factor in multivariate analysis. However, we indeed found that the nodal metastasis was closely correlated with the poorer prognosis in univariate analysis ($P < 0.0001$). It is worth noting that lymph node status, TNM stage and LVI were included in multivariate analyses for DSS and DFS in our study. As we known, nodal metastasis status is included in TNM staging for GC and there is a strong association between nodal metastasis status and TNM stage. Meanwhile, consistent with previous reports (Bu Z et al., Tumour Biol 2013, 34(2):1005-1012; del Casar et al., J Cancer Res Clin Oncol 2008, 134(2):153-161), our data revealed that the nodal metastasis status was significantly correlated with the presence of LVI in GC ($P < 0.0001$). Therefore, the effect of covariate mainly contribute to this negative result. In light of the Reviewer’s comments, we provided the above-mentioned explanations in our revised “Discussion” section (See the 330th line of page 15 and the 331st to 341st lines of page 16 in the revised manuscript).

2. From a tumor biology perspective, LVI in the primary tumor is presumably the beginning of systemic dissemination of cancer cells via either lymphatic or vascular
channels. Therefore, in patients with positive lymph nodes for metastasis, LVI is an inevitable event. The absence of identifiable LVI in these patients' tumors is merely a reflection of not enough sampling. Therefore, to study LVI impact in these patients is of little clinical significance and may confuse data analysis by creating unexpected bias.

Reply: It is true that LVI in the primary tumor is presumably the beginning of systemic dissemination of cancer cells via either lymphatic or vascular channels. In the present study, the presence of LVI was detected in 349/771 (45.3%) in patients with lymph nodal metastasis. Similar to our finding, Bu Z et al. showed the incidence of LVI detected by H&E staining was 49.3% (34/69) in resectable T2 GC patients with lymph node metastasis (Bu Z et al., Tumour Biol 2013, 34(2):1005-1012). Moreover, Kim et al. showed that the incidence of LVI was 59.8% (52/87) in GC patients with lymph node metastasis underwent curative resection by immunohistochemistry (Kim et al., J Surg Res 2010, 162(2):177-183). LVI is not always observed in GC patients with lymph node metastasis, regardless of which detection method is used. Actually, in our study, the tumors were sampled as many sections as possible (at least three sections per tumor available) for pathological evaluation. Several reasons may contribute to the absence of identifiable LVI. First, the biology process of systemic dissemination of cancer cells via either lymphatic or vascular channels is complex and has not been well elucidated. LVI is merely a morphological indicator, incapable of reflection of the whole biology process. Secondary, blood and/or lymph vessel might be obstructed by cancer cells too much to be recognized. At last, the number of sections available is another course. Therefore, the absence of identifiable LVI in lymph node-positive patients’ tumors might not only attribute to sampling. There will be interesting to study why the absence of identifiable LVI was found in metastatic GC. Further research to address this issue is certainly in order.

3. On the contrary, the impact of LVI in patients without nodal metastasis is of great clinical significance and interest. If the authors can draw a definitive conclusion form
this respective study by focusing their analysis on comparing DFS and CSS in patient groups with or without nodal metastasis, it will provide new evidence for risk stratification of potential recurrence and prediction of survival. It will be useful addition to the current TMN staging system.

**Reply:** We were quite in agreement with the reviewer’s view. In fact, we have assessed the impact of LVI detected by routine H&E staining on DSS and DFS in 361 GC patients without metastasis. Our data supported the conclusion that the presence of LVI is also a risk factor for recurrence and an independent factor of poor survival in nonmetastatic GC after curative resection. These findings have been submitted to a journal for peer review ([Int J Surg Pathol. 2015 in submission](#)). Thank you for your good suggestion!

4. It is quite encouraging but also surprising to see the plotted survival curves (Figure 2 and 3) are much better compared to the published SEER data (with greater than 20% survival in 100 month follow up period even for Stage 3 and 4 patients with or without LVI). The authors may want to discuss the potential causes for the difference. In addition, it will make the data more meaningful and clear to interpret if the authors would show separate curves for each stage rather than to combine two stages in one curve. The two curves for tumors without vascular invasion in figure 2B and figure 3C have unexpected drop at the end presumably due to data entry error in the analysis. Please check and correct.

**Reply:** Thanks for the Reviewer's suggestion! In the present study, 5-year DSS rate was 51.0% for all stages, 70.5% for stage I-II, 36.1% for stage III-IV. Similar results were shown in previous studies (Lu et al. J Oncol. 2012; 2012: 641218; Zheng et al. BMC Cancer. 2014 Apr 29;14:300). However, the published SEER data showed 5-year relative survival for GC in the United States was 28.3% for all stages, 64.1% for localized stage, 28.8% for regional stage. In fact, it has been suggested that the prognosis of patients with GC in Asian is better than that in Europe and US. Two potential causes for the difference were proposed. Firstly, the survival advantage of Asian ethnicity continued to hold after control was used for other well-known
prognostic factors (Wang et al., Ann Surg Oncol. 2015 Jan 29; Lui et al., Dig Dis Sci. 2014 Dec;59(12):3027-34). In addition, the surgical quality may contribute to the high survival rate in Asia. Gastrectomy with D2 lymphadenectomy is the standard treatment for GC patients in China. Several clinical trials also have confirmed the survival benefit for D2 lymph node dissection (Songun et al., Lancet Oncol. 2010 May;11(5):439-49). Meanwhile, due to the high incidence rate in China, the surgeon has abundant experience in surgery skills. In light of the Reviewer’s suggestions, we added the above-mentioned explanations in our revised “Discussion” section (See the 342nd to 352nd lines of page 16 and the 353rd to 356th of page 17 in the revised manuscript).

In accord with the Reviewer’s good suggestion, separate curves for each stage have been showed in the revised Fig. 3.

In light of the Reviewer’s comments, we have carefully checked the process of data entry in the analysis, and found no data entry error.

5. Since the authors did not attempt to separate lymphatic vs. vascular invasion, a term of lymphovascular invasion (LVI) should be used and replace the vascular invasion (VI) that is used in the current text to avoid confusion.

**Reply:** Thank you for the Reviewer's suggestion, the term “vascular invasion (VI)” has been replaced by “lymphovascular invasion (LVI)” (See the multiple places in the revised manuscript).