Peer Review File

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Reviewer A

Your manuscript "Organ heterogeneity and prognosis in isolated metastases of gastric cancer" characterizes the profile of single metastatic sites in patients with gastric cancer. According to your findings, patients with bone metastasis have poor survival in comparison with those with metastasis in other sites such as liver or lung. It is a secondary data analysis using information from the Surveillance, Epidemiology, and End Results (SEER) source. Though the potential contribution of this study to their field, I would like to comment on some concerns.

Major comments

1. Table 1 includes Unknown (missing) information as a different category for variables such as Marital Status, Surgery Treatment, Radiotherapy treatment, or AJCC T stage. Should this data be treated as missing (non-available) instead of categorized information? I think that it could affect regression calculations. So, it must be defined as missing.

Reply 1: We greatly appreciate the voluntary work of the reviewers and editors, and your comments indicate a thorough understanding of our work. This study, based on the SEER database, uses propensity score matching to control for confounding factors and the floating absolute risk method to investigate prognostic factors for the absolute risk of gastric cancer. The SEER database is currently one of the highest quality disease databases in the world, providing valuable research data for epidemiology, with numerous high-quality studies already published(Brar et al., 2020; Dibble et al., 2023). However, the issue of missing data is common in both high-quality public databases and real-world cohorts. We carefully considered this issue during our analysis. Excluding a large number of missing variables and samples would significantly reduce sample size and statistical power and introduce bias, while strategies for imputing missing values may not be appropriate. Therefore, in this study, we assigned dummy variables to missing values, defining them as the 'unknown' group. The use of dummy variable assignment is a commonly adopted method in clinical research(Brar et al., 2020; Chu et al., 2023; Dibble et al., 2023). Thank you for your guidance and concern.

2. According to the results, some variables are not associated with overall or disease-free survival in the analysis. So, they could be excluded from the multivariate analysis. Otherwise, some results could be overestimated.

Reply 2: Thank you for your suggestions. I greatly appreciate your meticulous attention to our research methods. Multivariate analysis is a commonly used method to control for confounding factors and to ascertain the true relationship between variables and outcomes. There are several approaches for selecting variables to include in a multivariate analysis: including all variables (full model approach), including outcome-related variables, including variables with p-values

below a certain threshold, and using AIC/BIC rules. Selecting variables for multivariate analysis based on univariate p-values is contentious, with commonly used thresholds being 0.05(Zandberg et al., 2021), 0.1(Farhangfar et al., 2014; Panitchote et al., 2019), 0.2(Dujardin et al., 2020), and 0.25(Adane et al., 2022). However, using p-value thresholds for variable inclusion may result in excluding important variables from the multivariate analysis. Some critical outcome-related variables might not show significant statistical differences in univariate analysis due to confounding factors, leading to inadequate control of confounders and failing to reveal the true relationship between study variables and outcomes. Similarly, as the reviewer mentioned, including too many variables in multivariate analysis may cause the observed relationships to be specific to the dataset used. There are examples where overfitting with too many variables in small datasets results in poor performance when validated with external datasets(Chowdhury and Turin, 2020). Peduzzi et al. suggested that for survival analysis, there should be at least 10 events per variable to ensure stable estimates(Peduzzi et al., 1995). In our study, we included 4297 participants, with 3749 deaths and 548 survivors, and included 15 variables in the multivariate regression analysis. This results in an average of approximately 249.9 events per variable, far exceeding the 10 events per variable threshold, ensuring the stability of our results while avoiding the risk of omitting important variables. Therefore, we included all variables in our multivariate regression analysis. Of course, we are very willing to heed your advice and continue to refine our research methods in future studies.

Minor comments

3. Please describe all abbreviations in their first mention, even in the abstract. For instance, PSM (Propensity score matching).

Reply 3: Thank you for your suggestions. We have made the corresponding revisions, providing the full names and abbreviations at their first mention in both the abstract and the main text of the manuscript. Your comments have been very helpful in improving the quality of our paper.

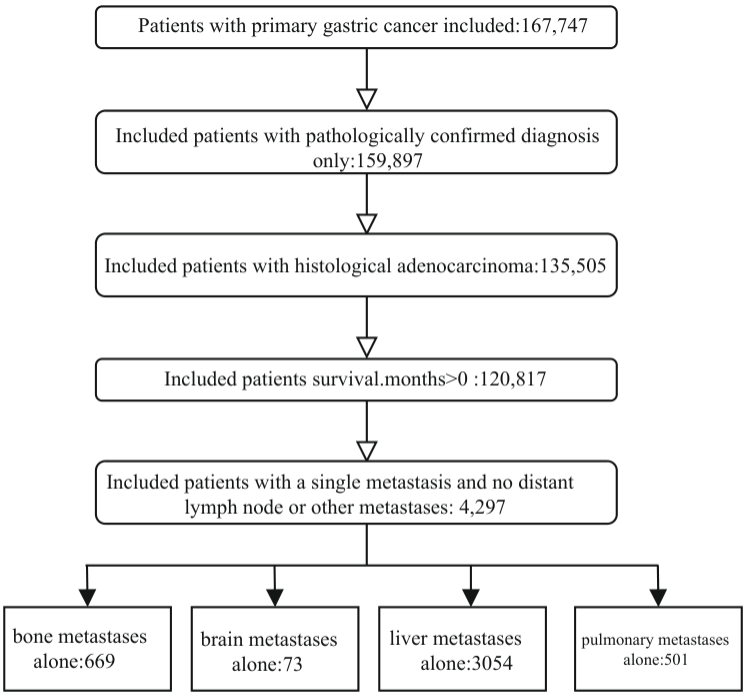
Changes in the text: We have provided the full term and abbreviation upon first mention and removed redundant full terms or abbreviations. These changes can be found on page 6, line 96; page 8, line 128 (removed the full term for PSM); page 7, line 122; page 13, line 234 (removed the full terms for OS and DSS); page 13, line 237 (removed the abbreviation for NCCN); page 13, line 249 (removed the full term for SREs); page 14, line 256 (removed the abbreviation for DIC); and pages 14, lines 263-266 (removed the abbreviations for PDGF, OPG, and HGF). In addition, we carefully checked the article and made further revisions to address inappropriate wording and grammatical issues. These revisions did not change the meaning of the original text. In order to reflect the specific changes we made, in addition to the "manuscript.docx", we also uploaded an additional document titled "Grammar Error Correction of Manuscript.docx", which reflects the changes we made.

Reviewer B

1. The term “solitary metastases” may lead to misunderstandings. Reviewer understood that solitary metastases mean single organ metastases in this study.

Reply 1: We greatly appreciate you raising this issue. We apologize for any confusion caused by the term "solitary metastases." To minimize any misunderstanding, we have changed "solitary metastases" to "single organ metastases" in the manuscript. Title changed to“**Heterogeneity and prognosis of single organ metastases in gastric cancer”.**
Changes in the text: We have accepted the reviewers' suggestions and changed "solitary metastases" to "single organ metastases" on page 2, lines 25 and 26,line34,line42; page 3, line 46; page 5, line 60, line74, line76 and line78; page 6, line 83, line92, line93 and line100; page 7, line103 and line105; page 9, line149, line156 and line158; page 12, line214 and line215; page 16, line312 and line314.

2. In this study, were patients with peritoneal metastasis or distant lymph node metastasis excluded? Or were patients collected regardless of peritoneal or distant lymph node metastasis? If former, were patients with such metastasis completely excluded? The authors should clearly describe this issue.
Reply 2: Thank you for your reminder. Your attention to the inclusion and exclusion criteria of the study and your emphasis on clear presentation highlight the importance of rigor in scientific research, and I fully agree and appreciate your reminder. When collecting data, we consulted the relevant descriptions in the SEER database. The SEER data dictionary mentions Met.Distant (Identifies whether distant lymph node(s) are an involved metastatic site) and Met.Others (Identifies any type of distant involvement not captured in the Mets at DxBone , Mets at Dx-Brain , Mets at Dx-Liver , Mets at DxLung, and Mets at Dx-Distant LN fields. It includes involvement of other specific sites and more generalized metastases such as carcinomatosis. Some examples include but are not limited to the adrenal gland, bone marrow, pleura, malignant pleural effusion, peritoneum, and skin). Therefore, in our study, we have already restricted the inclusion of patients with distant lymph node metastasis and peritoneal metastasis. To further clarify the study population, we have provided corresponding supplementary explanations in the methodology section and Supplementary Figure 1. Once again, thank you for your valuable comments.
Changes in the text: On page 7, line 123 and line 124, of the Methods section, we have made the corresponding additions. Additionally, we have revised Supplementary Figure 1 and provided supplementary explanations following the figure:



Supplementary Figure 1. Patient inclusion flow chart. Other metastases:include but are not limited to the adrenal gland, bone marrow, pleura, malignant pleural effusion, peritoneum, and skin.

3. Reviewer was interested in the results that bone metastases were associated with worse survival in patients with single organ metastases. However, the survival curves between patients with bone metastases and other metastases look very similar. Is there any clinical significance in the difference of survivals which were much shorter than those in the Eastern countries? Furthermore, patients with single organ metastases definitely underwent different types of treatment. Some liver metastases were resected, but no bone metastases were resected. Is there any significance in comparison among patients with different backgrounds? What should physicians do, referring to the results of this study? Reviewer believes that survival outcomes may depend on the number of metastases even though metastases exist in only one organ. Reviewer doubts that the metastatic organ determines survival.

Reply 3: Thank you very much for your valuable critique of my research conclusions. We agree with the reviewers that factors such as treatment methods, the extent of metastases within a single organ, and patient characteristics may act as confounding variables when exploring the prognosis of patients with single-organ metastasis. We are well aware that the resection of metastatic lesions impacts survival, and we have always strived to minimize the influence of these factors. In our study, we included covariates such as age, race, radiotherapy/surgery, and chemotherapy, and systematically included all relevant variables into the multivariate analysis to adequately adjust for confounding factors. Given that retrospective observational studies cannot eliminate the impact of confounding factors through "a priori" randomization, we intend to employ propensity score matching as a method for 'post hoc' randomization to enhance control over confounding factors. Furthermore, we recognize that establishing a control group might also introduce bias. Therefore, we calculated the floating absolute risk for single-organ metastasis using the floating absolute risk method. By controlling for bias through multivariate and multi-method

approaches, we consistently observed that single bone metastasis in gastric cancer is a risk factor for poor prognosis across all three methods. Of course, as the reviewers mentioned, this study is based on the SEER database, which mainly includes survival data from Western populations. Further validation is needed to determine if analogous conclusions apply to Eastern populations. We are currently collecting a cohort of gastric cancer patients with single-organ metastasis. However, since multiple organ metastases are often present when distant metastasis of gastric cancer is observed, the number of patients with single-organ metastasis is relatively small. Therefore, it amassing a sufficient dataset from Eastern patients to investigate the prognostic patterns of single-organ metastasis in gastric cancer will be a time-consuming process. We are actively working on this and have also mentioned the limitations of our study being based on Western cohorts, which require further exploration and validation in Asian populations. Thank you again for your guidance and suggestions. They are invaluable to me and represent a significant direction for our future research.

4. Reviewer understands that multivariate analysis is one of methods to match patients' backgrounds. Thus, multivariate analysis after PSM matching may be unproper. Either multivariate analysis or PSM matching may be enough to analyze prognostic factors.

Reply 4: Thank you very much for your valuable comments on my research methods. Simplifying processes and refining details are crucial for high-quality research. I would like to explain a bit more in detail. As mentioned in Question 3, multivariate analysis can control for confounding factors to some extent and explore the true relationship between single organ metastasis and prognosis in gastric cancer patients. However, retrospective observational studies cannot eliminate the impact of confounding factors through "a priori" randomization, which may lead readers to question the conclusions. We also used propensity score matching for "post-hoc randomization" to further control for confounding factors, which plays a decisive role in enhancing the reliability of the conclusions. Additionally, considering that setting up a control group might also introduce bias, we calculated the floating absolute risk of single organ metastasis using the floating absolute risk method. Through multivariable and multimethod control of bias, we consistently observed that solitary bone metastasis in gastric cancer is a prognostic risk factor across three methods. Therefore, the use of multivariate analysis and propensity score matching rigorously validates our hypothesis and ensures that the research conclusions stand up to scrutiny, increasing the robustness of the conclusions. I greatly value each of your suggestions and hope to take this opportunity to delve deeper and find the most effective solutions that meet the research needs. Thank you for prompting me to further reflect on and optimize my research design.

5. Supplementary Figure 2 is not required. It has no information to be presented.

Reply 5: Thank you for your suggestions. You are a very responsible reviewer. We have removed Supplementary Figure 2 and incorporated its content into the main text. Your keen insight has not only improved the quality of the report but also provided us with valuable guidance on handling details.

Changes in the text: We removed Supplementary Figure 2.