Peer Review File

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Reviewer A

This is an interesting and potentially significant finding, given the challenges of monitoring response and tumor control with standard MRI.

There are some questions to address to make this more informative, and help the reader understand the extent of this finding to guide further study.

(1) The focus is on FLR but in looking at the data absolute fibrinogen itself seems more at least similarly associated with outcome. Is it just that fibrinogen-lymphocyte ratio tends to be higher with elevated fibrinogen no matter what the denominator lymphocytes are. Or could lymphocyte count alone be as informative.

Reply1: Thank you for your valuable comments and insights regarding our study on the Fibrinogen-Lymphocyte Ratio (FLR) and its association with outcome. We appreciate your thoughtful observations.We agree that the absolute fibrinogen levels themselves appear to be associated with the outcome, and we acknowledge that the FLR may not be the sole determinant of this association. The FLR combines both fibrinogen and lymphocyte count, and it is possible that elevated fibrinogen levels play a significant role in influencing the FLR, leading to higher FLR values, regardless of the lymphocyte count.Additionally, we acknowledge that lymphocyte count alone can provide informative insights into the immune response and potentially impact the outcome. Lymphocytes play a crucial role in modulating the immune system, and alterations in their count can reflect the overall immune status of the patient. In fact, we have discovered the huge impact of radiotherapy on lymphocytes during the research process, and relevant research results have been published (DOI: 10.1080/02648725.2023.2197331). Because the research content is relatively large, it is not shown in this article.The FLR value was first proposed by Fan et al. in a study of esophageal cancer in 2019. Subsequently, some people have studied the prognostic relationship between FLR and lung cancer, head and neck cancer, and liver cancer. However, there have been no relevant reports in glioma. This may also be an innovation of this article.

(2) This included 104 of 669 pts treated during the time period. This may introduce bias. How were the included patients selected?

Reply2: Thank you for your valuable feedback. To clarify, the selection of patients for inclusion in our study was based on specific criteria to ensure the relevance and validity of our findings. The inclusion criteria were predetermined and aimed to capture a representative sample of patients who underwent radiotherapy during the specified time period. Inclusion criteria: (1) age ≥18 years; (2) Eastern Cooperative Oncology Group (ECOG) score ≤2 points; (3) documented complete pathology report or glioblastoma diagnosis by pathologic consultation in our hospital(The histopathological diagnosis of glioblastoma is based on the 2016 WHO classification of tumors of theCNS); (4) no previous surgery or postoperative chemotherapy and radiotherapy; (5) blood and biochemical indices that were less than two times the upper limit of normal (ULN) before radiotherapy; and (6) no administration of bevacizumab or other anti-tumour drugs during radiotherapy. Exclusion criteria: (1) occurrence of postoperative

infection, or hyperthermia; (2) complications such as coagulative dysfunction, haemorrhagic disorder, autoimmune disease, or other severe comorbidities; and (3) incomplete radiotherapy, or insufficient radiation dose. We appreciate your valuable feedback, please let us know if you have any further suggestions or concerns. Thank you once again for your time and consideration.

(3) Mean brain and brainstem dose may only be associated with poor outcome because the tumors are larger or in a bad location.

Reply3: Thank you for your insightful comment regarding the association between mean brain and brainstem dose and the outcome in our study. There is no doubt that larger tumors or poorly located tumors are associated with poor prognosis. Our study wanted to investigate whether fibrinogen changes are related to radiotherapy-related dose-volume factors. The results show that the volume of the radiotherapy target volume and the dose volume of the organs at risk will significantly affect the changes in fibrinogen, and a larger tumor irradiation volume can expose more circulating cells to radiation, resulting in more lymphocytes. destruction, resulting in severe lymphopenia.

(4) The potential for fibrinogen and fibrinogen-like proteins to suppress the anti-neoplastic immune response would merit comment in the discussion, as a hypothesis explaining this observation.

Reply4: Thank you for your suggestion regarding the potential role of fibrinogen and fibrinogen-like proteins in suppressing the anti-neoplastic immune response. We agree that this hypothesis could provide valuable insights and help explain the observations in our study. Indeed, accumulating evidence suggests that fibrinogen and related proteins may play a role in modulating the immune response within the tumor microenvironment. Fibrinogen can promote tumor growth and metastasis through various mechanisms, including immune evasion and suppression. It can contribute to the formation of a fibrin-rich matrix, which can act as a physical barrier to immune cell infiltration and impair the anti-tumor immune response.In light of this hypothesis, we plan to expand the discussion section of our paper to include a thorough examination of the potential mechanisms by which fibrinogen and fibrinogen-like proteins may suppress the anti-neoplastic immune response. We will review relevant literature and discuss the implications of these findings in the context of our own results.By incorporating this hypothesis into our discussion, we aim to provide a more comprehensive understanding of the observed association between fibrinogen and the outcome, and shed light on the potential immunological implications of our findings.We greatly appreciate your valuable input, which has prompted us to consider an important aspect that can enhance the interpretation and clinical significance of our study. If you have any further suggestions or specific references that you believe would be valuable for us to include, please feel free to share them.Thank you for your time and thoughtful feedback.

Changes in the text: The modified content can be found on page 13 of the word file (Page number in revision status), and additions have been made in the references.

(5)There is literature associating extent of lymphopenia with volume treated that is specific to the brain. You do cite important literature related to other tumor types.

a.Huang J, DeWees TA, Badiyan SN, Speirs CK, Mullen DF, Fergus S, Tran DD, Linette G,

Campian JL, Chicoine MR, Kim AH, Dunn G, Simpson JR, Robinson CG. Clinical and Dosimetric Predictors of Acute Severe Lymphopenia During Radiation Therapy and Concurrent Temozolomide for High-Grade Glioma. Int J Radiat Oncol Biol Phys. 2015 Aug 1;92(5):1000-1007. doi: 10.1016/j.ijrobp.2015.04.005. Epub 2015 Apr 8. PMID: 26025775.
b.Yovino S, Kleinberg L, Grossman SA, Narayanan M, Ford E. The etiology of treatment-related lymphopenia in patients with malignant gliomas: modeling radiation dose to circulating lymphocytes explains clinical observations and suggests methods of modifying the impact of radiation on immune cells. Cancer Invest. 2013 Feb;31(2):140-4. doi: 10.3109/07357907.2012.762780. PMID: 23362951; PMCID: PMC3991115.

Reply 5: We sincerely appreciate the valuable comments. We appreciate your suggestions to add more information to enhance the clarity and comprehensiveness. Based on your suggestions, we have made changes to the Discussion section and added these two valuable references that you suggested. We believe that with your suggestions, the quality of our manuscripts will be greatly improved. Thank you again for your valuable feedback.

Changes in the text: Please refer to page 17 of the Word file (Page number in revision status), and additions have been made in the references.

Reviewer B

Overall, this is an excellent topic and interesting manuscript. The temporal information is key and novel in the glioblastoma literature. I recommend the following major edits, below, be incorporated into the manuscript.

Page numbers listed related to PDF page not footer page.

1. Change title to “Prognostic significance of alterations in fibrinogen level and fibrinogen-to-lymphocyte ratio after radiotherapy on survival outcomes in glioblastoma”

Reply 1: Thank you for your suggestion regarding the title of our manuscript. In light of your recommendation, we would be more than happy to make the requested change to the title. We appreciate your guidance in this matter.

Changes in the text: Please refer to page 1 of the Word file manuscript and page 1 of the Tltle page (Page number in revision status).

2. Change short running title to “fibrinogen and fibrinogen-to-lymphocyte ratio after radiotherapy in glioblastoma”

Reply 2: Thank you for your suggestion regarding the short running title of our manuscript. In light of your recommendation, we would be more than happy to make the requested change to the title. We appreciate your guidance in this matter.

Changes in the text: Please refer to page 1 of Title page (Page number in revision status).

3. Change title on page 2 to be the same as page 1.

Reply 3: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.

Changes in the text: Please refer to page 1 of the Word file manuscript (Page number in revision status).

4. Remove the word “incident” in describing glioblastoma throughout

Reply 4: In accordance with your request, we have deleted the word "incident" in four places in the article.

Changes in the text: Please refer to page 1,2,4,5 of the Word file manuscript (Page number in revision status).

5. Page 2 Line 10- change the word “enrolled” since this is a retrospective study

Reply 5: According to your request, we changed "enrolled" to "included".

Changes in the text: Please refer to page 1 of the Word file manuscript.

6. Page 3 line 24- remove the word “result”

Reply 6: According to your request, we have deleted "result".

Changes in the text: Please refer to page 2 of the Word file manuscript.

7. Recommend a more robust introduction to fibrinogen within the introduction and it’s relevance in cancer/if possible specifically in glioblastoma

Reply 7: Thanks to the reviewer's suggestion. I have made supplements in introduction.

Changes in the text: Please refer to page 4 in introduction of the Word file manuscript (Page number in revision status).

8. Recommend stating your primary objective more clearly- last sentence on page 3.

Reply 8: Thanks to the reviewer's suggestion. According to your request, we have made changes to the last sentence in the introduction.

Changes in the text: Please refer to page 4 in introduction (the last sentence) of the Word file manuscript.

9. Remove the word enrolled on line 50 and replace with term relating to retrospective nature of study.

Reply 9: Thanks to the reviewer's suggestion. According to your request, we changed "enrolled" to "included".

Changes in the text: Please refer to page 5 of the Word file manuscript (Page number in revision status).

10. Please mention inclusion criteria #3 in the discussion.

Reply 10: Thanks to the reviewer's suggestion. We have added to the Discussion section.

Changes in the text: Please refer to page 14 of the Word file manuscript (Page number in revision status).

11. Why include inclusion criterion #5? Please mention in discussion.

Reply 11: Thanks to the reviewer's suggestion. We have added to the Discussion section.

Changes in the text: Please refer to page 14,15 of the Word file manuscript (Page number in revision status).

12. Please clarify inclusion criterion #6. Aren't the patients receiving concurrent chemoradiotherapy?

Reply 12: Thanks to the reviewer's suggestion. We have made changes to inclusion criterion #6.

Changes in the text: Please refer to page 5 of the Word file manuscript (Page number in revision status).

13. For exclusion criteria, did criterion #2 exclude those with deep vein thromboses? This is not a small percentage of patients with gbm.

Reply 13: Patients with deep vein thrombosis are not excluded, but patients with congenital or acquired coagulation disorders, bleeding disorders, and autoimmune diseases are excluded. If they suffer from deep vein thrombosis but their blood and biochemical indicators, and coagulation function indicators are less than two times the upper limit of normal values, times, they can all join the group.

14. Page 5 Line 73- please spell out the word "one"

Reply 14: Thanks to the reviewer's suggestion. According to your request, we changed "1" to "one".

Changes in the text: Please refer to page 6 of the Word file manuscript (Page number in revision status).

15. Page 5 Radiotherapy section- why were the CTs done after 4 hour fasting?

Reply 15: Because enhanced CT positioning requires intravenous injection of iodinated contrast agent, some patients may have contrast agent allergies and intolerance, such as nausea and vomiting. The purpose of fasting is to prevent aspiration during this test, which could lead to serious adverse events.

16. Page 5- Were the patients with glioblastomas immobilized with long masks that included immobilization of their shoulders? I haven't found this to be standard so want to make sure this is not an error.

Reply 16: Yes, for conventional cranial radiation therapy, we use a mask that covers from the top of the head to below the clavicle. This type of mask provides excellent immobilization, as shown in the image below. However, for hypofractionated radiotherapy, considering the requirements of the equipment, we would use a smaller mask that covers from the top of the head to the neck.

17. Page 6- Were MRIs used for planning as well?

Reply 17: Thank you for your valuable input. In our center, we introduced the first MRI-guided positioning machine in 2022. Prior to that, we used to fuse diagnostic MRI images taken in the same position as the CT images for target delineation. I made changes on page 5.

Changes in the text: Please refer to page 6 of the Word file manuscript.

18. Page 6 Lines 97 and 98- please change to "monitor units were recorded"

Reply 18: Thank you very much for your valuable feedback. We have made the requested

changes as your requirements.
Changes in the text: Please refer to page 7 of the Word file manuscript (Page number in revision status).

19. Page 6 Line 101- please change to Stupp scheme

Reply 19: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.
Changes in the text: Please refer to page 7 of the Word file manuscript (Page number in revision status).

20. Page 7 Line 116- Can you check this correct? Only 62% of patients completed more than 4 cycles of adjuvant temozolomide. This seems low.

Reply 20: Thank you for your comment. We have thoroughly reviewed the data, and we can confirm that the information presented on page 7, line 116, is correct. Our findings indicate that only 62% of patients completed more than 4 cycles of adjuvant temozolomide. We understand that this percentage may appear low, but it accurately reflects the completion rate observed in our study population. We believe this information highlights an important aspect of treatment adherence and could potentially be further explored in future research.

21. Page 7 Line 117- Please clarify your follow up range.

Reply 21: Thanks to the reviewer's suggestion. After careful inspection,we changed "6" to "60".
Changes in the text: Please refer to page 8 of the Word file manuscript (Page number in revision status).

22. Page 9 Line 163- Please put the word “table 2” in parentheses.

Reply 22: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.
Changes in the text: Please refer to page 10 of the Word file manuscript.

23. Page 11 Line 203 – Change to “head and neck tumours”

Reply 23: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.
Changes in the text: Please refer to page 12 of the Word file manuscript (Page number in revision status).

24. Page 12 Line 231- Change glioma to either “high grade glioma” or “glioblastoma” here and below unless you are including low grade gliomas as well

Reply 24: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.
Changes in the text: Please refer to page 12, 13, 14, 15, 18 of the Word file manuscript (Page number in revision status).

25. Page 13 Line 256- Spell out MLC for non-radiation audience understanding.

Reply 25: Thank you very much for your valuable feedback. We have made the requested

changes as your requirements.
Changes in the text: Please refer to page 15 of the Word file manuscript (Page number in revision status).

26. Page 14 Line 273- missing a space in “count reduction”
Reply 26: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.
Changes in the text: Please refer to page 16 of the Word file manuscript (Page number in revision status).

27. Page 14 Line 280- please change “irradiation dose” to “radiation dose”
Reply 27: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.
Changes in the text: Please refer to page 16 of the Word file manuscript (Page number in revision status).

28. Page 14 Line 281- please clarify what you mean by “segmentation mode”
Reply 28: Thank you very much for your valuable feedback. We have added the definition of “segmentation mode” based on your suggestion.
Changes in the text: Please refer to page 16 of the Word file manuscript (Page number in revision status).

29. Page 16 line 314- please state “research hotspot” in a different way
Reply 29: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.
Changes in the text: Please refer to page 19 of the Word file manuscript (Page number in revision status).
Once again, we would like to express our heartfelt gratitude for your diligent efforts and the time you have dedicated to this review. We truly appreciate your valuable contributions.

Other notes:
-Recommend that you spell out dose and fractionation beyond “Stupp scheme” for clarity.
Reply: Thank you very much for your valuable feedback. We made additions to the radiotherapy methods on page 7.
Changes in the text: Please refer to page 7 of the Word file manuscript (Page number in revision status).

-Capitalization of dosimetric ROIs is inconsistent throughout. Please make this consistent.
Reply: Thanks to the reviewer's suggestion. We have modified the capitalization in many places in the article.

-Smallest font on figures is hard to read
Reply: Thanks to the reviewer's suggestion. We have re-uploaded the figures in high resolution.
Changes in the text: Please refer to page 25,26,27,28 of the Word file manuscript (Page number

in revision status).

-Unclear how “change” in factors levels was accomplished- difference, fold change?

Reply: According to the RTOG guidelines, the requirements for organs at risk, such as the brain and brainstem, during radiation therapy for glioblastoma are not very detailed. We want to comprehensively determine whether these parameters have an impact on our study. Therefore, we will gradually set the parameters for these ROIs in a gradient manner.

-Change term post-operative residue to- postoperative tumor volume throughout

Reply: Thanks to the reviewer's suggestion. We have change the term post-operative residue to- postoperative tumor volume throughout.

-Please integrate primary objective of study into discussion and recommend ending with a last summary paragraph that is more reflective of initial goals. The last paragraph seems disconnected.

Reply: Thank you very much for your valuable feedback. We have made the requested changes as your requirements.

Changes in the text: Please refer to page 18, 19 of the Word file manuscript (Page number in revision status).