Author's response to reviews

Title: Pre-treatment neutrophil-to-lymphocyte ratio is associated with neutrophil and T-cell infiltration and predicts clinical outcome in patients with glioblastoma

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Version: 2
Date: 4 July 2015

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

(Manuscript number: MS: 1611648523170215)

We would like to thank you for giving us the opportunity to revise our manuscript. We have carefully taken reviewer’s comments into consideration in preparing our revision, which resulted in a paper that is clearer and more rigorous. Below is our point-by-point response to reviewer’s comments.

1. In the Manuscript Body, Introduction has been renamed to Background.

2. Our study was approved by the institutional review board of The First Hospital of China Medical University. This information has been added in the revised paper.

Response to Reviewer Richard M Bambury:

Thanks for your careful review. We have revised our paper according to your comments.

1. As suggested by the reviewer, in keeping with prior literature and prognostic nomograms, age was additionally analyzed as a categorical variable (≤60 years vs. >60 years), and the results were presented in Table 1 and 3.

2. According to the reviewer’s suggestion, KPS was additionally analyzed as a categorical variable (≤70 vs. 80-100), and the results were shown in Table 1 and 3.
3. As suggested by the reviewer, in Table 2 variables have been marked with * (assessed as categorical variables) or ** (assessed as continuous variables). This has been stated in the table legend.

4. According to the reviewer’s suggestion, the paragraph describing ROC curves and the former Figure 3 have been removed.

5. As suggested by the reviewer, the former Figure 2 has been split into two figures with D as a separate figure (new Figure 3) highlighting the NLR-PLR correlation.

6. The statement “Pre-treatment NLR levels were correlated with 2-year survival rates” has been removed.

7. In Figure 2, NLR and PLR were assessed as continuous variables. This information has been added in the figure legend.

8. In Table 1, the differences between patients with pre-treatment NLR <4 and ≥4 were compared in sex, age, tumor size, KPS, degree of resection, BMI, MGMT status, pre-treatment PLR and OS. P value was calculated using chi-square test for categorical variables and using Student’s t-test for continuous variables. This information has been added in the table legend.

9. Previous studies reported that metabolic syndrome (1, 2) and hypertension (3, 4) can potentially affect the measurement of NLR. Thus, these confounding factors should be considered in studies of glioblastoma patients (5). As suggested by the reviewer, these
literatures have been cited in the revised paper.

10. According to the reviewer’s suggestion, the conclusion has been rewritten. The correlation between peripheral blood NLR and tumor-infiltrating neutrophils/ T-cells, and the prognostic significance of NLR independent of MGMT status have been emphasized.

11. A 30% residual tumor was commonly used as a cut-off point to differentiate degree of resection by us and other researchers (6, 7). And these literatures have been cited in the revised paper.

Response to Reviewer Panagiotis Karagiannis:

Thanks for your careful review. We have revised our paper according to your comments.

1. We agree with the reviewer that patients with KPS <70 should receive supportive medication prior surgery. Fortunately, at our hospital, blood sample collection is the first thing after admission and is usually before medication. Moreover, as we stated in the “Study Population”, only patients with pre-treatment blood examination were included in this study, and those lacking these data were excluded.

2. For glioblastoma patients progressing after prior standard therapy, there is no established chemotheraphy regimen available (8). In this study, after tumor progression, patients received re-operation, re-irradiation, traditional Chinese medication, bevacizumab, lomustine or various other chemotherapies, and some patients only received
palliative treatment. The number of patients receiving each treatment was very limited. If we took all these factors into survival analysis, the analysis would be complicated and inaccurate, which would also deviate from the main subject of this paper. Furthermore, many important studies did not include second line treatment in OS analyses (7, 9). However, in this study, the post-progression salvage treatments were heterogeneous, which may affect the survival analysis. This is a limitation of our study, and we have discussed this limitation in the paper.

3. As we stated in the “blood examination” section, the blood draw was performed the first day after surgery. And we agree with the reviewer that, the postoperative increase of NLR and PLR may be caused by the stress of surgery and the medical interventions before blood sample collection, and we have discussed this in the paper.

4. We agree with the reviewer that in some studies PFS (progression free survival) can also be used for prognostic evaluation. However, for glioblastoma patients receiving standard radio-chemotherapy, the identification of pseudo-progression and true progression is very difficult, which leads to inaccurate PFS assessment. Especially for early cases, because of insufficient understanding of pseudo-progression, rather than RANO criteria we used the Macdonald Criteria to assess tumor progression, which resulted in
incorrect judgement and PFS assessment (10). Thus, in this study, rather than PFS, we used OS for prognostic evaluation to obtain more reliable results.

5. As we stated in the paper, previous important studies used a 400× magnification to assess the CD3\(^+\) T-cells infiltration (11, 12) and a 200× magnification to assess neutrophils infiltration (13, 14) in glioblastoma. In keeping with prior literature we used the same magnification in this study.

6. In Figure 4D, the 0.016 \(P\) value indicates significant differences among the five survival curves in global comparison. In multiple comparison, patients with <10 and 10-20 neutrophils infiltration survived significantly longer than patients with 20-50 and 50-100 neutrophils infiltration (\(P<0.05\)). However, the survival difference between patients with >100 neutrophil infiltration and the other groups was insignificant. We agree with the reviewer that the limited number of cases in this high infiltration group may affect the significance. Nevertheless, when we use 20 neutrophils infiltration as a cut-off point, we find that patients with \(\geq 20\) neutrophils infiltration survived significantly shorter than patients with <20 neutrophils infiltration (mean OS 13 months vs. 20 months, log rank \(P=0.021\), Figure 4E). Although the main subject of our study was not to explore the prognostic role of neutrophil infiltration, our results suggested that
increased neutrophil infiltration was correlated with shorter survival in glioblastoma patients. And as suggested by the reviewer, mean OS for each group was indicated in the revised figure.

7. In this study, although we examined the prognostic role of PLR, the main subject is the prognostic significance of NLR. And we found that although in univariate analysis PLR was associated with patient survival, PLR was not an independent prognostic factor in glioblastoma. To keep the paper concise and focused, these results of PLR were presented in Table 1-3 in detail, and briefly described in the “Pre-treatment NLR is superior to PLR as a prognostic factor in glioblastoma” paragraph as suggested by the reviewer.

8. According to the reviewer’s suggestion, the scale bar in Figure 4A for CD3⁺ T-lymphocytes has been labeled.

9. Figure 4B and C are showing that high neutrophil infiltration (number of neutrophils $\geq 10/200 \times$ HPF) and low CD3⁺ T-cell infiltration (number of CD3⁺ T-cells $\leq 20/400 \times$ HPF) were more frequent in patients with pre-treatment NLR $\geq 4$ than in those with pre-treatment NLR $< 4$ (69.4% vs. 36.9%, $P < 0.001$ and 59.2% vs. 38.8%, $P = 0.019$, respectively). $P$ value was calculated using chi-square test. As suggested by the reviewer, these have been more clearly indicated in Figure 4B and C.

10. “KPS” has been added before “Karnofsky Performance Score” in
table legend of Table 2.

11. As suggested by Reviewer Richard M Bambury, ROC analysis has been removed from the paper along with former Figure 3.

12. As suggested by the reviewer, some of the figure legends have been rewritten. More information and description have been added.

13. The reviewer’s suggestion is very inspiring. However, since the components of T-cells are very complex, the study of T-cell subpopulation infiltration is another big topic. In this study, we took the first step to establish the relationship between systemic inflammatory markers and local inflammatory infiltration. And in future studies, we will further explore the specific role of T-cell subsets including CD8⁺, CD4⁺ and FoxP3⁺ T-cell.

Thanks for the careful review and we are looking forward to your reply.

Best wishes.

Dr Anhua Wu

References


