Author's response to reviews

Title: Preoperative neutrophil-to-lymphocyte ratio is an independent prognostic marker in patients with laryngeal squamous cell carcinoma

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Author's response to reviews:

Dear Editors:

We thank you and the two distinguished reviewers for their critical and insightful comments concerning our manuscript entitled “Preoperative neutrophil-to-lymphocyte ratio is an independent prognostic marker in patients with laryngeal squamous cell carcinoma” (MS: 1755169606157600). Those comments are valuable and helpful for revising and improving our manuscript. We have studied the comments carefully and have made corrections which we hope to meet with approval. The responses to the reviewer’s comments are as listed as follows. We have also improved the quality of written English carefully.

Reviewer: 1

MAJOR REVISIONS REQUIRED

1. Introduction

1) 72 – Please rephrase line to a combination of surgery, radio-, and chemotherapy

Response: Thank you for your advice. We have revised it in the paper according to your suggestion. (line 72, page 4)

2) 73 to 74 – What epidemiological study? Please cite source. What does the authors mean by “improved little”? Are they referring to survival outcomes?

Response: We added the reference in the revised manuscript. (line 74, page 4) “improved little” means that the treatment outcome for LSCC doesn’t improve significantly. The survival outcome mainly refers to 5-year survival rate.

3) 77 to 84 – Unclear what the background behind neutrophil to lymphocyte ratio; what kind of history does it have in head and neck cancer? What are previous findings? It’s not made clear what the reasoning for this particular study is.
Response: We have made this clear in the revised manuscript. Kawata et al indicated that lymphocyte infiltration around the tumor associated with a better prognosis of HCC [1], whereas neutrophil in tumor stroma associated with a poor prognosis [2]. An elevated neutrophil-to-lymphocyte ratio (NLR), the ratio of absolute neutrophil count to absolute lymphocyte count, may reflect a systemic inflammatory response [3]. Perisanidis et al showed that high neutrophil-to-lymphocyte ratio was an independent marker of poor disease-specific survival in patients with oral cancer [4]. NLR has been shown to be an effective prognostic marker in many solid tumors [5-11]. Kum et al showed that neutrophil-to-lymphocyte ratio elevated in squamous cell carcinoma of larynx compared to benign and precancerous laryngeal lesions [12]. To our knowledge, the prognostic value of NLR in laryngeal squamous cell carcinoma has not been reported. We thought that whether NLR could predict prognosis of LSCC or not. So we performed this study.

We have added this information in the revised manuscript.


4) 83 to 84 – Grammatical mistakes
Response: We have revised the grammatical mistake. And the manuscript was reviewed again to check grammar mistakes. (line 85-86, page 4)

2. Methods
1) 94 – Grammatical Error
Response: We have revised the grammatical mistake. And the manuscript was reviewed again to check grammar mistakes. (line 94, page 5)

2) 95 to 97 – Were patients who had palliative treatment included? Please specify whether treatment with curative intent was an inclusion factor.
Response: We are sorry to make this unclear. In our study, patients who had palliative treatment weren’t included in this study. All patients accepted curative treatment. We have added the inclusion criteria in the revised manuscript. (Line 102-103, Page 5)

3) 97 – Clarify what is meant by “chronic infectious diseases”
Response: we are sorry to make the inclusion criteria unclear. For our study, the inclusion criteria should be “no evidence of sepsis” [1]. We have changed it in the revised manuscript. (line 97-98, page 5)


4) 100 – Specify what is meant by no preoperative steroids. Does the authors mean to say no steroids ever or within what time frame does there need to be from steroid administration to inclusion. If there is a time frame, what is the justification for that time frame?
Response: we are sorry to make this unclear. No Patients had the history of taking steroids in the study. (line 101, page 5)

5) 102 – Unclear as to why NLR is calculated this way; where is the precedent or previous study that showed this to the way to be calculated?
Response: NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. #line 103-104, page 5)

All studies of NLR adopted such calculation [1-5].


6) 103 to 105 – Procedural breakdown belongs in the results section
Response: According to the reviewer’s suggestion. We have changed it in the revised manuscript. (line 138-140, page 7)

7) 107 to 109 – Please change “date of surgery” for DFS and OS calculations to “date of treatment” as not all patients had surgery upfront as the primary modality for their tumors. Also if patients received RT or Chemotherapy; what doses were used for RT and which chemotherapy agents were used? Most patients will not tolerate the full course of chemotherapy, what did the author use as a “cutoff” for acceptance that the patients received chemotherapy?
Response: For our study, the inclusion criteria were: ·····(7) no pre-operative treatments such as radiotherapy or chemotherapy·····. All patients only received surgery treatment in our study. So we think that “date of surgery” for DFS and OS calculations is reasonable.

3. Statistical Analysis
1) 118 to 120 – It’s unclear what variables was included in the cox-regression analysis; in this particular study, matters of TNM staging, age, gender, comorbidities as well as treatment status should all play a part in effecting the survival status.
Response: We are sorry to make this unclear. Factors analyzed by univariate analysis with p < 0.05 were included in multivariate Cox-regression analysis. We have revised it in the revised manuscript. (line 115-116, page 6). If patients had distant metastasis, they would be excluded in our study. Meanwhile, single T staging and lymph node metastasis were listed as separated variables. Hence#TNM staging including T staging, lymph node and distant metastasis wasn’t listed as a separated variable in our study. We analyzed the effect of age and gender to prognosis by univariate analysis. The results showed that age and gender were not associated with prognosis. Factors analyzed by univariate analysis with p < 0.05 were included in multivariate Cox-regression analysis.
age and gender were not included into multivariable Cox-regression analysis.

4. Results

1) Demographic Data

136 to 137 – The authors need to make these clear about “Smoking” and “drinking” histories. Pack years? Amount of alcoholic drinks per day? Last time they smoked or drank?

Response: We are sorry to make these unclear. We defined smoking history as 20 pack-years or more, drinking history as drinking more than 1000 gram-years or more[1,2]. We have changed it in the revised manuscript. (line 136-138, page 7)


2) 155 – Please provide SD as well as range and median for follow-up

Response: We are sorry that we didn’t provide mean ± SD of follow-up. The mean ± SD of follow-up was 54.1 ± 23.4 months. We have added it in the revised manuscript. (line 157-158, page 8)

3) 171 – the authors likely means histological grade instead of “historical grade”

Response: According to reviewer’s suggestion, we have revised it as histological grade in the revised manuscript. (Line 175, Page 8)

4) 173 – It’s unclear what the authors meant by “clinical grade” do they mean the TNM staging done clinically? If so does that mean the TNM staging system otherwise used in the paper is pathological? It's also unclear as to why the clinical grade was not included in the multivariate analysis

Response: The clinical stage is different from TNM staging system. But the clinical stage was based on TNM staging. The clinical stage has five stages, details as follow:

0 stage Tis N0 M0
#stage T1 N0 M0
#stage T2 N0 M0
# stage T3 N0 M0# T1 N1 M0# T2 N1 M0# T3 N1 M0
#A stage T4a N0 M0# T4a N1 M0# T1 N2 M0# T2 N2 M0# T3 N2 M0# T4a N2 M0
#B stage T4b any N M0# any T N3 M0
#C stage any T any N M1

T classification and Lymph node metastasis were listed as separate variable in
our study. However, Clinical stage was a comprehensive variable including T stage and lymph nod metastasis. To avoid the repeated impact of variable, clinical stage was excluded from the multivariable analyses.

5) 176 to 182 – It’s interesting that T staging was not prognostic of DFS but was in OS. This bring back to the question of why treatment modalities was no included in the cox-regression analysis, given that early and late stage laryngeal cancer was included in this study, it’s pivotal that treatment modalities are included. Without this valuable piece of information, it is in my opinion that the analysis is flawed and yields a confounded result.

Response: The reviewer made an important point here. Our results showed that advanced T classification was an independent poor prognostic factor of OS but wasn’t in DFS. In our study, the inclusion criteria were: -(7) no pre-operative treatments such as radiotherapy or chemotherapy; (8) patients were accepted curative treatment. All patients received only surgery, no any treatment. The treatment modalities include only surgery in the study.

6) Tables – It’s confusing why 60 is used as the cutoff for age differentiation within this study? Where is the supporting evidence/literature to show that this has effect on survival?

Response: 60 years old was used as the cutoff for age differentiation with many studies about LSCC [1-3]. It has been confirmed by many studies that age has no effect on NLR [4-7]. So 60 years old was the cutoff for age differentiation in our study.


5. Discussion
1) 206 to 210 – As the author states that optimal cut-off value for NLR is not accepted; it’s certainly not established within the head and neck cancer literature. Perhaps a study to first establish evidence of optimal cut-off for head and neck cancer in general and in particular as a prognostic factor in a disease site where treatment and prognosis is well established (i.e. Oral Cavity) before an undertaking in this study would be justified.

Response: The reviewer has made a very good point here. The cutoff value of NLR is not consistent in many studies [1-4]. The cut-off value for NLR is different in hepatocellular carcinoma [5-8]. Perisanidis et al showed that the optimal cutoff of NLR was 1.9 in the oral cancer [4]. It has been determined by Rassouli et al that the optimal cutoff of NLR was 3.0 in head and neck squamous cell carcinoma [9]. The above two study is only two research on the prognostic value of NLR in head and neck cancer. However, the prognostic value of NLR in laryngeal squamous cell carcinoma has not been reported, Hence we performed the study.


2) 215 to 220 – The study by Kim et al, has a much different goal as compared to this study, as such it is unclear what kind of comparisons the authors are trying to
establish between the two papers.
Response: We used this paper to only suggest that NLR was elevated in the laryngeal squamous carcinoma. Our aim is not to compare the two papers.

3) 221 to 223 – What studies?
Response: We have added relative references in the revised manuscript. (line 223, page 11)

6. Comments:
1) It is unclear the role of NLR in head and neck cancer or any cancer in general. The literature surrounding this possible prognostic maker is still controversial and is yet to be established. The role of NLR in particular in head and neck cancer has not been looked at in any large scale study in relation to prognosis. Therefore, a initial study to look at the possibility of NLR’s role in head and neck cancer in general as a prognostic factor needs to be established before any further studies can be undertaken. This study in particular does not address the issues of how NLR is optimally calculated? When is the preoperative values taken? The cut-off value is not clearly proven and the authors themselves state that there is no universally accepted value. Therefore, without that, one cannot ascertain as to the applicability of a cut-off value within this cohort of patients compared to another.

Response: The reviewer made a very good point here. The role in hepatocellular carcinoma and gastrointestinal stromal tumor is very clear by many studies [1-6]. However, the role of NLR in head and neck cancer is unclear. So we performed the study to evaluate the role of NLR in LSCC.

We are sorry to make this unclear. The optimal cutoff score of preoperative NLR was defined by using receiver operating characteristic (ROC) curve analysis [2, 3, 6, 7]. The cutoff value was that point closest to both maximum sensitivity and specificity. (line 147-153, page 7)

<table>
<thead>
<tr>
<th>NLR Sensitivity Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1401 48.1% 71.9%</td>
</tr>
<tr>
<td>2.1657 48.1% 73.0%</td>
</tr>
<tr>
<td>2.1744 44.2% 73.0%</td>
</tr>
</tbody>
</table>

Routine blood tests were performed on the day before surgery. NLR was defined as the absolute neutrophil count divided by the absolute lymphocyte count. (line 103-104, page 5)

The cutoff value is not clearly proven, because the prognostic value of NLR in laryngeal squamous cell carcinoma has not been reported so far. We firstly reported the prognostic value of NLR in LSCC. However, it is noted that this study is limited by its retrospective nature and the relatively small size in a single-center. Further multicenter, large prospective studies are needed to confirm the finding.


2) One of the main flaws of the methods of this study is the lack of treatment modalities. Because the study encompasses a wide TNM stage of laryngeal cancer, the lack of treatment modalities within the Cox-regression analysis is a major confounder that renders the analysis and the conclusions of the study flawed in drawing its conclusions.

Response: The reviewer has made a very good point here. For our study, the inclusion criteria were: ···(7) no pre-operative treatments such as radiotherapy or chemotherapy···. All patients received only surgery, no any treatment. The treatment modality have only surgery in the study. Treatment modalities in LSCC include surgery, radiotherapy, chemotherapy, or a combination therapy.

3) There is also a lack of comorbidity and performance scores which need to be included in the multivariate analysis as they are another source of major confounder within the analysis. This is especially true given that NLR is proposed as an “inflammatory marker” that has a role in patient immunity and overall well-being status.

Response: The reviewer has made a very good point here. This is especially true given that NLR is proposed as an “inflammatory marker” that has a role in patient immunity and overall well-being status. To minimize some comorbidities influence on NLR, patients with comorbidites, such as chronic inflammatory diseases or no evidence of sepsis, hematological disorders or treatment that could result in a elevated NLR, for example, administration of hematopoietic agents such as granulocyte-colony stimulating factor (G-CSF) within 1 month before surgery autoimmune disease or treatment with steroids were excluded in the study. (line 95-103, page 5)
4) There is also serious doubt that even if NLR is a true prognostic marker for head and neck cancer, what the utility of it’s clinical value is. There is currently so many prognostic values including TNM staging system. What utility does NLR add on top of these factors? How does it affect the treatments and effects on patients from a clinical stand-point.

Response: The reviewer made a good point here. Perisanidis et al showed that high neutrophil-to-lymphocyte ratio was an independent marker of poor disease-specific survival in patients with oral cancer [1]. It has been confirmed that NLR is an independent predictor of recurrence in head and neck squamous cell carcinoma [2]. The above study was the only two study on the prognostic role of NLR in head and neck cancer. Our study showed that NLR was a prognostic marker for LSCC. Our study adds evidence on the prognostic role of NLR in head and neck cancer.

There are many prognostic factors such as kallikrein-related peptidase 6 (KLK6)[3], MicroRNA-9[4], LncRNA-AC026166.2-001 and RP11-169 D4 .1-001[5] and TNM stage. It is time-consuming and at high cost to detecting KLK6, MicroRNA-9 and LncRNA-AC02 6166.2-001 and RP11-169 D4 .1-001. And it is not easy to carry out widely. TNM stage need to be pathologically proved and is often not available. However, NLR is a simple and easy-to-obtain marker. It is very easy to widely apply in the clinic.

The clinical value is to help clinicians support intensive follow-up surveillance and adopt personalized adjuvant therapies for patients with LSCC who were at high risk for recurrence after surgical resection. We have added this information in the revised manuscript. (line 247-249, page 12)


Reviewer 2
Minor Essential Revisions

1) Transglottic is described as a subsite of laryngeal cancer. Generally laryngeal cancers are classified as Glottic, Subglottic and Supraglottic by the AJCC.
Please describe your definition of transglottic and why it is a separate subsite.
Response: We thank the reviewer for the constructive suggestion. We totally accept the reviewer’s suggestion. We re-separated as the locations of tumors included 80.2% (113/141) glottic, 17.0% (24/141) supraglottic and 2.8% (4/141) subglottic according to the AJCC. We have revised this information in the revised manuscript. (line 140-141, page 7. Table 1 and 2)

2) How many patients were treated with radiation and/or chemotherapy prior to surgery?
Response: For our study, the inclusion criteria were: ···(7) no pre-operative treatments such as radiotherapy or chemotherapy···. (line 101-102, page 5). In our study, no patients received radiation and/or chemotherapy prior to surgery.

3) Was NLR followed over time after surgery?
Response: The reviewer made a good point here. Because our study is a retrospective study, we did not collect these data. This is limitation of this study. This offers us a great idea in our future clinical research.