Peer Review File

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Reviewer A

Comment 1: First, in the title the term "predictive role" is misleading since the current study only examined the prognostic role of NLR, not prognosis predictive accuracy. The title needs to indicate the clinical research design of this study, i.e., a retrospective cohort study.

Reply 1: Thank you for this comment. we deleted the term "**predictive**" and changed the title as "**A Single Center Retrospective Study Assessing the Prognostic Significance of Pre-treatment Neutrophil/Lymphocyte Ratio in Locally Advanced Nasopharyngeal Carcinoma**" (see Page 1, line 1~3).

Comment 2: Second, the abstract needs some revisions. The purpose needs to briefly indicate the potential clinical significance of this research focus and what the knowledge gap is in relation to this focus. The methods need to describe the inclusion criteria of eligible subjects, assessment of baseline clinical factors including NLR, and follow up procedures. The results need to briefly describe the baseline clinical characteristics of the sample. The conclusion needs comments for the clinical implications of the findings.

Reply 2: We agree with the reviewer's comment. Based on your reminder, I revised the abstract carefully. We add "In light of the growing evidence suggesting the impact of inflammatory parameters on the survival of individuals with cancer," in the objective (see Page 2, line 22~23). In the method, we describe the inclusion criteria of eligible subjects, and describe the assessment of baseline clinical factors including NLR, and follow up procedures (page 2, line 28-33). In the result, we simplified the description of other risk factor and highlighted the importance of NLR (page 2, line 39-46). In the conclusion, we commented the clinical implications of the findings (page 3, line 48-53).

Third, in the introduction of the main text, the authors need to review what has been known on the prognostic role of NLR cancers, as well as other known inflammatory biomarkers, and explain why the NLR, not other biomarkers deserved to be studied. The authors also need to explain the potential mechanisms of NLR as a prognostic factor in cancer. The rationale "provide a basis for guiding clinical treatment" was vague and unclear, I suggest the authors to specify the potential significance of this study.

Reply 3: Thank you for this comment. In the introduction, we added the potential mechanisms of NLR as a prognostic factor in cancer "However, the underlying molecular mechanisms need further understanding. One crucial factor could be the association of elevated NLR with a tumor microenvironment that promotes tumor progression, potentially contributing to an unfavorable prognosis."(page 4, line 80-83), and we illustrated the other known inflammatory biomarkers that affect the prognosis " Inflammatory markers, including platelet-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR), and systemic immune-inflammation index (SII),"(page 3, line 73-75), and give the reason that NLR is most easily measured and the cost of NLR is effective "The measurement of peripheral NLR through routine blood examinations is a simple and cost-effective method." (page 4, line 77-83), besides, previous studies mainly focus on talking about the value of NLR in OS or PFS but rarely on the role of NLR in local failure recurrence and distant metastasis separately. In this study, the potential effect of NLR in local or distant metastasis pattern would be analyzed "However, the role of NLR as a prognostic marker of local-regional recurrence survival or distant failure-free survival (DFFS) LANPC is rarely reported. The potential effect of NLR in local or distant failure patterns still needs further investigation. The current study collected baseline data of pre-treatment NLR in patients with LANPC to observe the prognostic risk factors affecting OS, PFS, DMFS, and local failure-free survival (LFFS). The current study collected baseline data of pre-treatment NLR in patients with LANPC to observe the prognostic risk factors affecting OS, PFS, DMFS, and local failure-free survival (LFFS)." (page 4, line 86-91). We deleted the vague and unclear sentence "provide a basis for guiding clinical treatment" (page 5, line 89-91).

Fourth, in the methodology of the main text, please accurately describe the clinical research design of this study, the assessment of baseline clinical factors, and sample size estimation procedures. In statistics, the authors need to describe the procedures for ascertaining the independent prognostic role of NLR, including the adjustment of potential confounders. It seems that the authors identified prognostic factors but this is not the focus of this study.

Reply 4: We agree with the reviewer's comment and made the corresponding adjustment in accordance with the requirement (see Page 4, line 94~103). The sample size was determined

to be ten times greater than the number of variables. (see Page 6, line 143~144). We used univariate and multivariate Cox regression analyses to confirm the independent prognostic role of NLR (see Page 10, line 194~209).

Reviewer B

The paper titled "PROGNOSTIC AND PREDICTIVE ROLE OF PRETREATMENT NEUTROPHIL/LYMPHOCYTES RATIOIN LOCO-REGIONAL ADVANCED NASOPHARYNGEAL CARCINOMA" is interesting, which explored the prognostic and predictive role of neutrophil lymphocyte ratio (NLR) in patients with locally advanced nasopharyngeal carcinoma (NPC) before IMRT. The authors conclude that an increased NLR indicated detrimental outcomes for patients with loco-regional advanced nasopharyngeal carcinoma. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) *Neutrophil-to-lymphocyte ratio (NLR) has been used to predict the prognosis of patients with many tumors, including nasopharyngeal carcinoma (NPC) [Int J Biol Markers. 2022, 37(3):270-279; Cancer Manag Res. 2019, 11:8269-8275]. I hope the author can elaborate on the innovation of this study, which is the difference from previous reports.*

Reply1: Thank you for this comment. In the article" [Int J Biol Markers. 2022, 37(3):270-279; Cancer Manag Res. 2019, 11:8269-8275]", the enrolled patients were NPC with stage II. And the difference of our research from other previous articles is that they rarely focus the role of NLR on distant metastasis.

2) *The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Prognostic significance of neutrophil-lymphocyte ratio in multiple myeloma patients, Transl Cancer Res 2018;7(1):88-96.". It is recommended to quote the articles.*

   Reply 2: Thank you for your reminding, we cited the article in the introduction (see Page 4, line 77 ~78).

3) *There are still some weak points in this paper. It is suggested that the author increase the possible mechanism analysis about the prognostic and predictive role of NLR in nasopharyngeal carcinoma. This is more conducive to support the conclusions of this study.*

Reply 3: Thank you for your reminding, we added the possible mechanism analysis in the discussion."The underlying mechanisms of correlation between NLR and poor prognosis of tumor are not fully understood. However, an elevated NLR is indicative of either an enhanced neutrophil count and/or a reduced lymphocyte count. Neutrophils are a type of inflammatory

cells that contribute to various stages of tumor development by producing cytokines, including oncostatin M, hepatocyte growth factor, and transforming growth factor- β (TGF- β). Furthermore, neutrophils enhance tumor angiogenesis by releasing angiogenic factors, including angiopoietin-1, vascular endothelial growth factor, and fibroblast growth factor-2. Furthermore, lymphocytes mediate immune surveillance and help in the elimination of tumor cells." (see Page 11, line 208 ~216).

4) *In the abstract, the "Purpose" should be changed into "Background". And the "Background" is too simple. It is suggested to add relevant contents.*

Reply 4: We agree with the reviewer's comment. We add "In light of the growing evidence suggesting the impact of inflammatory parameters on the survival of individuals with cancer," in the purpose (see Page 2, line 22~23).

5) *The content stated in this paper is too old; mainly because most of the references are 5 years ago. Please refer to the relevant literature published in recent years to update the content of the paper.*

Reply 5: Thank you for your reminding, we have refreshed the references. I have updated the reference as follows:

1. Delete the references from "TCR-23-528-CL-Review version with line numbers (for your reference)":

[1] An, X., Ding, PR., Wang, FH, *et al.* Elevated neutrophil to lymphocyte ratio predicts poor prognosis in nasopharyngeal carcinoma. Tumor Biol. 32, 317–324 (2011). https://doi.org/10.1007/s13277-010-0124-7. Epub 2010 Oct 30. PMID: 21052888.

[4] Jin Y, Ye X, He C, *et al.* Pretreatment neutrophil-to-lymphocyte ratio as predictor of survival for patients with metastatic nasopharyngeal carcinoma. Head Neck. 2015 Jan;37(1):69-75. doi: 10.1002/hed.23565. Epub 2014 Feb 28. PMID: 24327524.

[5] Tan T, Lim WT, Fong KW, *et al.* Concurrent chemo-radiation with or without induction gemcitabine, Carboplatin, and Paclitaxel: a randomized, phase 2/3 trial in locally advanced nasopharyngeal carcinoma. Int J Radiat Oncol Biol Phys. 2015 Apr 1;91(5):952-60. doi:10.1016/j.ijrobp.2015.01.002. PMID: 25832687.

[6] Bensouda Y, Kaikani W, Ahbeddou N, *et al.* Treatment for metastatic nasopharyngeal carcinoma. Eur Ann Otorhinolaryngol Head Neck Dis. 2011 Apr;128(2):79-85. doi: 10.1016/j.anorl.2010.10.003. Epub 2010 Dec 21. PMID: 21177151.

[7] Razak AR, Siu LL, Liu FF, *et al.* Nasopharyngeal carcinoma: the next challenges. Eur J Cancer. 2010 Jul;46(11):1967-78. doi: 10.1016/j.ejca.2010.04.004. Epub 2010 May 5. PMID: 20451372.

[8]Liao LJ, Hsu WL, Wang CT, *et al.* Prognostic impact of pre-treatment neutrophil-to-lymphocyte ratio (NLR) in nasopharyngeal carcinoma: A retrospective study of 180 Taiwanese patients. Clin Otolaryngol. 2018 Apr;43(2):463-469. doi: 10.1111/coa.12992. Epub 2017 Oct 9. Erratum in: Clin Otolaryngol. 2018 Dec;43(6):1644. PMID: 28950051.

[16] Jones D. ICRU Report 50—Prescribing, Recording and Reporting Photon Beam Therapy. Medical Physics 1994;21:833-4.

[17] Morgan-Fletcher LS. Prescribing, recording and reporting photon beam therapy (supplement to ICRU report 50) (Report 62). Br J Radiol 2001;74:294.

[18] Chang H, Gao J, Xu BQ, *et al.* Haemoglobin, neutrophil to lymphocyte ratio and platelet count improve prognosis prediction of the TNM staging system in nasopharyngeal carcinoma: development and validation in 3,237 patients from a single institution. Clin Oncol (R Coll Radiol). 2013 Nov;25(11):639-46. doi: 10.1016/j.clon.2013.07.004. Epub 2013 Jul 31. PMID: 23910226.

[21] Templeton AJ, McNamara MG, Šeruga B, *et al.* Prognostic role of neutrophil-to-lymphocyte ratio in solid tumors: a systematic review and meta-analysis. J Natl Cancer Inst. 2014 May 29;106(6):dju124. doi: 10.1093/jnci/dju124. PMID: 24875653.

[22] Gunn GB, Villa RD, Sedler RR, *et al.* Nasopharyngeal carcinoma metastasis to the pituitary gland: a case report and literature review. J Neurooncol. 2004 May;68(1):87-90. doi: 10.1023/b:neon.0000024750.93572.ce. PMID: 15174525.

[26] Chua DT, Ma J, Sham JS, *et al.* Long-term survival after cisplatin-based induction chemotherapy and radiotherapy for nasopharyngeal carcinoma: a pooled data analysis of two phase III trials. J Clin Oncol. 2005 Feb 20;23(6):1118-24. doi: 10.1200/JCO.2005.12.081. Epub 2005 Jan 18. PMID: 15657403.

We added the references in the "revision manuscript" as follows:

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F: **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries**. *CA Cancer J Clin* 2021, **71**(3):209-249.

8. Tang LL, Chen YP, Chen CB, Chen MY, Chen NY, Chen XZ, Du XJ, Fang WF, Feng M, Gao J *et al*: **The Chinese Society of Clinical Oncology (CSCO) clinical guidelines for the diagnosis and treatment of nasopharyngeal carcinoma**. *Cancer Commun (Lond)* 2021, **41**(11):1195-1227.
9. Chen YP, Ismaila N, Chua MLK, Colevas AD, Haddad R, Huang SH, Wee JTS, Whitley AC, Yi JL, Yom SS *et al*: **Chemotherapy in Combination With Radiotherapy for Definitive-Intent Treatment of Stage II-IVA Nasopharyngeal Carcinoma: CSCO and ASCO Guideline**. *J Clin Oncol* 2021, **39**(7):840-859.

10. Jiang Y-T, Chen K-H, Yang J, Liang Z-G, Qu S, Li L, Zhu X-D: **Establishment of a Prognostic Nomogram for Patients With Locoregionally Advanced Nasopharyngeal Carcinoma Incorporating TNM Stage, Post-Induction Chemotherapy Tumor Volume and Epstein-Barr Virus DNA Load**. *Frontiers in Oncology* 2021, **11**.

16. Zuo H, Zhai L, Liu X, Gao H, Xu P: **Prognostic significance of neutrophil-lymphocyte ratio in multiple myeloma patients**. *Translational Cancer Research* 2018, **7**(1):88-96.

18. Xu C, Chen YP, Liu X, Li WF, Chen L, Mao YP, Zhang Y, Guo R, Zhou GQ, Tang LL *et al*: **Establishing and applying nomograms based on the 8th edition of the UICC/AJCC staging system to select patients with nasopharyngeal carcinoma who benefit from induction chemotherapy plus concurrent chemoradiotherapy**. *Oral Oncol* 2017, **69**:99-107.

20. Ou D, Wang X, Wu M, Fen X, Yujiao L, Chaosu H, Xiayun H: **Prognostic value of post-radiotherapy neutrophil-to-lymphocyte ratio in locally advanced nasopharyngeal carcinoma**.*Strahlentherapie und Onkologie* 2020, **196**(3):252-261.

31. Pan XB, Huang ST, Zhu XD: **Neutrophil-to-lymphocyte ratio predicts the prognosis of stage II nasopharyngeal carcinoma**. *Cancer Manag Res* 2019, **11**:8269-8275.

6) In line 23, "According" should be changed into "according". Please check carefully.

Reply 6: Thank you for your reminding, we have revised this sentence.