## PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf**) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Prognostic value of derived neutrophil-to-lymphocyte ratio (dNLR) in non-small cell lung cancer patients receiving immune checkpoint inhibitors: a meta-analysis |
| **AUTHORS** | Yang, Tao; Hao, Lizheng; Yang, Xinyu; Luo, Changyong; Wang, Guomi; Lin Cai, Caroline; Qi, Shuo; Li, Zhong |

## VERSION 1 – REVIEW

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| **REVIEWER** | ÖĞÜŞ, Ersin<br>Baskent University , Biostatistics |
| **REVIEW RETURNED** | 28-Jan-2021 |

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| **GENERAL COMMENTS** | Nice work |

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| **REVIEWER** | Reuter, Simon Bertram<br>Nastved Hospital, Respiratory Medicine |
| **REVIEW RETURNED** | 17-Feb-2021 |

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| **GENERAL COMMENTS** | Dear Authors,<br>Thank you for this exciting manuscript. Your study is focusing on an important topic, namely choosing which patients benefit from ICIs.<br>In general, the manuscript is well written and contains all the vital information reporting a meta-analysis.<br>In the results section:<br>Page 9, line 49-52: "Subgroup analysis was performed by ethnicity; the results showed that dNLR was a negative predictor for NSCLC both in Asian (HR = 1.57, 95% CI 0.97–2.54; P = 0.068) and European or American patients (HR = 1.45, 95% CI 1.15–1.84; P = 0.002)". For Asians, the 95% CI includes 1, and the P-value is non-significant.Fr<br>Furthermore, this needs to be addressed in the discussion.<br>In addition, HR for PFS and OS are higher using low dNLR cut-offs than a high cut-off; this needs further elaboration in the discussion.<br>In the discussion, furthermore needs to be commented.<br>Did the included studies provide information about other diseases, previous treatment, among others, that could influence the dNLR?<br>Page 13, line 52-56 "Several limitations of our meta-analysis require careful consideration. First, the eligible studies were all retrospective, so retrospective biases may influence the accuracy of results". Retrospective biases, in particular, needs to be further elaborated. In general, the consequences of only including retrospective studies needs to be explained more carefully.<br><br>Kind Regards |

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| **REVIEWER** | Zhang, Yiwei<br>University of Michigan, Department of Statistics |
| **REVIEW RETURNED** | 15-Mar-2021 |

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| **GENERAL COMMENTS** | The paper made contributions on calling researchers' attention on the prognostic effect of dNLR in ICI therapy for NSCLC using meta-analysis on 8 studies. My main question is whether this significant association between pretreatment dNLR with OS/PFS also holds for chemotherapy or combination of ICI and chemotherapy. If dNLR only predicts clinical response to immune therapy, this can be a potential biomarker as companion diagnostic. Some other questions/suggestions are as follows.<br>1. I suggest replacing “elevated dNLR” to “higher dNLR”, since elevated dNLR is more common when post-treatment dNLR is mentioned.<br>2. I suggest providing more information about ICI. The ICIs in the 8 selected studies were not discussed in the background.<br>3. For the study without HR CI (authored by Russo A), the author should elaborate how HR CI is estimated. I noticed the samples size for this study is very small (n=28). I wonder whether any value is added to include this study in the meta-analysis.<br>4. In the statistical analysis section, the authors stated that “I2 > 50 % and P < 0.05 in the Cochran’s Q test were considered to indicate significant heterogeneity, and the random effects model was applied to calculate the pooled HRs”. However, a random effects model was adopted due to I2=50.5% and P=0.059 for analysis of association between pretreatment dNLR and PFS. Why the random effects model was selected even if the p-value criteria did not meet?<br>5. I suggest including CI for I2, because the width of the I2 CI informs about the accuracy of the true heterogeneity estimation.<br>6. I don’t understand how the subgroup analysis was conducted using univariable and multivariable analysis and I don’t see the results for univariable analysis in Table 3. |

## VERSION 1 – AUTHOR RESPONSE

Reviewer Reports:
Reviewer 1:
Thank you very much for your affirmation of this article, and we will make any efforts to improve it.
Reviewer 2:
Thank you very much for your comments for this article which helped us put forward such useful suggestions, and we will make a point-by-point response to explain.

1. In the results section: Page 9, line 49-52: "Subgroup analysis was performed by ethnicity; the results showed that dNLR was a negative predictor for NSCLC both in Asian (HR = 1.57, 95% CI 0.97–2.54; P = 0.068) and European or American patients (HR = 1.45, 95% CI 1.15–1.84; P = 0.002)". For Asians, the 95% CI includes 1, and the P-value is non-significant. Furthermore, this needs to be addressed in the discussion.

Response: Thank you for your careful review, and I'm sorry for this problem. Based on your comments, we have revised this part accordingly and further explained in the discussion section.

2. In addition, HR for PFS and OS are higher using low dNLR cut-offs than a high cut-off; this needs further elaboration in the discussion. In the discussion, furthermore needs to be commented.

Response: Thank you very much for your valuable question. This great review comment prompt us to reevaluate the results of subgroup analysis based on dNLR cut-off value. We found a mistake in the previous analysis, which has now been corrected. We mistakenly extracted the post-treatment data that should be the pre-treatment data in study authored by Prelaj A (PMID: 32245624; doi: 10.1016/j.cllc.2019.11.017). The results of reanalysis show that the pooled HR was 1.72 (95% CI 1.49–/span>1.99, P < 0.001) for cutoff value ≥ 3 group and 1.48 (95% CI 1.15-1.90, P = 0.002) for cut-off value < 3 group in OS-related studies, and pooled HR was 1.33 (95% CI 1.14-1.55, P < 0.001) for cutoff value ≥ 3 group and 1.51 (95% CI 1.01-2.26, P = 0.043) for cut-off value < 3 group in PFS-related studies. In the current results, HR for OS are higher using high dNLR cut-offs than a low cut-off, and the difference of HR for PFS between high- and low- cut-off subgroups is not obvious. In addition, your important question makes us think deeply about the problem of dNLR cut-off value, and explain it in the discussion section.
Thank you very much for your nice question again, and we are sorry for this mistake caused by our carelessness.

Original table in study Prelaj A, PMID: 32245624

3. Did the included studies provide information about other diseases, previous treatment, among others, that could influence the dNLR?

Response: Thank you for your useful question, we reviewed the original papers and found that the included studies did not provide information about other diseases, previous treatment, among others, that could influence the dNLR. In the future research, we will pay more attention to this aspect.

4. Page 13, line 52-56 "Several limitations of our meta-analysis require careful consideration. First, the eligible studies were all retrospective, so retrospective biases may influence the accuracy of results". Retrospective biases, in particular, needs to be further elaborated. In general, the consequences of only including retrospective studies needs to be explained more carefully.

Response: Thank you for pointing this out, this is a very important issue. We have explained the retrospective biases and are more cautious in drawing conclusions.
Reviewer: 3
Thank you very much for your guidance on our manuscript, which has further improved the quality of our manuscript.

1. My main question is whether this significant association between pretreatment dNLR with OS/PFS also holds for chemotherapy or combination of ICI and chemotherapy. If dNLR only predicts clinical response to immune therapy, this can be a potential biomarker as companion diagnostic.

Response: Thank you very much for your question, it's a very insightful question. Nowadays, more and more studies have found that some peripheral inflammatory indexes are related to the prognosis of lung cancer patients, such as platelet to lymphocyte ratio (PLR), neutrophil to lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), etc. these studies mainly focus on the relationship between that inflammatory indexes and the prognosis of immunotherapy. In addition, some studies have found that these inflammatory indexes are closely related to the prognosis of chemotherapy, targeted drugs and other comprehensive treatment too. The results of meta-analysis of Zimu Wang et al showed that pretreatment NLR is a promising prognostic indicator for NSCLC patients receiving systemic therapy, including chemotherapy, immunotherapy and targeted therapy (PMID: 31367535 DOI: 10.21037/tlcr.2019.06.10). Yan Wang et al found that pretreatment LMR may be a useful prognostic marker in NSCLC patients receiving systemic therapy, including surgery, CRT, EGFT-TKI (PMID: 31319409 DOI: 10.1159/000501726). It can be inferred that the study of the relationship between dNLR and the prognosis of lung cancer patients receiving systemic therapy is also of great significance. However, dNLR is a relatively new indicator, and the number of related studies is relatively small. In future studies, we will continue to pay attention to the research progress in this area, and timely supplement and update our research.

2. I suggest replacing “elevated dNLR” to “higher dNLR”, since elevated dNLR is more common when post-treatment dNLR is mentioned.

Response: Thank you very much for your correction and reminder, and we have replaced “elevated dNLR” to “higher dNLR” in the uploaded manuscript.

3. I suggest providing more information about ICI. The ICIs in the 8 selected studies were not discussed in the background.

Response: Thank you very much for your suggestion, and we have revised the background in the uploaded manuscript.

4. For the study without HR CI (authored by Russo A), the author should elaborate how HR CI is estimated. I noticed the samples size for this study is very small (n=28). I wonder whether any value is added to include this study in the meta-analysis.

Response: Thank you very much for your reminding, we have added the calculation formula to the manuscript. DNLR is an inflammatory index that has been paid attention to recently. When we searched the literature, we found that the relevant studies were published in the last 2-3 years, and the number of relevant studies was relatively small. Therefore, we cherish every study that meets our inclusion criteria, and make full use of their data.

5. In the statistical analysis section, the authors stated that “I2 > 50 % and P < 0.05 in the Cochran’s Q test were considered to indicate significant heterogeneity, and the random effects model was applied to calculate the pooled HRs”. However, a random effects model was adopted due to I2=50.5% and P=0.059 for analysis of association between pretreatment dNLR and PFS. Why the random effects model was selected even if the p-value criteria did not meet?

Response: I'm very sorry for this is a mistake. We have replaced it with fixed effect model and reanalyzed it. Thank you very much for your careful examination.

6. I suggest including CI for I2, because the width of the I2 CI informs about the accuracy of the true heterogeneity estimation.

Response: Thank you very much for your comments, we have calculated the 95%CI for I2 according to the method provided in the paper authored by Julian P T Higgins and added it to our manuscript. (Quantifying heterogeneity in a meta-analysis. Statist. Med. 2002; 21:1539–1558 PMID: 12111919 DOI: 10.1002/sim.1186)

7. I don’t understand how the subgroup analysis was conducted using univariable and multivariable analysis and I don’t see the results for univariable analysis in Table 3.

Response: We are sorry that we did not state our subgroup analysis intention clearly. In our study, the univariable and multivariable subgroups grouping method are based on different ways to get HR value in the original research. Among all eligible studies, except for one study, other studies provided HR values obtained from multivariate Cox regression analysis. We performed subgroup analysis according to univariate and multivariate analysis, there was only one study in univariate subgroup, so the combined results were not shown.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Reuter, Simon Bertram<br>Nastved Hospital, Respiratory Medicine |
| **REVIEW RETURNED** | 04-May-2021 |

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| **GENERAL COMMENTS** | Dear Authors,<br>Thanks for the revision of the manuscript, I still have some comments and questions.<br><br>1. Did the included studies provide information about other diseases, previous<br>treatment, among others, that could influence the dNLR? |

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| | Response: Thank you for your useful question, we reviewed the original papers and<br>found that the included studies did not provide information about other diseases,<br>previous treatment, among others, that could influence the dNLR. In the future<br>research, we will pay more attention to this aspect.<br><br>This then needs to be mentioned in the discussion, as well as how it might impact the results.<br><br>The biases mentioned in the discussion page 14 are still not sufficient, e.g. is there recall bias in the included studies? How is selection bias influencing the results (relating to the above comment)?<br><br>Page 10, A fixed effects model was applied due to relatively satisfactory homogeneity (I2=18.6%, 95% CI -71.4%-61.4%, P = 0.283).<br>Ether is the I2 or the CI95 wrong.<br><br>Another reviewer mentioned that:<br>" I don't understand how the subgroup analysis was conducted using univariable<br>and multivariable analysis and I don't see the results for univariable analysis in<br>Table 3.".<br>I still don't understand that.<br><br>Lastly, the manuscript needs language improvement.<br>Kind Regards |

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| **REVIEWER** | Zhang, Yiwei<br>University of Michigan, Department of Statistics |
| **REVIEW RETURNED** | 02-May-2021 |

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| **GENERAL COMMENTS** | The authors have clearly replied my previous comments. I don't have additional comments. |

**VERSION 2 – AUTHOR RESPONSE**

Reviewer Reports:

Reviewer 3:

The authors have clearly replied my previous comments. I don't have additional comments.

Dear Dr. Yiwei Zhang:

Thank you very much for your valuable comments and approval.

Reviewer 2:

Dear Dr. Simon Bertram Reuter:

Thank you very much for your careful review and valuable comments. It is also a pity that your requirements were not fully met in the last revision. This time, we will try our best to revise the draft

and hope to get your approval. Finally, thank you again for your comments, which greatly improved the quality of our manuscript.

1. Did the included studies provide information about other diseases, previous treatment, among others, that could influence the dNLR?

Response: Thank you for your useful question, we reviewed the original papers and found that the included studies did not provide information about other diseases, previous treatment, among others, that could influence the dNLR. In the future research, we will pay more attention to this aspect.

This then needs to be mentioned in the discussion, as well as how it might impact the results.

The biases mentioned in the discussion page 14 are still not sufficient, e.g. is there recall bias in the included studies? How is selection bias influencing the results (relating to the above comment)?

Response: Thank you very much for reminding us that we have added this section to the discussion (at the end of the fourth paragraph of the discussion section, the text marked in yellow). In addition, we also supplement the discussion on retrospective bias (at the fifth paragraph of the discussion section, the text marked in yellow). Thank you very much for your question, which improved the level of our discussion section.

2. Page 10, A fixed effects model was applied due to relatively satisfactory homogeneity (I2=18.6%, 95% CI -71.4%-61.4%, P = 0.283).

Ether is the I2 or the CI95 wrong.

Response: Thank you very much for your reminding. There is no error in our I2 and its CI 95%, but which is ambiguous in the presentation form. In the previous manuscript, we used "-" to indicate the range. Here we want to indicate that the range is from "minus 71.4% (-71.4%)" to "61.4%". Now we have used “~” to indicate the range. (I2=18.6%, [95% CI (-71.4%~61.4%)])

In addition, we calculated the 95%CI for I2 according to the method provided in the paper authored by Julian P T Higgins (Quantifying heterogeneity in a meta-analysis. Statist. Med. 2002; 21:1539–1558 PMID: 12111919 DOI: 10.1002/sim.1186).

3. Another reviewer mentioned that:" I don’t understand how the subgroup analysis was conducted using univariable and multivariable analysis and I don’t see the results for univariable analysis in Table 3.".

I still don't understand that?

Response: We're sorry that we didn't explain this point clearly before. Among all eligible original studies, the prognostic value of dNLR was assessed with Cox model for survival, including univariate and multivariate Cox regression analysis. Among eight eligible studies, except for one study (Russo A 2018), other seven studies provided HR values obtained from multivariate Cox regression analysis. Meta analysis of HR values of all studies may cause some biases, so we performed subgroup analysis according to univariate and multivariate analysis, there was only one study in univariate subgroup, so the combined results were not shown. Some published meta-analyses also use subgroup analysis when facing similar situations, such as Yupeng G 2020 (Revealing the prognostic landscape of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in metastatic castration-resistant prostate cancer patients treated with abiraterone or enzalutamide: a meta-analysis PMID: 32034294, DOI: 10.1038/s41391-020-0209-3), Nan D 2016 (The Prognostic Value of PLR in Lung Cancer, a Meta-analysis Based on Results from a Large Consecutive Cohort PMID: 27703265 DOI: 10.1038/srep34823) et al.