PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Neutrophil-to-Lymphocyte Ratio as a Predictor of Mortality in Intensive Care Unit Patients: A Retrospective Analysis of the Medical Information Mart for Intensive Care III Database
AUTHORS	Wu, Xie; Luo, Qipeng; Su, Zhanhao; Li, Yinan; Wang, Hongbai; Liu, Qiao; Yuan, Su; Yan, Fuxia

VERSION 1 – REVIEW

REVIEWER	Shen, Yanfei Dongyang People's Hospital, Intensive Care Unit
REVIEW RETURNED	29-Jul-2021

GENERAL COMMENTS	The author investigated predictive value of NLR in critically ill patients, using data from a online database. The major findings are that NLR can predict hospital mortality, and both low and high NLR are associated with increased mortality rate. I have several concerns. Major issue, Both NLR and PLR are considered as novel inflammatory indexes in many cohorts, such as COVID-19, AKI, sepsis, ARDS. In the current study, mixed patients in several ICUs were included. As heterogeneous has become an important issue in ICU, simply investigating the overall predictive value of NLR in the whole ICU cohort may increase the bias risk. Subgroup analysis should be considered. Line 33 the definition of abnormal value should be provided. In my opinion, extreme high WBC (such as >200) may be affected by potential hematological disorders, which may bias the predictive value of NLR. In addition, are patients with confirmed hematological disorders included in the current study? More clear inclusion criteria should be described. Percentage of missing values should be described. In the statistical analysis, the selection of confounding factors seems arbitrary. All continuous data were presented as medians with IQR. Please be consistency (page 8, line 18). Result Line 52 the AUC of NLR alone was poor. Actually, this may be a result of the significant heterogeneity within mixed ICU patients. In addition, the author should explain why using 6 and 1 as the cut-off values for NLR.

	Page 10, line 9. The author reported that patients with extreme low NLR was also associated with poor outcome. We noted in the supplementary figure, the mortality in category 0-1 was significantly higher than that in category 1-2. As only 580/21822=2.6% patients were in this category, whether this finding was affected by other factors needs to be investigated. The baseline comparisons within these three groups should be presented, including neutrophil, lymphocyte, etc. Page 10, Line 36 the c-index increased from 0.798 to 0.789 after adding NLR to the SAPS score. Although statistically significant (due to the large sample size), the clinical significance may be very small. This should be described. Line 46 the subgroup analysis could help us to understand the predictive value of NLR. However, dividing patients according diagnosis instead of ICU types may be more relevant. Discussion I suggest the discussion should be revised after the above issues been resolved. In addition, we noted several studies also investigated the predictive value of NLR in mixed ICU patients. The difference with these studies should be addressed. such as PMID: 25598149, PMID: 31166439, PMID: 33299038

REVIEWER	Sinto, Robert University of Indonesia
REVIEW RETURNED	15-Aug-2021

GENERAL COMMENTS	- p3, line 21-22: is this a cross sectional study, while authors tried to follow subjects outcome (including the "time span")? - No specified explanation on participant consent, ethics approval available. - p7, line 7: the AUC of NLR is "only" 0.609; a value of AUC that could not be classified as good value of prognostic predictor, although this value is the best among the other inflammation marker tested in this study. How could this justified as basis for further statistical analysis test (adding it to the well known predictor)? Or on the other hand, authors should conclude that NLR is actually a "weak" predictor of ICU outcome? - p12, Please elaborate more (line 50), the scientifically plausible reason for choosing SAPS II from other existing ICU prognosis score (i.e. APACHE, SOFA, etc). - The addition of NLR to SAPS II improves AUC from 0.789 to 0.798. Is this improved AUC is clinically significant alongside with the necessity to revised the well known " SAPS II", although statistically significant ($p<0.05$)? Does this, on the other hand, reveal the not usefulness of adding NLR to existing SAPS-II criteria? - Please elaborate more how do patient comorbidity confounded the prognostic ability of NLR to predict outcome (cancer progression, inflammatory disease, cardiovascular disease)?

	- Authors need to further explain the clinical application of their finding, since authors could not construct a new scoring system (p13 line 40) - Please elaborate the relationship of this manuscript with previous publication (reference no 7); since authors used the same database (MIMIC-III); How big does the redundancy exist?

VERSION 1 – AUTHOR RESPONSE

Response to reviewers:
Reviewer: 1

Dr. Shen,
Thanks for your important and detailed comments, and we have made some modifications according to your comments, the details are as follows:

1. Comment: Both NLR and PLR are considered as novel inflammatory indexes in many cohorts, such as COVID-19, AKI, sepsis, ARDS. In the current study, mixed patients in several ICUs were included. As heterogeneous has become an important issue in ICU, simply investigating the overall predictive value of NLR in the whole ICU cohort may increase the bias risk. Subgroup analysis should be considered.

Reply: Thanks for your suggestion. Although subgroup analysis was also considered in our study, but we divided patients according to ICU types, and we agree with your opinion that it would be more relevanto divide patients based on diagnosis. Therefore, we did another subgroup analysis based on the main diagnosis, the results are shown in Table 4. However, although we conducted a subgroup analysis, in-depth analyses were not undertaken as it was not the aim of our study.

2. Comment: Line 33 the definition of abnormal value should be provided. In my opinion, extreme high WBC (such as >200) may be affected by potential hematological disorders, which may bias the predictive value of NLR. In addition, are patients with confirmed hematological disorders included in the current study? More clear inclusion criteria should be described.

Reply: Thank you so much for your suggestion, the abnormal value was defined as exceeding a certain standard value. This manuscript mentioned it but it may not be clear enough, so we have modified this point to make the definition clearer (Page 6, Line 18-19). “Abnormal values in this study were defined as extreme outliers, that is WBC count >400 × 109/L, NLR >100, and PLR >8,000.” Since the purpose of our research was to study the general applicability of NLR to predict ICU mortality, all patients over 16 years of age were included in this study, including patients with confirmed hematological disorders. This selection criteria refer to previous study (PMID: 25598149). We have rewritten the inclusion and exclusion criteria to make ieasier to understand. (Page 6, line12-15)

3. Comment: Percentage of missing values should be described.

Reply: Thank you for your suggestion, and we have added a description of missing values in the manuscript “The missing values of all selected variables are less than 10%, so we replaced the missing observations with the mean values.” (Page6, line20-22).

4. Comment: In the statistical analysis, the selection of confounding factors seems arbitrary. All continuous data were presented as medians with IQR. Please be consistency (page 8, line 18).

Reply: Thank you so much for your detailed suggestions, confounding factors was selected based on the p value in the univariate analysis and their clinical significance. We have added this sentence in the method section “we adjusted for variables with a p value less than 0.2 in the univariate analysis or clinically significant” (Page 8, line 2).
We have presented all continuous data as medians with IQR (Page 8, line 15).

5. Comment: Line 52 the AUC of NLR alone was poor. Actually, this may be a result of the significant heterogeneity within mixed ICU patients. In addition, the author should explain why using 6 and 1 as the cut-off values for NLR.

Reply: We quite agree with your opinion. We included mixed patients in several ICUs in our study, and the resulting heterogeneity may be the main reason for the low AUC value of NLR. However, the main purpose of this study was to find an easy-to-obtain and universal inflammation index, so we did not limit the inclusion criteria to only a certain diagnosis just to increase the AUC value.
The reason for choosing 1 and 6 as the cutoff values are described in the results section. “The in-hospital mortality rates for different NLRs are shown in Supplemental Figure 1. We found that both a high (>6) and low (≤1) NLR were associated with a higher mortality rate. Therefore, we selected the NLR as our best inflammatory marker, with cut-off values of 1 and 6.” (Page 9, line 7-10)

6. Comment: Page 10, line 9. The author reported that patients with extreme low NLR was also associated with poor outcome. We noted in the supplementary figure, the mortality in category 0-1 was significantly higher than that in category 1-2. As only 580/21822=2.6% patients were in this category, whether this finding was affected by other factors needs to be investigated. The baseline comparisons within these three groups should be presented, including neutrophil, lymphocyte, etc.

Reply: We really appreciate your rigorous scientific attitude, and we agree with your suggestion that the baseline comparisons within these three groups should be presented. We have supplemented these results in Supplemental Table 2. There are statistical differences in the baseline data between the three groups, but the influence of these confounding factors can be eliminated by multivariate regression.

7. Comment: Page 10, Line 36 the c-index increased from 0.798 to 0.789 after adding NLR to the SAPS score. Although statistically significant (due to the large sample size), the clinical significance may be very small. This should be described.

Reply: Thank you so much for your comment. We have added this paragraph in the discussion section. “Although SAPS II is well-known, it lacks inflammatory indicators. As an easy-to-obtain, sensitive inflammatory indicator that does not increase the financial burden of patients, NLR has been reported by may previous studies and has high clinical significance. That’s why we tried to add NLR to

the SAPS II to evaluate whether it can increase its predictive performance. Although the AUC value does not increase very much from a numerical point of view, since this study is qualitative rather than quantitative, it is enough to illustrate the clinical value of NLR, and also provide a certain reference for the future studies." (Page 13, line 15-22)

8. Comment: Line 46 the subgroup analysis could help us to understand the predictive value of NLR. However, dividing patients according diagnosis instead of ICU types may be more relevant.

Reply: This suggestion is very important, and we have already accepted it and re- analyzed the subgroups based on the diagnosis (Table 4).

9. Comment: I suggest the discussion should be revised after the above issues been resolved. In addition, we noted several studies also investigated the predictive value of NLR in mixed ICU patients. The difference with these studies should be addressed. such as PMID: 25598149, PMID: 31166439, PMID: 33299038
Reply: Thank you so much for reminding me, and we have already revised the discussion. The differences between this manuscript and previous studies have been mentioned in many places in discussion. Such as "Many previous studies have overlooked the possibility of a low NLR leading to a poor prognosis" (Page 11, line 16); "The major strengths of our study are the large sample size and the inclusion of all ICU patients without selection bias. Further, we noticed that the mortality rate was also elevated in patients with a low NLR. More importantly, we found that adding the NLR to the SAPS II could improve its predictive power for ICU mortality…" (Page 14, line1-5).

Reviewer: 2

Dr. Robert Sinto,
Thank you so much for your constructive suggestions, and here are the responses point by point.

1. Comment: - p3, line 21-22: is this a cross sectional study, while authors tried to follow subject outcomes (including the "time span")?

Reply: Thank you very much for pointing out our mistake, this study is not a cross-sectional study but a case-control study, and we have revised this error. In addition, the secondary outcomes of this

study seem to have a time span, such as death in 30 or 90 days. But this study only cares about whether death occurs, when it occurs is not the focus of this study, that's why we chose logistic regression instead of cox regression.

2. Comment: No specified explanation on participant consent, ethics approval available.

Reply: Thank you for your reminder, the ethical statement is at the end of the manuscript. (Page 15, line 11)

3. Comment: p7, line 7: the AUC of NLR is "only" 0.609; a value of AUC that could not be classified as good value of prognostic predictor, although this value is the best among the other inflammation marker tested in this study. How could this justified as basis for further statistical analysis test (adding it to the well known predictor)? Or on the other hand, authors should conclude that NLR is actually a "weak" predictor of ICU outcome?

Reply: Thank you so much for your comments. We included mixed patients in several ICUs in our study, and the resulting heterogeneity may be the main reason for the low AUC value of NLR. However, the main purpose of this study was to find an easy-to-obtain and universal inflammation index, so we did not limit the inclusion criteria to only a certain diagnosis just to increase the AUC value. We also did some efforts to illustrate the clinical significance of NLR. On the one hand, we added NLR to the well-known predictor to see whether it can increase the predictive power. On the other hand, we added a subgroup analysis based on diagnosis to reduce the bias. Although the AUC of NLR is not very high, it is very clinically significant, and we hope that our research results can attract the attention of more clinical experts.

4. Comment: p12. Please elaborate more (line 50), the scientifically plausible reason for choosing SAPS II from other existing ICU prognosis score (i.e. APACHE, SOFA, etc).

Reply: Thank you for your advice, SOFA includes platelet count and APACHE contains WBC count, only SOFA lacks inflammatory indicators, that's why we chose SAPS. We have added this part in the discussion section. "Commonly used ICU prognosis score include APACHE, SOFA, SAPS and so on. In this study, the SAPS was chosen because of its lack of inflammatory indicators." (Page 13, Line 7-8)

5. Comment: The addition of NLR to SAPS II improves AUC from 0.789 to 0.798. Is this improved AUC clinically significant alongside with the necessity to revised the well-known" SAPS II", although statistically significant ($p<0.05$)? Does this, on the other hand, reveal the not usefulness of adding NLR to existing SAPS-II criteria?

Reply: Thank you so much for your comment. Although SAPS II is well-known, it lacks inflammatory indicators. As an easy-to-obtain, sensitive inflammatory indicator that does not increase the financial burden of patients, NLR has been reported by many previous studies and has high clinical significance. That's why we tried to add NLR to the SAPS II to evaluate whether it can increase its predictive performance. Although the AUC value does not increase very much from a numerical point of view, since this study is qualitative rather than quantitative, it is enough to illustrate the clinical value of NLR, and also provide a certain reference for the future studies. And we have added this paragraph in the discussion section. (Page 13, Line 15-22)

6. Comment:
Please elaborate more how do patient comorbidity confounded the prognostic ability of NLR to predict outcome (cancer progression, inflammatory disease, cardiovascular disease)?

Reply: Thank you for your suggestion, we apologize for not paying enough attention to this problem in the previous manuscript. In this revision, we did a subgroup analysis based on the diagnosis (Table 4). Through the subgroup analysis, we can understand the prognostic ability of NLR in different comorbidities.

7. Comment:
Authors need to further explain the clinical application of their finding, since authors could not construct a new scoring system (p13 line 40)

Reply: Thanks for your reminder. We added this sentence in the manuscript in page 14, line 12 “however, it has also attracted the attention of clinicians to be wary of abnormal NLR values”. In addition, the clinical application is also mentioned above in page 14, line 3-5 “More importantly, we found that adding the NLR to the SAPS II could improve its predictive power for ICU mortality, which is an important prompt for future scoring systems and may be of particular interest to critical care specialists”.

8. Comment:
Please elaborate the relationship of this manuscript with previous publication (reference no 7); since authors used the same database (MIMIC-III); How big does the redundancy exist?

Reply: Thank you for your question. Although the data is extracted from the same database, the research object and the main purpose are all different. Previous studies have focused on the impact of NLR on a certain type of disease, for example, sepsis is studied in Reference 7, so they only included those patients diagnosed with sepsis in MIMIC III. While the main purpose of this study was to find an inflammatory index that is universally applicable to all patients in ICU, so we included all adult patients admitted to ICU. More importantly, due to different grouping methods, previous studies have only noticed that the prognosis is poor when the NLR is high, while this study also found that a low NLR also increases the mortality rate.

VERSION 2 – REVIEW

REVIEWER	Shen, Yanfei Dongyang People's Hospital, Intensive Care Unit
REVIEW RETURNED	09-Oct-2021

GENERAL COMMENTS	Thank you for your revision. All my concerns are addressed except for Figure 1. I dont why, but I didnot see figure 1 in the revised manuscript. Please be sure you have uploaded it.

REVIEWER	Sinto, Robert University of Indonesia
REVIEW RETURNED	09-Oct-2021

GENERAL COMMENTS	Authors have tried to perform the manuscript revision. Further major concern, especially on the discussion part should be further commented as follow: 1. Line 13: Authors chose SAPS due to its lack of inflammatory indicators (perhaps due to its simplicity), but then try to add inflammatory indicators to the existing SAPS (make the prognosis score becoming more complex with the result of clinically not important increase of AUC from 0.789 to 0.798). Please explain more on this contradictive statement. 2. Please elaborate more the statement in page 13: "since this is qualitative rather than quantitative". Authors perform quantitative statistical analysis that meet the quantitative nature of the study. Please explain more the "qualitative" nature of study that was stated by the authors, and how "the qualitative" nature of study could suggest a statistically insignificance finding as an important convincing statement as was concluded by authors in the conclusions? In addition to the AUC of NLR is "only" 0.609; a value of AUC that could not be classified as good value of prognostic predictor. Please explain the rationalization of not concluding that NLR is a "weak" predictor of ICU outcome? 3. Please add flow diagram (how many patient records that were screened, excluded, included, etc). Page 6: since authors perform replacement of the missing value, please further describe whether the missing values were missing at random. Additional table to support the statement of type of missing values is needed.

VERSION 2 – AUTHOR RESPONSE

Response to reviewers:

Reviewer: 1

Dr. Shen,

Thank you very much for your constructive suggestions, which has significantly raised the quality of the manuscript. We have no idea why you can’t see Figure 1 in the previous revision. However, we make sure that all figures of this version have been uploaded. Thanks again for your reminder.

Reviewer: 2

Dr. Robert Sinto,

Thank you very much for your comments, which really help us a lot to improve our manuscript, especially on the discussion part. And here are the responses point by point.

1. Comment: Line 13: Authors chose SAPS due to its lack of inflammatory indicators (perhaps due to its simplicity), but then try to add inflammatory indicators to the existing SAPS (make the prognosis score becoming more complex with the result of clinically not important increase of AUC from 0.789 to 0.798). Please explain more on this contradictive statement.

Reply: Thank you so much for your suggestion. SAPS II had no inflammatory indexes, so we tried to add one to see if it can improve its predictive ability. And we only added one inflammatory indicator to the existing model, so we believe that it does not mae the model much more complicated. More importantly, the main purpose of this study was not to construct a new prognostic model, but to clarify the importance of NLR. To illustrate the clinical significance of NLR, we calculated not only the AUC, but also the NRI and IDI. the results of the NRI and IDI analyses indicated that the addition of NLR significantly improved the prediction ability. We have added these explanations in the discussion (Page 12, Line 12-20).

2. Comment: Please elaborate more the statement in page 13: "since this is qualitative rather than quantitative". Authors perform quantitative statistical analysis that meet the quantitative nature of the study. Please explain more the "qualitative" nature of study that was stated by the authors, and how "the qualitative" nature of study could suggest a statistically insignificance finding as an important convincing statement as was concluded by authors in the conclusions? In addition to the AUC of NLR is "only" 0.609; a value of AUC that could not be classified as good value of prognostic predictor. Please explain the rationalization of not concluding that NLR is a "weak" predictor of ICU outcome?

Reply: Thank you very much for your comments. We consider this research is qualitative is because we did not assign scores to NLR and added it to the exiting SAPS II to form a new model. Importantly, the goal of this study was not to create a new prognostic model but illustrate the relationship between NLR and prognosis.We have explained it in the limitation “We can only infer that the addition of the NLR can improve the performance of the SAPS II because the NLR scores cannot be directly included in the SAPS II to construct a new scoring system……” (Page 13, Line 12-17). If the statement is not clear or needs any form of modification, please don’t hesitate to contact us.

Although NLR has the best predictive ability among the inflammatory markers of the routine blood tests, we have to admit that NLR alone is a weak predictor. This may be related to that the prognosis of ICU patients are affected by many other confounding factors. However, after translating NLR into categorical variable and adjusting for the confounding factors, NLR showed a good ability to predict ICU outcomes. Thanks again for your suggestion and we have made some explanation in the discussion (Page 10, Line 12-17).

3. Comment: Please add flow diagram (how many patient records that were screened, excluded, included, etc). Page 6: since authors perform replacement of the missing value, please further

describe whether the missing values were missing at random. Additional table to support the statement of type of missing values is needed.

Reply: Thank you for your suggestion, and we have added a flow diagram in the manuscript, as shown in Figure 1. For missing values, we removed cases with missing values for blood routine test data, then “There were no missing data except for age (missing for 0.2% of cases, n=42), and it was missing at random, so we replaced it with the mean value.” We have added this sentence in the first paragraph of the results section.

VERSION 3 – REVIEW

REVIEWER	Shen, Yanfei Dongyang People's Hospital, Intensive Care Unit
REVIEW RETURNED	26-Oct-2021

GENERAL COMMENTS	All my concerns have been adequately addressed.

REVIEWER	Sinto, Robert University of Indonesia
REVIEW RETURNED	23-Oct-2021

GENERAL COMMENTS	Author has addresses our previous concern and add revision in the manuscript.