PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Prognostic value of lymphocyte to monocyte ratio and neutrophil to lymphocyte ratio in follicular lymphoma: a retrospective cohort study
AUTHORS	Lee, Shing Fung; Luque, Miguel-Angel

VERSION 1 – REVIEW

REVIEWER	Luis F. Porrata Mayo Clinic, USA
REVIEW RETURNED	05-Jun-2017

GENERAL COMMENTS	The methodology is sound and the appropriate references were cited. Overall very good quality manuscript

REVIEWER	Luigi Marcheselli Italian lymphoma foundation, at Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia (Modena, Italy)
REVIEW RETURNED	14-Jun-2017

GENERAL COMMENTS	Major comments: 1) In literature has been reported some cut-points for LMR/NLR in B-cell lymphomas (especially for LMR), so would be more useful validate the proposed thresholds in an external database (evaluate the reproducibility), rather than calculate new cut-points. 2) About the cut-off. The mortality is a time to event data, thus the authors should find the cut-off from a) Cox model (for example AUC according to Heagerty approach) or b) at fixed time of follow-up (for example at 5 or 10 years of follow-up, excluding from logistic regression patients censored before 5 or 10 years). 3) Did the authors check the functional form (linear, quadratic, logarithmic …) of LMR/NLR with the log(HR) in PFS and OS? 4) Given the small sample size and the small number of events, the reproducibility of the cut-off obtained in this study is questionable. The authors could try to evaluate the stability of the cut-point by means of bootstrap techniques. 5) Did the authors check the interaction between rituximab use and LMR/NLR levels? In other word, LMR/NLR show a homogeneous effect in patients treated with or without rituximab?

	Minor comments: 1) Figure 1. Please, adding the censoring symbol in the Kaplan-Meier curves. 2) Statistical method. Perhaps the “ranges (minimum, maximum)” are associated at the continuous variables, not at categorical variables.

REVIEWER	Luigi Marcheselli Italian lymphoma foundation, at Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia (Modena, Italy)
REVIEW RETURNED	14-Jun-2017

GENERAL COMMENTS	The manuscript needs a major improvement. Major comments: 1) In literature has been reported some cut-points for LMR/NLR in B-cell lymphomas (especially for LMR), so would be more useful validate the proposed thresholds in an external database (evaluate the reproducibility), rather than calculate new cut-points. 2) About the cut-off. The mortality is a time to event data, thus the authors should find the cut-off from a) Cox model (for example AUC according to Heagerty approach) or b) at fixed time of follow-up (for example at 5 or 10 years of follow-up, excluding from logistic regression patients censored before 5 or 10 years). 3) Did the authors check the functional form (linear, quadratic, logarithmic …) of LMR/NLR with the log(HR) in PFS and OS? 4) Given the small sample size and the small number of events, the reproducibility of the cut-off obtained in this study is questionable. The authors could try to evaluate the stability of the cut-point by means of bootstrap techniques. 5) Did the authors check the interaction between rituximab use and LMR/NLR levels? In other word, LMR/NLR show a homogeneous effect in patients treated with or without rituximab? Minor comments: 1) Figure 1. Please, adding the censoring symbol in the Kaplan-Meier curves. 2) Statistical method. Perhaps the “ranges (minimum, maximum)” are associated at the continuous variables, not at categorical variables.

REVIEWER	Naoto Tomita Division of Hematology and Oncology, Department of Internal Medicine, St. Marianna University School of Medicine, Japan None
REVIEW RETURNED	16-Jun-2017

GENERAL COMMENTS	The authors described that high LMR at diagnosis and high NLR at relapse had prognostic impact in patients with FL. I think this conclusion is not reliable because of the following reasons. 1. Treatment regimen is not uniform. If the authors draw any conclusions by using LMR or NLR, the treatment should be uniform in this relatively small cohort analysis. 2. In the article summary, the authors stated " Our study included patients without exposure to rituximab so that our result is also applicable to regions where rituximab is less accessible.". This is incorrect. To prove this, they must analyze in patient cohort treated without rituximab only. This is also applied in RT alone group. 3. The data is not validated in an independent cohort. Reproducibility is needed to establish a new risk factor.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name: Luis F. Porrata
Institution and Country: Mayo Clinic, USA
Competing Interests: None

Comment: The methodology is sound and the appropriate references were cited. Overall very good quality manuscript.

Reviewer: 2

Reviewer Name: Luigi Marcheselli
Institution and Country: Italian lymphoma foundation at Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia (Modena, Italy)
Competing Interests: None declared

SF Lee et al, after analyzed 88 patients with follicular lymphoma, in an observational retrospective study, proposed a LMR and NLR as prognostic factors, with cut-off of 3.43 and 2.78, respectively.

The manuscript needs a major improvement.

Major comments:

1) In literature has been reported some cut-points for LMR/NLR in B-cell lymphomas (especially for LMR), so would be more useful validate the proposed thresholds in an external database (evaluate the reproducibility), rather than calculate new cut-points.

Response: We recognise the importance of an external validation set, but we do not have access to independent data. Thus, we have decided, in line with your suggestion, to use cross-validation to evaluate the area under curve (AUC). After fitting the binary logistic regression models, the predictive performance was assessed via the AUC. AUC was estimated for a sample (the test sample) that is independent of the sample used to predict the dependent variable (the training sample) using 10-fold cross-validation. This strategy allows us to generate a more realistic estimate of predictive performance in absence of an external validation set.

We used the Stata user written command cvAUROC which implements k-fold cross-validation for the AUC for a binary outcome after fitting a logistic regression model. We added a new reference in our statistical methods for the cvAUROC Stata command:

"Luque-Fernandez, MA; Maringe, C; Nelson, P; (2017) CVAUROC: Stata module to compute Cross-validated Area Under the Curve for ROC Analysis after Predictive Modelling for Binary Outcomes. EconPapers."

2) About the cut-off. The mortality is a time to event data, thus the authors should find the cut-off from:
a) Cox model (for example AUC according to Heagerty approach) or
b) at fixed time of follow-up (for example at 5 or 10 years of follow-up, excluding from logistic regression patients censored before 5 or 10 years).

Response: Thank you for your suggestion. We recognize that accounting for time and censoring is important to evaluate LMR/NLR performance to classify individuals according to their vital status at the end of follow-up. Based on the Shen and Yuan paper, we fitted two weighted binary logistic models including time as covariates. Weights were computed to adjust for the inverse probability of censoring. We also added the reference highlighted below:

Reference: Shen W, Ning J, Yuan Y. A direct method to evaluate the time-dependent predictive accuracy for biomarkers. Biometrics. 2015;71(2):439-449. doi:10.1111/biom.12293.

3) Did the authors check the functional form (linear, quadratic, logarithmic …) of LMR/NLR with the log(HR) in PFS and OS?

Response: Yes, we did. In our methods section, we stated that we developed a sensitivity analysis to evaluate the robustness of our findings in the multivariate analysis. We assessed departs from linearity and the function of LMR/NLR. We now have stated it in the methods section.

4) Given the small sample size and the small number of events, the reproducibility of the cut-off obtained in this study is questionable. The authors could try to evaluate the stability of the cut-point by means of bootstrap techniques.
Response: Thank you for this insightful comment. Following your suggestion, we used cross-validation techniques to assess the performance of the cutoff values.

5) Did the authors check the interaction between rituximab use and LMR/NLR levels? In other word, LMR/NLR show a homogeneous effect in patients treated with or without rituximab?
Response: Thank you for your comment. Yes, we evaluated the interaction between rituximab and LMR/NLR levels. However, no conclusive evidence can be extrapolated given the reduced sample size for the secondary analysis.

Minor comments:

1) Figure 1. Please, add the censoring symbol in the Kaplan-Meier curves.

Response: Thank you. We have modified our Kaplan-Meier curves according to your suggestion.

2) Statistical method. Perhaps the “ranges (minimum, maximum)” are associated at the continuous variables, not at categorical variables.

Response: Thank you for pointing this out. We have amended our manuscript according to this comment.

Reviewer: 3

Reviewer Name: Naoto Tomita
Institution and Country: Division of Hematology and Oncology, Department of Internal Medicine, St. Marianna University School of Medicine, Japan
Competing Interests: None

The authors described that high LMR at diagnosis and high NLR at relapse had prognostic impact in patients with FL. I think this conclusion is not reliable because of the following reasons.

1. Treatment regimen is not uniform. If the authors draw any conclusions by using LMR or NLR, the treatment should be uniform in this relatively small cohort analysis.

Response: Thank you for your comment. We agree that it is an important limitation. We have added this important statement as a limitation in our discussion section.

2. In the article summary, the authors stated “Our study included patients without exposure to rituximab so that our result is also applicable to regions where rituximab is less accessible.”. This is incorrect. To prove this, they must analyze in patient cohort treated without rituximab only. This is also applied in RT alone group.

Response: Thank you for pointing this out. We have deleted this sentence from our discussion section.

3. The data is not validated in an independent cohort. Reproducibility is needed to establish a new risk factor.

Response: We recognize the importance of an external validation set but we do not have access to an independent data set. Thus, we have decided, in line with your suggestion, to use cross-validation to evaluate the AUC. After fitting the binary logistic regression models, the predictive performance was assessed via the area under the curve (AUC). AUC was estimated for a sample (the test sample) that is independent of the sample used to predict the dependent variable (the training sample) using 10-fold cross-validation. This strategy allows us to generate a more realistic estimate of predictive performance in the absence of an external validation set.

We used the Stata user written command cvAUROC which implements k-fold cross-validation for the AUC for a binary outcome after fitting a logistic regression model. We added a new reference in our statistical methods for the cvAUROC Stata command:

“Luque-Fernandez, MA; Maringe, C; Nelson, P; (2017) CVAUROC: Stata module to compute Cross-validated Area Under the Curve for ROC Analysis after Predictive Modelling for Binary Outcomes. EconPapers.”

Reviewer: 4

Reviewer Name: Linda Fabris
Institution and Country: Md Anderson cancer center, US
Competing Interests: none

This study is well done and the analysis of NLR is a quite new potential prognostic factor for patients with FL. I find the analysis detailed and comprehensive, the statistical analysis is powerful.

Comment: The only minor point is the limited analysis of potential limitation of the study and also of potential bias that could have influenced the results. I would add a more extensive description in the discussion section.

Response: Thank you for your suggestions. We have expanded the discussion of the potential limitations and biases in the manuscript (paragraph 4 onwards)

Once again, we thank the Editor and reviewers sincerely for all the comments, and hope that we have addressed all concerns to their satisfaction in our revised manuscript.

VERSION 2 – REVIEW

REVIEWER	Luigi Marcheselli Italian Lymphoma Foundation (Fondazione Italiana Linfomi), at University of Modena and Reggio-Emlia, Italy
REVIEW RETURNED	24-Aug-2017

GENERAL COMMENTS	The authors have improved the manuscript. Only two minor comments 1) the authors reported some estimates for post-progression survival: is it survival from the date of progression/relapse to date of death or last follow-up for censored patients? If yes, the authors should add this definition in “statistical methods”. 2) Along the manuscript the authors use the acronym HR and RR. They should use always HR.

REVIEWER	Naoto Tomita Division of Hematology and Oncology, Department of Internal Medicine , St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan
REVIEW RETURNED	25-Aug-2017

GENERAL COMMENTS	The manuscript is generally well-revised.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Reviewer Name: Luigi Marcheselli
Institution and Country: Italian lymphoma foundation at Department of Diagnostic, Clinical and Public Health Medicine, University of Modena and Reggio Emilia (Modena, Italy)
Competing Interests: None declared

The authors have improved the manuscript.

Only two minor comments
1) The authors reported some estimates for post-progression survival: is it survival from the date of progression/relapse to date of death or last follow-up for censored patients? If yes, the authors should add this definition in “statistical methods”.

Response: Thank you for pointing this out. Yes, it is. We have revised the “Materials and Methods” session accordingly.

2) Along the manuscript the authors use the acronym HR and RR. They should use always HR.

Response: Thank you for your suggestion. We have revised the manuscript accordingly to avoid confusion.

Reviewer: 3

Reviewer Name: Naoto Tomita
Institution and Country: Division of Hematology and Oncology, Department of Internal Medicine, St. Marianna University School of Medicine, Japan
Competing Interests: None

Comment: The manuscript is generally well-revised.

Response: We thank the Reviewer for this overall comment. Your valuable suggestions have allowed us to revise the manuscript critically.

Once again, we thank the Editor and reviewers sincerely for all the comments, and hope that we have addressed all concerns to their satisfaction in our revised manuscript.