PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Prognostic value of MiR-219-5p in relation to mortality in patients with small cell lung cancer: a retrospective, observational cohort study in China
AUTHORS	Cao, Zhijun; Zhang, Jigang; Zhang, Xiaohui; Xiang, Mengqi; Xu, Zhihua; Wu, Xiangmei

VERSION 1 – REVIEW

REVIEWER	Nojima, Masanori The Institute of Medical Science, the University of Tokyo, Center for Translational Research
REVIEW RETURNED	21-Jun-2022

GENERAL COMMENTS	No major statistical problems, but insufficient verification of clinical usefulness. Please respond to the following points. 1. Figure 3 should be shown on a logarithmic scale. 2. Was the nomogram in Figure 4 based on LASSO regression? If it was LASSO, describe the model parameters in detail. 3. To test the clinical usefulness of the nomogram, divide the cases into 3-4 groups according to the results of the nomogram application and then perform Kaplan-Meier analysis. This should be done for both training and test (validation) sets. 4. The specificity of the green line appears to partially exceed 100%. It is strange that it goes to the left of the origin. 5. The clinical impact is not considered high because the data is old (2015 is the most recent), and generalizability is not clear. For biomarkers derived from cancer cells, they may be more strongly associated with recurrence and PFS than with overall survival, which is more susceptible to other factors. Have these outcomes been considered? The significance of miR-219 may be clearer if its association with clinicopathological features is assessed.

REVIEWER	Zhou, Qiang Suining Central Hospital, Oncology
REVIEW RETURNED	31-Aug-2022

GENERAL COMMENTS	In the presented manuscript entitled “MiR-219-5p decrease the risk of cancer-related mortality in patients with small cell lung cancer”, the authors estimate the patients' risk factors in SCLC. They found that MiR-219-5p decreased the risk of cancer-related mortality in patients with SCLC. Nomogram based on multivariate analysis demonstrated good accuracy in estimating the risk of overall mortality. The study included 133 patients with SCLC and collected data from 86 patients at other hospitals for external

	validation. Statistical methods were rational, and the project is evidence-based. Here are some minor comments: 1. Currently, articles related to external validation are retrospective studies, and they need to comply with TRIPOD standards, and we advised authors to improve their papers according to STROBE standards and to upload TRIPOD reports. 2. The selection of predictors and the development of scores and the use of a Nomogram are not described in the method section. 3. How the cut-off value is selected for the numeric variables in the article is suggested to be displayed by the authors in the method section. 4. Additionally, the authors should carefully revise the manuscript for grammar, inappropriate/confused expressions, and spelling mistakes. For example. 'although nomograms' should be 'although nomogram'...

REVIEWER	Ca, Dinghua Yangzhou University
REVIEW RETURNED	03-Sep-2022

GENERAL COMMENTS	The authors investigated the association between miR-219-5p level and the prognosis of SCLC patients. The results showed that the mortality in group with high miR-219-5p level (≥ 1.50) was 74.6%. Patients with high miR-219-5p level (P <0.001, HR=0.36), immunotherapy (P <0.001, HR=0.44), PNI score >47.9 (P =0.01, HR=0.45) remained statistically factors for better OS and regarded as independent protective factors. This work is potentially interesting, but there are some concerns that need to be addressed as follows. Major comments 1) As the authors mentioned that microRNA plays a significant role in cancers. Dysregulation of miRNA has been shown to occur in various cancers. Did the authors detect the concentration of other important miRNAs, which are known to play important roles in the development of cancer. Please explain the reasoning for selecting only miR-219-5p for the investigation in this study. 2) This study was a 5-year follow up, and the authors revealed the mortality rate of the subjects. The authors should indicate if any of the patients enrolled died from any other causes. If there are deaths among the subjects, the authors should indicate if the miRNA is associated with the cause of death. 3) Please specify the diagnostic criteria for SCLC. 4) Figure 3 showed multivariate Cox regression analyze for OS. It seems that other characteristics have higher risk per the hazard ratio. Please give a reason. 5) Please give a rational in the manuscript why you adjust for age and gender (and not other variables) in table 2. You don´t mention this adjusted analysis is the Results section. Please comment on this or remove it. 6) Please include an explicit sample size assessment. Saying that you have done a calculation is not the same thing as presenting it. You don't need to write out what software you used. Minor comments

	1) Please indicate the full phrase for the abbreviation "OS" when it first appears in the abstract.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Masanori Nojima, the Institute of Medical Science, the University of Tokyo
Comments to the Author:
No major statistical problems, but insufficient verification of clinical usefulness. Please respond to the following points.

1. Figure 3 should be shown on a logarithmic scale.
Response: Thanks for your suggestion. We have added the logarithmic scale in Figure 3.

2. Was the nomogram in Figure 4 based on LASSO regression? If it was LASSO, describe the model parameters in detail.
Response: Thanks for your reminder. LASSO regression is suitable for filtering variables composed of high-throughput data, for example, to filter predictor variables among variables above 100 is often used in LASSO regression. Our data involved few variables, so we directly applied single-factor and multi-factor Cox regression analysis to screen variables.

3. To test the clinical usefulness of the nomogram, divide the cases into 3-4 groups according to the results of the nomogram application and then perform Kaplan-Meier analysis. This should be done for both training and test (validation) sets.
Response: Thanks for your reminder. We divided the training and control groups into four groups with Nomogram total scores of 40-60, 60-80,80-100, and 100-120, respectively, and plotted survival curves and performed Kaplan-Meier analysis, which was found to be statistically significant. (Page 13, Lines 233-237; Figure S1a, S1b)

4. The specificity of the green line appears to partially exceed 100%. It is strange that it goes to the left of the origin.
Response: Thanks for your reminder. Sorry, there was a problem with the axes in Figure 5, we have fixed it and re-uploaded Figure 5.

5. The clinical impact is not considered high because the data is old (2015 is the most recent), and generalizability is not clear. For biomarkers derived from cancer cells, they may be more strongly associated with recurrence and PFS than with overall survival, which is more susceptible to other factors. Have these outcomes been considered? The significance of miR-219 may be clearer if its association with clinicopathological features is assessed.
Response: Thanks for your reminder. Thank you very much for your suggestion, as this batch of data is from around 2015, it does have some limitations, which we have explained in the discussion section. We will carry out a deeper study in the future.

Reviewer: 2
Dr. Qiang Zhou, Suining Central Hospital
Comments to the Author:
In the presented manuscript entitled “MiR-219-5p decrease the risk of cancer-related mortality in patients with small cell lung cancer”, the authors estimate the patients' risk factors in SCLC. They found that MiR-219-5p decreased the risk of cancer-related mortality in patients with SCLC. Nomogram based on multivariate analysis demonstrated good accuracy in estimating the risk of overall mortality. The study included 133 patients with SCLC and collected data from 86 patients at

other hospitals for external validation. Statistical methods were rational, and the project is evidence-based. Here are some minor comments:

1. Currently, articles related to external validation are retrospective studies, and they need to comply with TRIPOD standards, and we advised authors to improve their papers according to STROBE standards and to upload TRIPOD reports.
Response: Thanks for your reminder. We have uploaded the TRIPOD reports.

2. The selection of predictors and the development of scores and the use of a Nomogram are not described in the method section.
Response: Thanks for your reminder. We have added these to the method section. (Pages 7-8, Lines 155-159)

3. How the cut-off value is selected for the numeric variables in the article is suggested to be displayed by the authors in the method section.
Response: Thanks for your reminder. We use the median as the cut-off value. We also added it to the method section. (Page 5, line 106)
4. Additionally, the authors should carefully revise the manuscript for grammar, inappropriate/confused expressions, and spelling mistakes. For example. 'although nomograms' should be 'although nomogram'...
Response: Thank you for your reminder. This revised manuscript has been edited and proofread by native speakers.

Reviewer: 3
Dinghua Ca, Zhenjiang People's Hospital
Comments to the Author:
The authors investigated the association between miR-219-5p level and the prognosis of SCLC patients. The results showed that the mortality in group with high miR-219-5p level (≥ 1.50) was 74.6%. Patients with high miR-219-5p level (P <0.001, HR=0.36), immunotherapy (P <0.001, HR=0.44), PNI score >47.9 (P =0.01, HR=0.45) remained statistically factors for better OS and regarded as independent protective factors. This work is potentially interesting, but there are some concerns that need to be addressed as follows.
Major comments
1) As the authors mentioned that microRNA plays a significant role in cancers. Dysregulation of miRNA has been shown to occur in various cancers. Did the authors detect the concentration of other important miRNAs, which are known to play important roles in the development of cancer. Please explain the reasoning for selecting only miR-219-5p for the investigation in this study.
Response: Thank you for your good suggestions. Recent studies have showed that miR-219-5p could participate in the progression and development of various cancers. To demonstrate the prognostic role of miR-219-5p in SCLC, we hope that this study can prove the relevant role of miR-219-5p, and lay the foundation for the next step of mechanism research. In the next experiment and analysis, we will further demonstrate the correlation between miR-219-5p and patients with SCLC. Therefore, we selected miR-219-5p for the investigation in this study.

2) This study was a 5-year follow up, and the authors revealed the mortality rate of the subjects. The authors should indicate if any of the patients enrolled died from any other causes. If there are deaths among the subjects, the authors should indicate if the miRNA is associated with the cause of death.
Response: Thank you for your recommends. Among the 133 patients in our study, 1 patients died of massive hemorrhage of unknown causes and 1 patient died of car accident. According to the relevant professional knowledge and literature reference, the death caused by these causes is not related to microRNA. Therefore, these dead patients have no impact on our overall results, that is, there is no impact on the relationship between microRNA and prognosis of SCLC.

3) Please specify the diagnostic criteria for SCLC.
Response: Thank you for your kind and careful comments. The clinical manifestations, cell morphology and therapeutic response of small cell lung cancer (SCLC) are significantly different from other types of lung cancer. 40 years ago, SCLC and non-small cell lung cancer (NSCLC) were distinguished. The most common types of SCLC are squamous cell carcinoma, adenocarcinoma, large cell carcinoma and other rare types. Although SCLC is associated with smoking, people who never smoke also develop lung adenocarcinoma. SCLC is less sensitive to chemotherapy and radiotherapy than non-small cell lung cancer. The treatment of SCLC should be based on histological type, stage, general health status and complications. When SCLC is suspected, the focus is to confirm the diagnosis and determine the scope and extent of the disease. The main diagnostic points are as follows: medical history, physical examination, routine laboratory evaluation, chest X ray, enhanced CT scan of chest and upper abdomen, biopsy. As well as experienced pathologists to check, distinguish small cells or non-small cells. Most lung tumors can be diagnosed by light microscopy. Immunohistochemistry and electron microscopy are helpful for Histotyping. In this study, the diagnosis of SCLC was confirmed by histopathological examination and other aspects.

4) Figure 3 showed multivariate Cox regression analyze for OS. It seems that other characteristics have higher risk per the hazard ratio. Please give a reason.
Response: Thank you for your comments. In the general population and in patients with SCLC, microRNA has consistently been shown to be associated with better or worse outcomes. However, miR-219-5p belong to microRNA, and other clinicopathological parameters belong to many other different types. There is no comparability between different types of parameters, that is, there is no way to compare which is more important or which is more influential. In our study, we mainly studied the effect of the expression of miR-219-5p on the prognosis of SCLC. It seems that other characteristics had higher risk per the hazard ratio. However, in the future research, we can carry out the research on the influence of this type on the prognosis of SCLC to further explore the mechanism of SCLC.

5) Please give a rational in the manuscript why you adjust for age and gender (and not other variables) in table 2. You don´t mention this adjusted analysis is the Results section. Please comment on this or remove it.
Response: Thank you for your good suggestions. Because age and gender may cause bias, we further check whether the data and results have an impact by calibrating age and gender.

6) Please include an explicit sample size assessment. Saying that you have done a calculation is not the same thing as presenting it. You don’t need to write out what software you used.
Response: Thank you for your kind comments. Sample size assessment was conducted by the software NCSS-PASS version 11.0 (https://www.ncss.com/software/pass/). We analyzed the data by Log-rank and Cox-regression. We entered into the part of survival-Logrank and survival-cox-regression of the PASS, then set the Power (1-beta) and Alpha (Significance Level) as 0.90 and 0.05 respectively. Finally, we calculate the sample size and found the number of patients we enrolled was larger than the sample size. The report of the sample size assessment was displayed in Supplemental Material Part II.

Minor comments
1) Please indicate the full phrase for the abbreviation "OS" when it first appears in the abstract.
Response: Thank you for your kind and careful comments. We have indicated the full phrase for the abbreviation "OS" when it first appears in the abstract. Please check the revision. (Page 2, line 45)

VERSION 2 – REVIEW

REVIEWER	Nojima, Masanori The Institute of Medical Science, the University of Tokyo, Center for Translational Research
REVIEW RETURNED	12-Oct-2022

GENERAL COMMENTS	1. Response: Thanks for your suggestion. We have added the logarithmic scale in Figure 3. → There is still no logarithmic scale in Figure 3. Scale of hazard ratio for the forest plot should be changed. When logarithmic scale applied, the points of scale would be set to 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 (if base unit is 2). 2. Response: Thanks for your reminder. We divided the training and control groups into four groups with Nomogram total scores of 40-60, 60-80,80-100, and 100-120, respectively, and plotted survival curves and performed Kaplan-Meier analysis, which was found to be statistically significant. (Page 13, Lines 233-237;Figure S1a, S1b) → Thank you for accepting my opinion. These are expected ones. Good dose-response relationship is observed.

REVIEWER	Zhou, Qiang Suining Central Hospital, Oncology
REVIEW RETURNED	01-Oct-2022

GENERAL COMMENTS	The authors have addressed all the comments.

REVIEWER	Ca, Dinghua Yangzhou University
REVIEW RETURNED	01-Oct-2022

GENERAL COMMENTS	No further comments.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

1. Response: Thanks for your suggestion. We have added the logarithmic scale in Figure 3.

→ There is still no logarithmic scale in Figure 3. Scale of hazard ratio for the forest plot should be changed. When logarithmic scale applied, the points of scale would be set to 0.125, 0.25, 0.5, 1, 2, 4, 8, 16 (if base unit is 2).

Response: Thanks for your suggestion. We have revised the Figure 3.

VERSION 3 – REVIEW

REVIEWER	Nojima, Masanori The Institute of Medical Science, the University of Tokyo, Center for Translational Research
REVIEW RETURNED	02-Nov-2022

GENERAL COMMENTS	The scale itself was appropriately revised in Figure 3, but even if they are on a logarithmic scale, it could be better that the original hazard ratios are used for the bottom ticks, such as 0.25, 0.5, 0, 1, 2, 4, 8 instead of -2, -1, 0, 1, 2, 3. Please correct that in the final proofreading. Re-review is not required.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1
Comments to the Author:
The scale itself was appropriately revised in Figure 3, but even if they are on a logarithmic scale, it could be better that the original hazard ratios are used for the bottom ticks, such as 0.25, 0.5, 0, 1, 2, 4, 8 instead of -2, -1, 0, 1, 2, 3. Please correct that in the final proofreading. Re-review is not required.
Response: Thanks for your suggestion.We have revised the Figure 3 based on your suggestion. Please check the revision.