

Peer Review File

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Reviewer A

I have some comments that I hope the authors will consider.

1. While pancreas cancer retains a terrible prognosis, some of the survival statistics seem to be outdated.

Reply 1: We have modified some updated data about survival and change reference 2.

- Page 5 line 55-58.
Changes in the text: “Despite advances of recent years in the oncological management of pancreatic cancer, remains one of the most lethal neoplasms with a 5-year survival rate ranges 5% to 15% and an OS rate of 6% (2), mainly due to early recurrence or progression of the disease despite optimal treatment;”
- Page 17 line 332.
Changes in the text: “2. Puckett, Y., & Garfield, K. (2022). Pancreatic Cancer. In StatPearls. StatPearls Publishing.”

2. The use of the N-to-L ratio has been researched before. The authors comment on this but it is not clear to me how the present work either adds to or distinguishes itself from prior work. Are there instances they discovered where the ratio indicates something the CA 19-9 does not, or is it useful in instances of CA 19-9 nonproducers?

- Reply 2: NLR reflects the response of inflammatory cells in the host which play an important role in tumor development, whereas CA 19-9 reflects the intrinsic properties of cancer cells and the low positive predictive value.

3. The authors state that a higher ratio means more aggressive treatment is indicated. What does this mean, exactly; what is more aggressive? And how would this impact a better outcome?

Reply 3: Locally advanced disease requires “more aggressive treatment” which means neoadjuvant treatment with chemotherapy and/or radiation, which affects the quality of life of patients compared to surgical resection. We added the meaning of “more aggressive treatment”.

- Page 11 line 203-206.
Changes in the text: “Therefore, it is of utmost importance to identify prognostic biomarkers that will help us to select patients who may benefit from more aggressive treatment strategies (neoadjuvant chemotherapy and/or radiation) and closer follow-up to prevent and diagnose early recurrences respectively.”

4. Does the ratio change overtime and does this result in an improved outcome?

- Reply 4: No, NLR is a static cut-off in our study. Better results only when patients are detected in early stages.

Reviewer B

The paper titled "Combination of Neutrophil-to-Lymphocyte Ratio and Serum CA 19-9 as a Prognostic Factor in Pancreatic Cancer" is an original research article that focuses on the prognostic significance of combining neutrophil-to-lymphocyte ratio (NLR) and serum CA 19-9 levels in patients with pancreatic cancer. This retrospective study, which analyzed 153 pancreatic cancer patients from 2013 to 2018, aims to assess the prognostic value of pre-treatment NLR and serum CA 19-9 levels, both separately and combined. The study found that high NLR and high CA 19-9 values were independently associated with poor overall survival (OS) and progression-free survival (PFS). A novel classification system combining these biomarkers showed better prognostic significance than when used individually. The study suggests that this combination could be a useful tool in predicting outcomes and guiding treatment strategies in pancreatic cancer patients.

Abstract:

1. Consider adding specific statistical values (e.g., hazard ratios, p-values) to the results section for clarity.

Reply 1: We added statistical values.

- Page 2 line 35-44.

Change in the text: "The cut-off value determined for NLR was 2.4 and for serum CA 19-9 was 553 U/mL. Survival analysis showed that the 5-year overall survival (OS) was 9% in patients with low-NLR compared with 2% for patients with high-NLR ($p=0.008$), and 5-year progression-free survival (PFS) was 5.7% in patients with low-NLR compared with 1.3% in patients with high-NLR ($p=0.007$). For patients with low-CA-19.9, 5-year OS was 8.5% compared with 0% for patients with high-CA-19-9 ($p=0.002$), and 5-year PFS was 4.1% in patients with low-CA 19-9 compared with 0% in patients with high-CA 19-9 ($p=0.005$). Classification groups created showed that 5-year OS in Group 1 (low-NLR and low-CA19-9) was 11.8% compared with 1.9% for patients in Group 2 ($p<0.001$) (either one or both high-NLR or CA 19-9), and 5-year PFS was 8.6% in Group 1 and 0% in Group 2 ($p=0.001$)."

Introduction:

1. Please expand on the pathophysiological link between NLR, CA 19-9, and pancreatic cancer progression to provide a more comprehensive biological rationale for using these markers.

Reply 1: We added information on the pathophysiology of pancreatic cancer and biomarkers and changed references from 4-13.

- Page 5-6 line 65-76.

Changes in the text: "CA 19-9 is a cell surface glycoprotein complex, produced by the ductal cells of the pancreas and other organs. It is overexpressed in a wide range of benign

diseases such as cholestasis and malignant diseases, mainly in pancreatic ductal adenocarcinoma (13-14). It has been used as useful biomarker in diagnosis, assessment of resectability, monitoring response to treatment, prognosis, and surveillance in pancreatic cancer (4-7). It reflects biological aggressiveness and is a predictor of hematogenous dissemination, micrometastatic and metastatic disease since CA 19-9 plays a role in malignant cell-adhesion to endothelial cells and transmigration (8,9).

CA 19-9 values are a valuable prognostic marker in patients undergoing curative resection or neoadjuvant chemotherapy. Previous studies have reported the association between CA 19-9 levels and survival in pancreatic cancer. Therefore, postoperative follow-up is important since an increase in serum levels of CA 19-9 predicts the recurrence of pancreatic cancer (10-12).”

- Page 17 line 335-336.
Added in the text: “4. Lee, T., Teng, T. Z. J., & Shelat, V. G. (2020). Carbohydrate antigen 19-9 - tumor marker: Past, present, and future. World journal of gastrointestinal surgery, 12(12), 468–490.”
- Page 17 line 337-339.
Added in the text: “5. Dogan M, Algin Efnan, Guven ZT, et al. Neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, neutrophil-platelet score and prognostic nutritional index: do they have prognostic significance in metastatic pancreas cancer? Curr Med Res Opin 2018; 34: 857-863.”
- Page 6 line 77-81.
Added in the text: “NLR is one of the most studied indicators of systemic inflammatory response. Neutrophils are known to infiltrate tumors, contributing to the tumor microenvironment for secretion of cytokines, while lymphocytes induce cell death. NLR has been increasingly valued as a fundamental prognostic factor in the different stages of the disease, suggesting that high levels of NLR reflect unresectable disease or progression depending on the case (15-16).”
- Page 19 line 362-365.
Added in the text: “15. Shin, K., Jung, E. K., Park, S. J., Jeong, S., Kim, I. H., & Lee, M. A. (2021). Neutrophil-to-lymphocyte ratio and carbohydrate antigen 19-9 as prognostic markers for advanced pancreatic cancer patients receiving first-line chemotherapy. World journal of gastrointestinal oncology, 13(8), 915–928.”
- Page 19 line 366-369.
Added in the text: “16. Iwai, N., Okuda, T., Sakagami, J., Harada, T., Ohara, T., Taniguchi, M., Sakai, H., Oka, K., Hara, T., Tsuji, T., Komaki, T., Kagawa, K., Yasuda, H., Naito, Y., & Itoh, Y. (2020). Neutrophil to lymphocyte ratio predicts prognosis in unresectable pancreatic cancer. Scientific reports, 10(1), 18758.”

Methods:

1. Clarify the criteria used for patient selection, including any exclusion criteria.

Reply 1: We added selection criteria Page 7 line 104-106 and exclusion criteria are on the page 7 line 106-108.

- Page 7 line 104-106.

Changes in the text: We included patients with histologically confirmed PDAC, over 18 years, complete file with serum levels of lymphocytes, neutrophils and CA 19-9.

2. On page 6, line 97, standardize the notation to “CA19-9” for consistency.

- Reply 2: We changed Ca19-9 for CA19-9. Page 6 line 97.

3. The manuscript would benefit from a clearer explanation of the statistical methods employed, particularly in determining cutoff values for NLR and CA 19-9.

Reply 3: We added a detailed explanation about statistical methods.

- Page 8 line 133-135.

Added in the text: The optimal cut-off values were first determined for the entire cohort and later it was identified independently within the subgroups. The best cutoff value of NLR was 2.4, which was comparable with previous studies and the cutoff value of CA19-9 was 553 U/mL.

4. Include a statement about handling missing data or outliers in the analysis.

- Reply 4: In our analysis, outliers were not removed.

Results:

1. Consider adding subgroup analyses, if applicable, to explore the effects in different patient populations, particularly in subgroups like patients who underwent surgery, chemotherapy ± radiotherapy only, and best supportive care only.

- Reply 1: We analyzed the treatment only in the univariate and multivariate analysis; in another study we could analyze a cut-off point according to the treatment received.

2. A detailed analysis of the survival outcomes for each of the four possible combinations based on the cutoffs for CA19-9 and NLR would be informative. Additionally, the rationale behind the decision to evaluate only two groups should be clarified in this study.

- Reply 2: The analysis of survival is on Page 10-11 line 167-190. About the evaluation of 2 groups, we considered groups for bad and good prognostic.

Discussion:

1. Provide an explanation sufficient to convince the reader that this study did not use other strong prognostic factors such as performance status, clinical stage, presence of metastases, or treatment strategy.

- Reply 1: We did it, we used factors such ECOG-Ps, clinical stage, tumor size, presence of metastases, tumor differentiation grade and vascular involvement. Table 2 and 3.

2. We should consider whether the cutoff values were reproducible by treatment strategy or by stage.

- Reply 2: The cut-off values were reproducible by stage because the serum values were collected at diagnosis.

3. Compare the results of this study with the existing literature, highlighting similarities and differences in cutoff values and prognostic impact.

Reply 3: We compared our study with another similar literature.

- Page 12 lines 221-225.
Changes in the text: “The prognostic significance of NLR in pancreatic cancer was explored by another studies and is matching with our cut-off (2.4), the other levels that ranged from 2.62 to 3.74. In general, all the results reveal that a high NLR at the time of diagnosis or post-treatment could be an independent indicator of poor prognosis and is associated with metastatic stage in patients with pancreatic cancer (16,30-31).”
- Page 20 lines 403-405.
Added in the text: “30. Onoe S, Maeda A, Takayama Y, et al. The Prognostic Impact of the Lymphocyte-to-Monocyte Ratio in Resected Pancreatic Head Adenocarcinoma. Med Princ Pract. 2019;28(6):517-525.”
- Page 21 lines 406-408.
Added in the text: “31. Shin K, Jung EK, Park SJ, Jeong S, Kim IH, Lee MA. Neutrophil-to-lymphocyte ratio and carbohydrate antigen 19-9 as prognostic markers for advanced pancreatic cancer patients receiving first-line chemotherapy. World J Gastrointest Oncol. 2021;13(8):915-928.”

Conclusion:

1. Reinforce the potential clinical utility of combining NLR and CA 19-9 in pancreatic cancer prognosis.

Reply1: We added some information for clinical utility of the parameters.

- Page 16 line 307-312.
Added in the text: “Although a specific cut-off value has not been established, our study has demonstrated the prognostic utility of the combination of NLR and CA19-9, when both parameters are elevated, they have worse survival than patients with both parameters below the cut-off point and assume disease in advanced stages.”

2. Offer recommendations for how these findings might be integrated into current clinical workflows.

Reply 2: We added some recommendation about when it is useful to implement these parameters.

- Page 16 line 312-313.

Added in the text: Furthermore, the implementation of these parameters is a great option since they are very practical to calculate in daily clinical practice.

General Suggestions:

1. The paper would benefit from thorough proofreading to correct any typographical or grammatical errors.

Reviewer C

This manuscript 'Combination of Neutrophil-to-Lymphocyte ratio and serum CA 19-9 as a prognostic factor in pancreatic cancer' is an original article presenting the efficacy of NLR plus CA19-9 to predict prognosis of pancreatic cancer (PC).

Prediction of pancreatic cancer prognosis is useful for the determination of a therapy plan. Until now, some biomarkers have been reported as useful, for example: NLR, LMR, CA19-9, PNI, etc. In this manuscript, the authors emphasize the improved usefulness of a combination of CA19-9 and NLR compared to solo CA19-9 and solo NLR. This result is a new finding. If in fact true, it is very exciting.

My suggestions are follows.

Major

1. The authors stated that a combination of CA19-9 and NLR was a better predictor than solo CA19-9 and NLR. Although there were no results to show better usefulness of the combination group. All of solo NLR, solo CA19-9, and a combination were excellent predictive factors of PC prognosis with a significant difference. There were different P-values between the three groups, but these differences could not show the superiority or inferiority between the groups. Accordingly, the authors should show the difference between these groups using another statistical method (For example: study of sensitivity, specificity, and accuracy for predicting 12-month life expectancy).

- Reply 1: The X-tile method is a useful tool in data analysis for stratifying continuous variables in survival studies, widely used in medical oncology research and other fields analyzing survival or continuous response data. X-tile provides a systematic way to divide a continuous variable into groups, helping identifying patterns or associations that might not otherwise be evident. Therefore, we decided to continue using this statistical test in our study "Combination of Neutrophil-to-Lymphocyte ratio and serum CA 19-9 as a prognostic factor in pancreatic cancer."

2. Authors should further discuss their reasoning for the superiority of the combination group over solo CA19-9 and solo NLR.

Reply 2: We did it, see Conclusions section, page 16 line 308-313.

Minor

1. Manuscript English is inadequate and an English editing service should be utilized. As examples, the following expressions need revision.

L38 com-pared->compared

L69 Traditionally -> Traditionally,

L96 Informed consent from patients was waived due to the retrospective nature.

- Authors should provide a notification on their homepage or via posters at the hospital.

- Reply L96: Due to retrospective nature of our article, the Investigation Committee at “Instituto Nacional de Cancerología” approved “no need” for informed consent. We attached the official document in previous email.

L119 chi-squared or Fisher exact test -- Chi-squared or Fisher’s exact test

L126 Ca19-9 - CA 19-9

L128 chi-squared -- Chi-squared

L161 The median and 1-, 3- and 5-year OS were 13.2 months (11.2 – 15.1) and 58.7%, 15.1%, and 9.0% respectively in low-NLR group

----The median OS, 1-, 3-, 5-year survival rates were -----

- Reply 1: changes in the expressions above were made.
2. In the discussion section, the authors state the same facts repeatedly. As such, the discussion can be further refined.
- Reply 1: changes were made.