January 4, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14682-review.doc).

Title: A novel immunological and nutritional-based prognostic index for gastric cancer

Author: Kai-Yu Sun, Jian-Bo Xu, Shu-Ling Chen, Yu-Jie Yuan, Hui Wu, Jian-Jun Peng, Chuang-Qi Chen, Pi Guo, Yuan-Tao Hao, Yu-Long He

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14682

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer. The responses are included below. Comments are indicated in bold, followed by our replies.

Reviewer #1

1. **The study performed by Sun et al. is very interesting, but it needs to be generally shortened and revised for some clarifications, as below specified.**

Reply: Thanks for your appreciation and comments. We have tried our best to shorten the manuscript to make it more concise and have revised the manuscript as you suggested.

2. Major comments:

a. For admission of the authors themselves the analysis includes also patients underwent palliative resection (R+ 123 and M+ 158): these patients had a (statistically significant) higher rate of poor nutritional status. Moreover, R resection is a well-known independent prognostic factor for gastric cancer (as resulted also in the multivariate models of this study). This is a great bias for the correct interpretation of the real prognostic impact of the nutritional scores.

Reply: Thanks for your comments. As you concern, it is true that we have to minimize the bias from other resources (eg. patients underwent palliative resection, R resection, et al) in order to correctly interpret the real prognostic impact of the nutritional scores. Therefore, in our original manuscript, we have applied the propensity score analysis to adjust the influence of any potential factor involved in both group selection and patient survival (eg. age, sex, pathological stage, et al.) in the univariate analysis. And these factors might have included resectability as you concerned. Moreover, the method of addressing bias with propensity score analysis has also been performed in many other papers. Examples are the following:

1. Wong MC, Tam WW, Lao XQ, et al. The incidence of cancer deaths among hypertensive patients in a large Chinese population: A cohort study. Int J Cardiol 2015; 20;179:178-85.
2. Shen SL, Fu SJ, Chen B, et al. Preoperative aspartate aminotransferase to platelet ratio is an independent prognostic factor for hepatitis B-induced hepatocellular carcinoma after hepatic resection. Ann Surg Oncol 2014;21(12):3802-9.
3. Hirokawa F, Kubo S, Nagano H, et al. Do patients with small solitary hepatocellular carcinomas without macroscopically vascular invasion require anatomic resection? Propensity score analysis. Surgery 2015;157(1):27-36.

b. The variables included in the last rows of the Table 1 (from "postoperative complications") are not conditions associated to the poor nutritional status, but actually they are direct sequelae of the poor nutritional status. They are not to be included in this table.

Reply: Thanks for your concerns. The postoperative complications we included in **Table 1** indicated any potential postoperative complication in a broad sense such as bleeding, anastomotic stricture, adhesive intestinal obstruction, deep venous thrombosis, hypostatic pneumonia, fistula and so on. These complications are not exclusively associated with poor nutritional status as they can be caused by other factors such as poor operational skills or incomplete postoperative nursing. Therefore, it seemed reasonable that we got the result: postoperative complications are not conditions associated to the poor nutritional status. However, in our study, we showed that severe postoperative complications were significantly associated with poor nutritional status as you suggested. It indicated that poor nutritional status might lead to severe postoperative complications and this finding was useful in clinical practice. However, our results might require prospective studies with larger sample size to further confirm.

Minor corrections:

a. Abbreviations in the "Novelty of the study" are not explained;

Reply: We are sorry to have missed the abbreviations in this part and now we have added the explanations to the corresponding abbreviations.

b. Some language mistakes;

Reply: We are terribly sorry to have made some language mistakes. We have gone through the entire manuscript again and corrected the mistakes as necessary.

c. In "Methods" section ("patients" part) delete "...for whom this was indicated and the type of gastrectomy was determined in a departmental meeting": this decision generally occurs in the intraoperative phase;

Reply: Thanks for your recommendation. We have deleted this sentence as you suggested.

d. In "Methods" section ("patients" part) it is unhelpful the last sentence

(otherwise the authors should present an diagram flow with the excluded patients);

Reply: Thanks for your concern. As you suggested, we have deleted the last sentence of "**Patients**"part in "**Methods**"section.

e. In "Methods" section ("data" part) the sentence "Events occurring within 30 days..." should be included in the "Statistical analysis" part;

Reply: Thanks for your suggestion. We have included the sentence "Events occurring within 30 days…"in the "**Statistical analysis**"part.

f. In "Methods" section ("Statistical analysis" part) the authors should avoid the term "independent prognostic value" for the univariate analysis;

Reply: We are sorry to have made these interpreting errors. In the revised manuscript, we have re-stated the corresponding sentences by avoiding the term "independent prognostic value" for the univariate analysis.

g. Authors should add a table on concordance between the different nutritional scores.

Reply: Thanks for your recommendation. We have added a new table (**Table 1**) on concordance between the different nutritional scores to make it more concise and readable.

h. Remove Table 4 and Figure 3;

Reply: Thanks for your recommendation. To make the manuscript more concise, **Table 4** and **Figure 3** have been removed in the revised manuscript as you suggested.

i. In figure 2 remove graphs E-L;

Reply: Graphs E-L have been removed from **Figure 2**.

l. In the tables it is not never included the symbol for "higher (or lower) and

equal".

Reply: We are terribly sorry to have missed the symbol of "equal" in some spaces. We have corrected these errors in the corresponding tables.

m. In the tables the authors used Fish's exact test without any specification.

Reply: Thank you for detecting this problem. After carefully checking the data and performing the statistical analysis again, we found that in the χ^2 analysis of association between PNI and ALB, 0 cells (0.0%) have expected count less than 5. Therefore, there was no need to apply Fish's exact test and we just need to perform the regular Pearson χ^2 analysis. We have correspondingly deleted this text in **Table 2**.

n. Discussion is to be significantly shortened.

Reply: Thanks for your concern. The **Discussion** part might be too long as originally we not only hoped to discuss our findings with the results of other literatures but also to demonstrate potential mechanisms behind our findings and then prospect into future researches and clinical application. Now, as you suggested, we have tried our best to shorten the **Discussion** part.

Reviewer #2

This study, while interesting, is not sufficient to be published in its current state. The authors compared the prognostic significance of different immuno-nutritional indices including PNI, NLR and PLR in a large cohort of gastric cancer patients, and proposed a new index-Canton score, which is superior to other indexes in predicting OS. There are some questions and comments as following:

Reply: Thanks for your appreciations and comments. We have endeavored to improve the manuscript as you suggested.

Major Revisions:

1. **The inclusion and exclusion criteria of patient enrolment were not provided**

in detail. How about the patients coexisting with other malignancies or synchronous immune diseases, such as syphilis or hyperthyroidism?

Reply: Thanks for your questions. It is true that we have not provided the exclusion criteria of patient enrollment in detail. As you suggested, we have added more details to the enrollment criteria in "**Methods**" section ("**Patients**" part). Patients with other malignancies or synchronous immune diseases were actually excluded in our original manuscript. In terms of the inclusion criteria of patient enrollment, we have stated several sentences in "**Methods**" section ("**Patients**" part). Examples are as the following: we enrolled 632 patients with *histologically proven gastric cancer who underwent gastrectomy between January 1998 and December 2008 at the First Affiliated Hospital of Sun Yat-Sen University*. They were all *aged over 18 years*, and *complete clinical and laboratory data were available* in each case.

2. **The authors said that PNI was an independent prognostic factor for OS in gastric cancer, especially in patients with advanced disease, however they didn't mention whether these patients received albumin supporting treatment before surgical resection, as transfusion of albumin could directly influence the level of PNI. How to invade or minimize this influence?**

Reply: Thanks for your concerns. On the one way, the blood tests such as the level of serum albumin and total lymphocyte count were obtained in the first or the second day of patient admissions when they have not received any treatment. Therefore, the level of PNI was generated by the patients'baseline nutritional and immunological status without the influence of other supporting treatments such as transfusion of albumin. On the other way, prospective clinical studies have failed to find any benefit for transfusion of albumin, therefore, the preoperative albumin supplementation could not significantly influence the patients'overall survival (OS). In light of these two aspects, the preoperative transfusion of albumin did not influence either the level of PNI or patients'OS, thus, the association between PNI and OS was not biased due to this factor. In order to clarify this issue, we have added some explanations about this issue in the "**Methods**"section ("**Data**"part) and the"**Discussion**"section.

3. **In this paper, the authors declared that PNI, NLR, PLR were associated with OS, and PNI showed independent prognostic significance for OS. After combining PNI, NLR, PLR, ALB, and PLT to generate several new indices and comparing them, they found index with the greatest prognostic significance was a combination of PNI, NLR, and PLT, and they refer to this as Canton score. Two questions should be answered.**

1) How about the prognostic value of index including PNI, NLR and PLR?

Reply: Thanks for your question. Actually, we have originally estimated the prognostic values of different derived indices but we did not provide the corresponding results in the manuscript for saving thesis length. As you concerned, we have provided the relevant methods in the "**Methods**" section ("**Statistical analysis**" part) and all the results of other derived indices in the Supporting information (**Table S1**). The AUC of index including PNI, NLR and PLR was 0.679 (end-of follow up), 0.638 (12-month), 0.651 (36-month) and 0.647 (60-month).

2) How to compare these derived indices? Why you choose index combining PNI, NLR, PLT as the greatest prognostic factor? The methods and results should be provided.

Reply: Thanks for your questions and comments. Actually, we have originally estimated and compared the prognostic values of different derived indices but we did not provide the corresponding results in the manuscript for saving thesis length. We first calculated the areas under the ROC curves (AUC) of all the derived indices (a total number of 15) to compare the predictive ability of each index in different time points (the end of follow-up, 12 months, 36 months and 60 month), respectively. Then, we found two indices with the greatest prognostic significance than other combinations (**Table S1**). They were the combination of PNI, NLR and PLT and the combination of PNI, NLR, PLR and PLT. With the advantage of convenience, the combination of PNI, NLR and PLT, which we referred to Canton score, was chosen as the novel prognostic index considering there was no significant difference between

these two derived indexes. Next, we further compared the predictive value of Canton score with that of PNI by comparing their AUC using the z-test. The difference was considered significant if p value is less than 0.05. As you concerned, we have provided the relevant methods in the "**Methods**" section ("**Statistical analysis**" part) and all the results of other derived indices in the Supporting information (**Table S1**).

Table S1. The areas under the receiver operating characteristics curve (AUC) for survival of gastric patients based on all the derived prognostic scores at the end of follow-up, or after 12, 36, or 60 months.

Item	All survival		12 months		36 months		60 months	
	AUC	P value	AUC	P value	AUC	P value	AUC	P value
PNI	0.630	<0.001	0.602	<0.001	0.621	<0.001	0.614	<0.001
NLR	0.613	<0.001	0.593	<0.001	0.587	<0.001	0.588	<0.001
PLR	0.611	<0.001	0.580	0.002	0.592	<0.001	0.593	<0.001
PLT	0.573	0.004	0.564	0.013	0.562	0.007	0.566	0.005
PNI&NLR	0.535	0.493	0.502	0.968	0.476	0.570	0.487	0.776
PNI&PLR	0.477	0.656	0.480	0.644	0.435	0.123	0.455	0.309
PNI&PLT	0.498	0.970	0.493	0.867	0.469	0.468	0.449	0.251
NLR&PLR	0.477	0.647	0.447	0.229	0.459	0.329	0.472	0.522
NLR&PLT	0.487	0.796	0.466	0.437	0.488	0.772	0.461	0.382
PLR&PLT	0.440	0.240	0.448	0.237	0.448	0.219	0.442	0.190
PNI&NLR&PLR	0.679	<0.001	0.638	<0.001	0.651	<0.001	0.647	<0.001
PNI&NLR&PLT	0.684	<0.001	0.655	<0.001	0.657	<0.001	0.654	<0.001
PNI&PLR&PLT	0.668	<0.001	0.634	<0.001	0.647	<0.001	0.646	<0.001
NLR&PLR&PLT	0.660	<0.001	0.627	<0.001	0.629	<0.001	0.632	<0.001
PNI&NLR&PLR&PLT	0.685	<0.001	0.647	<0.001	0.657	<0.001	0.655	<0.001

4. **The Canton score combined PNI, NLR and PLT, the authors defined item that PNI > 48, NLR < 1.83 and PLT < 3 × 10^{11}/L as value 3, which was the highest value of Canton score. (Table 3) Moreover, the authors found that higher Canton score indicating higher risk of death both in univariate and multivariate analysis. (Line 10-16 Page 14 and Table 5) However, in the section of Survival in Results (Line 1-4 Page 12), the authors showed that high PNI, low NLR and low PLR were associated with better OS (Figure 1 and Table 2). Combining with this conclusion and the definition of Canton score, it seemed that higher Canton score might have better survival,**

although the factor enrolled was PLT, instead of PLR. So two questions should be answered.

1) **In Table 2, the authors showed that PLT was associated with OS in univariate analysis, which had better survival? High or low group?**

Reply: First of all, we are terribly sorry to have made such confusions to you. Actually, all the confusions were from the mistakes we made in the definitions of Canton score in **Table 3**. The correct definitions of Canton score were as the following:

Items	Value of Canton score
PNI ≥ 48, NLR≤1.83 and PLT≤3 × 1011/L	0
PNI ≥ 48, NLR ≤ 1.83 and PLT>3 × 1011/L	1
PNI ≥ 48, NLR>1.83 and PLT≤3 × 1011/L	1
PNI<48, NLR ≤ 1.83 and PLT ≤ 3 × 1011/L	1
PNI ≥48, NLR >1.83 and PLT>3 × 1011/L	2
PNI<48, NLR≤1.83 and PLT >3 × 1011/L	2
PNI<48, NLR >1.83 and PLT ≤ 3 × 1011/L	2
PNI<48, NLR>1.83 and PLT>3 × 1011/L	3

Therefore, higher Canton score indicated higher risk of death both in univariate and multivariate analysis which was not contradicted to the conclusion that high PNI, low NLR and low PLR were associated with better OS. In this sense, low level of PLT was associated with better OS.

2) **How to explain the contradiction described above? Merely due to the difference of PLT and PLR?**

Reply: We would like to express our apologies to make this confusion for our carelessness again. Actually, all the contradiction was from the mistakes we made in the definitions of Canton score in **Table 3**. The correct definitions of Canton score were as the following:

Items	Value of Canton score
PNI ≥ 48, NLR≤1.83 and PLT≤3 × 1011/L	0
PNI ≥ 48, NLR ≤ 1.83 and PLT>3 × 1011/L	1
PNI ≥ 48, NLR>1.83 and PLT≤3 × 1011/L	1
PNI<48, NLR ≤ 1.83 and PLT ≤ 3 × 1011/L	1

PNI ≥48, NLR >1.83 and PLT>3 × 1011/L	2
PNI<48, NLR≤1.83 and PLT >3 × 1011/L	2
PNI<48, NLR >1.83 and PLT ≤ 3 × 1011/L	2
PNI<48, NLR>1.83 and PLT>3 × 1011/L	3

Therefore, higher Canton score indicated higher risk of death both in univariate and multivariate analysis which was not contradicted to the conclusion that high PNI, low NLR and low PLR were associated with better OS.

5. **The authors said that the maximum sensitivity, specificity, and agreement rate of Canton score for predicting prognosis were 84.6%, 34.9%, and 70.1%. However, the specificity was too low which greatly limited its application in clinical practice.**

Reply: Thanks for your concern. It is true that the specificity was relatively lower compared to the sensitivity, however, we respectfully think that the sensitivity was more meaningful in its application in clinical practice and the low specificity did not limit its application too much due to the following two reasons. First, the sensitivity as high as 84.6% was very sensitive to help us identify those patients with poor prognosis with Canton score before surgery and led us to give them preoperative medical treatments to achieve better nutritional and immunological status. Second, preoperative nutritional and immunological supporting treatment would not bring harm to patients with low level of PNI even though they were not necessarily with poor prognosis due to the low specificity of PNI. We respectfully agreed that it is an ideal situation that PNI holds both the high sensitivity and specificity. However, in our manuscript, we focused on the PNI's capability of detecting patients with risks while the low sensitivity has its limitations to some extent but not significant.

Minor Revisions:

1. **The authors said patients were routinely followed up, are there any patients lost to follow up? This should be mentioned.**

Reply: Thanks for your concern. In our study, there are 43 patients lost to follow up and the follow-up rate reached 93.2%. And this information has been added in the

Results part.

2. **In Table 1 and Table 2, patients whose WBC=11*10^9/L or ALB=35g/L or PLT=300*10^9/L or CEA=5ng/ml were divided into which groups? High or Low? These information should be described accurately.**

Reply: Thanks for your suggestions. We have divided WBC=11*10^9/L or ALB=35g/L or PLT=300*10^9/L or CEA=5ng/ml to the corresponding groups. These information have been added in **Table 1** and **Table 2**.

3. **In Table 2, Multivariate analysis revealed that patients with R1R2 resection had better survival (HR=0.485, 95% CI: 0.266-0.883, p=0.018) comparing with those patients received R0 resection. However, in Table 5, the results showed that patients with R1R2 resection had worse survival (HR=1.567, 95% CI: 1.204-2.040, p=0.002) comparing with those patients received R0 resection. The authors should check these data carefully.**

Reply: After carefully checking the data, we are terribly sorry to have made the reporting errors in **Table 2**. The accurate interpretation was that multivariate analysis revealed that patients with R1R2 resection had worse survival (HR=2.062, 95% CI: 1.133-3.759, p=0.018) comparing with those patients received R1R2 resection. And this finding was consistent with the results showed in **Table 5** that patients with R1R2 resection had worse survival (HR=1.567, 95% CI: 1.204-2.040, p=0.002) comparing with those patients received R0 resection.

4. **In Figure 4, the authors showed that the AUC at these four points were significantly greater for Canton score than for PNI (p = 0.022, p = 0.030, p < 0.001, and p = 0.024, respectively), however, these p values were inconsistent with the description in the paper (Line 7-10 Page 14). The author should carefully check these data.**

Reply: We have checked the data and performed the statistical analysis again to confirm the results. Finally, we found that the contradiction came from the

inconsistent order of p values between Figures and the texts of manuscript. In **Figure 4**, the order of the p values of AUC was the end of follow-up, 12-month, 36-month and 60-month, however in the text of manuscript, we reported the p values in the order of 12-month, 36-month, 60-month and the end of follow-up. Therefore, we have corrected the text values in the manuscript to correspond to **Figure 4**.

5. **In Table 5, the authors demonstrated that Canton score was an independent prognostic factor in multivariate analysis. However, the variables enrolled in the univariate and multivariate analysis should be described, not only the results.**

Reply: Thanks for your suggestions. We have originally estimated all the variables in the univariate and multivariate analysis but we did not describe all the results in **Table 5** for saving thesis length. However, as you hoped us to describe all the variables enrolled in both analyses, we have supplemented the results of the rest of variables in a new **Table S2.** We only kept the results of two variables in the current **Table 4** because another reviewer recommended us to reduce the length of tables as much as possible.

6. **This sentence "We found that the 5-year OS of patients with a low PNI and stage II or III disease was significantly shorter, but no significant association was found between PNI and OS, although the 5-year OS of patients with a low PNI was slightly shorter" (Line 21-22 Page 16 and Line 1-2 Page 17) should be well organized and rewritten.**

Reply: Thank you for pointing out this mistake. We have rewritten this sentence as the following: We found that for patients in stage II or III, 5-year OS was significantly shorter in the group of low PNI, however, for patients in stage I or IV, no significant association was found although the 5-year OS of patients with a low PNI was slightly shorter.

7. **The numbers of tables and figures should be simplified, removing the**

unnecessary charts, for example, Table 4 or Figure 4. What's more, the section of Discussion should be shortened.

Reply: Thanks for your recommendation. The **Discussion** part might be too long as originally we not only hoped to discuss our findings with the results of other literatures but also to demonstrate potential mechanisms behind our findings and then prospect into future researches and clinical application. However, to make the manuscript more concise, we have removed **Table 4** and tried our best to shorten the **Discussion** section.

Reviewer #3

1. Overall this is an interesting study and the work is generally clearly presented and described.

Reply: Thanks so much for your appreciation to our manuscript. We have endeavored to improve our manuscript as you commented in the following.

2. However the manuscript would benefit from a reduction in length. A lot of detailed data is presented and I recommend that the number/size of tables/figures is reduced. Table 1 is too long and the rows presenting data on postoperative complications, infectious, surgical, medical and severe complications should be removed as these are not in themselves prognostic factors but rather are manifestations of these factors. Also simplify figure 2 by focusing on the PNI data (remove figs E-L) and remove figure 3 and table 4 as the data can be adequately described in the text.

Reply: Thanks for your valuable advices. We have tried our best to shorten the manuscript to make it more concise and readable. In terms of the number of tables/figures, we have removed **Figure 2 (E-L)**, **Figure 3** and **Table 4** as you suggested. Moreover, we have deleted some data on infectious, surgical and medical from **Table 1** as we agreed that these are not in themselves prognostic factors but rather are manifestations of these factors. However, we respectfully did not remove that data on postoperative complication and severe postoperative complication in

Table 1 due to the following two reasons: first, our study focused on the long-term prognosis and the short-term prognosis such as postoperative complication was actually one of the prognostic factors of long-term prognosis; second, we originally planned to investigate the association between PNI and postoperative complication, thus we included these two factors in **Table 1**. We sincerely hoped that we have made sense in this issue.

3. Under statistical analysis, section page 9 paragraph 2, remove the reference to an independent prognostic value in univariate analysis as univariate analysis does not identify independent factors.

Reply: We are sorry to have made these interpreting errors. In the revised manuscript, we have re-stated the corresponding sentences by avoiding the term "independent prognostic value" for the univariate analysis.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely,

Yulong He

Yulong He, M.D. Ph.D.

Division of Gastrointestinal Surgery, The First Affiliated Hospital, Sun Yat-Sen

University, Guangzhou, 510080, P. R. China.

Phone: +86.20.28823389.

Fax: +86.20.28823389.

E-mail: ylh@medmail.com.cn.