Author's response to reviews

Title: Preoperative platelet-lymphocyte ratio is superior to neutrophil-lymphocyte ratio as a prognostic factor for soft-tissue sarcoma

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Author's response to reviews:

Dear reviewers,

Thank you for your nice comments and suggestions. We have carefully read and accordingly revised the manuscript. Following is a point to point response to the editor’s and reviewer’s comments.

We hope we have addressed all of the questions and concerns satisfactorily and the revised manuscript is acceptable for publication in BMC Cancer.

Best regards.

Sincerely yours,

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Comments and answers:
Editor’s Comments:

1.) Copyediting: We recommend that you copyedit the paper to improve the style of written English. If this is not possible, you may need to use a professional language editing service. For authors who wish to have the language in their manuscript edited by a native-English speaker with scientific expertise, BioMed Central recommends Edanz (www.edanzediting.com/bmc1). BioMed Central has negotiated a 10% discount to the fee charged to BioMed Central authors by Edanz. Use of an editing service is neither a requirement nor a guarantee of acceptance for publication. For more information, see our FAQ on language editing services at http://www.biomedcentral.com/authors/authorfaq/editing.

Answer: Thank you very much for your kindly recommendation. According to your comments, we have had our manuscript polished by Edanz to improve the written English.

2.) Abstract Background needs additional context.

Answer: Thank you for your valuable suggestions. We have added additional context in abstract background section, page 3, from line 43 to line 45.

3.) Remove tables from the supplement files and include them at the end of the main manuscript file.

Answer: We have removed tables from the supplement files and included them at the end of the main manuscript file.

Reviewers’ comments

Reviewer 1: Vijaya Bhatt

Major Compulsory Revisions

1. The results of this study is different than those of the study by Szkandera et al (British Journal of Cancer (2013) 108, 1677–1683). In their study of 260 soft-tissue sarcoma patients, Szkandera et al demonstrated that elevated preoperative neutrophil/lymphocyte ratio was an independent prognostic factor in the multivariate analysis. This is also shown in another study: Biomarkers. 2012 Sep;17(6):539-44. Interestingly, the study by Azab et al (Med Oncol (2013)30:432), although in breast cancer patients, concluded that "Pretreatment neutrophil/lymphocyte ratio is superior to platelet/lymphocyte ratio as a predictor of long-term mortality." Similarly, Azab et al in colon cancer patients (Cancer Biomark. 2014;14(5):303-12) demonstrated similar findings.

-The authors should thoroughly discuss such prior literature, compare with their study in terms of results and any unique design and provide potential explanations for the discrepancy in their results.

Answer:

We would like to thank you very much for your interesting comments and
suggestions. We have discussed these prior literature, compared their studies with ours and provided potential explanations in the discussion section, page 11, from line 213 to line 231.

Our study demonstrated that PLR not NLR was an independent prognostic factor in the multivariate analysis which were inconsistent with those prior studies listed above. However, these studies used different cancer populations, different NLR and PLR cut off values from our study, and they used different median age and different histologic proportions of subtypes from ours, which might hinder comparability of their results with ours.

In addition, NLR can be affected by the atherosclerotic risk factors such as smoking, alcohol consumption, metabolic syndrome[1] and even the performance status may play a significant role. It would be better if Szkandera et al and Idowu et al mentioned these variables that may contribute to the clinical outcome in multivariate analysis. The difference of multivariate analysis model made the difference of these results. Szkandera et included sex, tumor site, tumor depth, tumor grade, tumor size, tumor necrosis, resection margins, tumor stage, adjuvant chemotherapy, adjuvant radiotherapy and NLR (<3.58 vs #3.58)[2] in the multivariate analysis, while Idowu et al included age, sex, anatomic location, grade, tumor size, surgical margin and NLR(<5 vs #5)[3]. In our study, we included age, gender, performance status, smoking history, diabetes mellitus, cardiopulmonary disease, tumor depth, tumor stage, tumor site, adjuvant radiotherapy, adjuvant chemotheraphy, and NLR(<2.5 vs #2.5)( additional file 4: Table S4)

Azab et al (Med Oncol (2013)30:432) conducted the first studies to evaluate the predictive value of NLR vs PLR in breast cancer and colorectal cancer. They compared NLR and PLR in the lymphocyte subsets analyses according to Harell’s C-index ranges when both NLR and PLR were significant predictors of mortality in the multivariate models [4]. They realized that the limitation of population—“About 90% of our patients were Caucasians, limiting the usage of NLR, PLR ratios in other ethnicity.”[5]

Some published studies concluded the results consistent with our findings. Smith et al[6] reported PLR, not NLR, as a predictor of mortality in pancreatic cancer. Asher et al[7] showed that PLR, not NLR, to be a significant predictor of mortality in ovarian cancer. In conclusion, multicenter and prospective studies including more potential confounding factors should be conducted to investigate the problem in the future.

2. Two large systematic review and meta-analysis [Cancer Epidemiol Biomarkers Prev. 2014 Jul;23(7):1204-12. and J Natl Cancer Inst. 2014 May 29;106(6)] have analyzed the role of similar markers. These should be referenced and discussed.

Answer:

We have referenced and discussed these two meta-analyses relatively in the Background section, page 5, from line 89 to line 90 and discussion section, page 11, from line 208 to line 210.
3. The following study has demonstrated that elevated preoperative systemic inflammatory markers predict poor disease-specific survival in localized soft tissue sarcoma: Ann Surg Oncol. 2014 Mar;21(3):778-85. This study shows that elevation of multiple markers was a more significant prognostic factor than elevation of a single marker. Also, histologic grade was strongly correlated with inflammatory marker values. The study is also important since it utilizes disease-specific survival in a specific subset of soft tissue sarcoma. This study should be referenced and discussed.

Answer:
We have referenced and discussed this study in the discussion section, page 13, from line 241 to line 247

4. In the discussion section, the authors should describe the implications of study. In other words, how does PLR compare to other prognostic factors such as tumor grade/size? Can the use of PLR enhance current prognostication?

Answer:
Thank you for your instructive suggestions, we have described the implications of this study in the discussion section, page 12, from line 232 to line240

5. The authors should briefly discuss about different cutoff values used in different studies. This is important for clinical practice. What cutoff values does a clinician use to identify whether a ratio for NLR or PLR is elevated?

Answer:
Thank you for your valuable advices. Different studies used different cutoff values, it depends on sample size, population and other confounding factors. It is well known that racial variation affected the cutoff value. Caucasians have higher peripheral blood neutrophil and lower lymphocyte counts compared to Asians[8]. An NLR>5 was considered elevated in accordance with some earlier reports based on caucasian population[3, 9, 10],while some Asian population based studies applied NLR >2.5~4 as cutoff point[11-13]. As to PLR, some reports made the 150 and 300 as the cutoff points[11, 14], some studies identified the ideal cutoff value applying receiver operating curve and the cutoff points were also close to 150 or 300[15, 16]. Our study had mostly Asian patients and we determined the optimal cutoff value for PLR 133.915 (AUC: 0.640, 95%CI=0.541-0.739#P=0.005), and for NLR 2.5 (AUC: 0.632, 95%CI=0.533-0.731,P=0.009). For clinical practice, we considered that a multicenter and large sample size study is needed to evaluate the optimal cutoff value, We have briefly discussed this part in the discussion section, page 12 from line 226 to line 231.

6. It is important to realize that a single test done within 7 days before surgery or other therapy has limitations. Further, these ratios may differ based on age, smoking status, presence of infection or inflammatory conditions etc. A recent study demonstrate that neutrophil lymphocyte ratio may vary by race (Average
values and racial differences of neutrophil lymphocyte ratio among a nationally representative sample of United States subjects. PLoS One. 2014 Nov 6;9(11):e112361.) At the least, these limitations should be acknowledged.

Answer:

We concur with you that a single test done within 7 days before surgery and other therapy has limitations. Some potential confounding factors may contribute to the clinical outcome. Thus, we recorded and incorporated factors such as smoking status, performance status, co-morbidities (Table 1). Before we included patients, we excluded patients presented with active infection.(In the method section, page 5 from line 100 to 102). Further, we acknowledged that population is another limitation as more than 95% of our patients were Asians. We also mentioned it in the discussion section, page 13, from line 247 to line 248.

7. The study population is heterogeneous e.g. the authors have used different types of soft-tissue sarcoma and at different stages. Only some of the patients received radiotherapy. The use of adjuvant or neoadjuvant chemotherapy is not mentioned. Since prognosis may differ because of these factors, this may be another limitation of the study.

Answer:

In the methods section, page 6, from line108 to line109, we mentioned that “Adjuvant chemotherapy was administered in 39 patients (17.6%), and adjuvant radiotherapy treatment in 65 patients (29.3%). We included these factors in univariate and multivariate analysis (Table3 and Table4). We are sorry that we have not mentioned the use of adjuvant or neoadjuvant chemotherapy specifically in our original manuscript. We have added the use of adjuvant chemotherapy in methods section, page 6 from line109 to line110. Because the subjects included were patients who underwent extensive and radical resection and their blood count data are collected before surgery within 7 days. Thus, any neoadjuvant chemotherapy before surgery may affect the data on blood count. We have mentioned this in the method section, page 5, from line99to line100

8. The authors should discuss about the followings: inclusion/exclusion criteria, method of data collection e.g. chart review/use of database, staging evaluation, indication of surgery for stage IV disease and adjuvant radiotherapy in their patient population. The use of adjuvant or neoadjuvant chemotherapy and indication should be mentioned.

Answer:

We have discussed the inclusion/exclusion criteria, method of data collection, staging evaluation, indication of surgery for stage IV disease and adjuvant radiotherapy in their patient population in the method section, page 5, from line99to line113.

Since any neoadjuvant chemotherapy before surgery may affect the data on blood count. We included all patients had confirmed STS, and none had received
chemotherapy before collection of the blood count data. To date, it has not been concluded any clear indication for the use of adjuvant chemotherapy. Available evidence from meta-analyses and randomized clinical trials suggest that postoperative chemotherapy improves relapse-free survival in patients with STS of extremities. However, data regarding OS advantage are conflicting. (NCCN guidelines Version1.2014 Soft tissue sarcoma, MS-7.) In our study we advocated patients with high risk factors (FNCLCC G3, nerves and vessels infiltration, tumor size >10cm) to have adjuvant chemotherapy and doxorubicin-based combination chemotherapy regimens were mostly used. We have supplemented the use of adjuvant chemotherapy in the method section, page 6 from line 109 to line 110.

9. The overall survival also depends on the presence of comorbid conditions. This is very important in potentially curative disease. The unavailability of data regarding comorbid conditions, cause of death or disease specific survival is another limitation. I understand that the authors have included DFS, which is useful. In the same note, what was the source of data regarding vital status (alive or dead) at last follow-up?

Answer:

We are aware that co-morbidities might influence the clinical outcome and we showed this important information in Table 1, and incorporated potential confounding factors including diabetes mellitus and cardiopulmonary disease in univariate and multivariate analysis (Table 3 and Table 4). On the other hand, we re-checked the chart of cancer registry and found 5 patients deaths were due to other causes. Deaths confirmed to be caused by STSs were treated as an end point for disease-specific survival and the deaths due to other causes were regarded as censored observations. Then, we evaluated the 5-year disease-specific survival in low PLR group (87.2%) and high PLR group (65.5%) relatively. While the 5-year overall survival in low PLR group and high PLR group were 85.5% and 60.2%. We acknowledged that the data regarding disease specific survival was important for analysis, which we mentioned in the discussion section, page 13 from line 241 to line 247.

The follow-up examinations were provided by the follow-up programme of an independent department in Sun Yat-sen University in regular intervals (3-month intervals in years 1-3, 6-months intervals in years 4-5, and 12-month intervals in years 6-15 after diagnosis.) We have mentioned that in the method section, page 6 from line 113 to line 116.

Minor Essential Revisions

1. Mean absolute neutrophil count should be mentioned.

Answer:

We have mentioned this information in the result section, page 8 from line 144 to line 145.

Discretionary Revisions
1. The author may consider replacing the term "predictive" with "prognostic" in the title.

Answer:
Thank you for your advice. We have replaced the term “predictive” with “prognostic” in the title.

Reviewer 2: George Malietzis

Major Compulsory Revisions

The whole manuscript is built upon the superiority of PLR over NLR but there are issues with the Multivariable analysis. The authors did not report the HRs for the tutor grade and size in tables 3 and 4 and no justification has been given. It seems to me that the sample size and the events number are not allowing the regression model to account for all the variables. If that is the case then the authors need to explain that in the limitation are reanalyse with inclusion only the statistical significant factors from the univariate analysis.

Answer:
Thank you for your nice comments. The stage our patients was classified according to the American Joint Committee on Cancer (AJCC) 7th Edition, 2010 [17]. The evaluation of the stage is decided by multiple factors including primary tumor size (T), regional lymph Nodes(N), distant metastases(M), and tumor grade. The reason that we excluded the factors of tumor size and tumor grade in the multivariate anlaysis is to eliminated the influence of statistical colinearity. We have mentioned it in the result section, page 9, from line 162 to line 165.

As you have mentioned tumor size and tumor grade, which are very important variables, we developed another multivariate model including these two factors, which demonstrated elevated PLR still showed significant association with poor clinical outcome (Additional file 1-2).

Minor Essential Revisions

P-values for the ROC analysis are missing

Answer:
We have supplemented this information in the result section, page 8 from line 146 to line 148.

Reviewer 3: Joanna Szkandera

Major Revisions

1. This paper needs major English revisions!!! The authors switched between
present tense and past tense. Furthermore, they used abbreviations that were explained first, e.g. DFS, and in the next sentence the abbreviation was written out in full. Due to language problems, some parts of the manuscript are confusing, e.g.:

Answer: Thank you very much for your kindly recommendation. According to your comments, we have had our manuscript polished by Edanz to improve the written English.

1) Title: “The preoperative platelet-lymphocyte-ratio is superior to neutrophil lymphocyte ratio as a predictive factor on soft-tissue sarcoma” should be „The preoperative platelet-lymphocyte-ratio is superior to neutrophil-lymphocyte-ratio as a predictive factor on soft-tissue sarcoma“

Answer:
Thank you for your careful reading of our manuscript. We are very sorry for our written errors. According to your nice suggestions, we have corrected this in the title.

2) Abstract, page 3, Methods, line 50: “Use Kaplan-Meier curves....” should be “Kaplan-Meier curves were used...”

Results, page 3, line 54: “As to multivariate analysis which included tumor site, AJCC stage, only high PLR was still identified...” shouldn’t it be “As to multivariate analysis, tumor site, AJCC and PLR were identified as...”?

line 60: “However, in multivariate analysis including tumor site, AJCC stage, only the PLR were...” shouldn’t it be “In multivariate analysis, tumor site, AJCC stage and PLR were also related to decreased DFS”?

Both mistakes should also be corrected in the results section, page 9, paragraph 2, e.g.: “As to multivariate analysis which included age, gender, tumor site, AJCC stage, adjuvant radiotherapy and PLR and NLR, PLR was still identified as independent prognostic factors of poor clinical outcome.” and “In addition, PLR was independently associated with DFS in multivariate analysis including age, gender, tumor site, AJCC stage, adjuvant radiotherapy and PLR and NLR.”

Answer:
We have corrected this sentence in the abstract, page 3, line 48.

And we have made the expression more clearly in abstract section, page 3 line 52, 56 and in results section, page 8, from line 157 to line 159.

3) Background, page 4, line 79: “… with not adjuvant radiation…” should be “…without adjuvant radiation…”

page 5, line 99: “Raised platelet counts was indicated inferior survival....” Should be “Raised platelet counts indicated inferior survival…”

line 109: “To date, PLR has been identified the prognosis in different cancers as a...” should be “To date, PLR has been identified as a prognostic factor in different cancers.”
page 6, line 112: “NLR has been considered the prognosis value in…” should be “NLR has been shown to have prognostic value in..”

Answer:
We have corrected these words in background section, page 4 line 64; page 4 line78; page 5 line 86; page 5 line88.

4) Methods, Subjects, page 6, line 128: “Adjuvant radiotherapy was administered in 39 patients, and adjuvant radiotherapy treatment in 65 patients.- what is the difference between those two treatments??

line 129, “The follow-up examinations are provided…” should be “The follow-up examinations were provided…”

Statistical analysis, page 7, line 154: “…grade, adjuvant radiotherapy, AJCC stage on OS and DFS.” Should be “grade, adjuvant radiotherapy and AJCC stage on OS and DFS.”

Answer:
Thank you very much for your careful work. We are very sorry for the clerical error. We wanted to state that “Adjuvant chemotherapy was administered in 39 patients (17.6%), and adjuvant radiotherapy treatment in 65 patients (29.3%)”. We have corrected this sentence in the method section, page 6 from line 108 to line 109 and line 113. In the statistical analysis, we have corrected in page 7 line 133..

5) Results, page 8, line 166: “….classified into different subtypes which are show in…” should be “….classified into different subtypes which were show in…” page 9, line 179:” The reason that we exclude the factors of tumor grade and tumor size…” should be “The reason why factors such as tumor grade and tumor size were excluded…”

Answer:
We have corrected these statements in the results section, page 8 line 142, page 9 line163.

6) Discussion, page 11, line 227: “…from the platelets resulting to angiogenesis” should be “….from the platelets resulting in angiogenesis”

Answer:
We have corrected this sentence in the discussion section, page 10 line199.

2. As tumor depth is a well known prognostic factor in soft tissue sarcomas, it should be added in Table 1 and included in the univariate analyses in Table 3 and 4.

Answer:
We have added this important factor in Table 1 and included it in the univariate and multivariate analysis in Table 3 and 4.
3. In the introduction and at the end of the discussion, the authors state that there is no study regarding the significance of PLR in soft tissue sarcoma. The authors may wish to read and also discuss the following paper: Szkandera J, Gerger A, Liegl-Atzwanger B, Absenger G, Stotz M, et al. The lymphocyte/monocyte ratio predicts poor clinical outcome and improves the predictive accuracy in patients with soft tissue sarcomas. Int J Cancer. 2014 Jul 15;135(2):362-70. doi: 10.1002/ijc.28677. In this study we also evaluated the prognostic significance of PLR on clinical outcome in soft tissue sarcoma patients and found statistically significant associations in univariate, but not multivariate analyses.

Answer:
We are very sorry to have missed such important paper. We have referenced this paper and discussed it in the discussion section, page 11 from line 213 to line 222.

The authors have done a nice analysis predicting accuracy in soft tissue sarcoma. What's important, they developed a nomogram by supplementing the L/M ratio to the well-established Kattan nomogram and evaluated the predictive accuracy of this novel nomogram by applying calibration and Harrell's concordance index (c-index)[18]. However, compared with their findings, we used different cancer populations and different NLR and PLR cut off values, which might hinder comparability our results with theirs. In addition, there are some potential confounding factors that should be incorporated in the multivariate analysis in the future prospective studies.

Minor revisions:
1. Please include the information on the duration of the follow-up.
2. HR and 95% CI should to be rounded to 2 decimal places.

Answer:
Thank you for your nice suggestions. We have added the duration of the follow-up in the result section, page 7 from line 141-142.
As you advised, HR and 95% have been rounded to 2 decimal places instead of 3 decimal places.

Reviewer 4: Mairead McNamara

Major Compulsory Revisions:

1. General comment:
There are many grammar errors throughout the manuscript which at times alter meaning of the text. It would benefit from review and editing by a person with expertise in the field of interest and a good command of the English language.

Answer:
Thank you very much to point out the grammar issues in our manuscript.
According to the comments from you and the editors, we polished the manuscript with a professional assistance in writing.

2. Background:

Line 87 in background section mentions methylated RASSFIA. This should read RASSF1A and needs to be corrected (figure 1, not I).

In line 88, it is stated that “Osteopontin (OPN) in serum was determined to be a prognostic factor (I presume for survival?) for soft tissue sarcoma”. Please state if elevated or decreased levels of osteopontin are prognostic.

Line 109, it is stated that “To date, PLR has been identified the prognosis in different cancers as a reliable and easily accessible marker.....” – this sentence is very unclear and should probably read “To date, high PLR has been associated with poor prognosis in different cancers and is a reliable and easily accessible marker....”.

Answer:
In line 70 in background section, we have corrected RASSFIA to RASSF1A.
In line 71, we have stated that---high serum osteopontin is correlated with poor prognosis in STS.
In line 86, we have altered the words to express more clearly.

3. Methods (Subjects subsection):

Line 128 and 129 are confusing: It reads “Adjuvant radiotherapy was administered in 39 patients (17.6%), and adjuvant radiotherapy treatment in 65 patients (29.3%). Radiotherapy is mentioned in both parts of this sentence. Should one refer to chemotherapy?. Please clarify.

Answer:
Thank you very much for your kindly reminding of our written errors. We wanted to express “Adjuvant chemotherapy was administered in 39 patients (17.6%), and adjuvant radiotherapy treatment in 65 patients (29.3%)”. We are very sorry to have made the clerical error. We have corrected this sentence in the method section, page 6, from line 108 to line 109.

4. Statistical analysis:

Line 144 states “The optimal cut-off value for the lipid profile was calculated by applying....”. I presume this is incorrect and should refer to the platelet-lymphocyte ratio?.

Answer:
Thank you very much for your kindly reminding of the incorrect words. It should refer to the platelet-lymphocyte ratio actually. We have corrected this word in the method section, page 6 line 122.

5. Results:

Was performance status of patients recorded or included in
univariable/multivariable analysis? There should be some mention of this as it may contribute to outcome.

Answer:
According to your comments, we have reviewed our database and recorded and included this important factor in univariate and multivariate analysis (Table3 and Table4).

6.Discussion:
In line 238, it is stated that “A combined index using platelet and lymphocyte counts has been investigated as a prognostic factor for some cancers”. Examples of references are then given. There is a published systematic review and meta-analysis on “Prognostic role of platelet to lymphocyte ratio in solid tumors: a systematic review and meta-analysis”, Cancer Epidemiol Biomarkers Prev 23:1204-12 by Templeton AJ et al which would warrant inclusion here as a reference given the inclusion of over 12,000 patients in this study addressing the current topic.

Answer:
We have referenced and discussed this paper in the discussion section, page 11 from line 208 to line 210.

Minor Essential Revisions:

Abstract:
1. The use of STS without a prior definition within the first line of methods section of the abstract is a little confusing and should be amended.

Answer:
We have add a prior definition in the line 47, page 3 of Abstract section.

2. Figures:
Please provide a description of low and high PLR in figure legend. Is this higher and lower than cut-off as mentioned in results section?

Answer:
We have provided a description of low and high PLR in figure legend in line 494-500, page 20. We have also mentioned that PLR higher than cut-off value (133.915) was associated with poor survival.

References


