Author's response to reviews

Title: Neutrophil/Lymphocyte Ratio has no predictive or prognostic value in breast cancer patients undergoing preoperative systemic therapy

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Author's response to reviews: see over
Dear Editor-in-Chief,

Please find enclosed a revised manuscript entitled “N/L Ratio has no predictive or prognostic value in breast cancer patients undergoing preoperative systemic therapy” by Christoph Suppan, Vesna Bjelic-Radisic, Marlen La Garde, Andrea Groselj-Strele, Katharina Eberhardt, Hellmut Samonigg, Hans Loibner, Nadia Dandachi and Marija Balic for consideration for publication in BMC Cancer. We strongly believe that we were able to address all the issues raised by reviewers and we hope that this revised manuscript is now suitable for publication. Please note that all changes made to the manuscript have been highlighted in red.

Regarding to the three peer reviews received on 17 September 2015 we would like to give a point-by-point response to the concerns.
Reviewer 1: Jennifer M Giltnane

Ad major compulsory revisions

Ad 1) The paper should be refocused on one clinical subtype of breast cancer. Although cohort numbers will be smaller, not doing so ignores strong evidence that clinical subtypes have different natural histories with systemic neoadjuvant therapy. Given improved presurgical responses to systemic therapy, yet overall poor prognosis, I suggest triple negative breast cancer, but this is up to the authors' discretion.

We appreciate reviewer's suggestion regarding the subgroups. However, we do not agree that the paper should focus only on one subgroup of patients. This is a retrospective cohort of preoperatively treated breast cancer patients. We have now added an additional analysis with respect to intrinsic subgroups and no subgroup could be identified where N/L Ratio was predictive of pCR. This was now included in the results section line 217-222 and we included a new table 4. Thus, we can conclude that focusing only on one subgroup would not change the main statement of the paper.

Ad 2) In my opinion, the paper uses an outdated method (modified Sinn regression score) to score pathological response to neoadjuvant therapy. A 2013 paper comparing multiple methods showed that regression of the tumor in the lymph node is key factor in prediction of long-term response. The authors should re-score the cohort with at least one other method that incorporates lymph node status.

We strongly appreciate reviewer's suggestion regarding the scoring of pathological response. We used this method since during the time period of the treatment of patients the Sinn regression score was used. As suggested by the reviewer we have included an additional analysis where we defined pCR as no invasive tumor rest in the primary tumor and lymph nodes (ypT0isypN0 or Sinn score 3 and 4 and ypN0). Importantly, there was no difference in the predictive value of NL Ratio for the new definition of pCR. We have included as reference the pooled analysis by Cortaazar et al.

These results were now included in the methods section line 155-157, and in the results section line 214-216. We have also added a new table 3b with the new definition of pCR, and the table 3 with the old definition is now table 3a.

Ad Minor Essential Revisions
1. There are scattered typos, including non-English spelling of figure legends, to be corrected.

We carefully revised the manuscript and corrected typos where needed.

Ad discretionary revisions:

1. The authors reference Krenn-Pilko et al., 2014; authors who evaluated the effect of preoperative platelet-to-lymphocyte Ratio and found it to be significant for long-term outcome. This seems like an easy, obvious addition to the study at hand that would take minimal additional work.

We do appreciate the suggestions by the reviewer, that it would be a nice addition to the study to add the preoperative platelet-to-lymphocyte ratio, however our analysis was designed to address the value of NL Ratio alone, and further analysis is out of the scope of our study.

2. The authors also reference Loi et al., 2014, and others in independent analyses, showing the utility of evaluating lymphocytic infiltration in the assessment of response in triple negative breast cancer. Given these findings, it would an admirable addition to the current study to validate this hypothesis.

The same is the case also with the suggestion regarding the primary tumor analysis and lymphocytic infiltration.

Reviewer 2 Andrea Mastro
no comments needing revision

Reviewer 3: John Hughes

Ad minor essential revisions:

Both 'tumor' and 'tumour' appear in the text and 'Faktor' in Table 2.

The decimal point in tables should be a full stop (.) not a comma (,).

Please remove the duplicated sentence at line 232 starting "Many groups ... ".

The suggested changes were made accordingly.

Ad major compulsory revisions:
All discrepancies between the data reported in Table 1 and the text must be corrected.

i. Age, menopausal status, type of carcinoma and number of treatment cycles are in the text but not in the table.

Age at the very least should be in the table.

ii. The table and text report different percentages for example:

Histology cT2 Text: 53 Table: 53.1.
Her2 positive Text: 18.3 + 6.0 = 24.3 Table: 24.2.

iii. the total number of patients was N = 298. Laboratory data were available for 247. Table 1 should report N, the total number for of patients recruited, 298 and n = 247 the number analysed but sub-totals in Table 1 differ as follows:

Parameter sub-total Tumor Size 281 Histology 294

Tumor Grading 268
Her2 Status 252
ER Status 288
PR Status 288
Lymph node Status 291 Neoadjuvant Therapy 298 pCR 294

Response to Therapy 386[sic]

Why are these sub-totals different? The analysis was supposed to be based on 247 derived from an initial recruitment of 298.

The authors need to define an evaluable population. The text promises 247 derived from the 298 recruited.

We appreciate the suggestion by the reviewer and apologize for the discrepancies in the numbers. These were now carefully revised and corrected. For easier reading and better understanding we now show only results for patients where also NLRatio was analysed (N=247). Only in the material and methods section we now have stated that the original group was 298.

i. Age, menopausal status, type of carcinoma and number of treatment cycles are now in the text and in the table.

ii. The differences for histology and Her2positive were also corrected.

iii. We have corrected the number discrepancies as already stated above.

Ad 2)
Statistics for NLR should be tabulated and possibly separate summaries for neutrophil and lymphocyte.

We appreciate this suggestion, however, in our opinion a separate summaries for neutrophils and lymphocytes does not add value, because it was not in the scope of our study. We aimed to analyse the value of the NLratio for prediction and prognosis of our cohort.

Ad 3)

All patients who remained disease free should be treated as either censored or lost-to-follow up if they have stopped attending evaluations (see Collett D. Modelling Survival Data in Medical Research. Chapman and Hall, in particular Figures 1.1 and 1.2).

In this study for all patients who progressed the time of progression was interval censored. For patients who did not miss any evaluations the size of this interval was between 3 months and one year. If a patient missed one evaluation then the interval could be two years. The authors appear to have simply used the date of evaluation as the date of progression. This is inconsistent and unsatisfactory.

Can the authors please define an appropriate time to disease progression that is consistent for all patients?

There must have been patients who were disease free and stopped attending evaluations. These patients were lost-to-follow up. Can the authors please provide a definition for lost-to-follow up patients?

The number of patients who progressed, remained disease free and were lost-to-follow up is essential information that must be tabulated so that readers can assess the reliability of these data.

Are the authors confident that the duration of the study is not unreasonably long?

We appreciate this very valuable suggestion of the reviewer 3 that the definition of time to disease progression was missing. We now added the definition of the DFS as the time in months from the date of biopsy to the first documented recurrence during the follow up or the date of the final documentation. This was added in lines 167-168. Therefore, the time of progression was not interval censored, but defined as the first documented recurrence. The median follow up time was also included in the methods section line 165, and was 123 months.

We hope that these clear definitions now answer all the issues raised by the reviewer.

Ad 4)
Table 2 presents the results of the univariable logistic regression. This table is not comprehensible without reading the text to discover how response to therapy was defined and Table 1 for the number of patients. But as stated above the numbers presented for Sinn scores must be wrong assuming the stated recruitment of 298 is correct.

We appreciate the comment of the reviewer regarding the table 2. We have now corrected table 1 and included the definition of response, and added this in a footnote in tables 2 and 3 a and b. The numbers were also corrected, as stated above.

Ad 5)

Table 3 suffers from the same omissions as Table 2. It is not possible to know with any confidence how many patients were included in this analysis. The report initially promises data for 298 patients this is then reduced to the 247 who have available laboratory values, a further reduction then occurs in the text to 29 patients with pCR and 218 who did not achieve pCR, a total of 237.

None of the preceding values agree with Table 1 where pCR status is presented for 294 patients. Data for 1 in 5 patients has gone missing. Where did all these data go?

As already stated above, we appreciate these suggestions by the reviewer. We have now clarified the numbers and corrected them accordingly across the paper and have included a new version of the table1 and correct tables 2 and 3 (a and b)

Ad 6)

Apart from NLR there is no clear statement either in the table or in the text of which other covariates were fitted in the multi-varable model. For multivariable models all the covariates should be presented in some way even in a footnote.

This article suffers from many inconsistencies between tables and text which undermines the credibility of the analysis. There must be 100% agreement between text and tables. This is difficult to achieve if tables have been created by cutting and pasting/copying by hand which is certainly the case here.

Cutting and pasting tables is no longer necessary as tables and text can be created together programmatically.

We recognize that tables and numbers had discrepancies, due to the total number of patients included in the study (298) and the number of patients with available N/L Ratio was available (247). We have now corrected these numbers and also the tables as stated above. We have also included the number of missing data in the table 1. Therefore, we now have 100% agreement between the data presented. We have also included the number of patients in each category in table 5 (previously table 4)
Herewith we hope that we have addressed all the issues raised by the reviewers sufficiently.

Sincerely yours,
Christoph Suppan

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