Author's response to reviews

Title: Plasma MMP2 in combination with MMP9 and TIMP1 improves the non-invasive detection of transitional cell carcinoma of the bladder

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Author's response to reviews: see over
RE: Resubmission of original work manuscript:
No. 1736728878568775

New Title:
“Plasma MMP2 in combination with MMP9 and TIMP1 improves non-invasive detection of transitional cell carcinoma of the bladder”

Andrea Staack, Steffen Badendieck, Dietmar Schnorr, Stefan A. Loening, Klaus Jung
From the Department of Urology, University Hospital Charité, Humboldt University of Berlin, Germany

Dear Members of the Editorial Board,
Dear Dr. Roddam,
Dear Dr. Sier,

Thank you for reviewing our manuscript named above a second time. We provided further recommended chances in this letter and made corrections within the text. We appreciate the opportunity you gave us for resubmission of our manuscript to BMC Urology.

Sincerely,

Dr. med. Andrea Staack
### Reviewer’s report: Dr. Andrew Roddam

**Minor essential revisions:**

1. *It is suggested to change Table I to simplify this table to make it more accessible to readers. The reviewer is certain that if appropriate control were made then some of the significant associations presented would no longer remain significant.*

   In regards to this suggestion we have changed Table 1 and eliminated the subgroups with superficial, invasive, G1, G2, and G3 bladder tumors to help the reader focusing on the main groups. Doing this helped to avoid unnecessary statistical comparisons.

   Considering the reviewer’s concern we re-evaluated the statistical results, which are newly presented in Table I. In our new analysis we are comparing three groups (controls, results from patients with non-metastasized bladder cancer and from patients with metastasized bladder cancer) and applying the Kruskal-Wallis test, where we are now taking multiple comparisons into the account. Using the Kruskal-Wallis test we overall received also statistical significant results like we did using the Mann-Whitney test. Under this calculation the comparison between non-metastasized and metastasized bladder cancer for TIMP1 and the comparison between non-metastasized bladder cancer and the control group for MTC1 were not significantly different.

   Our study results are of exploratory nature and we believe further corrections of the outcome results would not be useful. In this respect, we agree with the view of Bender and Lange (BMJ 1999; 318:600) that exploratory studies should be analyzed without multiplicity adjustment.

   Changes are made in the text:
   - Page 11, line 5-7;
   - Page 12, line 9-19.

2. *The reviewer stated that it is not necessary to display the full correlation matrix in Table 2; two decimal places are only necessary.*

   The required changes have been made in Table 2. For a better overview we switched the last lines (T-Stage and Grading) to the beginning of this table.

3. *The reviewer is concerned over a degree of overfitting and a high degree of co-linearity between the markers, which became correlated.*

   As suggested we added a cautionary note in the text, which states the concern of a high degree of co-linearity of combining markers with the highest degree of
correlation.

Page 21, line 10-14:
“To some degree there might exist an overfitting in the resulting data of the two virtual mROC classifiers because MMP9 and TIMP1 in the two best combinations are the markers with the highest correlation to each other. Further prospective studies should prove the usefulness of these marker combinations.”

<table>
<thead>
<tr>
<th>4. The reviewer asks for to reduce the number of decimal places for the AUC to 2. Multiple comparisons are being made in this table but no adjustment is given to the p-values for significance.</th>
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<tbody>
<tr>
<td>We have reduced the number of decimal places in Table III.</td>
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<td>Additionally, as AUC comparisons could only be performed as pair wise comparisons (MedCalc program, version 8.2), we adjusted the P values by the sequentially rejective Bonferroni approach. The statistical values are given in Table III. Adjusting our results with the Bonferroni method the P values for MTC1 and MMP9 in comparison with AUC 0.5 remained no longer statistically significant. Applying the Bonferroni test to the pair wise AUC comparison with the AUC for MMP2 (Table III) did not change the previously calculated significances.</td>
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<td>Page 11, lines 5-7; Page 14, lines 14-15.</td>
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<table>
<thead>
<tr>
<th>5. The reviewer suggests a simplification of Table IV.</th>
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<tbody>
<tr>
<td>We made the suggested changes in Table IV and present only data for 95% sensitivity and specificity.</td>
</tr>
<tr>
<td>Changes are made in the text: Page 14, line 21-23 and page 15, line 1.</td>
</tr>
</tbody>
</table>
**Reviewer’s report: Dr. Cornelis Sier**

- **General**

_The reviewer requests additional visualization of the results._

As requested we provided an additional figure with dot-plots for the most successful combinations with MMP2 as the best single marker (Figure 3) for better visualization. Healthy controls are presented by black dots and bladder cancer patients by blue dots with MMP2 concentration given on the x-axis and the MMP values on the y-axis. We hope that these figures better visualize and support the data of improved specificity shown in Table III.

Changes are made in the Figure legend:
Page 31, line 26-32,
and in the text at page 15, line 9-12.

- **Major compulsory revisions**

1. _The authors should explain why the median values for the patients also changed from the previous version._

The reviewer is right; our headline to the first table in the initial paper was misleading. The initial labeling was wrong in the first manuscript. We labeled the table with “data for the control group are presented as arithmetic means with the 5th and 95th percentiles; data for all patients with TCC (BCa, mBCa) are presented as median values with minimum and maximum values.” Instead, we presented in this initial table the means with the percentiles for the controls, but also the means for the bladder cancer groups, but with their ranges.

In our previous submitted table (as well as in the recent one) we changed the labeling and corrected the data in this table. Data are now corrected to the median values with their ranges for the control and the tumour groups. We apologize for this confusion.

2. _The discussion seems still too superficial. The major parts are filled with a repetition of the results section and the reason why plasma was used in this study and not serum. The latter point was already perfectly explained in the introduction._
We have changed and edited the discussion in order to explain the impact of the scientific background for MMPs and TIMPs further. In regards to the reviewer’s comment we deleted the explanation about the advantages using plasma instead serum in the discussion since we have discussed this point already in the introduction. In our opinion, the remaining points in the discussion are necessary for a better comparison to other authors.

Page 16, line 16-23; page 17, line 1-14; Page 19, line 10-12 (was deleted); Page 20, line 4-11.

- **The reviewer listed minor essential revisions.**

1. A suggestive sentence in the result section of the abstract should be changed: “The median MMP2 concentration was elevated in all patient groups with TCC in comparison to controls.”

   We have changed this sentence in the result section of the abstract (page 3, line 20-21).

2. “Non-invasive tumour detection” as mentioned in the abstract could be interpreted in two ways and should be avoided.

   Changes are made in the conclusion section of the abstract at page 4, line 6 to: “MMP2 is a statistically significant marker in blood plasma for bladder cancer detection with an increased diagnostic value in combination with MMP9 and TIMP1.”

3. “p” is missing in Table I for mBCA/TIMP1

   A correction has been made in Table I.

4. **Figure 1 should mention that the healthy controls are included.**

   We added this sentence in the figure legend of figure 1:
   Page 31, line 7-8.