Author's response to reviews

Title: TIMP-1 and VEGF-165 serum concentration during first-line therapy of ovarian cancer patients

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Author's response to reviews: see over
Dear Dr. Norton,

Please find enclosed the revised manuscript 1731802324307383 entitled “TIMP-1 and VEGF-165 serum concentration during first-line therapy of ovarian cancer patients”.

The resubmission is accompanied by a point-by-point response to the editor’s and reviewers’ remarks. We incorporated the suggestions and the revised manuscript highlights the changes we have made.

We hope that the manuscript is now acceptable for publication in BMC Cancer.

Yours sincerely,

Sven Mahner
We truly appreciate the editor’s and reviewers’ comments and suggestions regarding our manuscript 1731802324307383.

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Editorial Board:
1. Please can you also include mention of the fact that you received your ELISA kits for no cost from Siemens Healthcare Diagnostics, in your Competing interests section.  
   Reply:  
   We acknowledged the fact that the ELISA kits were provided at no cost from Siemens Healthcare Diagnostics in the competing interest section.

2. Please also confirm that you had ethical approval for your study and include a statement to this effect in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.  
   Reply:  
   The use of medical records, serum and tumor tissue was approved by the ethics committee of the Medical Board Hamburg (reference number #190504). This statement has been included in the methods section of the manuscript.

3. We recommend that you copyedit the paper to improve the style of written English.  
   Reply:  
   The manuscript has been redacted as suggested.

Reviewer 1 (David Hardisson):
4. Last line in Conclusions in the Abstract (“Angiogenesis related serum markers could help to optimize therapy in this context”) should be deleted. I find this sentence too speculative.  
   Reply:  
   The last line of the conclusions in the abstract has been deleted as suggested by the reviewer.
5. The Methods section regarding the quantitative analysis of serum VEGF-165 and TIMP-1 levels is too repetitive.

**Reply:**
We have revised the methods section of our manuscript and combined the descriptions regarding the quantitative analysis of VEGF-165 and TIMP-1 levels according to the reviewer’s recommendation.

6. The histopathology of tumors should be detailed in patient characteristics. Was there any correlation between histological type of tumor and TIMP-1 or VEGF-165 serum values?

**Reply:**
We thank the reviewer for pointing out this lack of detail in our manuscript. The histopathologic subtype of tumors has been added to Table 1 (patient characteristics). However, the number of non-serous tumors (1 endometroid ovarian cancer, 1 clear cell ovarian cancer) was too low for correlation analysis of different subtypes with TIMP-1 or VEGF-165 serum values.

7. How was the response to chemotherapy evaluated? In my opinion, it would be of interest to correlate the results of the quantification of CA-125, TIMP-1, and VEGF-165 serum values with the response to chemotherapy (partial response versus complete response).

**Reply:**
In the current study, response was evaluated with the biomarker CA-125. Except for one patient, all subjects responded to first-line treatment. The median decrease of CA-125 in responding patients was 95% (range 54%-100%). The use of computed tomography or other imaging modalities would have been limited by the fact that the majority of patients in our study had no index lesions after surgery. In accordance with international guidelines, image-based response evaluation was not performed. This information has been added to the methods and result section. As suggested by the reviewer, we analyzed the correlation between VEGF-165 and TIMP-1 and response. Results are shown in the new Table 5 and the methods section has been adapted. The one patient without CA-125 response did not present
significantly different levels of TIMP-1 and VEGF-165 concentrations compared to the other patients.

8. The authors should clearly indicate if all serum samples were collected at the same time in the different groups (before surgery-after surgery and before chemotherapy-during chemotherapy-after chemotherapy). This is especially relevant in the last two groups: during chemotherapy (chemotherapy consists in six cycles of carboplatin-paclitaxel along several weeks) and after chemotherapy (serum levels will not be probably the same one week than four weeks after finishing chemotherapy).

Reply:
The reviewer is raising an important point that we now describe more clearly in the revised manuscript. Serum samples were collected within 7 days before surgery (1), within 7 days after surgery (2), within 3 weeks of the third cycle of chemotherapy (3) and during the first follow-up visit 3 months after the last cycle of chemotherapy (4). This information was added to the methods section of our manuscript. We agree with the reviewer’s remark that serum levels of tumor-markers might still change during the first weeks after chemotherapy, and it would be interesting to assess this hypothesis in a future project.

9. Did the authors find any correlation between CA-125, TIMP-1, and VEGF-165 serum values and some of the clinicopathologic variables included in the study such as lymph node metastasis or grading (grade 2 versus grade 3 tumors).

Reply:
When analyzing the correlation between CA-125, TIMP-1 and VEGF-165 serum values and clinicopathologic variables we also examined the role of lymph node metastasis and grading. However, no correlations were observed and these results were not shown in the primary manuscript to restrict the length of the text and improve legibility of the tables. We added the requested information to the new Table 4 of the revised manuscript. To comply with the request of reviewer 2, we also simplified Table 3 and moved the results of the correlations between serum markers and age, residual tumor and ascites to Table 4.

10. In the Discussion, first paragraph, last two lines, the authors indicate “… a potential prognostic and predictive role…”. From the data presented in the study no
predictive role in the evaluation of these markers can be clearly established. This statement should be, therefore, changed to reflect only the potential prognostic value of TIMP-1 and VEGF-165.

Reply:
We appreciate the suggestion and have changed the statement. The discussion and also the conclusions of the abstract have been changed accordingly.

11. If tissue samples of the tumors are available, it would be very interesting to confirm the results of the study by immunohistochemistry against VEGF and TIMP-1.

Reply:
We agree with the reviewer that a confirmation of the serum results by immunohistochemistry would be interesting. However, in the current serum study, tissue samples were not systematically collected. Few published studies assessed the expression of VEGF and TIMP-1 in tumors of the ovary. Huang et al. for example could show overexpression of TIMP-1 in 89% of serous ovarian cancers in a systematic analysis of 90 patients with epithelial ovarian tumors (Huang et al. Gynecologic Oncology 77, 369-376, 2000). Regarding VEGF, overexpression has also been demonstrated in the majority of patients (e.g. Shen et al. British Journal of Cancer 83, 196-203, 2000 and Duncan et al., reference 29 of our manuscript).

12. Minor spelling errors should be corrected (i.e., Abstract, Background, line 3: ("TIMP-1 in" instead of "TIMP-1 is").

Reply:
The spelling error has been corrected. Please see response to point 3 above.

Reviewer 2 (Lindy Durrant):
13. My main criticism of the study is that serum markers must be used to assess individual patient’s response to therapy and therefore all statistics should have been done for paired samples and assessed by a non parametric ranking test. The results should also be presented as linked data for each patent so it is possible to assess how many patients showed an increase in TIMP-1 and VEGF-165 serum concentrations post surgery and a decrease post chemotherapy.
Reply:
We fully agree with the reviewer on the importance of the selection of statistical tests in serum marker studies. In fact we did use a non parametric ranking test (Wilcoxon test for paired data) to assess samples at consecutive time-points for individual patients. The description of this test was specified in the methods section of the manuscript.
We appreciate the suggestion to present linked data for each patient and added Figure 2 demonstrating the changes of marker concentrations for individual patients.

14. It is unclear if TIMP-1 and VEGF-165 serum concentrations reflect tumour burden or biologic responses. Post surgery the increase in TIMP-1 and VEGF-165 serum concentrations could be due to the biology of wound repair. However, it is less clear whether there is a significant drop in TIMP-1 and VEGF-165 serum concentrations during chemotherapy when compared to before surgery or just a return to normal levels following wound repair. This needs clarifying. Normal levels of TIMP-1 and VEGF-165 serum concentrations need to be presented for an appropriate group of aged matched controls as it is difficult to evaluate if there is any elevation in cancer patients.

Reply:
The reviewer is raising a very interesting point. The increase of TIMP-1 and VEGF-165 serum concentration can indeed be due to the biology of wound repair, as we have discussed in our manuscript. However, when comparing serum concentrations during and after chemotherapy with the ones before surgery, there still remains a significant decrease of TIMP-1 and VEGF-165. We added this information to the results section of the manuscript. A “normal level” of TIMP-1 and VEGF-165 serum concentration has not been defined. This has also been discussed in our manuscript (3rd and 4th paragraph of the discussion) and was our rationale to use median values for the analyses. We assume that a longitudinal measurement of individual patient levels - using each patient as their own control – might be a better approach than the assessment of absolute values at single time-points. Following the suggestion of the reviewer we emphasized this aspect in an additional paragraph at the end of the discussion section.
15. Similarly CA-125 is a good predictor of response to chemotherapy in 80% of patients the role of new serum markers is therefore in the 20% of patients where CA-125 is not indicative of response. The authors should show in this group of patients whether TIMP-1 and VEGF-165 serum concentrations where useful in predicting responses.

Reply:
Please see response to point 7 above.

16. Table 3 is unintelligible. It is unclear why TIMP-1 and VEGF-165 serum concentrations were correlated with age? There are multiple comparisons leading to a type 1 statistical error. P values need to be adjusted for multiple comparisons. PFS and OS should be presented as Kaplan-Meir plots. How do the authors explain that TIMP-1 after CTX was highly significant with overall survival but not progression free survival?

Reply:
To improve legibility of Table 3 we moved the results of the correlations between serum markers and age, residual tumor and ascites to a new Table 4. In ovarian cancer, age has previously been demonstrated to be of prognostic relevance (reference 10 of our manuscript) and was therefore included in the correlation analyses of our study.

The purpose of our study was to generate hypotheses about the role of TIMP-1 and VEGF-165 serum concentrations during first-line treatment of ovarian cancer. Applying Bonferroni-correction to adjust for multiple comparisons is not common practice in this setting. To confirm the results of our study in an independent patient cohort, Bonferroni-correction would of course be mandatory. If correction for multiple comparisons was applied in the present study, an adjusted p-value of 0.001 would have to be regarded as statistically significant and only CA-125 after chemotherapy would remain as predictor of progression-free survival.

As suggested by the reviewer we are now presenting PFS and OS as Kaplan-Meier plots for CA-125 after chemotherapy, TIMP-1 after chemotherapy, VEGF-165 before chemotherapy and VEGF-165 after chemotherapy. These plots visualize the prognostic relevance of the markers and are shown in the additional Figure 3. Regarding the non-significant impact of TIMP-1 after chemotherapy on progression-free survival Figure 3 also demonstrates a trend of unfavourable disease-free
survival in patient with elevated TIMP-1. Statistical significance was most likely not reached due to the limited power of the analysis.

17. Tumour types should be added to Table 1 as ovarian cancer has now been shown to be a diverse group of cancer with very different molecular profiles and biological outcome.  
Reply:  
Please see response to point 6 above.