Author's response to reviews

Title: Plasmacytoid variant of bladder cancer defines patients with poor prognosis if treated with cystectomy and adjuvant cisplatin-based chemotherapy

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

Many thanks for your e-mail from the 31st of October and the possibility to revise our manuscript. In addition we would like to thank the reviewers for their supportive efforts. Please, let me stress the point, that the reviewers’ helpful suggestions helped us to improve the overall quality of the manuscript.

The AUO-AB 05/95 trial was published in the Journal of Clinical Oncology in 2005. The trial was conducted in the 90’s and was at this timepoint not prospectively registered as this was not required for performing and publishing randomized and controlled trails at that time. The trial is one of the largest trials on adjuvant chemotherapies in bladder cancer and highly cited over the years. Moreover, it served as a basis for different high-ranking publications on bladder cancer as it reports on a very valuable patient cohort. Therefore we kindly ask you to give us the possibility to report on our interesting and important results despite the lacking registration.

In the following section, we would like to address all issues raised by the reviewers point by point.

All changes exceeding minor grammatical revisions have been highlighted in the main manuscript.

Reviewer 2 (Giovanni Lughezzani):

The authors examined the survival of patients treated with cystectomy and adjuvant cisplatin CT for bladder cancer according to different histological subtypes.

Major compulsory revisions

- The authors state that patients with locally-advanced bladder cancer were included in the analysis. From table 1, it appears that also some patients with pT1
and/or pN0 disease were included. I think the authors should provide better information about the inclusion criteria used for enrolling patients for the current study.

*Answer: Thank you for this remark. The total number of patients with T1 disease was only 5 patients (2.5%), moreover the indication criteria for the AUO trial was locally advanced bladder cancer either pT3 /pT4 or pN1. All patients were treated according to current guidelines.*

- The authors present a series of patients that were subjected to different adjuvant CT schemes. These different schemes may act as confounders in the overall survival analysis performed by the authors. Are there any data available on the survival outcomes of patients subjected to these different treatments? Are the toxicity profiles similar between these schemes? Were there any early events related to CT?

*Answer: The AUO-trial showed that adjuvant chemotherapy with CM and MVEC is equally effective in this setting. Moreover, chemotherapy with GC is the current standard in chemotherapy, because it is regarded to be equally effective as MVEC with less toxic side effects. Multivariate Cox’s regression hazard analysis of our cohort was adjusted to the chemotherapeutic regime and it showed histology as an independent risk factor that is not influenced by different chemotherapies.*

- I do not understand why the authors used overall survival as the outcome to be evaluated. Overall survival may be affected by several variables besides tumor characteristics (e.g. age, comorbidities etc.). Besides providing data on patients comorbidities on table 1, the authors should analyze the impact of histological...
subtypes on cancer-specific survival, as it may represent the best outcome to be evaluated to test the prognostic impact of tumor aggressiveness itself.

Answer: We added in the discussion that overall survival as endpoint is a limiting factor of our analysis. We do not have data for all patients concerning cancer-specific survival but we have them for overall survival. Therefore statistical analysis with all known data (overall survival) should give more reliable results. In addition chemotherapy treatment can result in comorbidity and can finally affect overall survival. Therefore we think that overall survival is acceptable as endpoint of our study.

When we consider cancer specific survival we see in Kaplan Meier analysis mean survival rates for PUC patients of 35.5 months, for UC patients of 67.8 months and for MPC patients of 64.2 months but this is not significant (P=0.22; log rank test). In the multivariate Cox’ regression hazard analysis (adjusted to age, sex, tumor grade, tumor stage, lymph node and metastases status, type of chemotherapy) we find a 2.0-fold increased risk of tumor-related death for PUC patients compared to MPC patients (not significant, P=0.26) and an 1.8-fold increased risk of tumor-related death for PUC patients compared to UC patients (not significant, P=0.15). We have added these data to the revised manuscript.

- It appears that the percentages are missing in Table 1. In addition, Table 1 should report the p-values obtained by comparing the different variables for each of the three histological subgroups. The statistical test used for evaluating these comparisons should also be reported in the methods section.

Answer: We added the percentages in the table. The p-values obtained by comparing the different variables for each of the three histological subgroups were
added. The statistical tests (Fisher’s exact test and t-test) are mentioned in the table legend and in the material and method section. In addition we added the findings to the Result section:

“A significant difference for chemotherapy treatment between the different histotypes (P=0.043; Fisher’s exact test; table1) is detected this difference is reasoned by the fact that only UC patients but not MPC or PUC patients have been treated with gemcitabine and cisplatin. However, when we consider only M-vec and Cm treatment regimens no significant difference between the different histotypes is detected (P=0.451; Fisher’s exact test; data not shown).”

- The authors report that plasmacytoid patients showed significantly worse overall survival relative to patients with other histologies in Kaplan-Meier analysis (p=0.013). The authors should clarify the test use for such a comparison (I assume it is the log-rank test). In addition, they should provide to separate p-values referring to the comparison between PUC-UC and PUC-MPC, respectively.

Answer: Yes, we applied log-rank test for the comparison in the Kaplan-Meier analysis. We have added this to the Material and Method section. Since we think multivariate Cox’s analysis is more meaningful than the univariate one (Kaplan-Meier analysis), we have analyzed the comparison between PUC-UC also in a multivariate Cox’s regression hazard analysis. We detected that PUC patients had a 2.4-fold (95% CI: 1.3-4.4; p=0.006) increased risk of death compared with UC patients. We have added this finding to the Result section.

- The authors should provide a table (Table 2) reporting the results of their multivariable Cox regression analysis. Specifically, it would be interesting to
know whether there were any other independent predictors of survival besides
tumor histology. In addition, the authors report a significant overall survival
difference between PUC and MPC patients only (p=0.045). Therefore, I assume
that there were no significant differences between PUC and UC patients. This
finding is somehow surprising. Do the authors have any explanation for this?
Again, what about testing cancer-specific survival?

Answer: Reviewer 1 asked to integrate the table with the Cox’s regression backward
model (formerly Suppl. table now table 2) into the manuscript. This table includes
the data asked by reviewer 2. We see for the histological subtype and for the lymph
node status a significant correlation with overall survival when all
clinopathological parameters are included in the model. However, in the backward
Cox’s regression model the significance for the lymph node status disappears
(becomes a trend towards significance) already at step 2. However, the histological
subtype remains significantly associated with the risk of death.

There was a significant difference between the PUC and the UC patients, i.e. PUC
patients had a 2.4-fold (95% CI: 1.3-4.4; p=0.006) increased risk of death compared
with UC patients but no significant difference in risk of death between UC and MPC
patients was detected. We have added this new finding to the result section and
thank the reviewer for this helpful comment.

**Reviewer 1 (Jo Cresswell)**

A histological study linked to a trial of radical cystectomy combined with adjuvant
chemotherapy for locally advanced bladder cancer. The original trial results were
published in 2005 so the follow up is lengthy.
The authors have analysed results according to histological subtype and suggest that plasmacytoid urothelial carcinoma has a worse prognosis than either conventional urothelial carcinoma or micropapillary variant.

The study is of interest as it identifies a subgroup of cancers with a particularly poor prognosis. These subtypes are rare so this report is one of the larger case series. This is of value to the treating urologist as it suggests that aggressive treatment with early cystectomy is warranted. None of the patients received neoadjuvant chemotherapy so we cannot draw any conclusions regarding its value. Nor can we compare to a group without adjuvant chemotherapy.

1) The main limitation of the study, as the authors acknowledge is the small numbers of subtype cancers which is inevitable. This may explain the somewhat unexpected result that micropapillary variant (MPC) have better survival than conventional urothelial cancer (UC) and plasmacytoid (PUC). This is particularly surprising given that all of the MPC had T3 or T4 disease and more than half were node positive. Urologists will not be reassured that MPC has a better prognosis than previously thought based on these small numbers.

PUC on the other hand, seemingly has a very poor prognosis and studies such as this may provide evidence for aggressive treatment of this disease at the early stages, and indicates insensitivity to chemotherapy.

Methods:

2) The authors should comment on the cut-off value of 50% used to define the different subtypes. It would be interesting to see the correlation with prognosis for different proportions of subtypes.
Answer: The new histological subtypes of bladder cancer are first described in the 2004 WHO classification. There is still no consensus among the pathologist what would be the optimal cut off to define a specific subtype because nearly all cases show areas of conventional UC. Most studies used a cut off of 50%, so we used this cut off in the present study. We think this cut off is reasonable, because the majority of the tumor shows the specific histological subtype which defines a potentially poorer prognosis. We did not evaluate 10% or 30% cut off levels. However, very few cases showed such a minority component. Therefore, the 50% cut off is a reasonable one for the time being to define histological variants of UC which are clinical relevant.

3) A further limitation is the retrospective review of histology by a single pathologist. Assessment of the different subtypes will involve significant interobserver variability and this weakens the study. It would be interesting to see the correlation with histological report from a second pathologist blinded to the initial report.

Answer: As there is still no consensus among the pathologist about the best cut off to define variant histology interobserver variability could affect the quality of the results. We added this information in the discussion.

Results:

1) The table included as a supplementary file is very important to the readers interpretation of the results, and should be included in the main article.
Answer: We included the table (table 2) in the main text as suggested by the reviewer.

2) The percentages are missing for many of the values in the table

Answer: Thank you for this remark. We added all percentages to the table for a better understanding of the data.

Minor corrections:

Abstract

1) Consider "has gained more attention" last line of background

Answer: We corrected the sentence as suggested.

Results

2) Paragraph 1 includes median age of patients but mixes up months with years

Answer: Thank you for this remark. We corrected months to years in this sentence.

Discussion

3) "gathers well known" consider "accumulates"

Answer: We changed the sentence to: „As for PUC we could show in the largest series described to date, that on the one hand this subtype of urothelial carcinoma accumulates prognostic unfavourable molecular features, like such as the loss of CK20, a high proliferation index and p53 accumulation, and as well as on the other hand exhibits characteristic molecular features, like such as a complete loss of membranous E-cadherin expression [4],” like suggested by the reviewer.

4) "exhibitings“ should be "exhibits"
Answer: We changed the sentence as indicated. Please, see answer to remark 3.

Survival curves

5) No legends are included. The authors should explain what is meant by "censored"

Answer: Sorry for the missing legends of the survival curves. We added them to the figures for a better understanding. Censoring of patients (marked with a cross) means mathematically removing a patient from the survival curve at the end of his/her follow-up time. This explanation was added to the figure legend.

Reviewer 3 (Theodorus Van der Kwast)

Reviewer's report:

Major compulsory revisions:

1) There seems to be an overrepresentation of plasmacytoid variant of UC (n=18, about 10%), and it is not clear where these patients originate from: part (166) is from the AUO-AB05/95 trial, but 39 seem to come from elsewhere. I would like to see the distribution of variants in the two populations. Could there be a bias explaining why a) PUC pts. are younger and b) have a different prognosis? Explain why this overrepresentation of PUC in this series!

Further, please mention in the discussion that overall survival is measured as outcome parameter which is of course a limitation.

Answer: Thank you for these critical remarks. As the AUO trial analysed survival of patients with locally advanced bladder cancer we see a selection of patients with assumable more aggressive diseases than on average. This is probably the reason of the overrepresentation of PUC in this series. As shown in table 1 all cases of PUC
came from the AUO-AB 05/95 trial comparing CM and MVEC. The trial included patients with pT3/pT4 or pN1 disease. Our experience with these histological variants is that they are frequently locally advanced. Looking at the clinopathologic parameters there was no unfavourable patient selection of PUC patients compared to UC patients. In fact there were less pT1 tumors and less pN1-3 tumors in PUC. Moreover in the PUC cohort there were less female patients, what is regarded as a negative prognostic factor in muscle invasive bladder cancer. Regarding age at diagnosis it seems that PUC patients are somewhat younger, but this information is limited by the small number of patients included. We added in the discussion that measurement of overall survival is a limitation of the study („Another limitation of our results is the measurement of overall survival in our series as this could be affected by several variables besides tumor characteristics.“). But as mentioned as answer for reviewer 2, we do not have data for all patients concerning cancer-specific survival but all data for overall survival. Therefore statistical analysis with all known data (overall survival) should give more reliable results in our opinion. In addition chemotherapy treatment can result in comorbidity and can finally effect overall survival. Therefore we think that overall survival is acceptable as endpoint of our study.

Minor essential revisions:

1) Supplementary table 1 is not complete, please fill out %. Now, it is almost unreadable.

   Answer: We completed the table by adding all the percentages as suggested.

2) Discussion is very lengthy and could be condensed substantially.
Answer: Thank you for this helpful comment. We condensed the discussion and tried to arrange it not so lengthy.

3) The title is somewhat inadequate: I would suggest that it includes plasmacytoid variant.

Answer: Thank you for this proposal. We changes the title of our manuscript to: „Plasmacytoid variant of bladder cancer defines patients with poor prognosis if treated with cystectomy and adjuvant cisplatin-based chemotherapy”

Specifics: 3) On page 7 please insert "PUC" before "patients" in the sentence ...using gemcitabine and cisplatin may occur at least in a subgroup of patients. ...Otherwise it is not clear for the reader.

Answer: We added „PUC“ in the sentence as requested.

4) In the supplemental table 1 grades include grade 2 and 3, apparently from the WHO 1973 system. Given the retrospective review, I would expect that they would have been graded using WHO 2004. lease explain in M&M which grading system was used.

Answer: We added in the material and methods: Grading was performed according to the WHO classification of 1973 and the one of 2004. According to the WHO classification of 2004 all of the tumors were classified as high-grade. This information was added in table 1.
5) Page 8 lower part: "Our results clearly show that MPC morphology is..... " I do not understand this statement in this context as the authors show that MPC has no impact on overall survival.

Answer: We removed this sentence from the discussion to improve the overall understanding of the text and to shorten the discussion.

The hot topic of our manuscript and its interest for your readership is further highlighted by the results of Dayyani et al. who published their results on poor prognosis of PUC patients in *The Journal of urology* during the review process of this manuscript („Plasmacytoid Urothelial Carcinomas - A Chemo-sensitive Cancer with Poor Prognosis, and Peritoneal Carcinomatosis“). We added a note (below Acknowledgements) in our manuscript and amended the references with the updated data.

After revising the manuscript according to your suggestions we would be very honored if you would find the manuscript suitable for publication in “BMC Cancer”.

Yours sincerely,

Bastian Keck for all authors