REBUTTAL

September 10, 2013

Dear Editor, dear reviewers

We would like to thank you for the careful review process and the opportunity to submit a revised version of the manuscript according to the reviewer's comments. Especially, we thank that we were allowed to have a longer revision time after contacting the editorial office. **We would like to respond point by point to the reviewer's concerns.**

Title: Late Biliary Complications in Human Alveolar Echinococcosis are associated with high mortality

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Reviewer 1 (Reviewed by 00001390)

The present study evaluated the role of late biliary complications in non-resectable alveolar echinococcosis (AE) under long-term chemotherapy with benzimidazoles, and showed that the occurrence of late biliary complications indicates a poor prognostic outcome. This study will provide useful information with intensive study for rare disease. I understand this study will be provisionally valuable one, however, I have some questions especially in statistical analysis.

Comment 1. When statistical analysis was done based on non-parametric method, results of numerical data are indicated as median (quartile), not as mean±SD shown in this manuscript.

Response to this point: As suggested, the results section has been changed and data are now presented as median (quartile)

Comment 2. Because follow-up periods of patients with late biliary complications was twice longer than that of control without late biliary complications with significance, the occurrence of late biliary complication may be influenced by follow-up periods? If patients with control group are followed-up more longer periods, late biliary complication possibly occur?

Response to this point: We agree with the reviewer and also feel this is a very valid point. It is impossible to answer this question precisely due to lack of specific data regarding this point. However, we believe that this is not of relevance for the observation. Patients in the control group had a maximum follow-up of 25.3 y, patients with biliary complications had a maximum follow-up of 26 y, thus showing a considerable overlap despite different mean (20.3 vs 11.3 y) and median (23.0 vs. 8.6 y) follow-up times. Furthermore, follow-up time was not associated with biliary complications in regression analysis. This, in our opinion, makes it unlikely that the different follow-up explains all the differences.

Comment 3. What is the independent risk factor for the occurrence of late biliary complication? To clarify this, multivariate statistics is needed?

Response to this point: We specifically like to thank the reviewer for this suggestion. This point has been profoundly discussed by the authors. In AE, there are no "parasite factors" known which would influence the therapeutic response (comparable to genotype or viral load as "viral factors" in chronic hepatitis C.) "Parasitic load" or PNM classification might be risk factors for lack of therapeutic response; unfortunately, in our patients with late biliary complications these data are lacking.

With these considerations in mind, we did a multivariate analysis, following the reviewer's suggestions. Regression analysis revealed that previous surgery is a risk factor for late biliary complications as might be expected. The association of length and type of benzimidazole treatment is difficult to interpret which is discussed in the manuscript. These new data are now shown in Table 3 and are explained in the text of the manuscript.

Comment 4. I think that deaths during follow-up until 2006 or survival should not be analyzed by the Mann-Whitney in table 1, but should be analyzed by Log-rank test in figure 2. Figure 2 showed that there were no significant difference between biliary complication group and control, that seems to be a final result of this study. I consider it is inappropriate to compare survival after diagnosis of the late biliary complication with

overall survival in the control group or complication group, that was shown in figure 2, because time course of these groups were essentially different. In conclusion, the present study showed that there were no significant differences in survival between biliary complication group and control.

Response to this point: We would like to thank the reviewer for this suggestion. We agree that the original Figure 2 was potentially misleading. Therefore, this figure was split into two separate panels. In the new Figure 2 only overall survival of the two patient groups will be directly compared (Fig. 2A); survival of patients after the occurrence of biliary complications is presented now in a separate panel (Fig. 2B). Statistical analysis for Fig. 2A is done using the Log rank test and lack of a significant difference clearly pointed out.

We also agree with the reviewer that the direct comparison of survival time of the group with biliary complications vs. the group without (Mann-Whitney test table 1) has indeed been inappropriate. This result is now removed from the table (and explained by a footnote).

We believe that, due to these modifications, the result of our will now be much clearer to the reader: there was no significant difference in survival for patients with and without biliary complications.

Reviewer 2 (Reviewed by 00068472)

General The authors address the important area of late biliary complications of alveolar Echinococcosis (AE). They evaluated the late biliary complications in non-resectable AE under long-term chemotherapy with benzimidazoles. The authors concluded that late biliary complications are common and are associated with poor prognosis and high mortality rate.

In my view the English needs some corrections. Overall, the presentation of the topic is confused.

Major Compulsory Revision:

1. General

1.1. Overall, the presentation of the topic is confused.

Response to this point: The manuscript was carefully edited. Parts of the manuscript were

rearranged and the wording and the language improved (compare point 1.2). We believe, these changes will greatly increase clarity and readability of the manuscript.

1.2. The English needs corrections.

Response to this point: The submitted revised version of the manuscript has been proof-read by a native English speaker.

2. Abstract

2.1. The Abstract is incomplete: the selection criteria, statistical methods are poorly defined.

Response to this point: We agree with the reviewer, the abstract has been modified for completeness and clarity.

2.2. The conclusions drawn appear to be not sufficiently supported.

Response to this point: the conclusion has been adapted to make it less speculative. Additional analyses have also been added (see point 3.2.) further expanding the manuscript.

2.3. As stated in the Cover letter, the authors should clearly state that instead a prospective cohort study, this is a retrospective analysis.

Response to this point: We agree on this point and state clearly in the revised version of the manuscript that this is a retrospective analysis.

3. Methods

3.1. The authors must explain in more detail the selection (inclusion/exclusion) criteria of patients.

Response to this point: In the methods section, the patient selection process (inclusion, exclusion criteria) has been clarified in the text similar to figure 1.

3.2. The potential confounding variables were not taken sufficiently into consideration.

Response to this point: We agree that this is an important consideration and this concern has also been raised by reviewer 1. A linear regression analysis is now included in the manuscript addressing this point (please compare our response to comment 3 of reviewer 1).

3.3. Is there any difference in outcome in association with the type of benzimidazole treatment?

Response to this point: The type of benzimidazole treatment was considered in our linear

regression analysis. However, we believe that benzimidazole subgroups were too small to allow for a meaningful analysis (even though significant results were obtained). In the text the reader is cautioned not to over interpret this result (please also compare our response to comment 3 of reviewer 1).

4. Results:

4.1. Overall, the presentation of results is confused.

Response to this point: We carefully edited the manuscript on many occasions to improve readability and clarity of the manuscript. We believe, the structure of the new manuscript is now more readable

4.2. They should discuss separately the liver-bilary related and non-related mortality.

Response to this point: The discussion concerning this point has been changed.

4.3. The most common biliary complications during long-term chemotherapy were the following: late-onset cholangitis, sclerosing-cholangitis-like lesions, hepaticolithiasis, affection of the common bile duct, secondary biliary cirrhosis. Liver biopsy has been done in all complications? What does it mean exactly: "sclerosing-cholangitis-like lesions" or "affection of the common bile duct"?

Response to this point: The terms "sclerosing-cholangitis-like lesions" or "affection of the common bile duct"? are explained more precisely in the revised version of the manuscript. Liver biopsies were not performed routinely in these patients, especially not to prove biliary cirrhosis. This has been added in the methods section as well.

5. Discussion

5.1. The conclusions drawn appear to be not sufficiently supported, therefore are a little speculative.

Response to this point: The conclusion has been adapted to make it less speculative.

5.2. They should discuss in more detail several limitations of their study.

Response to this point: We agree on this point and discuss the limitations in more detail in the discussion.

5.3. The potential predictive and prognostic factors for late biliary complications should be added and discussed.

Response to this point: Following the reviewers suggestions a Pearson's correlation and linear regression analysis is now included in the manuscript. Our data are summarized in

the new table 3. We also like to refer to our response to comment 3 of reviewer 1.

The manuscript has been improved according to the suggestions of reviewers:

References and typesetting were corrected.

We hope that, with these changes,the manuscript becomes valuable for publication in the *World Journal of Gastroenterology*.

Sincerely yours,

Dr. P. Frei , PD Dr. S. Vavricka and co-authors

September 10, 2013