Author's response to reviews

Title: Interleukin-22 predicts severity and death in advanced liver cirrhosis: A prospective cohort study

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Author’s response to reviews: see over
Dear Professor D’Souza,

thank you for considering a revised manuscript of “Interleukin-22 predicts severity and death in advanced liver cirrhosis: A prospective cohort study” for potential publication in *BMC Medicine*. We are pleased that reviewer #1 had only minor points of criticism and found our study carefully performed and well written. We are also grateful to reviewer #2 for the valuable suggestions to improve our manuscript. We have carefully revised our manuscript according to each of the comments of the reviewers. Please find attached the revised manuscript with the changes underlined and a point by point response. We hope that the revised manuscript is now suitable for publication in *BMC Medicine*.

With kind regards

Bernd Kronenberger       Heiko Mühl       Albrecht Piiper
Point by point response

**Reviewer #1: Aldo J. Montano-Loza**

1. Spelling of “bilirubin” on page 10, first para.

   The spelling error is now corrected.


   The term “aminotranspeptidase” was replaced by “aminotransferase”.

**Reviewer #2: Ashwani Singal**

We appreciate that the reviewer found the idea novel and thank for his suggestions to improve the manuscript. All concerns were carefully addressed and included in the revised manuscript:

1. **Background:** Poorly developed and does not stretch to specific aims of the paper. For example authors themselves rightly point out protective role of IL-22 and fail to provide sufficient background information on which the study hypothesis is based.

   We agree with the reviewer that background information and development of the hypothesis could be improved and have performed efforts to fulfill this.

   Background information is given on page 5, para 2 and 3, and page 17, para 2 and 3. The description and development of the hypothesis was improved (page 5, para 3).

2. **Methods:** a) It is not clear whether elevated levels of IL-22 are taken at the baseline or any time during the follow up, b) not clear how the survival was recorded as many patients would die outside the hospital, and c) did no one drop out of the study and this seems really surprising for me especially for sick
patients as included in this study

a) **Time of sampling of IL-22**
The time of blood sampling is clarified in the revised manuscript. All samples for IL-22 baseline were collected at study inclusion (page 8, para 1).

b) **Recording of survival and c) dropout**
As explained on page 9, para 2 the follow-up period started at study inclusion and first blood collection for IL-22 quantification and ended at patient’s time of death, liver transplantation or last contact. Only death was recorded as event. All patients were seen every 4-12 weeks at the liver center of the hospital of the J.W. Goethe University. At the end of the study the patient’s physician was contacted. In patients who were lost to follow-up before the end-of study, the time in the study ranged from study inclusion to last contact.

3. Results: a) **Tables and figures are redundant. For example figure 2 and 5 could be combined into one figure and figure 7 and 8 may not be needed,**

a) **Tables and Figures.**
The manuscript contains two tables. Table 1 contains patient characteristics and is highly necessary. Table 2 shows the correlation of IL-22 with hematological and biochemical parameters. The reviewer is correct that some data in the tables were repeated in the manuscript text. The redundant passages were removed in the text of the revised manuscript page 11, para 1; page 15, para 1 and 2.

The reviewer suggested combining Figures 2 and 5. Figure 2 shows the comparison of IL-22 between healthy controls and the overall cohort. Figure 5 shows IL-22 levels in the subgroups according to liver disease etiology. We agree with the reviewer that Figure 5 may not be needed as it contains negative results (no association between etiology of liver disease with IL-22 levels). Because the result is reported in the text, we removed Figure 5 from the revised version of our manuscript.

The reviewer states that Figures 7 and 8 may not be needed. Figure 7 shows the comparison of IL-22 levels in patients with different forms of liver disease related complications. As this
Figure illustrates a major finding of the study, we believe that this Figure is important and should not be removed.

Figure 8 shows the correlation between IL-22 and the MELD score. This is also an important observation which should be illustrated. As both Figures are important in our opinion, the Figures were not removed. Nevertheless, redundant information in the text is now removed (page 15, para 1).

b) Results of cox regression analysis
The results from multivariate Cox regression are now completely shown on page 16, para 1, including HR and CI and P values.

c) Correlation of IL-22 with AFP
The correlation between IL-22 and AFP was calculated. However, it was not significant (r=0.018, P>0.2). This is now included in Table 2.

4. The significance of the study is not convincing. Is it possible that high levels are just a reflection of severity of liver disease?
A major finding of the present study was that elevation of systemic IL-22 is associated with the presence of liver related complications. The reviewer is right that this important finding was not pointed out in the discussion. A statement that high levels of IL-22 may reflect liver disease is now included in the present manuscript (page 18, last sentence of para 1).

5. Does IL-22 clear through the liver and what is its pharmacokinetics?
IL-22 was not administered in the present study, therefore pharmacokinetics cannot be determined. In the literature there is no information about IL-22 elimination. In the present study we investigated whether IL-22 levels are stably increased during follow-up or whether IL-22 levels were only transiently increased. We observed that IL-22 levels remained elevated in the majority of the patients (Figure 3, page 12, last para).

The mechanism of IL-22 elimination is not yet clarified. It can be assumed that IL-22 levels are associated with increased production, and reduced hepatic or renal elimination. This point is now addressed on page 18, last sentence of para 2.
6. It would have been better if authors looked at the c-statics of IL-22 and compared to established prognostic model MELD score.

The study was planned in cooperation with the Institute of Biostatistics and mathematical Modeling of the J. W. Goethe University and was designed to perform survival analysis by Cox regression analysis. As described above the time in the study ranged from inclusion to last contact or death. Death was recorded as event. This type of analysis appears most appropriate to analyze differences in survival according to a baseline parameter.

The estimated area under the observed receiver operating characteristics, also referred as c-statistic, is a statistical procedure which has also been generalized for use in survival analysis. According to the reviewer’s suggestion, we included a ROC analysis to compare MELD score and IL-22 in the revised manuscript (page 16, last para). In agreement to the Cox regression analysis, IL-22 significantly discriminated patients who died from those who survived the follow-up period (AUC 0.682, CI [0.560-0.805], P=0.010) while the MELD score did not discriminate patients who died during follow-up vs. those who survived (AUC 0.611, CI [0.470-0.752], P=0.118).

7. Pearson calculation may not be the right way to look at as IL-22 has not been shown by the authors as a continuous score in this study like MELD score. Rather prognostic value of IL-22 was shown at a cut off of 18.

Pearson calculation was not performed in the present study and we agree with the reviewer that this test would not have been appropriate. Instead in the manuscript the non-parametric Spearman calculation was performed. Furthermore, the correlation between IL-22 and the MELD score was performed with continuous IL-22 and continuous MELD score. The correlation is illustrated in Figure 7 of the revised manuscript.

8. In addition language is tardy at many places. For example Blood sampling heading should be replaced with collection of blood samples, decompensated liver disease heading in table 1 should be liver related complications, liver disease heading in table 1 should be etiology of liver disease, cell death heading in table 2 should be liver enzymes to name a few!

The addressed terms were replaced. The manuscript has extensively been checked for errors by experienced scientists proficient in English language.