Author's response to reviews

Title: Association of response to hepatitis B vaccination and the survival in dialysis patients

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

Following your letter regarding the manuscript “Association of response to hepatitis B vaccination and the survival in dialysis patients” submitted to BMC Nephrology for publication, we are sending the rebuttal letter explaining the changes performed on the manuscript. The changes incorporate the suggestions of the reviewers and we used the tracked changes with color text. We found the comments very helpful and constructive. We have addressed all the changes recommended by the reviewers and we are confident that the new version of the manuscript is easier to understand and clearly defined. The revisions are addressed below.

**Reviewer:** Gabriel Almroth

1. **Abstract: Background:** Line 2; As described recently.

   Page 2 line 3
   We have corrected the sentence as “As described recently, “ accordingly.

2. **Abstract-Results:** Line 2; There was no significant association between immune response (not difference)

   Page 2 Line 12
   We have corrected the sentence as” There was no significant association between immune response and 5-year survival rate (p =0.600)…”

3. **Abstract-Background:** Line 3; followed by infection as second cause. Line 18; Proneness to infection (not easily infection)

   Page 3 Line 6
   We have changed the sentence as “……, with infection the second leading cause” accordingly.

   Page 4 line 1
   We have changed the sentence as “……from proneness to infection to
impaired immune response to vaccination”.

4. Methods: The retrospective design of the study is of importance and similar prospective studies are suggested in the discussion part.

Thanks for this suggestion. We have added in Page 14 line 13 “The results of our study need to be replicated in prospective studies of using larger populations of chronic, stable dialysis patients.”.

4. Reference numbers 8-10 are written in small, why?

It is an error during processing this manuscript. We have corrected it accordingly.

5. Statistical analysis: The statistical tests were performed with an established program and the models chosen are motivated in text and figures.

We really thanks for this comment.

6. Results:
The most common cause of mortality was (unusually to the general population of dialysis patients) infection instead of cardiovascular death and the cause of death could be given in more detail for the 19 patients who died from infection. As discussed malnutrition and age were associated with increased mortality. The results of the predictive model of infection-cause mortality in dialysis patients shown in table 3 and figure 2 are very interesting and deserves publication.

We have added the detailed causes of 19 patients who died from the infection. In page 6 line 16 “The most common cause of death was infection (54.3%; 19 patients, including 8 cases of severe pneumonia, 4 cases of peritonitis, 2 cases of infective endocarditis, and 1 case of either meningitis, diabetic foot infection,
septic arthritis, urosepsis, and septicemia)

7. The discussion part is well written. Why are the references 11-13 again given in small types? Examples of language errors: In the present study instead of in present study and Recent review has pointed out instead of has point out. Also In the last parts of the discussion "causing instead of casing and "Interestingly we did not find any associations between... instead of the associations". The dysregulations of various cytokines might be better language than deregulations. High levels of IL-2 instead of higher levels of IL-2 etc. From the findings above rather than from above findings. In the study of Kimmel et al they suggest that better T-cell function rather than only "better T-cell function..."Dyslipidemia is spelled wrongly and regarding the infection-cause mortality is better than regard the infection-cause mortality. Our present findings rather thanour present finding.

It is a processing error of the draft that Ref 11-13 in small types. In page 9 line 4, we have corrected to “[11-13]. We really thanks for the correction of the language errors. In page 9 line 12, we corrected as “ In the present study, …” In page 10 line 1, we corrected as” A recent review indicated…” In page 10 line 15, we corrected as” responsible for….” In page 10 line 16, we corrected as “ …an association…” In page 11 line 4, we corrected as “ …dysrgulation…” In page 11 line 12, we corrected as” …High levels of IL-2…” In page 12 line 6, we corrected as” These findings indicate that the laboratory immune parameter, especially the Th1/Th2 cytokine ratio that implies defective immunity, may be able to predict clinical outcomes,…” In page 13 line 15, we corrected as “ dyslipidemia..”

8. References, tables and figures are all acceptable

Thanks for this comment.
Thanks. We have sent for English editing before the submission of this revision.

Reviewer: Carlos Abaete de los Santos

Reviewer's report:

1) Comparing the "Conclusion" (found in the "Abstract") with the "Figure 1" looks like they do not combine, because the figure didn’t demonstrate differences in the survival curve when responders and non-responders were compared, contrarily to the statement registered by the authors in the text.

Thanks for this comment. This study aimed to investigate the association of response to hepatitis B vaccination and survival in dialysis patients. Our data showed the clinical immune response following HBV vaccination could not predict the all-cause mortality in dialysis patients. Thus, the figure 1 presented the curves of all-cause of mortality rather than the infection-cause mortality between responders and non-responder. We have addressed this point in the last section of the discussion as “In conclusion, we demonstrated that the non-response following HBV vaccination could predict the infection-cause mortality in dialysis patients. However, the clinical immune response following HBV vaccination could not predict the all-cause mortality in dialysis patients.” To avoid the possible misunderstanding, we have added “There was no significant association between the immune response to HBV vaccination and the 5-year survival rate “in the conclusion of abstract. Our results are definitely consistent with the conclusion.

Based on the results above, we further analyzed the association of response to hepatitis B vaccination and the cause of mortality. We noted that non-response following HBV vaccination, diabetes, and low level of albumin could predict the infection-cause mortality in dialysis patients (Table 3, figure 2). Thus, the conclusion in the abstract is consistent with our results.

2) Although the authors mentioned many times in the text the
presence of "infection-inflammation" they did not demonstrate its clinical or laboratory presence anytime.

End stage renal disease (ESRD) is a recognized status of chronic inflammation, as a result of hypercytokinemia (Girndt M et al, Kidney Int 2003, 63: S76-79). Our study enrolled ESRD subjects only, so it might be reasonable that our study population would be in status of chronic inflammation with varying degrees.

In clinical setting, ESRD status is easily prone to cardiovascular disease as well as infection. The acquired immune dysfunction in uremic milieu might be the chief pathogenic factor, mediating through CVD and infections, to cause the majority deaths in ESRD (Kato S et al, Clin J Am Soc Nephrol 2008, 3: 1526–33). Several studies have worked out the association between laboratory Data (i.e cytokines) and clinical outcomes in ESRD patients. Girndt et al have found the higher levels of IL-6 and TNF-α correlate with non-seroconversion to HBV vaccinations in HD patients (Girndt M et al, Kidney Int 1995, 47:559-65). Kimmel et al suggest that better T cell function and humoral immunity are associated with a survival advantage in HD patients (Kimmel PL et al, Kidney Int 1998, 54: 236-44).

In this study, we attempted to link the clinical findings (i.e. the immune response following HBV vaccination and the survival in ESRD populations). Thus, we have presented the clinical data about the mortality and infection-related mortality. Due to the study design, we do not check the cytokines profiles of our study subjects. Thus, we could not provide the laboratory parameters about inflammation in our study. Our current study provides further illumination for the complex associations among immune dysfunction and cause of mortality in ESRD patients. We might carry out future studies to clarify the relationship among the pattern of cytokines, immune response, and survival in ESRD patients. We really appreciate this viewpoint. Thanks.

3) As far as I could understand the authors showed completely different results, based in distinct statistical methods used and based its conclusions in the most convenient one.

All statistical analyses are conducted by a statistician who is one of our
authors. We have re-examined the statistic methods. We are sure that the statistic methods are appropriate and our results are definitely consistent with the conclusion.

**Quality of written English:** Needs some language corrections before being Published

Thanks. We have sent for English editing before the submission of this revision.

**Reviewer:** Seyed-Moayed Alavian

**Reviewer's report:**

1- we need more clear data regarding prevalence of diabetes at the onset of study and anti-HBV vaccination and finally at the end of study or dying and impact of all of these in response to HBV vaccine and loss of anti-HBs Ab. Thanks.

It is a valuable point since diabetic patients might have higher mortality and weakened immune response to HBV vaccination. Our previous reports (Liu et al, vaccine 2005; 23: 3957-3960) have showed that diabetes is not related to the immune response following HBV vaccination. Furthermore, we have assessed the decaying rate of anti-HBV Ab in dialysis population and noted that DM is not associated with decaying trend of anti-HBs Ab. (Lin et al, Vaccine 2011; 29:3738-41)

In this study, the prevalence of diabetes at the onset of study and anti-HBV vaccination is 32.0 % (diabetes: 50 patients, total: 156 patients). We have analyzed the interaction between DM and response to HBV vaccination \( (p=0.111) \) at the onset of study (table 1).

We have mentioned this on Page 6 line 10” The prevalence of diabetes at the onset of study or anti-HBV vaccination is 32.0 %. Univariate analysis indicated no significant difference of responders and non-responders in gender, presence of DM, presence of hepatitis C antibodies, hemoglobin, albumin, dialysis
modality, triglyceride, cholesterol, and several other relevant characteristics (Table 1)."

Further, at the end of study or dying, The prevalence of diabetes at the end of study or dying is 30% (diabetes: 36 patients, survival or dying: 120 patients). We found that no significant association between DM prevalence and loss of anti-HBs Ab (p=0.308).

To address this point, we have added the description on page 7 line 2 “By the end of the study, the prevalence of DM in all available subjects (including those that died during the study) was 30 %, but the impact of DM on the loss of anti-HBs Ab was not significant (p=0.308).

2- more clarification regarding malnutrition and validity of it.

In the study of Fernandez et al (Nephrol Dial Transplant 1996, 12; 1204-11), they noted that low level of albumin and low pre-dialysis serum urea are two most important factors closely and positively correlated to the response following HBV vaccination. Therefore, in their discussion, they concluded “malnutrition unfavorably influences the response to the HBV immunization in haemodialysis patients”. However, for more clear validation, we revised the “malnutrition” as “low level of albumin” in our discussion. We have added one reference in this section: Lowrie EG, Lew NL: Death risk in hemodialysis patients: The predictive value of commonly measured variables and an evaluation of death rate differences between facilities. Am J Kidney Dis 1990; 15: 458-82. The section was revised as “The study by Fernandez et al of 64 HD patients noted that low levels of albumin negatively influenced the response to HBV vaccination. In the study's survival analysis of 31 patients, non-responders had higher mortality and morbidity [30]. The authors did not adjust for albumin levels or age in the survival analysis of these HD subjects. However, our Cox regression survival analysis of 156 dialysis patients reveals that only old age and low levels of albumin have significantly higher morality but that non-response following HBV vaccination did not. Since low albumin levels are recognized as an independent factor of mortality in ESRD [31], we speculate that low albumin levels account for the concomitant impaired immune response following HBV vaccination and increased mortality in the Fernandez et al study”.

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.
Concerning the statistics, all statistical analyses are conducted by a statistician who is one of our authors. We have re-examined the statistic methods. We are sure that the statistic methods are appropriate and our results are definitely consistent with the conclusion.

Other additional changes;
1. We have changed the affiliation of Tsai Chen An.