Author’s response to reviews

Title: Mechanistic Study of the Cause of Decreased Blood 1,25-Dihydroxyvitamin D in Sepsis

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Dear editors,

We really appreciate the reviewers’ comments which aimed to improve the quality of our manuscript. We have now extensively revised the manuscript according to the comments. The revised parts related to the reviewers’ questions are highlighted in the main text of this manuscript. In addition, please also see the following point-to-point responses to the reviewers’ questions.

1. Reviewer 1:

1) In the results section: the authors start to discuss their results. These discussion parts should be taken into discussion or some to introduction part.
In the manuscript, we include rationales and brief descriptions of study designs before presenting specific data. We believe that this can help our readers to understand the contents better. Therefore, we would prefer to keep these parts.

2) Do the authors have the data regarding survi of sepsis patients? If yes, was there a difference between survivors and non-survivors?

Yes, these data are now included in Figure S1 and Table S2. From a time point different from that in our previous study of the same patient cohort (Nguyen HB et al., Plos One, 2013), our current data further support the previous finding, i.e. non-survivors when compared to survivors had significantly lower serum 1,25(OH)2D levels.

3) When was the serum sample was taken form sepsis patients? How long after the diagnosis of sepsis? And how did it affect the results?

We used the serum samples taken 3 hours after the diagnosis of sepsis. Patients might have received various treatments before the samples were taken. This could potentially affect the results.

4) Are there any other possible mechanisms that have been shown to be associated with low vitamin d levels? This issue may be discussed by the authors.

We have included the following discussion in our manuscript:

“Under normal conditions, serum 1,25(OH)2D levels are controlled by multiple mechanisms including genetic factors [31], serum IGF-1 levels [32, 33], serum FGF-23 levels [31], and serum 25(OH)D levels [32]. It has been shown that serum IGF-1 levels positively [32, 33] and serum FGF-23 levels negatively [31] correlate with serum 1,25(OH)2D levels. However, serum 25(OH)D levels do not correlate with serum 1,25(OH)2D levels [31, 34]. Serum 1,25(OH)2D levels change only when serum 25(OH)D levels change dramatically [34]. In our patient cohort, serum 25(OH)D levels at hospital admission were 21.5 ± 1.1 ng/mL [7] which are defined as vitamin D insufficiency but not deficiency [35]. Although the potential contribution of decreased serum 25(OH)D levels cannot be excluded, based on the foregoing previous findings, we reason that the decreased serum IGF-1 levels and increased serum FGF-23 levels are the major contributors to the suppressed serum 1,25(OH)2D levels.”
5) A correlation analysis can be made between 1,25 OH D2 levels, FGF23, IGF-1 and Cr to support their hypothesis.

We performed a correlation analysis and the data are presented in Figure 2.

Because kidney failure may directly impair kidney 1α-hydroxylase or indirectly via increased serum FGF-23 levels, serum creatinine levels were used for the correlation analysis. Using Spearman correlation analysis, our data showed that both serum levels of creatinine (Fig 2A, R=-0.333, p=0.006) and IGF-1 (Fig 2B, R=0.210, p=0.083) moderately correlated with serum 1,25(OH)2D levels. These data further support that multiple mechanisms including decreased serum IGF-1 levels and kidney failure/increased serum FGF-23 levels contribute to the suppressed serum 1,25(OH)2D levels in sepsis patients.

2. Reviewer 2:

1) GH has pulsatile secretion and in healthy subjects, very low levels of GH exist during the day without indicating GH deficiency. Thus GH measurement during any time does not give adequate message regarding GH dynamics. Serum IGF-1 level is more informative on the GH axis.

We agree with the reviewer’s comment that GH measurements at a specific time may not adequately provide information regarding GH dynamics. However, our data do suggest that there is an impairment in the expression and signaling of GHR in liver. Therefore, our data support that GH in sepsis patients is not sufficient to maintain normal serum IGF-1 levels.

2) Serum PTH level should be evaluated simultaneously with actual serum calcium level. The authors should give simultaneous serum calcium levels during the measurement of serum PTH level (may be given as an additional figure in Figure 1)

We have added the serum calcium levels in Figure 1C. Similar to the mouse data (Fig 3C), sepsis patients had significantly decreased serum calcium levels.

3) The numeric data are presented as figures. It would be better to give the data also as tables.

The numeric data are now included in Table S1-3.
4) Figure 1E: Control subjects are healthy people !!. Why their serum creatinine level is also increased?

We agree that normal healthy control subjects should not have higher creatinine levels. We checked the original data and found that we used a kit for this specific measurement from a company different from that for other creatinine measurements. All the values from this specific kit trended higher. Since the main purpose of this data is to compare between normal healthy controls and sepsis patients, we performed a transformation by arbitrarily dividing all values with a factor of “2”. The newly transformed data are presented in Figure 1F. We hope that this is a scientifically acceptable method for data presentation under this specific scenario. In addition, this was a separated experiment. Therefore, data from the rest of this manuscript are not affected.

5) What was the serum 25 (OH)D levels? Were they replaced in case of deficiency?

We did not measure 25(OH)D levels in this study. This study used the same patient cohort that was used in our previous study (Nguyen HB et al., Plos One, 2013). In the previous study, 25(OH)D levels were measured and at hospital admission were 21.5 ± 1.1 ng/mL. Based on current definition, this sepsis patient cohort had vitamin D insufficiency. Please also see added discussion regarding this topic.

The vitamin D insufficiency in this patient cohort was not corrected via supplementation.

6) Six patients have chronic renal failure (CRF) as a co-morbidity. As the authors suggest, 1-alpha hydroxylase activity is necessary for active vitamin D synthesis which is mediated by several factors such as FGF-23, PTH, etc. Presence of CRF will affect 1,25 (OH)2D activity/level irrespective of sepsis. Thus, the authors should also give data without including those patients with CRF. By this way, we will see the sole/real effect of sepsis on the mentioned parameters.

We have now added this data in Fig S2. Overall, the data are similar to those presented in Figure 1.

7) Conclusion: This paragraph should not be the repeat of summary of the presented data. Instead, it should provide clinical significance of the study and guidance for future studies.

We have now modified the conclusion as suggested.