Author's response to reviews

Title: The impact of pretransplant 25-hydroxy vitamin D deficiency on subsequent graft function: An observational study

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Version: 2 Date: 13 January 2012

Author's response to reviews: see over
Dear editor and reviewers

We highly appreciate the detailed comments of the referees and editor on our manuscript “The impact of pretransplant 25-hydroxy vitamin D deficiency on subsequent graft function: An observational study” and consideration for publication. Point-by-point suggestions are quite helpful for the improvement of our paper and during a month, we made every effort to incorporate them in the revised manuscript by reanalysis and reconstruction of the collected data.

As below, I would like to clarify some of the points kindly addressed by the reviewers and sincerely hope that the reviewers and the editors will be satisfied with our responses to the comments and the revisions for our original manuscript.

Sincerely,

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P.S. As editor requested, we have stated “written informed consent was obtained from all subjects” in method section.
Reviewer's report

Title: The impact of pretransplant 25-hydroxy vitamin D deficiency on subsequent graft function: An observational study

Version: 1 Date: 10 November 2011

Reviewer: Kyra Borchhardt

Reviewer's report:
Hyunwook Kim et al describe interesting observations regarding graft function of 131 kidney transplant recipients after 36 months and pretransplant vitamin D deficiency.

Although the results and work are important and should be published there are major issues that should be discussed/changed or described in more detail:

Methods: The patient flow should be documented. How many patients received a kidney graft during that time period? How many recipients lost graft or went back to dialysis, or were lost to follow up. How many patients were excluded for given reasons et cetera. I suggest providing a figure.

Response: As you suggested, the patient flow titled “Study profile” is provided as figure 1 and its details are described in method section (page 6-7).

Results: Concomitant therapy of patients is no result- this belongs into the patient- methods section. There should also be an explanation why patients received one or the other drug. Bisphosphonates aren’t supplements. Patients receiving vitamin D supplements should be excluded from the study, as we neither do not know their increase in 25-OHD post-transplant nor its impact on graft function, that however is key.

Response: As you suggested, the description of concomitant therapy and bisphosphonates as supplements, and data of the patients receiving vitamin D supplements after kidney transplantation (25 patients) are eliminated from result section and tables. Indications for post-KD vitamin D supplements are also described briefly. And subsequently, we have re-analyzed all parameters in the remaining 106 patients and state renewed data in revised manuscript.

Patients were divided into two groups regarding 25-OHD concentrations. The patient population itself is very inhomogen as it seems you have patients with hypocalcaemia due to sec. hyperparathyroidism in preemptive KT patients- but also malnutrition in other patients with long term chronic dialysis. Please use therefore the albumin corrected calcium.

Response: As you suggested, for correcting for confounder such as malnutrition, we have calculated albumin-corrected calcium and entered into analyses, which are shown in method/result section (page 7, 12) and tables.

Predominant RRT modality was associated with 25-OHD levels. Could there be a bias in patient collective for CAPD? Is there a negative selection bias? This should at least
discussed.

**Response:** Due to small number of the participants, we cannot completely rule out the possibility of selection bias. However, there are a few articles that reasonably explain the mechanisms of that phenomenon, and furthermore, demonstrate a similar result to our data. Therefore, we have mentioned them in the revised manuscript with references (page 16-17, reference 21-22).

Acute rejection episodes were defined clinically and biopsy proven? Did you distinguish between rejection episodes successful treated versus sine effectu, or the type and severity of rejection? Please discuss.

**Response:** All acute rejection episodes described in our manuscript were diagnosed not by clinical signs alone but confirmed by biopsy. Therefore, we have clarified this in our manuscript. In addition, as you suggested, the details of rejection type, treatment courses, and outcomes of the patients have been also described in method section (page 7).

There is an ongoing RCT investigating this issue. Should be mentioned (Thiem et al, Trials 2009)

**Response:** As you suggested, we clearly have mentioned that trial in the end of conclusion and also listed it in references (page 20, reference 35).

**Level of interest:** An article of importance in its field
Reviewer's report

Title: The impact of pretransplant 25-hydroxy vitamin D deficiency on subsequent graft function: An observational study

Version: 1 Date: 25 November 2011

Reviewer: Neil Boudville

Reviewer's report:

Minor Essential Revisions

Comments to authors:
- can you describe seasons that 25OHD was measured as this will clearly influence the level

Response: As you suggested, we have analyzed and described the seasonal variation of pretransplant 25-OHD levels in revised manuscript. By analysis, we found that while there is a trend towards higher 25-OHD levels in summer/autumn than in spring/winter, but it does not reach statistical significance. Therefore, we have not included the seasonal factor in subsequent multivariate analysis (Figure 2, page 11-12)

- How many grafts were not functioning 36 months after transplant? What were the 25OHD levels in these grafts. It does not seem to make sense to me to exclude these ones.

Response: As you pointed, we totally agree that it is the most desirable to analyze all the data from all the screened participants without a missing value. However, during follow-up period, we faced several aspects of note as below:

1. There was a definite trend towards lower eGFR in the patients with 25-OHD deficiency than in control patients over the study period.
2. There also existed several differences in baseline characteristics between groups (control vs. 25-OHD deficiency) other than 25-OHD level.
3. Occurrence of the analyzable events was too low to apply the statistical method using a dichotomous variable as outcome. Therefore, we had to use the statistical method using a continuous variable as outcome.

To satisfy above numbered conditions, we had no choice other than “repeated measures analysis of covariance” as statistical method, which can only be used with an equal number of observations per subject and fixed periods between observations. Therefore, we had to eliminate the data of the patients who did not satisfy above requirements, such as the patients who experienced graft failure, had missing data of graft function, or were dead during study period. And we have introduced this statistical process briefly in revised manuscript (Figure 1, page 6, 9).
Does the relationship between 25OHD and eGFR still stand when the cutoff is higher and also if 25OHD is used as a continuous variable.

Response: As you suggested, we have tried to analyze the data using higher cutoff value for 25-OHD deficiency, but no significant relationship between 25-OHD and eGFR was found. The details are as below:

1. Cutoff value as ≤ 15 ng/ml:
   65.1% (69/106) of the patients were classified as 25-OHD deficiency and repeated-measures ANOVA analysis shows that there is no significant between-subjects effect (25-OHD deficiency vs. control) \[F(1, 104) = 0.813, P = 0.369\]

2. Cutoff value as ≤ 20 ng/ml:
   84.9% (90/106) of the patients were classified as 25-OHD deficiency and repeated-measures ANOVA analysis shows that there is no significant between-subjects effect (25-OHD deficiency vs. control) \[F(1, 104) = 0.383, P = 0.538\]

3. Cutoff value as ≤ 30 ng/ml:
   99.1% (105/106) of the patients were classified as 25-OHD deficiency. Therefore, no further statistical analysis is thought to be needed.

Further, there were also no significant correlations between 25-OHD and eGFR analyzed at one year interval, using 25-OHD as a continuous variable by Pearson’s correlation analysis. The details are as below:

- 25-OHD level between eGFR at 12 month \(r = 0.069, P = 0.481\)
- 25-OHD level between eGFR at 24 month \(r = 0.010, P = 0.920\)
- 25-OHD level between eGFR at 36 month \(r = 0.025, P = 0.798\)

This association between 25OHD and eGFR does not prove causation. There are considerable other explanations. The main way to tease this is is to perform an intervention study.

Response: We totally agree with your comments. There has been a considerable amount of experimental and clinical studies proposing their possible interactions and several representative findings are quoted in our manuscript (reference 6-10, 23). And as also mentioned in our manuscript, ongoing randomized controlled trial by Thiem at al. (ClinicalTrial.gov NCT00752401) is especially worth being expected to address the issue of the effect of vitamin D supplementary intervention on posttransplant outcomes.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.
Reviewer's report

Title: The impact of pretransplant 25-hydroxy vitamin D deficiency on subsequent graft function: An observational study

Version: 1 Date: 1 December 2011

Reviewer: Didier Ducloux

Reviewer's report:
Kim et al reported a study on the impact of pretransplant 25-OH D3 deficiency on subsequent graft function. Briefly, they included 131 patients who underwent kidney transplantation. They measured 25-OH D3 before transplant and defined 25-OH deficiency as a concentration < 10ng/ml. They found that 25-OH D3 deficiency was associated with female gender, low serum albumin level, and peritoneal dialysis. 25-OH D3 level was the only parameter associated with eGFR during the 36-month follow-up period.

I have major concern with this study. Even when the question is of interest, this study suffers from too many biases.

The number of patients included in the study is too low to reasonably answer this question.

Response: Thank you. We have acknowledged that limitations, which are mainly due to single center design. However, we hope that you would concede that there might be some advantages of single center study, such as consistency in treatment protocol and in delicate sample handling that is especially required in the study of this field and can contribute to reducing bias.

A number of confounding factors (donor age, cold ischemia, PRA, ...) are lacking. As a consequence, it is very difficult to interpret the association between 25-OH D3 level and graft function.

Response: Donor factors of age and sex have been analyzed and described in manuscript (page 12, Table 1-2). And as you can observe in our study, 99.1% (105/106) of donors were living donors and, among them, 72.4% (76/105) were living-related donors. Such donor selection has been a matter of culture and ethics in our country (although now changing). This can explain why during study period, we so frequently omitted the cold ischemia time and PRA test results, which have been not routinely assessed. Other possible confounding factors associated with kidney transplantation have been described in manuscript.

The results suggest that 25-OH D3 level is more important for graft function than acute rejection. I do not think that it is realistic.

Response: Thank you. We have acknowledged that limitations. The small number of the participants might also contribute to that result. However, a number of mechanisms have been proposed to demonstrate the immunological roles of vitamin D. Further clinical and experimental studies are needed to address a potential causal role of vitamin D in acute rejection.