Author’s response to reviews

Title: Relationship between parathyroid mass and parathyroid hormone level in hemodialysis patients with secondary hyperparathyroidism

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Version: 2 Date: 13 December 2014

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**Author’s response to reviews:** see over
On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript. We have studied the comments from the reviewers carefully and revised the manuscript as the reviewers suggested. The revised portions are highlighted in the new manuscript. We have tried our best to revise our manuscript according to the comments and we hope the correction will meet with approval.

Reviewer #1: Bjorn Meijers

Majors revisions

1. If I’m correct, the authors correlated PTH levels with each resected parathyroid gland separately. In my opinion, it would be more logical to calculate, for each patient, total weight and volume of all resected glands and correlate these measurements with PTH levels.

   1. I am sorry for the misunderstanding caused by our negligence. Just as the reviewer said, the volume and the weight in Figure 3 were calculated for each patient and were the total weight and volume of all resected glands. However, the data of the maximum diameter was got from one parathyroid gland no matter what the anatomical location of the parathyroid gland.

   1. As pointed out by the reviewer, I realized that it is really a problem worthy of attention and needs a more detailed description. Thus, we revised the manuscript and marked in red in revised paper. I hope the correction will meet with approval.

2. If I see the distribution of PTH (Figure 1C), I’m not sure that this is a normal distribution. Please use QQ test and Shapiro-Wilk test to more formally test the normality. If not, use non-parametric test statistics (Spearman instead of Pearson, …)

   1. Thank you for your valuable comments. Actually, before the correlation analysis, the normality test of the statistical data was conducted. The data was not shown in the manuscript.
1 Q-Q Plot

![Q-Q Plot of PTH](image)

1 P-P Plot

![P-P Plot of PTH](image)

<table>
<thead>
<tr>
<th>Tests of Normality</th>
<th>Kolmogorov-Smirnov&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Shapiro-Wilk</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>Statistic: .102, df: 223, Sig.: .000</td>
<td>Statistic: .936, df: 223, Sig.: .000</td>
</tr>
</tbody>
</table>

<sup>a</sup> Lilliefors Significance Correction

3. There was no correlation between gland measurements and PTH levels below 1000. The authors state that this may be due to the use of 2nd HPT treatments. How many patients were on calcimimetics or phosphate binders, and were there indeed differences in drug therapy use between the different PTH subgroups? Could you give the number of patients in each PTH subgroup, maybe the authors did not observe a
correlation in the lower PTH groups due to rather low number of patients?

1. We appreciate the concern very much. It’s really important to think about and analyze the use of 2nd HPT treatments in the results. However, because vitamin D analogs and calcimimetics were unavailable in mainland China during the study period, the most common drugs currently used to treat secondary hyperparathyroidism in China were calcium-based phosphorus binders and non-selective VDR activator medications which may cause hypercalcemia and hyperphosphataemia. Thus, although all the patients included were on calcium-based phosphorus binders and non-selective VDR activator, poor compliance and irregular medication were usually found because of the recurrent hypercalcemia and hyperphosphataemia. And that’s why we preferred parathyroidectomy than repeat medication.

1. Although the records of irregular usage of drugs were hard to statistic, we are very willing to provide the general data in accordance with the requirements. In the 70 patients with iPTH<1000pg/ml, 10 patients are currently taking calcium carbonate (all patients took calcium carbonate in the medication history); 68 patients are currently taking non-selective VDR activator (all patients once received repeated ictus treatment of non-selective VDR activator in the medication history); In the 153 patients with iPTH>1000pg/ml, 22 patients are currently taking calcium carbonate (all patients took calcium carbonate in the medication history); 95 patients are currently taking non-selective VDR activator (all patients received repeated ictus treatment of non-selective VDR activator in the medication history).

4. As there was a preoperative ultrasound evaluation of the parathyroid glands, was there also an estimation of volume… If so, what is the agreement between these measurements and those of the resected glands?

1. For each patient, the localization of parathyroid glands was evaluated preoperatively by using two-dimensional (2D) ultrasound. Because most of the parathyroid glands were irregularly shaped, estimates of volume seem to be difficult and our ultrasound doctors were not able to provide the volume data for us. For example:
1. On our direct experience, two-dimensional ultrasound imaging is a useful way to determine the location of parathyroid glands. However, the value of estimation of volume is limited. The surgical exploration for parathyroid glands and the surgical experience were crucial.

5. In 90% of cases there was a histologic nodular transformation of the parathyroid glands. Were there differences in volume and weight between these cases and the other glands without nodular transformation?

1. Thank you for your valuable comments. It was true that the volume was larger and the weight was heavier in the parathyroid gland with nodular transformation, especially with multiple nodules. However, since in one patient, there were some glands without nodular transformation and some glands with nodular transformation. From the perspective of individuality, we don't pay more attention to the problem.

6. Could you give some more information about the indications of PTX in your centre?

1. As mentioned above, vitamin D analogs and calcimimetics were unavailable in mainland China; the most common drugs currently used to treat secondary hyperparathyroidism in China were calcium-based phosphorus binders and non-selective VDR activator medications which may cause hypercalcemia and hyperphosphataemia. Besides, the ictus treatment of large dose non-selective VDR activator was recommended even when iPTH is < 1000 pg/mL. In this case, hypercalcemia and hyperphosphataemia which could ultimately lead to vascular
calcification and cardiovascular events were common seen, although iPTH could be repressed temporarily. Thus, we cannot help wondering should we still use repeated ictus treatment of non-selective VDR activator when the parathyroid gland is becoming larger, even if iPTH is < 1000 pg/mL. Based on the above point of view, we actually have broadened the indications of PTX in our centre.

1 Inclusion Criteria: 1) Patients receiving dialysis for more than 6 months; 2) serum intact PTH level >500 pg/mL on two or more occasions; and/or 3) Patients with parathyroid nodular or diffuse hyperplasia identified by ultrasound imaging or radioisotope scan; 4) Patients with symptomatic secondary hyperparathyroidism, such as bone and joint pain, pathologic fractures, severe pruritus, restless legs syndrome and so on.
Reviewer: Klaus Olgaard

1. The strenghts of the paper is the very large number of parathyroid glands examined. The result are, however, only very sparsely described and not even in enough details to support the conclusion above. The patients were evaluated by ultrasonography and parathyroid scintigraphy, the results of these examinations are unfortunately not provided and the accuracy of the methods are not calculated. Therefore, the above conclusion is only valuable when the PTX has been performed and will as such not unfortunately not provide any meaning in the clinical evaluation. Please, discuss this part in details in the Discussion and please modify your conclusion appropriately.

1. We appreciate the concern very much and we also noticed such issues. However, please allow me to make some explanation. For each patient, the localization of parathyroid glands was evaluated preoperatively by using two-dimensional (2D) ultrasound and parathyroid scintigraphy (termed Tc-99m 2 methoxy-isobutyl-isonitrile, 99mTc-MIBI). Because most of the parathyroid glands were irregularly shaped, estimates of volume seem to be difficult and our radiologists were not able to provide the volume data for us. For example:
I don't know whether this problem is due to our technical flaws. However, I guess it might be common when the department was just beginning to carry out this surgery. On our direct experience, two-dimensional ultrasound imaging and parathyroid scintigraphy were useful ways to determine the location of parathyroid glands. However, the value of estimation of volume is limited. The surgical exploration for parathyroid glands and the surgical experience were crucial. Thus, the points to my story were: 1) provide a general idea of the relationship between parathyroid mass and parathyroid hormone level for the surgeons which might add their experience. 2) provide an exact reference for the surgeons if they evaluated preoperatively by using three-dimensional (3D) ultrasound or other advanced imaging techniques; 3) we recommend early surgery treatment rather than the ictus treatment of large dose non-selective VDR activator. This problem is particularly severe in China. The volume parameters of parathyroid gland might be used as a much more appropriate guide for the indication of surgical treatment even when the parathyroid hormone was less than 1000pg/ml.

2. Figure 2A presents a very nice correlation between weight and size of the parathyroid glands ex vivo. The in vivo data from ultrasonography and parathyroid scintigraphy need however also to be provided, the accuracy and sensitivity of the methods evaluated and the results discussed in relation to the measurements of size.
and volume and plasma PTH levels.

1 Thanks to you comments and it is indeed a problem worthy of attention. As mentioned and showed above, the ultrasonography and parathyroid scintigraphy in our center were useful to evaluate the location of parathyroid glands, however, were not helpful to provide the volume parameters. The accuracy and sensitivity were difficult to analyze statistically.

3. The pathology presented of the parathyroid glands is very sparsely provided, as it is just mentioned that all patients had the diagnosis of nodular parathyroid hyperplasia. The material demands for a more detailed description, on glands with a single large nodulus, glands with several noduli and glands with eosinophil noduli, correlation to PTH levels, Was necrosis present in some of the large glands? Did the find any mitotic activity? etc.

1 Thank you for your valuable comments. Actually, in our studies, the nodular parathyroid hyperplasia was confirmed in three steps. Step 1: the formation of nodes was observed in a section of the resected parathyroid gland with the naked eye. Step 2: the pathologists provided the information of parathyroid hyperplasia for us. Step 3: immunohistochemical staining (such as PCNA) was carried out to study the morphological changes of parathyroid gland (As shown in Figure). However, because the topic of our article is not here, so we did not descript in detail in the Methods.

1 Necrosis can be seen in some of the large glands even with the naked eye.
4. The exact criteria for total PTX should be presented. Secondary HPT by itself is not an indication for PTX in dialysis patients.

1. As the reviewer pointed out, it is really true that parathyroidectomy is suggested in patients with CKD stages 3–5D with severe hyperparathyroidism (HPT) who fail to respond to medical/pharmacological therapy. However, the reality is complex in China. Because vitamin D analogs and calcimimetics were unavailable in mainland China during the study period, the most common drugs used to treat secondary hyperparathyroidism in China were calcium-based phosphorus binders and non-selective VDR activator medications which may cause hypercalcemia and hyperphosphataemia. Recurring hypercalcemia and hyperphosphataemia were high risk factors for vascular calcification and cardiovascular events. Thus, based on the above point of view, we actually have broadened the indications of PTX in our centre.

1. According the reviewer’s suggestion, we added the inclusion criteria and the exact criteria in the revised paper and marked in red. I hope the correction will meet with approval.

5. Why total PTX? and not partial PTX or autotransplantation?

1. We appreciate the concern very much and it is really a problem worthy of attention. Indeed, as shown in the previous studies, recommended surgical procedures were subtotal PTX and total PTX with autotransplantation. In 2008, when we first began this project in our center, the patients included all had undergone total PTX with autotransplantation (There were about 30 cases). However, after six months, about more than half of the patients have significantly elevated levels of parathyroid hormone. What’s more, the greater trouble is that the autotransplanted parathyroid gland tissues were not easy to remove. On our direct experience, the relatively small size of missed adenomatous glands could always lead to a high rate of re-operation in whom major anaesthesia risks are frequent. Thus, we took total PTX in our center from 2009. Until now, we didn't found significant side effects in the past three years. However, long-term effects still needs further study with longer follow-up times.

1. After carefully studying the reviewer’ comments, we guess Dr Olgaard might be a seasoned specialist in the field. We hope that we could establish more communication in future to exchange knowledge and experience in surgical treatment for secondary hyperparathyroidism.

6. What was the medical treatment that had been attempted before PTX and what was the relation to size and volume of the glands.
We appreciate the concern very much. It’s really important to think about and analyze the use of 2nd HPT treatments in the results. However, because vitamin D analogs and calcimimetics were unavailable in mainland China during the study period, the most common drugs currently used to treat secondary hyperparathyroidism in China were calcium-based phosphorus binders and non-selective VDR activator medications which may cause hypercalcemia and hyperphosphataemia. Thus, although all the patients included were on calcium-based phosphorus binders and non-selective VDR activator, poor compliance and irregular medication were usually found because of the recurrent hypercalcemia and hyperphosphataemia. And that’s why we preferred parathyroidectomy than repeat medication.

Although the records of irregular usage of drugs were hard to statistic, we are very willing to provide the general data in accordance with the requirements. In the 70 patients with iPTH<1000pg/ml, 10 patients are currently taking calcium carbonate (all patients took calcium carbonate in the medication history); 68 patients are currently taking non-selective VDR activator (all patients once received repeated ictus treatment of non-selective VDR activator in the medication history); In the 153 patients with iPTH>1000pg/ml, 22 patients are currently taking calcium carbonate (all patients took calcium carbonate in the medication history); 95 patients are currently taking non-selective VDR activator (all patients received repeated ictus treatment of non-selective VDR activator in the medication history).

Approx 1/3 of the patients had PTH levels < 1000 pg/mL and the PTH levels in this large subgroup did not correlate with glandular size and volume, what was the indication for PTX in these patients, how many had large glandular size and volume. From Figure 3 it is evident that even small glands can be combined with very high PTH levels.

As mentioned above, we actually have broadened the indications of PTX in our centre. According the reviewer’s suggestion, we added the inclusion criteria and the exact criteria in the revised paper and marked in red. I hope the correction will meet with approval.

From Figure 3 it is evident that even small glands can be combined with very high PTH levels. After carefully studying the comments, we checked the database to verify and this phenomenon indeed exists. Quite frankly, I have no idea either and could not have an explanation for you. I guess there might be some hyperfunctional nodule in the parathyroids.

The clinical indication for PTX is usually obvious, when the PTH levels is of several thousands. The indication is, however, sometimes difficult to establish, when PTH is < 1000 pg/mL, but it is just in this situation that the results of the present
investigation found that the volume parameters are not of any help. This needs to be stressed in the conclusion, and also in the Abstract. Both need to be modified.

1. After carefully studying the comments, we guess some confusion and misunderstanding might be due to our vagueness and ambiguity in discussion. As mentioned above, the most common drugs currently used to treat secondary hyperparathyroidism in China were calcium-based phosphorus binders and non-selective VDR activator medications. The ictus treatment of large dose non-selective VDR activator was recommended even when iPTH is < 1000 pg/mL. In this case, hypercalcemia and hyperphosphataemia which could ultimately lead to vascular calcification and cardiovascular events were common seen, although iPTH could be repressed temporarily. Thus, we cannot help wondering should we still use repeated ictus treatment of non-selective VDR activator when the parathyroid gland is becoming larger, even if iPTH is < 1000 pg/mL. In our opinion, the volume parameters of parathyroid gland might be used as a much more appropriate guide for the indication of surgical treatment. Based on the above point of view, we actually have broadened the indications of PTX in our centre.

1. However, your comments are constructive to me and we found that our manuscript still left much to be desired. We touched up the manuscript totally and I hope it will be fine this time. Please see revised manuscript.

9. I would suggest that the authors change the Title to “Relationship between parathyroid mass and parathyroid hormone ….” as they have it in the Running Title.

1. Thank you for your valuable comments. Your suggested title is really more appropriate. We have revised and marked in red in revised paper.

10. In “Selection of patients” the age of the patients included was > 18 years; In the Results the age range is from 16 to 70 years. Please clarify.

1. Thank you for your kindly reminds and this is my negligence. In order to avoid negligence, we touched up the manuscript totally again and I hope it will be fine. Please see revised manuscript.