Author's response to reviews

Title: A rare case of watery diarrhea, hypokalemia and achlorhydria syndrome caused by pheochromocytoma

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Author's response to reviews: see over
Answers to comments:

To Reviewer: Giovanni Conzo
1. The indicated citation was added as suggested (see Ref. 19).

2. The paper was revised and some language corrections were made by a native speaker.

To Reviewer: Jacques Lenders
1. The main issue why I am not completely convinced that this is indeed a pheochromocytoma is based on the following arguments:
   a. Pathology: only immunohistochemistry with chromogranin A was done. This and the elevated plasma chromogranin A argue of course for a pheochromocytoma but why couldn’t this be an other neuroendocrine tumor such as a carcinoid instead of a pheochromocytoma? The authors do also not describe the typical ‘Zellballen’ structure in the HE staining and I can not see this in the figure 2. Therefore I would like to see some other immunohistochemical marker such as synaptophysin or tyrosine-hydroxylase, showing that this is indeed a chromaffin cell tumor;

   Besides chromogranin A, synaptophysin has also been stained (see revised Fig.2B), other positive markers like vimentin, CD56 are also available if required.

   b. now the authors might bring in the argument that the plasma NMN was hugely elevated in the presence of an only marginally elevated MN. My explanation for this very high plasma NMN level is the extreme sympathetic activation due to severe dehydration and prerenal failure. (when was the plasma sample for NMN taken?). In addition, it is very unusual, although not impossible, to have such a large pheochromocytoma with such a marginally elevated plasma MN since one would expect also an increased epinephrine secretion;

   The plasma sample for NMN was taken after the vital signs were improved by huge volumes of fluid infusion, though BP was still low (100/60mmHg) and HR fast (95bpm), since dehydration and prerenal failure was never fully rectified before surgery.

   The measurement of MN and NMN was performed using LC-MS/MS in our lab. And based on our own experience, it had good performance with respect to sensitively, specificity and stability.

   It is very unusual, although not impossible, to have such a large pheochromocytoma with a marginally elevated plasma MN. To our
knowledge, VHL patients could have exclusively elevated NMN (see ref. 16 and 17). We have also previously diagnosed a VHL patient, who exhibited a marginally elevated plasma MN 96.8 pg/ml but severely elevated NMN 9621.6 pg/ml (unpublished data).

c. A last argument of the authors might be the positive PET/CT scan but this is not specific for pheochromocytoma.

It is true that positive PET/CT scan is not specific for pheo. However, since MIBG is not available in mainland China. At present, we are unable to provide further specific radiologic evidence.

2. Did the patients have any spells or other typical signs and symptoms indicating pheochromocytoma?

Typical signs and symptoms indicating pheochromocytoma were absent in this patient, probably masked by severe dehydration. Tachycardia persisted before surgery. However, it could also probably be caused by dehydration.

3. Line 71, page 4: What is Smecta? Please use the generic name of this drug.

Smecta has been replaced by the generic name “diosmectite”.

4. Please provide the normal ranges of all laboratory measurements to table 1. In addition, the serum albumin should be added in view of the abnormal calcium level.

We have added the normal ranges for each parameter as required. Serum albumin level was also added as required.

5. If the authors can provide more evidence that this indeed was a pheochromocytoma, I would suggest to add a table summarizing the most relevant features of all published cases.

To our knowledge, the most relevant feature of all published cases was severe diarrhea, typical of WDHA syndrome and found in all cases. Actually, the clinical manifestation of WDHA caused by pheo is mostly the same as WDHA caused by VIPoma. So I’m wondering whether a table summarizing the features is necessary.

6. Although the abnormal bone markers, suggesting more osteoclastic activity than osteoblastic activity, is interesting in the context of the increased VIP, the
absence of a bone biopsy limits the interpretation of these data. It is also not essential for this case. However I would use these data in the Discussion only to explain the hypercalcemia. Is there any relation between this disturbed bone formation versus resorption with the increased unexplained calcitonin levels?

We don’t think the moderately elevated calcitonin was strong enough to disturb bone formation versus resorption. We have previously diagnosed several medullary thyroid cancer patients, with much higher level of calcitonin compared with the present case. However, bone markers were mostly within normal range. For example, a previously diagnosed MTC patient had a calcitonin level of 593pg/ml. Her osteocalcin was 18(ng/ml) (6-24.7), β-CTX0.32(ng/ml)(0.04-0.78), P1NP 57.5(ng/ml) (9.1-76.2).

7. Was the thyroid nodule examined for calcitonin staining?

The thyroid nodule was examined and negative for calcitonin staining.

8. The length of the Abstract should be rigourously reduced from 2 pages to maximally 1 page.

The abstract is reduced as required.

9. Figure 2: panels B, C and E can be omitted since they are not essential here.

Panel B, C and E are removed. IHC for synaptophysin is added.