

Answering reviewers

Dear editor,

Thank you very much for giving us an opportunity to revise our manuscript. We appreciate the editor and reviewers very much for their constructive comments and suggestions on our manuscript entitled “Functionality is not an independent prognostic factor for pancreatic neuroendocrine neoplasms” (Manuscript No: 53637).

We have studied reviewers’ comments carefully and made a careful revision on the manuscript according to the reviewers’ detailed suggestions. All revised portions are marked in red in the revised manuscript. We would like to express our great appreciation to the editor and reviewers. Looking forward to hearing from you.

Kind regards.

Dr. Chunlu Tan

Corresponding author:

Name: Chunlu Tan

E-mail: chunlutan@163.com

Dear reviewers:

Thank you for your comments on our manuscript entitled “Functionality is not an independent prognostic factor for pancreatic neuroendocrine neoplasms” (Manuscript No: 53637). Those comments are very helpful for revising and improving our paper. We have studied the comments carefully and made corrections which we hope meet with approval. The main corrections are in the manuscript and below we present a point-by-point response to reviewers’ comments. (the replies are highlighted in blue).

Replies to the reviewers’ comments:

reviewer #1

1.How was the **sample size** determined? There should be **discussion about whether the study was powered enough to identify a prognosis difference between insulinoma, other functioning PNEN and non functioning**. As mentioned, there seems to be a trend favouring insulinoma.

Numerous studies have demonstrated correlations of functionality with prognosis when sample sizes were less than 100 cases [PMID: 27701882, PMID: 23194346, PMID: 27350059]. Therefore, we expected that any sample with a sample size over 100 should identify the correlation between functionality and prognosis in univariate analysis. The

focus of this study is to examine whether this correlation still exists after excluding the influence of other related components. However, there is no comprehensive approach to estimate the sample size of a retrospective cohort study utilizing multivariate Cox regression with median survival hardly reached. With the sample size increasing, nonfunctional tumors and other functional tumors gradually showed different prognoses from insulinomas. But after adjusted with risk factors which had the highest Wald statistics, the correlation between functionality and prognosis disappeared in the multivariate analyses. The results showed that the functionality itself does not affect the prognosis, but the factors (e.g., age, tumor size) which are related with functionality affect the prognosis.

reviewer #2

1. As stated in the manuscript, one database is SEER which is a US-based multicenter database using uniform criteria, while the other database is based on a single institution of different patient population in a different country, potentially with different guideline and management strategy. It is understandable that the authors want to combine the databases to construct a larger patient cohort, the inherent difference between the two databases makes any comparison difficult and less convincing. It would be more reasonable if the authors collaborate with different institutions in China to achieve a multicenter patient population.

The stage and grade of tumors were assessed according to the same guidelines (the 8th edition of the AJCC Cancer Staging Manual and WHO 2017 classification). Since the present study is a retrospective cohort study, the differences in management strategy or laboratory implementation (which could exist between West China Hospital database and SEER database) would also exist if we combined our database with another center. We analyzed West China Hospital database and SEER database separately to provide the results of single databases. In our database, functionality was not associated with prognosis in multivariate analysis. In the SEER database, functionality was not correlated with prognosis even in the univariate analysis.

2. It is kind of strange that the authors found that **neither T stage (China cohort) nor tumor grade (both cohorts) had prognostic significance, which is contradictory to the literature.** This needs to be explained.

In the Western China Hospital database, grade was an independent risk factor for the prognosis, and T stage tended to be related with prognosis in univariate analysis. In the SEER database, T stage was an independent risk factor for the prognosis, but grade was not related to prognosis. According to guidelines, the assessment of grade depends on mitotic count and Ki-67 index, with the cutoff value 2/10 HPF and 3%, respectively. However, the cutoff values that makes the most sense are still debatable. There was a trend of shorter survival time for patients with higher T stage in our small-sample cohort(N=205), and T stage turned out to be an independent prognostic factor in large-sample cohorts (SEER N=426, Total N=631), which is similar with the literature. On one hand, it indicated that T stage is indeed a factor that affects the prognoses of patients with pNET, on the other hand, the results showed the importance of sample size in cohort study. We stated it under the "discussion".

3. The cohorts appear to be limited to **well-differentiated neuroendocrine tumor, thus it should be stated as such, and use pNET rather than pNEN.**

We have made certain modifications to the “pNEN” in the text.

4. All p values should have at least one number, not all 0. e.g., **use $p<0.0001$ rather than $p=0.000$**

It may be confused and misleading that p-values was displayed as 0.000. Since the SPSS software shows 3 digits after the decimal point, we replaced “ $p=0.000$ ” with “ $p<0.001$ ”.

5. **The statement: "Tumors that secrete insulin, namely, insulinomas..." is inaccurate.** As mentioned by the authors, insulinoma is defined by a constellation of clinical syndrome, not just secretion of insulin.

This statement was modified as "Tumors that secrete insulin and cause endogenous

hyperinsulinemic hypoglycemia, namely, insulinomas...". According to previous guidelines, hormone related syndrome is the only basis to distinguish between the nonfunctional neuroendocrine neoplasm and several types of functional neuroendocrine neoplasm. However, immunohistochemical staining also displays the expressions of insulin/glucagon/gastrin/somatostatin in non-functional tumors. The reasonability of classification based on symptoms rather than gene expression needs to be further explored. The result of this study did not support the opinion that hormone related syndrome is an efficacious tool to classify tumors into groups with different prognoses.

6. **Most insulinomas are removed by enucleation, with no lymph node removed.** This may explain many cases staged as pNx (most are insulinomas). Alternatively, it **could be a result of insufficient pathologic examination.**

We have noticed that local excision may lead to a higher proportion of patients with no lymph nodes examined. However, the results of univariate analyses in the large sample still showed that regional lymph node metastasis (N1) is related to prognosis, and the hazard ratios indicated that Nx patients were more likely to be N0 patients. The literatures reported the presence of regional lymph node metastasis even with pNEN smaller than 2cm in diameter, so more lymph node dissection has been performed after 2010. We believe that regional lymph node dissection is a trend of surgical treatment of early pNEN which may prolong the survival.

7. The third paragraph under "discussion", line 7, **"Primary tumor" should be changed to "T stage".**

We did not find the phrase "Primary tumor" in the third paragraph under "discussion". In the fifth paragraph under "result" we change the "Primary tumor" to "T stage".

8. The running title is too long. **Recommend to change to: pNET functionality not prognostic.**

We thank the reviewer for the excellent suggestion and replace the running title with "pNET functionality not prognostic".

9. **Recommend further polishing** in English language. It is not easy to read.

We checked the spelling, grammar and punctuation again and revised the manuscript.

Once again, thank you very much for your constructive comments and suggestions which would help us both in English and in depth to improve the quality of the paper.

Kind regards,

Dr. Chunlu Tan

Corresponding author:

Name: Chunlu Tan

E-mail: chunlutan@163.com