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Title: "Clinicopathological Features of Nonfunctioning Pancreatic Neuroendocrine Tumors."

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Dear Editor

We wish to convey our heartfelt thanks for your response, dated April 12th, to our manuscript. We found the reviewers' kind and detailed suggestions very helpful and have revised the manuscript accordingly.

Please find the revised manuscript (with revisions underlined) and a letter that includes point-by-point responses to your and the reviewers' comments as well as a description of the corresponding edits to the manuscript. After that, we had *American Journal Experts* proofread our manuscript by. We are resubmitting this revised manuscript for publication in the *World Journal of Gastroenterology*. In the point-by-point responses, the page and line numbers are taken from the revised manuscript, which is not underlined.

We hope that these modifications meet your expectations. We would be pleased to address any further questions that you or your reviewers might have.

Sincerely yours,

Mariko Furukori, Masahiko Taniguchi, and Hiroyuki Furukawa

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Comments to the Reviewer #1

1) In a recent study by Hashim et al., even low-risk pNETs were shown to have the potential of positive lymph node status and to bear a worse survival rate if positive lymph nodes are detected (4.5 versus 14.6 years). Indeed, some authors (e.g. Tsutsumi et al.) also favoured regional lymphadenectomy for tumors greater than 15mm in size and for those located in the pancreatic head. However, the currently available, relative large case series do now allow conclusions on the omission of lymphadenectomy in pNETs. Rather, current literature seems to favor regional lymphadenectomy in the treatment of pNETs, regardless of the tumor size and G status, due its prognostic role. The authors should definitely discuss the implications of the findings of Hashim et al. (Annals of Surgery, 2014) and Tsutsumi et al. (J Gastroenterol 2012) for their study.

We appreciate your comments.

As you have mentioned, a recent study reported that even low-risk, small pNETs can have a positive lymph node status and a worse survival rate if positive lymph nodes are detected. Additionally, current research appears to favor regional lymphadenectomy in the treatment of pNETs. We understand the importance of lymphadenectomy, but whether this procedure is necessary for very small, asymptomatic tumors is questionable.

In the present study, we found that tumors with diameters of less than 10 mm showed no evidence of malignancy, were well differentiated, and lacked lymph node metastasis. Additionally, a previous study reported the following:

1) The incidence of lymph node metastasis increases with increasing tumor size. Reviews from the literature have reported that small tumors with diameters of less than 15 mm metastasize at a rate of 8%, which is low compared with the rate for large tumors.
2) Large tumor size is correlated with malignant potential, and smaller tumors tend to be well differentiated. Well-differentiated tumors have less malignant potential, grow slower, and have a better prognosis.
3) Compared with symptomatic NF-pNETs, tumors that are incidentally detected have a good prognosis and low risk of malignancy.

According to these reports and our results, we suggest that lymphadenectomy may be omitted in the future for small tumors with diameters of less than 10 mm, particularly for tumors that are incidentally detected. These findings require further investigation of a large number of small pNETs.

However, Hashim et al. reported that even tumors smaller than 10 mm in diameter metastasize

at a rate of 12%. We understand that the omission of lymphadenectomy needs to be carefully considered, and comments on this topic are included in the DISCUSSION.

Page 6, Line 21

Currently, the association between the tumor size and the incidence of lymph node metastasis is controversial. Hashim et al. reported that there is an increased probability of nodal metastasis when the tumor size is larger than 15 mm [9]. Tsutsumi et al. reported an increased prevalence of lymph node metastasis in patients with gastrinomas and non-gastrinoma who have tumor sizes of 15 mm or larger [27]. In contrast, Parekh et al. reported that the tumor size is not associated with lymph node metastasis [28]. A number of studies have reported that the incidences of lymph node metastases for patients with NF-pNETs smaller than 20 and 15 mm are 14.4% and 8%, respectively [2, 9, 27-30].

Page 6, Line 32

In the present study, one of the 8 patients with sporadic NF-pNETs had NEC with a tumor size of 22 mm. Except for the case with NEC, the direct invasion and metastasis to the lymph nodes was only observed in a relatively large tumor with a diameter size of 32 mm. Tumors smaller than 10 mm in diameter showed no signs of malignancy, were well differentiated, and lacked lymph node metastasis. Additionally, none of the cases had recurrence, including in the lymph nodes or direct metastasis, for more than 10 years after surgery. Lymphadenectomy may be omitted in the future after further investigation of a large number of small NF-pNETs. However, Hashim et al. reported that even tumors smaller than 10 mm metastasize at a rate of 12% [9]. Additionally, lymphadenectomy is often omitted for small pNETs that are larger than 10 mm in size; the possibility of lymph node metastasis may be underestimated in those cases. Omission of lymphadenectomy needs to be carefully considered with further study. Even when lymphadenectomy is omitted, long-term follow-up is essential because there is a risk of late recurrence. If malignancy is confirmed postoperatively, oncologically appropriate lymphadenectomy must be considered based on the factors that determine the malignant potential, such as the Ki67 index, tumor differentiation status, surgical margin, and vascular invasion such as lymphoductal, neural, and venous [20-21].

2) As also noted by the authors, the very small case load in their study does not allow reliable conclusions, at least not at the extent as put forward by the authors. For example, the case no. 3 on the Table 1 had a tumor size of 22mm but still had no lymph node metastasis. In the presence of such a small number of patients, it is possible to draw a big spectrum of conclusions, the majority of which may be wrong. Therefore, the authors should certainly consider including more patients and/or more carefully/conservatively formulating their conclusions.

We appreciate your comments.

As you have mentioned, we made too many broad conclusions.

We modified Title, the conclusions in the ABSTRACT and DISCUSSION sections as follows.

Title: Clinicopathological Features of Small Nonfunctioning Pancreatic Neuroendocrine Tumors

Page 2, in CONCLUSION, Line 15

Sporadic NF-pNETs smaller than 10 mm tend to have less malignant potential. These findings suggest that lymphadenectomy may be omitted for small NF-pNETs after further investigation.

Page 7, in DISCUSSION, Line 20

In summary, we found that small NF-pNETs tend to have less malignant potential. In the present study, six of 7 cases of sporadic NF-pNETs, except for a case with NEC, were small tumors (smaller than 10 mm diameter). These small tumors showed no evidence of malignancy, were well differentiated, and lacked lymph node metastasis. This finding indicates that lymphadenectomy may be omitted in the future for small NF-pNETs, particularly for those tumors that are incidentally detected after further investigation. When lymphadenectomy is omitted, long-term follow-up is essential, and additional resection should be considered if malignancy is confirmed postoperatively. The tumor size can easily be measured preoperatively, and further study is expected to find other factors for predicting the malignant potential of small NF-pNETs.

Comments to the Reviewer #2

Minor points:

1) How the authors characterize "free of recurrence"? Is it only radiologically (which modality?) and/or biochemically (CgA and/or PP and/or other related hormones)? The

biochemical values before the operation and during follow up should also be reported.

We appreciate your comments.
We confirmed that the patients were free from recurrence with a follow-up CT scan. We did not measure the blood level of CgA because this measurement is not covered by insurance in Japan. We updated the MATERIALS AND METHODS, RESULTS, and DISCUSSION sections.

In MATERIALS AND METHODS, Page 4, Line 6
The postoperative follow-up included clinical examination, the blood neuron specific γ-enolase (NSE) level, and contrast-enhanced computed tomography (CT) scanning. CT scans were performed every 6 to 12 months in the first year, then annually thereafter.

Page 4, in RESULTS, Line 9
None of the patients had a preoperatively elevated blood level of NSE.

Page 5, in RESULTS, Line 10
All of the patients are currently alive without disease recurrence according to radiological imaging.

Page 7, in DISCUSSION, Line 15
In the present study, CgA, PP, and other hormones were not measured; it is important to measure these hormones to identify recurrences during follow-up.

2) I suggest that the authors will clearly state that the well differentiated cases were sporadic NF pNETs (except the vHL case).

We appreciate your comments.
As you have mentioned, we only discussed well-differentiated, sporadic cases, except for the vHL case.
We have updated the RESULTS and DISCUSSION sections to address your comments.

Page 4, in RESULTS, Line 5

One patient with von Hippel–Lindau disease had previously undergone enucleation of the pNETs; the others were sporadic cases.

Page 6, in DISCUSSION, Line 32

In the present study, one of the 9 patients was diagnosed with von Hippel–Lindau disease, and this patient should be considered separately because the biological properties of sporadic pNETs and hereditary pNETs, such as MEN-1 and von Hippel–Lindau disease, are different with respect to the incidence, number of tumors, and prognosis.

1) ***It is recommended that the authors will include only well differentiated sporadic cases. NECs have a different pathophysiology and even small tumors can be aggressive including a potential of giving hematogenous seeding. Furthermore, by including only one NEC case it is not appropriate to draw safe conclusions.***

We appreciate your comments.

As you have mentioned, we only discussed well-differentiated sporadic NF-pNETs cases, including NEC. We have updated the RESULTS and DISCUSSION sections according to your comments.

In RESULTS, Page 5, Line 2

Six of the 7 patients with sporadic NF-pNETs had small tumors that were less than 10 mm in size; one patient with NEC had a larger tumor. Tumors that were less than 10 mm in size showed no malignancy, were well differentiated, and lacked lymph node metastasis.

In DISCUSSION, Page 6, Line 35

One of the 8 patients with sporadic NF-pNETs had NEC with a tumor size of 22 mm. Except for the case with NEC, the direct invasion and metastasis to the lymph nodes was only observed in a relatively large tumor with a diameter size of 32 mm. Tumors smaller than 10 mm in diameter showed no signs of malignancy, were well differentiated, and lacked lymph node metastasis.

Major points:

1) The number of well differentiated sporadic NF pNETs cases that are less than 10 mm is limited and no reliable conclusions can be drawn. More cases should be included. On the other

hand I understand that identifying NF pNETs <10mm has its limitations.

We appreciate your comments.
We cannot include more cases because there are no additional cases from our institution.
Instead, we updated the Title as well as the ABSTRACT and DISCUSSION sections as follows.

Title: Clinicopathological Features of Small Nonfunctioning Pancreatic Neuroendocrine Tumors

Page 2, in CONCLUSION, Line 15
Sporadic NF-pNETs smaller than 10 mm tend to have less malignant potential. These findings suggest that lymphadenectomy may be omitted for small NF-pNETs after further investigation.

Page 7, in DISCUSSION, Line 20
In summary, we found that small NF-pNETs tend to have less malignant potential. In the present study, six of 7 cases of sporadic NF-pNETs, except for a case with NEC, were small tumors (smaller than 10 mm diameter). These small tumors showed no evidence of malignancy, were well differentiated, and lacked lymph node metastasis. This finding indicates that lymphadenectomy may be omitted in the future for small NF-pNETs, particularly for those tumors that are incidentally detected after further investigation. When lymphadenectomy is omitted, long-term follow-up is essential, and additional resection should be considered if malignancy is confirmed postoperatively. The tumor size can easily be measured preoperatively, and further study is expected to find other factors for predicting the malignant potential of small NF-pNETs.

2) The identification of LN metastases may be a late event for pNETs due to their slow growing nature especial for tumors with a Ki67<3%. A longer (radiological and biochemical) follow up in the cases that did not undergo lymphadenectomy (eg cases 1 and 9) would strengthen the manuscript especially when the authors' postulation opposes the to date literature trend that favors lymphadenectomy.

We appreciate your comments.

As you have mentioned, long-term follow up is essential, especially when lymphadenectomy is omitted, because pNETs progress slowly, and there is a risk of late recurrence.
We have updated the DISCUSSION as follows.

Page 7, Line 9

Omission of lymphadenectomy needs to be carefully considered with further study. Even when lymphadenectomy is omitted, long-term follow-up is essential because there is a risk of late recurrence.

3) Other factors eg neural/angio-invasion and not only size may determine the malignant potential of a lesion.

Thank you for your comments.
As you have mentioned, other factors such as vascular invasion, tumor differentiation, and the Ki67 index may determine the malignant potential of pNETs. However, these factors are less likely help to determine which surgery is needed because they are not reliably available preoperatively. When lymphadenectomy is omitted, we must consider the oncologically appropriate lymphadenectomy in reference to the features of the surgical specimen.
We have updated the DISCUSSION section as follows.

Page 7, Line 11

If malignancy is confirmed postoperatively, oncologically appropriate lymphadenectomy must be considered based on the factors that determine the malignant potential, such as the Ki67 index, tumor differentiation status, surgical margin, and vascular invasion such as lymphoductal, neural, and venous [20-21].

We highly appreciate your valuable comments.
We have revised the manuscript according to the suggestions of the reviewers, and the references and typesetting were corrected.
The changes suggested by the reviewers have greatly improved the quality of our manuscript,

and we hope that you will find that we have satisfactorily addressed all the pertinent issues in the revised version. We are grateful for the opportunity to submit our revised manuscript, and we look forward to your positive reply.

Thank you again for considering our paper.

Sincerely yours,

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