Responses to reviewers' comments

Reviewer #1: This is a manuscript by Xu SS et al about tumor infiltrating platelets in pNETs. I have the following questions: Where the patients on therapy and if yes can It be named? I suggest that the following information will be presented: Size at different cut-offs, extend of disease (localized vs nodal/distant metastases) The authors present that the average score of platelets immunostaining density was assessed in each of 5 high-power fields (magnification 200×). Where these fields randomly selected? Can the authors please specify? Which were the negative and the positive tests performed? Was neutralization test performed? Can the authors please specify? Why the authors chose the cut off of 10% as positive? "positive referred to immunostained platelets distribution which accounted for ≥ 10% of intratumoral region". Why they did not choose another cut-off? Which was the species of the secondary antibody with 3,3'-diaminobenzidine chromogen was Secondary Antibody Kit (G1210-2-A, Servicebio, Wuhan, China), the kit is not available on the internet in English. Where levels of CgA or NSE measured and where they correlated to the author's results? Which was the biochemical profile of the non -functional pNETs (PP producing?, levels, correlation of the immunohistochemocal results to biochemical levels etc). Why the TIP marker should be used compared to other more validated markers eg Ki67 that its calculation is more standardized (See ENETS recommendation)? Microphotograph B. It seems to be more a background than a true immunoreactivity of specific cells. In general the authors do not convince that the marker included can be used as an alternative to more established markers. The 10% threshold that they present is not explained why it was chosen and the paucity of the marker results that its use is cumbersome in clinical praxis. I suggest that the authors will try with a Pathology or Oncology journal instead.

Answer: We really appreciate the reviewer for the constructive suggestions.

And as requested we have made point to point revisions.

1. Where the patients on therapy and if yes can It be named?

Thank you for your comments. All the patients received therapy in Fudan University Shanghai Cancer Center. We add this in the revised manuscript.

2. I suggest that the following information will be presented: Size at different cut-offs, extend of disease (localized vs nodal/distant metastases)

Thank you for your comments. The median size of tumor diameter is 3.8 (1.0, 10.5) cm. Sixty-six patients had localized tumor and 47 patients had tumor with nodal metastasis. As suggested by the reviewer, we added the information about the tumor size and extend of disease (localized vs nodal) into the Table 1 in the revised manuscript. However, all the patients were chosen by the selection criteria: "All the specimens were selected via pathologic diagnose as pNET without distant metastasis and other tumor history". Thus, all the patients received radical resection without distant metastases.

3. Where these fields randomly selected? Can the authors please specify? Which were the negative and the positive tests performed? Was neutralization test performed? Can the authors please specify?

Thank you for your comments. The immunostaining images in the whole slide of each case were evaluated under the low-power scanning magnification (×100). Hotspot images were defined as the areas in the tumor with the highest number of cells with immunoreactive staining. Under the high-power magnification (×200), 5 representative photographs of hotspot were captured to identify the numbers of TIPs. The results were independently reviewed by two clinical experience pathologists (Dan Huang and Cong Tan). Positive test were performed in the pancreatic adenocarcinoma which definitively had the staining of CD42b. Negative controls were treated identically but with the primary antibodies omitted. However, the neutralization test which is always used in virus or serum experiments was not performed in our study. As suggested by the reviewer, we add the detailed description in the revised manuscript.

4. Why the authors chose the cut off of 10% as positive? "positive referred to immunostained platelets distribution which accounted for ≥ 10% of intratumoral region". Why they did not choose another cut-off?

Thank you for your comments. The cut-off value of TIPs was chosen based on the previous tumor-related studies by others, such as gastric cancer[1], breast cancer[2], and pancreatic ductal adenocarcinoma[3]. In the study, we evaluated the values of 5%, 10% and 20% as the cut-off values for CD42b expression. The proportion of positive CD42b expression at the cut-off values of 5%, 10% and 20% were 60.18%, 47.79%, and 25.66% respectively. The *P* value for survival comparison between patients with positive CD42b expression and those with negative CD42b expression were 0.042, 0.005, and 0.771 respectively at the cut-off values of 5%, 10% and 20%. The cut-off value of 10% had the best survival discrimination and was chosen as the cut-off value. As suggested by the reviewer, we add the references and detailed description in the revised manuscript.

REFERENCES

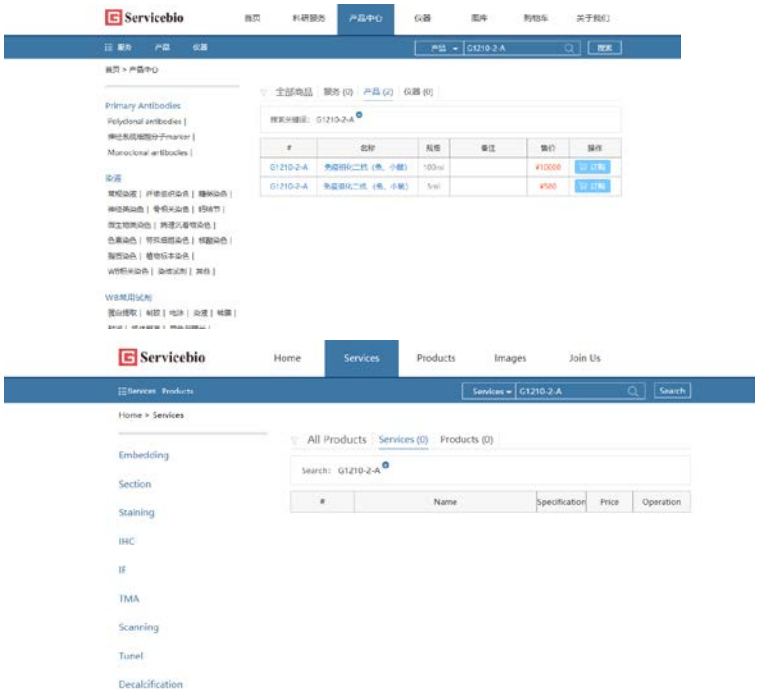
1 Saito H, Fushida S, Miyashita T, Oyama K, Yamaguchi T, Tsukada T, Kinoshita J, Tajima H, Ninomiya I, Ohta T. Potential of extravasated platelet aggregation as a surrogate marker for overall survival in patients with advanced gastric cancer treated with preoperative docetaxel, cisplatin and S-1: a retrospective observational study. *BMC Cancer* 2017; **17**(1): 294 [PMID: 28449652 DOI: 10.1186/s12885-017-3279-4]

2 Ishikawa S, Miyashita T, Inokuchi M, Hayashi H, Oyama K, Tajima H, Takamura H, Ninomiya I, Ahmed AK, Harman JW, Fushida S, Ohta T. Platelets surrounding primary tumor cells are related to chemoresistance. *Oncol Rep* 2016; **36**(2): 787-794 [PMID: 27349611 DOI: 10.3892/or.2016.4898]

3 Zhang SR, Yao L, Wang WQ, Xu JZ, Xu HX, Jin W, Gao HL, Wu CT, Qi ZH, Li H, Li S, Ni QX, Yu XJ, Fu DL, Liu L. Tumor-Infiltrating Platelets Predict Postsurgical Survival in Patients with Pancreatic Ductal Adenocarcinoma. *Ann Surg Oncol* 2018; **25**(13): 3984-3993 [PMID: 30171511 DOI: 10.1245/s10434-018-6727-8]

5. Which was the species of the secondary antibody with 3,3′-diaminobenzidine chromogen was Secondary Antibody Kit (G1210-2-A, Servicebio, Wuhan, China), the kit is not available on the internet in English.

Thank you for your comments. The product is only sold in China. There are differences between Chinese language website and English language website.



6. Where levels of CgA or NSE measured and where they correlated to the author's results? Which was the biochemical profile of the non -functional pNETs (PP producing?, levels, correlation of the immunohistochemocal results to biochemical levels etc).

Thank you for your comments. CgA and NSE are recommended for the diagnosis of pNET. However, the levels of CgA or NSE were not measured in these patients. Similarly, the level of PP was also not measured in this study. Thus, the relation of TIPs with CgA, NSE and PP were not evaluated in the study. It is a good advice to assess the relation of TIPs with CgA, NSE and PP and we will measure the levels of CgA, NSE and PP in future study to evaluate their relation with TIPs.

7. Why the TIP marker should be used compared to other more validated markers eg Ki67 that its calculation is more standardized (See ENETS

recommendation)?

Thank you for your comments. The marker of Ki67 represents the proliferative ability of tumor cells. It is one of the most important factors in the staging of pNET. The classification of G1 and G2 staging is partly dependent on the level of Ki67 (< 3% vs. 3%~20%). The prognostic value of Ki67 is verified by various studies. However, most researches mainly concentrated on the significance of tumor cells, less was focused on the importance of tumor microenvironment in pNET. Platelets are one of the important components of the tumor microenvironment. In this study, we demonstrated that the presence of CD42b+ TIPs in tumor tissue rather the level of platelets in serum were prognostic factor. In addition to the marker of Ki67 values, the presence of TIPs in tumor tissue independently predicted the recurrence and survival in pNET after resection. The presence of TIPs is an important marker complementary to the marker Ki67. It provides potential prognostic meaning of platelets in pNET and offers a clue to anti-platelet therapy for cancer therapy strategy.

8. Microphotograph B. It seems to be more a background than a true immunoreactivity of specific cells.

Thank you for your comments. As suggested by reviewer, we selected a more typical immunostaining picture to replace the microphotograph B in Figure 1.

9. In general the authors do not convince that the marker included can be used as an alternative to more established markers. The 10% threshold that they present is not explained why it was chosen and the paucity of the marker results that its use is cumbersome in clinical praxis.

Thank you for your comments. The cut-off value of TIPs was chosen based on the previous tumor-related studies by others, such as gastric cancer (PMID: 28449652), breast cancer (PMID: 27349611), and pancreatic ductal adenocarcinoma (PMID: 30171511). In the study, we evaluated the values of 5%, 10% and 20% as the cut-off values for CD42b expression. The proportion of positive CD42b expression at the cut-off values of 5%, 10% and 20% were 60.18%, 47.79%, and 25.66% respectively. The *P* value for survival comparison

between patients with positive CD42b expression and those with negative CD42b expression were 0.042, 0.005, and 0.771 respectively at the cut-off values of 5%, 10% and 20%. The cut-off value of 10% had the best survival discrimination and was chosen as the cut-off value. As suggested by the reviewer, we add the references and detailed description in the revised manuscript.

Reviewer #2: 1 To Authors This study develops a very interesting section on the biological behaviour of the pancreatic neuroendocrine tumors. The design of the research is rational; its development is correct in the methods, patients selection, histopathological assessment and statstical analysis. The results are believable and clearly showed. The Discussioni s complete, considering the most important characteristics of the topic. The references are many and up-to-date. In summary this is a very good work.

Answer: Thank you for your kind comments.

Reviewer #3: Title: Apt Abstract: Well elaborated giving a clear idea of the article in a nut shell. Introduction: Satisfactory. Materials and methods: Elaborately written with all essential details. Results: Satisfactory. Discussion: Good. Results: This section needs to be added with proper conclusions enlisted in it. References: Very good.

Answer: Thank you for your kind comments. As suggested by the reviewer, we added proper conclusion in the section “Results”.