Format for ANSWERING REVIEWERS

December 01, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6133-review.doc).

Title: The utility and safety of endoscopic ultrasound-guided fine-needle aspiration for resectable pancreatic cancer as a preoperative diagnostic modality

Authors: Taiki Kudo, Hiroshi Kawakami, Masaki Kuwatani, Kazunori Eto, Shuhei Kawahata, Yoko Abe, Manabu Onodera, Nobuyuki Ehira, Hiroaki Yamato, Shin Haba, Kazumichi Kawakubo, Naoya Sakamoto

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6133

The manuscript has been revised in accordance with the reviewer's suggestions:

1 The format has been updated.

2 Revisions have been made in accordance with the suggestions of the reviewer.

For reviewer # 00503867:

I thank the authors for detailing their experience with EUS-FNA of pancreatic adenocarcinomas. An excellent result in terms of diagnostic yield for FNA. I congratulate all the authors and HK for the performance of the EUS procedures and relatively good SE profile from EUS-FNA and the extensive follow-up of their patients. However there are several points that are concerning and need clarification and likely re-analysis of the data.

Thank you for your kind comments. We have revised our manuscript in accordance with your suggestions. In the revised manuscript, the edited text is in red font and is highlighted.

(1) The title should be "The utility and safety of EUS-FNA for resectable pancreatic cancer..." as this study only describes results for resectable pancreatic CA.

Response:

Thank you for your constructive comments. We have changed the title to "The utility and safety of endoscopic ultrasound-guided fine-needle aspiration for resectable pancreatic cancer as a preoperative diagnostic modality".

(2) The second last sentence in the "Core tip" paragraph is unjustifiable based on the current study. Was there false negatives in the nonEUS-FNA group? Although this is the one of the most important points about pre-operative EUS-FNA, this was not detailed at all in the manuscript.

Response:

We enrolled patients with pancreatic cancer, some of whom had undergone preoperative EUS-FNA. Thus, there were no false negative cases in the non-EUS-FNA group in this study. However, as the

reviewer mentions, false negative cases are rarely encountered in practice. EUS-FNA can also potentially reduce the incidence of inappropriate pancreatic surgery by providing an accurate diagnosis.

(3) There appears to be a significant selection bias in those patients selected to not have a EUS-FNA versus those that underwent pre-operative EUS-FNA. It was concluded that there is no adverse outcome to preoperative EUS-FNA. But there were significant differences b/w the two groups in RFS [742 vs 265] and OS [1042 vs 557] favouring EUS-FNA. This would suggest that there were inherent differences between the two groups and as such they cannot be directly compared in terms of survival. I assume that there was a selection bias in terms of which patients had an EUS but this was not described in the manuscript. The presented group characteristics were not much different apart from CEA levels but there was obviously other factors in play. This would be something that the authors need to examine further so that a fairer comparison can be made. The conclusions to the article are sound based upon the immediate complications from the actual procedure. Given so much effort was put into the survival curves of the two groups it is disappointing to say that these are not factually correct in terms of concluded that the FNA had long term safety (unless the FNA itself caused a survival benefit).

Response:

The CEA levels were not significantly different in this study. We performed both univariate and multivariate analyses, which revealed, that tumor size and administration of adjuvant chemotherapy were prognostic factors of relapse-free survival. However, tumor size, administration of adjuvant chemotherapy, *and* preoperative EUS-FNA were prognostic factors of overall survival. It is not possible that preoperative EUS-FNA itself was a favorable prognostic factor for overall survival. However, adjuvant chemotherapy was administered more frequently in the FNA+ group, reflecting a good performance status after recurrence. Thus, more patients could continue with second- or third-line chemotherapy in the FNA+ group than in the FNA- group.

(4) Finally given the differences I discussed in #3, it is possible that the authors may be able to draw out some novel factors from their data to help illustrate which patients tend to do better than others with apparent resectable pancreatic adenocarcinoma.

Response:

Thank you for your helpful comments. We performed multivariate analyses for overall survival and relapse-free survival, the results of which are summarized in Table 4 and Table 5.

Reviewer # 00160226:

The authors performed a retrospective review of all patients suffering from pancreatic cancer who underwent EUS-FNA. They concluded that the procedure is safe in patients with resectable pancreatic cancer. The argument of performing this procedure in resectable disease is the balance between the need to obtain cytology/histology for diagnosis vs complications in particular the risk of needle track dissemination.

I have a few questions to the authors:

Thank you for your kind comments. We have revised our manuscript in accordance with your suggestions. In the revised manuscript, the edited text is in red font and is highlighted.

Methods:

(5) How was it decided to perform FNA for these lesions? Was it due to the need to obtain cyto/histo diagnosis before starting chemotherapy?

Response:
Page 6, 2nd paragraph, lines 14–15:
As described in the original manuscript, we excluded patients who underwent neoadjuvant chemotherapy or chemoradiotherapy. We performed preoperative EUS-FNA if requested by the surgeon or physician.

(6) How much suction was applied during FNA?

Response:
Page 7, 3rd paragraph, lines 3–4:
As described in the original manuscript, the EUS-FNA needle was an Echotip® ultra (Cook Japan, Tokyo, Japan). The needle fits a 10 ml syringe to allow negative suction. We used this syringe for all EUS-FNA procedures in this study.

(7) Was an onsite cytopathologist a/v during the procedure

Response:
Page 8, 1st paragraph, lines 3–4:
As described in the original manuscript, an onsite cytopathologist participated in all EUS-FNA procedures during this study.

(8) What was the protocol for giving adjuvant chemo

Response:
Adjuvant chemotherapy consisted of gemcitabine at 1,000 mg/m^2 on days 1, 8, and 15 of a 28-day cycle or S-1 administered orally twice daily after meals at a dose of 80, 100, or 120 mg/day for body surface areas (BSAs) of <1.25 m^2, 1.25–1.5 m^2, or ≥1.5 m^2, respectively, for 28 consecutive days, followed by a 14-day rest. Thirty-eight patients in the FNA+ group received gemcitabine adjuvant chemotherapy, and 2 patients in FNA+ group received S-1 adjuvant chemotherapy. In the FNA- group, 12 patients received gemcitabine adjuvant chemotherapy and 2 patients received S-1 adjuvant chemotherapy.

Results:
(9) Can the authors postulate why the level of CEA was higher in the FNA- group. In principle, this may signify more advanced disease? However, if so, then this group should be the group that has more advanced disease and more underwent adjuvant chemo? But this was not the case as presented in the table for patient demographics.

Response:
Two patients had a very high level of CEA (242.3 and 197.9 ng/ml). Therefore, we repeated the statistical analysis of the tumor markers (CEA, CA19-9, SPan-1, and DU-PAN-2) using the Mann-Whitney U-test. We found no significant difference between the FNA+ and FNA- groups. We have added new data to Table 3. We also analyzed surgical outcome and tumor size, as well as the histological type and UICC stage of resected specimens of all enrolled patients. Once again, there was no significant difference between the FNA+ and FNA- groups. There was a selection bias with respect to adjuvant chemotherapy because this study was retrospective, and not all patients were followed up by our hospital after surgery.

(10) It was mentioned that specimens yield diagnosis for cytology or histology. How was this obtained and decided? Which needle provided histology?

Response:

The material obtained by EUS-FNA using a 19-, 22-, or 25-gauge needle is used for rapid onsite cytopathology. In brief, our policy is to obtain visible white material. The cytopathologist selects part of the white material to transfer to a glass slide for cytology and another part of material for formalin preservation to allow for histological examination.

(11) The patient with +ve cytology peritoneal lavage, could this be a result of EUS FNA? Did this patient suffer from recurrence?

Response:
This patient experienced recurrence in the peritoneum 233 days after surgery. It is difficult to demonstrate that EUS-FNA does not cause peritoneal dissemination.

Page 10, 1st paragraph, lines 4–7:
As described in the original manuscript, there was no sign of needle tract seeding based on the intraoperative findings. Furthermore, a CT scan taken before EUS-FNA revealed that the tumor already extended beyond the pancreas. We think this patient was at high risk of peritoneal dissemination regardless of EUS-FNA.

(12) What was the median FU time of patients?

Response:
The median time of all patients was 571.5 days.

(13) Perhaps the authors can perform a multivariate analysis of factors affecting survival with FNA as one of the covariate to strengthen the argument that FNA does not affect survival.

Response:
We performed univariate and multivariate analysis for overall survival in this study. These revealed that EUS-FNA had no adverse effect on surgical performance for pancreatic cancer.

Page 11, 1st paragraph, lines 9–13:
In addition, we performed univariate and multivariate analysis for overall survival (Table 4) and relapse-free survival (Table 5). The hazard ratios of EUS-FNA for overall and relapse-free survival were 0.46 ($P < 0.05$) and 0.46 ($P = 0.060$), respectively. This suggests that EUS-FNA was not an adverse prognostic factor for pancreatic surgery.

(14) Discussion:
The author should mention that although EUS FNA may or may not adversely affect survival, it may also be a surrogate outcome of the disease status of the patient and also the need to undergo chemotherapy.

Thank you for your helpful comments and in accordance with your suggestion, we have added the following text in the revised manuscript.

Page 12, 4th paragraph, lines 26-29:
Multivariate analysis revealed that tumor size and adjuvant chemotherapy were both prognostic factors for overall survival (OS) and relapse-free survival (RFS) in this study. EUS-FNA, however, was not a prognostic factor for OS and RFS. Thus, it is possible that patients in the FNA+ group benefited from the chemotherapy administered immediately after surgery.

Reviewer # 00183279
Although the superiorty of EUS in diagnosis and staging of pancreatic cancer has been proved in

many meta-analysis and the technique is still improving with the introduction of modern gadgetry like Elastography, Contrast Harmonic EUS, and FISH. Some precarious points in your study need to be mentioned and worked upon.

Thank you for your helpful and constructive comments, which have greatly helped us improve our manuscript. We have revised the text in accordance with your suggestions. In the revised manuscript, the edited text is in red font and is highlighted.

(15) Were all the lesion's in your study solid neoplasms, nothing is mentioned about the final histopathology of these tumors vis- a- vis the nature and the treatment received henceforth.

Response:

The final histopathological findings for these tumors are now summarized in Table 3. There was no significant difference between the FNA+ and FNA- groups.

(16) The selection bias on placing more patients in FNA+ group with neo-adjuvant chemotherapy is evident by a significant statistical enumeration. The study would have a significant impact if this bias was eliminated as in many studies the risk for tumor seeding and complications has been placed between 1.2%-4.4% , the percentage is too small to hold the selection bias. Gastrointest Endosc. 2003;58:690–695.

Response:

We excluded patients who underwent neoadjuvant chemotherapy. Because this study was retrospective and included relatively few patients, the selection biases in addition to neoadjuvant chemotherapy could not be eliminated.

(17) Enumeration needs on the method to Detect Relapse Free Survival (RFS) as the patients in non-neoadjuvant arm with FNA –ve faired better than FNA +ve. The study needs a multivariate analysis to assess the role of each variable on an individualized arm.

Response:

We performed a multivariate analysis for overall survival and relapse-free survival as shown in Table 4 and Table 5. This revealed that EUS-FNA itself was not a prognostic factor for relapse-free survival.

(18) The paper needs some EUS pictographs showing the lesion and the procedure as well as the post op imaging.

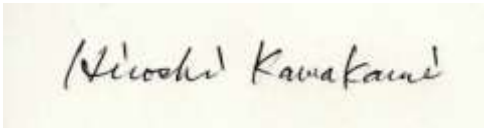
Response:

Thank you for your recommendation. However, we do not think that EUS pictographs or examples of post-operation imaging are necessary. EUS pictographs were typical of this procedure, and no needle puncture scars were visible in the resected specimens. Hence, we have not added EUS pictographs or post-operation images to the revised manuscript.

3 References and typesetting errors have been corrected.

Thank you again for considering our manuscript for publication in the *World Journal of Gastroenterology*.

Sincerely yours,



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