RESPONSE LETTER

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Title: Para-aortic node involvement is not an independent predictor of survival after resection for pancreatic cancer

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Dear Sir,

This is a response letter accompanying the resubmission of our manuscript "Para-aortic node involvement is not an independent predictor of survival after resection for pancreatic cancer".

First of all, we would like to thank all the reviewers for their valuable suggestions. In this letter, we report all the revisions we made according to the reviewers' comments. We have addressed each of the reviewers' suggestion and highlighted the revisions in the resubmitted manuscript in red color.

Kind Regards,

Prof. C. Sperti

1. Reviewer 0001832

"Was para-aortic node sampling done routinely in all patients or only in those with suspected metastasis?"

All patients underwent routinely para-aortic node sampling by harvesting the lymphocellular aortocaval tissue located below the left renal vein until the origin of inferior mesenteric artery (station 16b1).

We have added a sentence, see Section MATERIALS and METHODS, line 24: All patients underwent standard lymph node dissection (5,6,8a,12b1,12b2,12c,13a,13b,14a and 14b right lateral side, 17a,17b) and para-aortic sampling. Para-aortic nodes were excised by harvesting the lymphocellular aortocaval tissue located below the left renal vein until the origin of inferior mesenteric artery (station 16b1)

"I Would change the term "radicality" to margin involvement, since the resection is not more or less radical depending on margin status

We have changed the term "radicality" to margin involvement (See Section MATERIALS and METHODS, line 28: Curative resection was defined as tumor's resection with pathologically confirmed negative margins. R1 resection was defined as the presence of tumor ≤ 1mm from the margin, according to Leeds criteria)

2. Reviewer 03548113

"Regarding station 16b1, were all patients undergo the complete dissection of 16b1?Were some patients undergo just the sampling?"

All patients underwent routinely para-aortic node sampling by harvesting the lymphocellular aortocaval tissue located below the left renal vein until the origin of inferior mesenteric artery (station 16b1). We have added a sentence in Section MATERIALS and METHODS, line 24: All patients underwent standard lymph node dissection (5,6,8a,12b1,12b2,12c,13a,13b,14a and 14b right lateral side, 17a,17b) and para-

aortic sampling. Para-aortic nodes were excised by harvesting the lymphocellular aortocaval tissue located below the left renal vein until the origin of inferior mesenteric artery (station 16b1)

"What is LNF ratio, LNF status, and Paraaortic LNF status in Table 4 and 5? LNF is lymph node?

LNF is lymph node. We have revised the tables considering grading, stage, and PALNs (para-aortic lymph nodes status) (see Table 4 and 5)

- **"I agree that a few patients with PALN metastases can survive and this results are interesting. However, this study has a crucial problem about the statistical analysis. Authors showed that there were significantly correlation between stage and lymph node status, certainly. Therefore, these factors can't be included to the variables for multivariate analysis simultaneously. Furthermore, although lymph node ratio was not associated with poor survival by univariate analysis, why was lymph node ratio included to the variables for multivariate analysis? If the statistical analysis is performed correctly, PALN status may be the independent factor"**

We agree that lymph node status and margin involvement may affect statistical analysis. Therefore we have performed the multivariate analysis, excluding lymph node status, lymph node ratio and margin involvement. In this way, grading was confirmed to be the only independent predictor of disease-free and overall survival.

A sentence has been added in Section RESULTS- Multivariate analysis, line 3: When lymph node status, lymph node ratio, and margin involvement were excluded from multivariate analysis, grading was confirmed to be the only independent predictor of disease-free and overall survival ($p<0.0001$). Tables 4 and 5 have been changed according to reviewers suggestion.

3. Reviewer 01804834

- "The Results Section should be sub-headed in e.g. Univariable Analysis and Multivariable Analysis"

We have sub-headed the Result Section in Univariate Analysis, Multivariate analysis and Follow-up (See RESULTS Section)

- **"Some phrase- and language polishing should be done throughout the text, for example Abstract: AIM: "Lymph node involvement is an important prognostic factors for pancreatic cancer" should read Lymph node involvement is an important prognostic factor for pancreatic"; Methods " with para-aortic nodes dissection .." should read "..with para-aortic node dissection"; " mean and median number of pathologically assessed lymph nodes were 28 and 26, respectively (range 14-63) Range of mean or median? Only give the median number; " one hundred forty one patients recurred and died" should read (as I understand) "died after tumor recurrence"; Conclusion " but they were not independent prognostic index" should read " were not independent prognostic factors"**

1. **Abstract: AIM: "Lymph node involvement is an important prognostic factors for pancreatic cancer" should read Lymph node involvement is an important prognostic factor for pancreatic"**

See ABSTRACT, AIM: we have shortened the AIM section in "To analyze the importance of para-aortic node status in a series of patients who underwent pancreaticoduodenectomy in a single Institution"

2. **Methods " with para-aortic nodes dissection .." should read "..with para-aortic node dissection";**

See ABSTRACT, METHODS line 2 "with para-aortic node dissection for pancreatic adenocarcinoma"

3. **" mean and median number of pathologically assessed lymph nodes were 28 and 26, respectively (range 14-63) Range of mean or median? Only give the median number**

We have changed the sentence, giving only the median number. (See ABSTRACT-RESULTS: : median number of pathologically assessed lymph nodes was 26 (range 14-63); See RESULTS section, line 10: median number of pathologically assessed lymph nodes was 26 (range 14-63)).

4. **" one hundred forty one patients recurred and died" should read (as I understand) "died after tumor recurrence";**

See ABSTRACT-RESULTS, line 3: One-hundred forty-one patients recurred and died for tumor recurrence

- **In the discussion section, the following sentence "unfortunately, we have inadequate data on the number of lymph nodes removed, and their metastasis rate within 16a1, 16a2, 16b1 and 16b2 stations" should be explained/is difficult to understand in the context (at least to me)**

In the literature, there are not adequate data on the number of lymph nodes removed, and their metastasis rate within the subgroups 16a1, 16a2, 16b1 and 16 b2 . Several authors have reported 16b1 lymph node dissection as adequate for a better tumor staging and it should be included in a standard lymphadenectomy.

We changed two sentences in the DISCUSSION section to better explain this concept

4. Reviewer 00053888

- "The Authors have wisely acknowledged the short comings of their own study, they then go on to tell us the strengths of the study which are far out weighed by the weakness and this sentence can be removed"

The sentence "On the contrary, standardization of surgical technique (performed by the same surgical staff), postoperative treatment and adjuvant therapy was ensured for all patients".has been removed, as suggested by the reviewer.

5. Reviewer 02976802

- "However the authors should underline the novel aspects of their study. What do pancreatic surgeons learn from this additional study?"

Our study confirms that para-aortic node metastatic rate is relevant in pancreatic cancer patients. Survival is significantly decreased in patients with PALNs+, but PALNs involvement is not independent predictor of survival in these patients. Further large, prospective, multicentric studies are necessary to definitively determine the real role of PALNs involvement after resection for pancreatic adenocarcinoma. At the moment, it appears that the decision to perform pancreatic resection should not be taken on the basis

of para-aortic lymph node status only. We have added a sentence in the CONCLUSIONS section.

- "Do the authors have data on the morbidity of PALN resection, i.e. do they have cases without PALN resection for comparison. The potential benefit of the PALN resection regarding survival must be outweighed against the associated morbidity"

There was no operative mortality in this series, while overall morbidity rate was 41%: complications rate was not different between PALNs+ and PALNs- patients (41% and 39%, respectively), as well as pancreatic fistula rate (16% and 17%, respectively). Reoperation rate was not different in the two groups (5% and 6%, respectively). We have added a sentence in the RESULTS section.

- Why do the authors not have the number of positive (tumor-infiltrated) PALN? This would be an important measure tumor biology

The median number of positive para-aortic nodes was 3 (range 1-7). We have added a sentence in the RESULTS section.

- What is meant by radicality of resection? Do the Authors mean the R status?

Curative resection was defined as tumor's resection with pathologically confirmed negative margins. R1 resection was defined as the presence of tumor ≤ 1mm from the margin, according to Leeds criteria. We have added a sentence in the Material and Methods section.

- What was the follow-up time of the study?

The median follow-up of the study was 25 months (range 8-115). A sentence has been added in the RESULTS section – follow-up.

- What was the median survival time of patients with PALN+ status?

The median overall survival time of patients with PALNs+ was 18 months, while the median dise4ase free survival time was 8 months. We have added a sentence in the RESULTS section.

- Can the authors provide data on palliative patients and compare the survival outcome?

Eleven patients with para aortic node metastases underwent palliative bypass operation because of locally advanced or distant disease. Median survival in these patients was 9 months (range 2-28 months). A sentence has been added in section RESULTS.

- 151 patients in 12 years means approximately 12 patients per year. The Padua center probably had more pancreatoduodenectomies within this period. Was there a selection of cases, or did not all patients had a PALN dissection? If so, could there be a bias regarding the selection of cases for PALN dissection?

During the study period, 340 patients underwent pancreaticoduodenectomy for pancreatic neoplasms: after excluding IPMNs, endocrine tumors, cystic neoplasms, pancreatic metastasis, duodenal, ampullary, and bile duct cancers, 176 patients underwent pancreaticoduodenectomy for ductal adenocarcinoma of the pancreas. Of these, twenty-five patients who underwent resection after performing chemotherapy or chemoradiation for locally advanced pancreatic cancer before referral to our Department, were excluded from the study. In fact, as stated in the material and Methods section, no patient enrolled in this study underwent neoadjuvant therapy. Finally, 151 patients with pancreatic carcinoma were included in this analysis. (We have added a sentence in Material and Methods section, line 3 and in the RESULTS section as well)

- there are some misspellings typos throughout the manoscripyt (e.g. Introduction: However some Authors reported…)

See INTRODUCTION section, line 8: However, some Author reported…

6. Reviewer 00069105

- "Some more information about morbidity and relationship with survival could be interesting"

There was no operative mortality in this series, while overall morbidity rate was 41%: complications rate was not different between PALNs+ and PALNs- patients (41% and 39%, respectively), as well as pancreatic fistula rate (16% and 17%, respectively). Reoperation rate was not different in the two groups (5% and 6%, respectively).

We have added a sentence in the RESULTS section.

- Mistake in reference 6 (no year included):

See REFERENCES section, Refernce n° 6: Kayahara M, Nagakawa T, Ohta T, Kitagawa H, Ueno K, Tajima H, Elnemr A, Miwa K. Analysis of paraaortic lymph node involvement in pancreatic carcinoma: a significant indication for surgery? *Cancer* 1999; **85**:583–590. PMID: 10091731.

7. Reviewer 3261792

- First of all I have a comment about multivariate analysis. I think LN status or LN ratio and Para-aortic LN status were possible to be confounding factor each other because PALN+ cases are all node positive cases. You may need perform multivariate analysis using selected factor "grading, Radicality, LNF status" or "grading, radicality, para-aortic LN status". These factors not likely to be confounded each other

We performed Multivariate Analysis using selected factors as suggested (Grading, Stage and PALNs status). Multivariate analysis showed that tumor differentiation was the only independent predictor of long-term survival (Table 4), while grading and margin involvement were independent prognostic factors for disease-free survival (Table 5). When lymph node status, lymph node ratio, and margin involvement were excluded from multivariate analysis, grading was confirmed to be the only independent predictor of disease-free and overall survival ($p<0.0001$) (We have added a sentence in RESULTS section, Multivariate Analysis and we have revised TABLE 4 and 5)

- **I think there is a critical mistake of table 3. Is this data of table 3 right? It's possible that MST of all factor are exactly same between OS and DSF. Usually MST of DFS is shorter than that of OS. In fact the NST value of Para-aortic lymph nodes status in the Table 3 is different from that derived from survival curve shown in Fig. 2. If the data in table 3 are correct, all tumor recurrent cases died on the same day when recurrence is found. Is it possible?**

Data of Teble 3 were wrong. We apologize for the mistake, we have revised Table 3.

- **How about the results of multivariate analysis in the tumor grade 1-2 group? Is PLAN status independent prognostic factor or not?**

PALNs involvement is confirmed to be not an independent prognostic factor

- **There is wrong spelling in the chapter "Statistical analysis" Fischer's exact test =>Fisher's exact test**

See Statistical analysis Section, line 1: The χ^2 test or Fisher's exact test