Title: Intestinal-type of differentiation predicts favourable overall survival: confirmatory clinicopathological analysis of 198 periampullary adenocarcinomas of pancreatic, biliary, ampullary and duodenal origin

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Author's response to reviews: see over
Dear Members of the Editorial Board,

Hereby we submit the revised version of our manuscript (MS: 1522257449486003). We thank the reviewers for the thorough and detailed review of our manuscript and critical as well as helpful comments. Point-by-point answers are given below. Changes to the manuscript text have been marked in color.

Sincerely Yours,

Peter Bronsert, first author
Ulrich F. Wellner, corresponding author
Answers to the reviewers’ comments

Reviewer: Arne Westgaard

Reviewer’s Report: (not printed here).

Major compulsory revisions

(1) The definition of “periampullary adenocarcinoma” should be defined more precisely in the introduction of this manuscript. To get an accurate understanding of what the authors mean by this term, one has to combine the information given in the following parts of the manuscript: a) First sentence of “Introduction” section defining PDAC, DBDAC, AMPAC, and DUOAC as adenocarcinomas according to WHO classification (for which specific criteria to determine the separate origins apply).

Periampullary adenocarcinoma has been more precisely defined in the Introduction (see Page 3, line 3-9).

b) First sentence of “Patients and Data” defines “periampullary adenocarcinoma” as PDAC, DBDAC, AMPAC or DUOAC according to (a), although this implies that for example adenocarcinomas located to the pancreatic tail would be included by this definition.

The first sentence of “Patients and Data” was redefined (See Page 4, line 4 – 10)

c) “Periampullary cancer” (not adenocarcinoma) is then defined in the second sentence of the section “Histologic workup”, stating that “periampullary cancer was defined as comprising all pancreatic, distal bile duct, ampullary and duodenal cancers resected by pancreateoduodenectomy”. This is a common, pragmatic definition, although later in the manuscript the reader discovers that also resections starting by pancreateoduodenectomy and then advancing to total pancreatectomy were included in the definition. The whole manuscript should be revised to avoid such inconsistencies and to ensure simple access to core information without need of jumping to different parts of the manuscript.

The text has been rephrased and a clearer definition is now given at the beginning of the methods section to avoid misunderstandings. (see Table 1 – 5, Page 8, line 20; Page 9, line 7 – 8, 21; Page 10, line 5, 14, 21; Page 11, line 7, 21)

(2) Please inform about the name and type of institution in which the surgery and histopathological workup was performed.

The information has been added (see Page 4, line 10 – 14) (Some of the authors have recently moved to another institution, but surgery and follow-up was done at the departments of the University of Freiburg.)
(3) The authors might provide a flowchart figure to show the numbers of patients included and excluded, starting with the total number of patients identified from the prospective database, and in each step showing the numbers of patients that were excluded due to other diagnoses, lack of tissue material, perioperative death, or loss of follow-up (for example withdrawal of consent, death outside institution, migration to other parts of the country, etc). It could be assumed that follow-up was complete, although this is improbable if survival data was in fact extracted only from the hospital database, so I feel that this should also be commented in the manuscript.

A flowchart and description thereof has been added to the manuscript (see Figure 2., Page 8, line 7 – 11).

Follow-up was complete due to regular follow-up within the Comprehensive Cancer Center Freiburg, which is now mentioned in the text (see Page 8, line 14 – 15).

(4) Please describe the method of routine assessment of the tumour origin, and comment on why the ratio of pancreatic to non-pancreatic origin was higher than in other series that applied standardised histopathological workup of the specimens.

A more detailed description of the workup has been added ((see Page 5, line 4 – 19)

The possible reasons for a high rate of pancreatic origin are mentioned in the discussion (see Page 14, line 5 – 10).

Also the issue of misclassification of tumor origin is discussed (see Page 14, line 5 – 15).

(5) Please explain how duodenal cancers may be found to have pancreatobiliary-type differentiation.

Assessment of the histological subtype was performed in a blinded fashion, and resulted in classification to pancreatobiliary-like differentiation (see Page 9, line 21-22).

As now mentioned in the discussion: One might speculate that if INT type carcinoma can arise in the pancreas, vice versa PB type carcinoma may arise in rare instances in the duodenum due to common embryologic origin, but further evaluation is currently not possible given the exceeding rarity of DUOAC (see Page 13, line 14 -18).

(6) Please comment on the high frequency of R0 resections.

It is true that one has to be aware of the current issue of margin status assessment especially in pancreatic cancer. A detailed comment has been added to the discussion (see Page 14, line 16 – 22).

(7) Please restructure the text, tables, and figure legends in such a manner that the reader does not have to jump to different parts of the manuscript to understand how tests were performed, and the numbers of patients included for each test.
The manuscript text was restructured and more annotations and legends are given for the figures. We hope to provide better readability. (see Page 20 – 21, line 3 (p 20) – line 6 (p 21), and Page 1 – Page 21)

(8) Please rephrase the title. In my opinion, it is too vague, and not catchy enough to serve as a teaser to attract interested readers. This study is a (confirmatory) clinicopathological analysis of 198 pancreatoduodenectomies for periampullary adenocarcinoma, in which intestinal-type of differentiation predicts a favourable overall survival. I would prefer the title to reflect this message.

The title has been rephrased to "Intestinal-type of differentiation predicts favourable overall survival: confirmatory clinicopathological analysis of 198 periampullary adenocarcinomas of pancreatic, biliary, ampullary and duodenal origin" (see Page 1, Line 1-3)

(9) The conclusion in the abstract is incorrect and should be rephrased.

The conclusion has been rephrased to: "Intestinal type differentiation and lymph node ratio but not tumor location are independent prognostic factors in pooled analysis of periampullary adenocarcinomas. We conclude that differentiation is more important than tumor location for prognostic stratification in periampullary carcinomas." (see Page 2, line 23 – 26)

(10) Please add information to the figure legends as described above.

This has been done accordingly (see Page 20 – 21, line 3 (p 20) – line 6 (p 21))

Reviewer: William Greenhalf

Reviewer's report:

Major Compulsory Revisions

Given the relatively small numbers of patients involved it is absolutely essential to give the at risk figures underneath the Kaplan-Meyer curves in Figure 2.

The figures are now given in detail in Figure 3 and Table 4.
“Immunohistochemistry was carried out using commercially available antibodies for cytokeratin 7 (CK7), cytokeratin 20 (CK20), and caudal type homeobox 2 (CDX2)” I'd like to know supplier and catalogue number please.

The information has been added (see Page 7, line 4 – 8)

“Few patients had received neoadjuvant therapy before resection (7%)” This sounds like quite a lot to me. What was the breakdown of these patients? – i.e. how many were PDAC?

The information has been added. There were 13 PDAC and 1 AMPAC (Table 1 and Page 8, line 21 – 22).

Presumably the statement that “Presence of a precursor lesion was significantly associated with better survival.” Can only fit with “However, the subgroup of PDAC with associated IPMN did not show significantly better survival than PDAC without associated IPMN (p=0.538, Logrank test).” If the alternative cancer forms were both more associated with precursor lesions and better survival. Why not test this and say so? Although, perhaps this doesn’t fit nicely with the conclusion that “However, given the results of our study, the biologically valid and logistically preferable approach would be to distinguish between INT and PB differentiation rather than tumor location.”

This is correct and a more detailed comment including the testing has been added in the results section (see Page 11 - 12, line 25 (p 11) – line 1 (p 12)). We want to point out that associated IPMN did not constitute a bias for survival analysis and our study did not contain colloid pancreatic carcinoma arising from IPMN. In our opinion these results do not necessarily interfere with the conclusion.

Minor Essential Revisions

I think that it should be made clear that Pancreatic Ductal Adenocarcinoma is not the only form of “pancreatic cancer”. Can I suggest “The present WHO classification of tumors distinguishes between pancreatic (PDAC), extrahepatic (distal) bile duct (DBDAC).” should read “The present WHO classification of tumors distinguishes between pancreatic ductal adenocarcinoma (PDAC, referred to here as just pancreatic cancer), extrahepatic (distal) bile duct (DBDACL)

The text has been rephrased to avoid such inconsistencies (see Page 3, line 3 – 5).

“Another aspect is the question for the biological basis of the observed differences” should read “Another aspect is the question of the biological basis of the observed differences”.

The orthographic errors have been corrected (see Page 3, line 16)

“All cases with sufficiently available FFPE were included in the study” should read “All cases with sufficient available FFPE were included in the study.”

The orthographic errors have been corrected (see Page 4, line 17 – 18)
“a standardized protocol was followed for diagnostic workup of pancreatoduodenectomy specimen” should read “a standardized protocol was followed for diagnostic workup of pancreatoduodenectomy specimens”.

The orthographic errors have been corrected (Page 4, line 22 – 23)

Discretionary Revisions

This may be my ignorance – but I am not sure what is meant by “oral, aboral and deep resection margin”.

The description of the histological workup has been rephrased to provide better understanding (see Page 5, line 7 – 8).

I think LNR as an abbreviation for Lymph Node Ratio needs to be defined.

This has been corrected (Page 10, line 24).