November 21th, 2013

Dear Jin-Lei Wang, Director, Editorial Office,

Please find enclosed the edited manuscript in Word format (5089-review.doc).

Title: PATHOLOGIC HANDLING OF PANCREATODUODENECTOMY SPECIMENS: APPROACHES AND CONTROVERSIES.

Authors: M. Carmen Gómez-Mateo, Luis Sabater-Ortí, Antonio Ferrández-Izquierdo.

Name of Journal: *World Journal of Gastrointestinal Oncology*

ESPS Manuscript NO: 5089

First of all, we would like to thank you for the recommendations and the thorough analysis of the reviewers. Following their recommendations, we have revised the manuscript and modified the text accordingly. We think these modifications have made the paper and its conclusions clearer.

Answers to *Comments for author*:

Reviewer 1.

Major Comments.

Manuscript does not add to the difficulties of handling of pancreaticoduodenectomy except more confusion and controversy, and the case has semantic nature most of the time. By thorough analysis, it is clear that manuscript is highlighting the differences between authors and protocols as if pathologists are floundering through manners of handling which is not true regarding the nature of science and medical practice. Author explained the different ways of nomenclature of relevant margins and pointed out the need for standardization of nomenclature of the highly significant margin, pancreatic circumferential or radial margin. It is obvious it is not controversy but different ways of reading the same fact. Being the structure having many names may cause confusion to group of people but will not be opposing evidence. Differences in dissection protocols will not cause a problem if it is widely agreed about the prognostic factor that should be assessed. In the light of relative lack of independent prognostic factors for pancreatic carcinoma, it will be inacceptable to describe a protocol with shortage. None of the international protocols mentioned that it is not recommended to ink and submit the retroperitoneal and medial margins which are proved to be the most critical ones. The most important two topics that are hit, margin involvement and lymph node metastasis. There is a lack of consensus on margins in the term of final diagnosis but mentioning data like invasion of vascular, lymphatic, or perineural, in addition to least surgical margin in the microscopic description, is a usual practice. Writing the final diagnosis as involved margin or not is a matter of time depending on new large-scale approved data. I think the best example for this is the circumferential margin of rectal carcinoma. Furthermore, in view of the extremely poor prognosis of pancreatic cancer, clinicians sometimes expressed limited interest in a pathology report data, including the resection margin status. Author considered the lymph node ratio (LNR) more powerful prognostic marker ignoring the importance of lymph node location. Last, there is no data about handling protocol of PDAC at Hospital Clinico Universitario in Valencia, besides ambiguity of final assessment of R1 (margins involvement), i.e the hospital will consider indirect tumor invasion of vascular, perineural or lymphatic as R1 or not? And if the hospital protocol is prepared as a step towards standardized protocol, it lacks the guidelines relating to the minimum number of circumferential margin blocks. A very important point the manuscript does not touch completely is Whipple operation efficiency as an adequate operation for head of the pancreas duct cancer, in the light of involvement of lymph nodes along superior and inferior borders of body of pancreas, which are not removed in the Whipple procedure.

Another highly critical point is the variation that exists between pancreatic, ampullary, and CBD cancer in term of resection margin and lymph node metastatic locations.

Answer to Major Comments of reviewer 1:

Although traditionally most resections in pancreatic cancer are shown to be complete resections (R0), the prognosis is still, unfortunately, dismal. In recent studies many resections that were believed to be oncologically complete have, in fact, turned out to be R1 when the specimens were analyzed following a local standardized pathology protocol. This is an important problem since margin involvement is directly correlated with survival. Nevertheless there is still no international standardized protocol for the handling and management of specimens, for the definitions of the resection margins to be analyzed or for what is considered to be a positive margin. In our review we have aimed to point out the different approaches for this management and the controversies or differences that arise in the ways of handling the surgical pieces after duodenopancreatectomy.

It is true that all protocols recommend inking margins. The problem arises when slicing the specimen so that both anatomical relationships and margin involvement are clearly identified. For example, opening the specimen through the axis of the main pancreatic duct makes it very difficult to establishing tumor relationships with anatomical structures and to identify resection margins. Nevertheless, this is the procedure that was traditionally performed and is still carried out in some centers. In relation to vascular, lymphatic and perineural invasion at the resection margin, and despite the absence of clear evidence, some institutions such as The Royal College of Pathologists suggest that this should be considered R1 (indirect invasion) and should be clearly stated in the report. The tumor-node-metastasis (TNM) staging system of the American Joint Commission on Cancer (AJCC), however, considers the resection margin to be indirect R1 only when tumor cells are attached to or have invaded the vessel wall. To the contrary, The College of American Pathologists (2012) does not consider indirect involvement to be R1 and, consequently, they do not report it in their protocol. In answer to your question about indirect invasion, the protocol in our hospital considers vascular, lymphatic and perineural invasion to be R1 (indirect involvement), and for that reason it is included under the epigraph "involved". We have modified each definition to avoid misunderstandings (page 12 of the manuscript).

PATHOLOGIC REPORT OF PANCREATIC CARCINOMA AT H.C.U.VALENCIA
Involved:

- *Direct: tumor in contact with inked margin*

- *Direct: tumor ≤ 1 mm (specify distance:_____)*
- *Indirect (vascular, lymphatic or perineural) ≤ 1 mm*
- *Indirect lymph node metastasis ≤ 1 mm*

Regarding the comment on the importance of lymph node location, in our study we refer to regional lymph nodes since distant node metastases (inter-aorto-cava lymph nodes) are considered to be M1. TNM staging, 7th edition, considers N1 to be any involved node, regardless of the location. Neither The College of American Pathologists nor The Royal College protocols specifies which node is involved. We, however, propose the exact identification of the node station involved in our protocol. Additionally, several studies have found that lymph node ratio is a powerful prognostic marker, location notwithstanding (Berger AC, Watson JC, Ross EA, et al. The metastatic/examined lymph node ratio is an important prognostic factor after pancreaticoduodenectomy for pancreatic adenocarcinoma. Am Surg 2004;70:235-240; Sierzega M, Popiela T, Kulig J, et al. The ratio of metastatic/resected lymph nodes is an independent prognostic factor in patients with node-positive pancreatic head cancer. Pancreas 2006;33:240-245; and Pawlik TM, Gleisner AL, Cameron JL, et al. Prognostic relevance of lymph node ratio following pancreaticoduodenectomy for pancreatic cancer. Surgery 2007;141: 610-618).

We have also completed a brief guideline explaining the dissection protocol (Page 10 of the manuscript):

We propose the following steps for the dissection protocol:

1. *Leave the specimen 24-48h in formaldehyde for the correct fixation after opening through the antimesenteric border of the duodenum.*
2. *Explore the pancreatic anatomy in order to identify the different parts (head, body and tail) and give it the correct orientation in readiness for dissection. Identify the margins (circumferential resection margin composed of the PAM, PPM and PMM and the pancreatic transection margin, or PTM).*
3. *Ink the margins indicated in step 2 in different colors.*
4. *Slice the luminal margins (proximal gastric or duodenal and distal jejunal), bile duct margin (BDM), common bile duct or common hepatic duct margin and PTM.*
5. *Analyze the gastro-intestinal lumen to identify any ampullary or other lesions.*
6. *Following the European guidelines, slice the entire pancreatic head in a plane perpendicular to the longitudinal axis of the duodenum through the center of the ampulla. Identify the tumor, its size and relations to structures and its distance to the margins.*

7. *Continue slicing in parallel sections with a thickness of 5 mm in order to have samples of the tumor that show its relationship with the different anatomical structures (duodenum wall, ampulla...) and inked resection margins.*
8. *Separate a sample of non-neoplastic pancreas.*
9. *Identify lymph nodes from the different stations for individual analysis.*

In regards to the important point about Whipple procedure, according to The Royal College of Pathologists, the type of operation will depend upon the site and size of the tumor. Recent trials, single-center studies and a Cochrane Database Systematic Review did not show any difference in patient survival between standard PD versus pylorus-preserving PD, PD with or without vascular resection, and PD with or without extended lymphadenectomy. Nevertheless, our study focuses on the management and handling of surgical specimens and not on the surgical technique performed. (References: Diener MK et al. Pancreaticoduodenectomy (classic Whipple) versus pylorus-preserving pancreaticoduodenectomy (pp Whipple) for surgical treatment of periampullary and pancreatic carcinoma. (Cochrane Database Syst Rev 2008;16:CD006053; Lin PW, Lin YJ. Prospective randomized comparison between pylorus-preserving and standard pancreaticoduodenectomy. Br J Surg 1999;86:603–607; Seiler C et al. Randomized clinical trial of pylorus-preserving duodenopancreatectomy versus classical Whipple resection – long term results. Br J Surg 2005;92:547−556; Tran KT et al. Pylorus preserving pancreaticoduodenectomy versus standard Whipple procedure: a prospective randomized, multicenter analysis of 170 patients with pancreatic or periampullary tumors. Ann Surg 2004;240:738−745; Yekebas EF et al. En bloc vascular resection for locally advanced pancreatic malignancies infiltrating major blood vessels: perioperative outcome and long-term survival in 136 patients. Ann Surg 2008;247:300−309; Yeo CJ et al. Pancreaticoduodenectomy with or without distal gastrectomy and extended retroperitoneal lymphadenectomy for periampullary adenocarcinoma, part 2: randomized controlled trial evaluating survival, morbidity, and mortality. Ann Surg 2002;236:355–368; Pedrazzoli S et al. Standard versus extended lymphadenectomy associated with pancreatoduodenectomy in the surgical treatment of adenocarcinoma of the head of the pancreas: a multicenter, prospective, randomized study. Lymphadenectomy Study Group. Ann Surg 1998;228:508–517; and Farnell MB et al. The role of extended lymphadenectomy for adenocarcinoma of the head of the pancreas: strength of the evidence. J Gastrointest Surg 2008;12:651−656.)

In agreement with the reviewer, there is variation among carcinomas of the pancreas, ampulla de Vater and common bile duct in terms of resection margin and lymph node

metastatic locations. For pancreatic cancer, pathological prognostic factors include tumor size, tumor differentiation, lymph node metastases and resection margin status. For ampullary and CBD, tumor stage and lymph node involvement are the most important ones. Because similar principles can be applied to the pathological report of ampullary and CBD carcinomas, The Royal College of Pathologist included the 3 malignancies in the same dataset. The College of American Pathologists protocol, however, does not include tumors of the ampulla of Vater in the same installment. We think that the general rules of pathological handling of pancreatoduodenectomies should be used for the three types of tumors, although we have included some modifications in our protocol regarding pT classification (Page 12: 4. Primary tumor (TNM classification) [23]):

Tis	*Carcinoma in situ*
Pancreas	
T1	*Tumor limited to the pancreas, 2 cm or less in greatest dimension*
T2	*Tumor limited to the pancreas, more than 2cm in greatest dimension*
T3	*Tumor extends beyond the pancreas, but without involvement of the celiac axis or the superior mesenteric artery*
T4	*Tumor involves the celiac axis or the superior mesenteric artery*
Ampulla of Vater	
T1	*Tumor limited to ampulla of Vater or sphincter of Oddi*
T2	*Tumor invades duodenal wall*
T3	*Tumor invades pancreas*
T4	*Tumor invades peripancreatic soft tissues, or other adjacent organs or structures*
Distal extrahepatic bile duct	
T1	*Tumor confined to the bile duct*
T2	*Tumor invades beyond the wall of the bile duct*
T3	*Tumor invades the gall bladder, liver, pancreas, duodenum or other adjacent organs*
T4	*Tumor involves the celiac axis or the superior mesenteric artery*

Minor points

Comment 1 and Comment 2: Please describe ACP at its first usage. Please describe ADP at its first usage.

Answer to comment 1 and comment 2:

We have homogenized the terms ACP and ADP and have corrected them with the more appropriated term PDAC. We have described it in the first paragraph of the introduction.

"Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer affecting the exocrine pancreas, and it is the fourth leading cause of cancer death in both sexes in the USA [1]."

Comment 3. The statement "the opening of biliary and pancreatic ducts, horizontal section of the pancreas and transversal sections perpendicular to the ducts" should be further clarified.

Answer to comment 3:

We have simplified the statement to sum up the general idea of this method, the opening of the ducts (Page 7):

"Both the Armed Forces Institute of Pathology *(AFIP) in its 3rd edition [27], as well as Allen and Cameron in 2004 [30] suggested a way of handling specimens based on the opening of biliary and pancreatic ducts with sections perpendicular to the ducts. Recently, in their 4th edition, the AFIP [31] recommended performing perpendicular sections to the main duct. That notwithstanding, these sections would be tangential to the duodenal wall, thus making the analysis of the ampulla, distal pancreatic and bile duct difficult [5]."*

Comment 4. In the section -4. Margin involvement: R1 status, colorectal should be replaced with pancreatic.

Answer to comment 4:

We have modified our section 4. "Margin involvement" to clarify some points (page 8-9).

4. Margin involvement: R1 status

The lack of consensus on margins not only affects their nomenclature and inclusion in the pathological report, but also the definition of R1.

The role of margin involvement, and its prognostic relevance, has been well characterized in other cancer types such as rectal cancer. Verbeke, though, states that, "margin status in pancreatic cancer has been neglected" [5].

Resection margin involvement (R1) seems to be an important prognostic factor in pancreatic cancer, but R1 rates reported in the literature vary enormously. Rates as disparate as 16%

and >75% have been reported in different studies and, consequently, clinical outcome correlation has been observed in some of them, but not in all [5,6,15,35].

For the majority of American pathologists, there is a positive margin (R1) only when the tumor is directly in contact with the inked margin (0 mm clearance) [13,16,22,31,35]. For European pathologists, R1 margin involvement is established when the distance between the tumor and the resection margin is 1 mm or less [5,11,12,15,21]. This is called the "1 mm rule" and was taken from the R1 definition of rectal cancer assessment [21].

Another confusing circumstance is when there is no direct margin involvement by the tumor. Despite the absence of clear evidence, The Royal College of Pathologists suggests considering the incomplete excision to be an R1 resection if lymph node metastases or perineural/lymphovascular invasion is within the 1 mm limit (indirect invasion of R1) [5,11,21]. Conversely, according to the tumor-node-metastasis (TNM) staging system of the AJCC the resection margin is considered R1 indirectly only when tumor cells are attached to or invade the vessel wall [36] (Figure 3).

Reviewer 2

In this review article authors have discussed about the need to develop improved protocols to evaluate pancreatic specimens and their surgical margins that will be advantageous for prolong survival of pancreatic cancer patients. Currently, there is no general agreement on basic issues such as surgical margins or definition of incomplete excision of PDAC in spite of the availability of several guidelines for pathology handling of specimens. Authors have reviewed the problems and controversies that dealing with handling of specimens and resection margins. Authors have presented the protocol for pathology handling of duodenopancreatectomy specimens. Overall, the review is informative and suitable for publication.

However, there are some minor concerns which need to be addressed:

Comment 1. The data given in first paragraph of introduction from references 1 and 3 should be modified as more recent statistical reports are available now.

Answer to comment 1:

We have updated the statistical data with new reports from the published literature. For that reason, we have modified both paragraph 1 of the introduction (pag. 4), as well as the first line of the abstract (pag. 2) according to the recent reference "Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013.*CA Cancer J Clin*. 2013; 63:11-30. [PMID: 23335087 DOI: 10.3322/caac.21166]." We have also suppressed reference 3 and, in consequence, modified the rest of the references.

"Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer affecting the exocrine pancreas, and it is the fourth leading cause of cancer death in both sexes in the USA [1]. In that country, pancreatic cancer accounts for 3% of all new malignancies. It is estimated that 45,220 new cases will be diagnosed there during 2013, and it will be the cause of death for 38,460 patients [1]. Death rates for pancreatic cancer between 2005 and 2009 were 12.5 and 9.5 per 100,000 inhabitants (males and females, respectively) [1]. In Europe, pancreatic cancer accounted for 6.2% of deaths in 2012 (78,000 patients) [2]. The overall 5-year survival rate remains dismal, at around 5% [1].

Unfortunately, only 8% of pancreatic cancer patients are diagnosed in the early stages, and of those, only 20% are susceptible to surgical treatment [3].

Comment 2. Please give full name for abbreviations used in the beginning (ACP and ADP specimens under the heading "pathology management ….. pancreatic tumors").

Answer to comment 2:

We have homogenized the terms ACP and ADP and corrected them by the more appropriated term PDAC. We have described it in the first paragraph of the introduction.

"Pancreatic ductal adenocarcinoma (PDAC) is the most common cancer affecting the exocrine pancreas, and it is the fourth leading cause of cancer death in both sexes in the USA [1]."

Comment 3. References number 7 has not been cited anywhere in the manuscript.

Answer to comment 3:

We had omitted reference number 7 by mistake (now reordered as number 6). We have corrected this in paragraph 2 under the heading "Pathology management of resected pancreatic tumors":

"Despite the fact that resection margin status is a key prognostic factor, the rates of microscopic margin involvement (R1) vary enormously from study to study [6-10]."

Comment 4. There are several grammatical and typological errors please correct (ex. spelling of "Figura" as "Figure" under figure legends 1, 2 and 3).

Answer to comment 4:

We have reviewed and corrected all spelling errors in the manuscript:

- *"Figure 1: A: Pancreatoduodenectomy …"*
- *"Figure 2: Consecutive …"*
- *"Figure 3: A-C: Microscopic …"*

Comment 5. In figure legend 2, please correct "0,5" to "0.5" and in figure legend 3, give space between 1mm as 1 mm. Also provide space between 5-10mm under heading "differences in dissection protocols" and other places to make the text uniform throughout the manuscript.

Answer to comment 5:

We have corrected the entire measurement format to make it uniform.

- In figures 2 and 3: *"Figure 2: Consecutive parallel sections of 0.5 cm thickness following…"* and *"Figure 3: … D: Neoplastic cells within 1 mm of the resection margin colored in black …"*
- On page 7, under handling "differences in dissection protocols": *"The procedure performed by Westgaard et al. [12] consists of inking the retroperitoneal margin, performing a 5-10-mm-thick section parallel to this margin and serially slicing perpendicular to the ink."*
- On page 8, under handling "margin involvement": *"Another confusing circumstance is when there is no direct margin involvement by the tumor. Despite the absence of clear evidence, The Royal College of Pathologists suggests considering the incomplete excision to be an R1 resection if lymph node metastases or perineural/lymphovascular invasion is*

within the 1 mm limit (indirect invasion of R1) [5,11,21]. Conversely, according to the tumor-node-metastasis (TNM) staging system of the AJCC the resection margin is considered R1 indirectly only when tumor cells are attached to or invade the vessel wall [36] (Figure 3)."

All measurements in the pathologic report have also been corrected.

Comment 6. Please improve figure quality as scale and labeling are not visible.

Answer to comment 6:

We have improved the quality of scale and labeling of figures 1 and 2.

Comment 7. Extend your discussion to R0 status also under the heading "Margin Involvement" to differentiate and for better understanding of R0 and R1.

Answer to comment 7:

In agreement with your suggestion, we have extended our discussion in order to clarify the differences in margin involvement (page 8-9).

4. Margin involvement: R1 status

The lack of consensus on margins not only affects their nomenclature and inclusion in the pathological report, but also the definition of R1.

The role of margin involvement, and its prognostic relevance, has been well characterized in other cancer types such as rectal cancer. Verbeke, though, states that, "margin status in pancreatic cancer has been neglected" [5].

Resection margin involvement (R1) seems to be an important prognostic factor in pancreatic cancer, but R1 rates reported in the literature vary enormously. Rates as disparate as 16% and >75% have been reported in different studies and, consequently, clinical outcome correlation has been observed in some of them, but not in all [5,6,15,35].

For the majority of American pathologists, there is a positive margin (R1) only when the tumor is directly in contact with the inked margin (0 mm clearance) [13,16,22,31,35]. For European pathologists, R1 margin involvement is established when the distance between the tumor and the resection margin is 1 mm or less [5,11,12,15,21]. This is called the "1 mm rule" and was taken from the R1 definition of rectal cancer assessment [21].

Another confusing circumstance is when there is no direct margin involvement by the tumor. Despite the absence of clear evidence, The Royal College of Pathologists suggests considering the incomplete excision to be an R1 resection if lymph node metastases or perineural/lymphovascular invasion is within the 1 mm limit (indirect invasion of R1) [5,11,21]. Conversely, according to the tumor-node-metastasis (TNM) staging system of the AJCC the resection margin is considered R1 indirectly only when tumor cells are attached to or invade the vessel wall [36] (Figure 3).

Comment 8. The discrepancies and controversies in the techniques of tissue sampling may also exist. Please considered when developing a new protocol.

Answer to comment 8:

We are in total agreement with the reviewer. Discrepancies and controversies in sampling techniques will always exist. Achieving consensus on a protocol for the handling and management of pancreatoduodenectomy specimens, however, would provide a useful tool for making comparisons among the different studies and for homogenizing the criteria that establish the real status of resection margins.

Reviewer 3

The authors review the current practices in the workup up pancreatoduodenectomy specimens and present their own protocol. The english language needs major corrections.

Rejection

Answer to reviewer 3:

The new manuscript has been revised by two native English professional translators Ms. Landy Menzies and Mr. Mervin Eyler, both of whom have ample experience in medical literature.

We hope that the extensive modifications the manuscript has undergone will satisfy the requirements of reviewers 1 and 3, and that they will find our study as interesting and informative as reviewer 2 does.

Yours sincerely,

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