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TITLE:

DIPLOMA Approach for Standardized Pathology Assessment of Distal Pancreatectomy Specimens

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pathology, pancreas, surgery, pancreatic ductal adenocarcinoma, distal pancreatectomy, histopathology

SUMMARY:

The current study highlights a standardized approach to the macroscopic assessment of distal pancreatectomy specimens for pancreatic ductal adenocarcinoma, with special emphasis on the measurement of pancreatic dimensions and those of other organs, inking of margins, measurement of tumor size and proximity to margins, lymph node sampling and block selection.

ABSTRACT:

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal malignant cancers. A minority (20%) of PDACs are found in the pancreatic body and tail. Accurate pathology assessment of the pancreatic specimen is essential for providing prognostic information and it may guide further treatment strategies. The recent 8th edition of the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) staging system for pancreatic tumors has incorporated significant changes to tumor (pT) stage, which is predominantly based on tumor size. This change emphasizes the importance of careful block selection. Owing to the greater prevalence of tumors in the head of the pancreas, efforts are made to standardize the assessment of pancreatoduodenectomy specimens. However, consensus regarding the macroscopic assessment of distal (i.e., left) pancreatectomy specimens is lacking. The DIPLOMA approach includes the standardized measurement of pancreas and other resected organs, inking of relevant surgical margins and anatomical surfaces without removing covering layers of fat, measurement of tumor size (for T-stage), together with assessment of splenic vessel involvement (and other organs if present). All relevant margins are assessed, and relevant blocks are selected to confirm these parameters microscopically. The current protocol describes a standardized approach to the macroscopic assessment of distal pancreatectomy specimens. This approach was developed during several meetings with pathologists and surgeons during the preparation phase for an international multicenter trial (DIPLOMA, ISRCTN44897265), which focuses on radicality of distal pancreatectomy for pancreatic ductal adenocarcinoma. This standardized approach can be instrumental in the design of studies and will uniform reporting on the outcomes of distal pancreatectomy. The described technique is used in the DIPLOMA trial for pancreatic ductal adenocarcinoma but may also be useful for other indications.

INTRODUCTION:

Pancreatic ductal adenocarcinoma (PDAC) is associated with a very poor prognosis[1]. Surgery, in combination with (neo)adjuvant therapy remains the only curative treatment[2]. Following surgery, adequate histopathological assessment of the resected specimen is essential for prognostic stratification and in addition it may guide further treatment strategies[3]. Furthermore, the recent 8th edition of American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) staging system for pancreatic tumors has incorporated significant changes to tumor (pT) stage, which is predominantly based on tumor size[4,5]. While maximum tumor size is assessed macroscopically, adequate specimen sampling is required in order to corroborate these findings, particularly as chronic pancreatitis can mimic tumor appearance with the naked eye.

As the majority of pancreatic ductal adenocarcinomas (up to 80%) are encountered in the head of the pancreas, most of the literature is based on the assessment of pancreatoduodenectomy specimens[6,7]. In the United Kingdom, the Royal College of Pathologists (RCPath) have published datasets that provide evidence-based guidelines on the specimen handling, dissection and reporting of pancreatic cancer, with focus placed on the more common pancreatoduodenectomy specimens[8]. Nonetheless, international consensus regarding specimen grossing is still lacking and practice is still highly divergent between centers[6]. The equivalent process of standardizing pathology assessment of specimen originating from a distal (i.e., left) pancreatectomy is now of growing clinical interest.

The Distal Pancreatectomy, Minimally Invasive or Open, for malignancy (DIPLOMA, ISRCTN44897265) trial is an international multicenter, randomized controlled trial comparing open versus minimally invasive surgical approach for the management of PDAC of the pancreatic body and tail. The DIPLOMA pathology protocol has been developed as a means of standardizing pathology assessment and reporting for this trial. The protocol describes the assessment of distal pancreatectomy specimens, including specimen orientation, inking, lymph node sampling, assessment of splenic vessel involvement (and other organs if present), and block selection.

The described method was developed during four meetings of the DIPLOMA study group (April 2015 Manchester, December 2016 Amsterdam, May 2017 Mainz, and April 2018 Amsterdam) with highly experienced 20−40 surgeons and pancreatic pathologists from 10 countries across Europe. Discussions included the relevance of the various margins, the transection plane and especially the dissection plane between the posterior part of the body and tail.

Patient characteristics

A 79-year-old woman presented with an incidental finding of a 34 mm tumor in the body of the pancreas, which was suspicious for malignancy. The CT scan showed no radiological evidence of tumor involvement of major vascular structures or the presence of (distant) metastases. Only adjacent small sized lymphadenopathy was noted. The patient was discussed in the multidisciplinary team meeting where it was decided that she was eligible for surgery. An open radical distal pancreatectomy, splenectomy and wedge resection of the stomach was performed within the DIPLOMA trial.

Macroscopic assessment of distal pancreatectomy specimens and nomenclature of margins

The relevant margins that should be assessed in a distal pancreatectomy specimen include the transection margin, splenic artery and vein margins, posterior dissection margin, and additional margins in the case of multivisceral resections as shown in **Table 1**.

The transection margin is the surface where the pancreatic body was separated from the neck. Mainly in laparoscopic, but also in increasing numbers of open, surgical specimens, this margin is a linear staple line. The splenic artery and vein margins are in close proximity to the stapled transection margin and are marked with vascular clips or small staples. The posterior margin is the dissection plane between the posterior part of the body and tail of the pancreas and the frontal plane of the renal fascia, within the retroperitoneum. Between the anterior and posterior renal fascia is the perirenal space, within which lie the kidney and adrenal gland in a loose fibrofatty connective tissue compartment. The posterior dissection margin varies depending on the exact surgical procedure performed. This may include the anterior renal fascia, with or without the adrenal gland and posterior renal fascia[9,10]. While the anterior, peritonealized surface is not considered a surgical margin, tumor breaching of this surface is associated with an increased risk of local recurrence[3].

PROTOCOL:

The protocol followed the ethical guidelines of Southampton University Hospital NHS Foundation Trust. Informed consent was obtained for the use of the tissue for teaching and research purposes.

NOTE: The relevant steps are summarized in **Table 2** and the relevant materials in **Table of Materials**.

1. Specimen orientation

NOTE: In order to aid the histopathologist in accurate specimen orientation, ensure that the operating surgeon places orientation sutures to mark the posterior dissection plane, the splenic vessel margins, and the pancreatic transection margin.

1.1. Upon receipt, fix the specimen in formalin, at room temperature, for 24−48 h.

NOTE: Fixation may cause distortion of the specimen which makes accurate orientation difficult as the anterior peritoneal surface and retroperitoneal (posterior dissection) margin may have similar appearances. Furthermore, some centers take fresh tumor samples for research purposes. In this situation, care should be given to minimize distortion by inking, before fixation, one or more margins, if these are to be incised while the specimen is fresh.

1.2. Identify posterior dissection plane by orientation suture (placed by the operating surgeon) or by the presence of the spleen, which is lateral, and the splenic vessels on the postero-superior border, the suture marking the posterior dissection margin, and staples marking the (medial) transection margin. Identify also other margins/surfaces. Finally identify the spleen.

NOTE: In this specimen the anterior surface, transection margin, posterior margin, superior border, inferior border, and additional gastric wedge can be identified.

1.3. Ensure that the peripancreatic fat remains intact and attached to the specimen.

2. Measurements

2.1. Measure all dimensions in millimeters.

2.2. Measure the pancreas from medial to lateral (95 mm for this specimen), cranial to caudal (30 mm), and anterior to posterior (20 mm).

2.3. Measure the spleen also from cranial to caudal (110 mm for this specimen), anterior to posterior (60 mm), and medial to lateral (20 mm). In addition, weigh the spleen if possible (see the note below).

NOTE: If the tumor is in close proximity to the splenic hilum, the spleen should not be separated from the pancreas and weighing of spleen should be done en bloc with the pancreas.

2.4. Measure any potential additional organs, including the adrenal gland.

NOTE: In this protocol, the gastric wall was resected and measured. The wedge stomach measures 35 mm x 10 mm x 5 mm.

3. Inking

3.1. Having measured the pancreas and spleen in three dimensions, ink the posterior dissection margin, transection margin, anterior peritonealized surface, and additional organ margins using different colors. If the splenic vessel margins appear grossly involved, ink these in order to identify the true margin with 'en face' sections.

3.2. Though, not a requirement for margin assessment, ink the superior and inferior border of the pancreas for improved specimen orientation and to microscopically group lymph nodes into the relevant anatomical stations in single transverse sections on hematoxylin and eosin stained slides.

3.3. Furthermore, ink any additional margin (e.g., the gastric wall margin) with a different color.

NOTE: The specimen is inked in the following manner: the anterior peritonealized surface is inked in yellow, the gastric wall margin is inked in blue, the transection margin is inked in red, the superior border of the pancreas is inked in green in order to identify station 11 lymph nodes, the inferior border is inked in orange to identify station 18 nodes, and the posterior dissection margin is inked in black.

4. Dissection

4.1. Following measurement and inking of the specimen, use a blade to cut blocks from the transection and splenic vessel margins. Remove with the blade the staples in order to take an 'en face' section of the transection margin. Cut closely to the staple line to preserve as much of the proximity to the true margin.

NOTE: The staple line of the transection margin must be removed as metal staples cannot be cut by the microtome blades.

4.2. Cut with the blade a single 'en face' section of 3−5 mm of the transection margin of the splenic artery and vein. Alternatively, cut bread slices of 3−5 mm of the transection margin perpendicular to the stapled margin.

NOTE: The splenic artery and vein are in close proximity to the transection margin and are usually marked with clips. This can be used to give accurate distances of tumor to the margin.

4.3. Remove any additional margins using the blade.

NOTE: In the accompanying video, the staple line from the gastric wedge resection is removed.

4.4. Using a long knife slice the specimen from medial to lateral (transection margin to spleen) in 3−5 mm intervals, and number the slices (for example 1−15, with 15 slices). Do not remove any of the posterior pancreatic fat.

4.5. With the transverse sections of the specimen laid out from medial to lateral, record the number of tumors, including their location (body, and/or tail), appearance(s), and maximum tumor size(s).

NOTE: Unlike tumors of the pancreatic head where the significance of named vessel involvement is reflected in TNM staging, with superior mesenteric artery and/or coeliac axis involvement corresponding to pT4 disease, the clinical significance of splenic artery invasion remains under investigation[11]. For the purpose of future investigation, it is recommended to record the splenic artery and vein involvement within the macroscopic description, and to confirm it microscopically. The splenic artery lies in the superior border of the pancreas, while the vein runs along the posterior border of the pancreas.

5. Tumor assessment

5.1. Assess the tumor and its involvement of margins and/or (splenic) vessels.

NOTE: Here, the tumor is present in slices 9−14. The cut surface of the tumor is pale and homogeneous.

5.2. Measure the macroscopic tumor size in three dimensions (cranial to caudal, medial to lateral, and anterior to posterior).

NOTE: Here, the tumor measures 30 mm cranial to caudal plane, 25 mm anterior to posterior, and 13 mm medial to lateral. The measurement is based on slice thickness. Most commonly maximum tumor size is in the medial to lateral plane. This measurement is assessed adding up the slice thickness of each slide containing tumor. Distances to margins can be difficult to assess macroscopically as ductal adenocarcinoma of the pancreas have a highly dispersed growth[8,12]. On this basis, while an estimation of the distances to the margins can be recorded in the macroscopic description, corroboration of these findings microscopically based on adequate tumor sampling is important.

5.3. Slice the spleen with perpendicular section with 3−5 mm intervals starting from its outer convexity and proceed inwards towards the splenic hilum. In cases where the tumor is in close proximity to the spleen, keep the spleen intact with the specimen and take section in continuity between the tumor and spleen.

6. Tissue sampling

6.1. As is done for pancreatoduodenectomy specimens, rationalize block selection to demonstrate tumor type, size, large vessel and other organ involvement, nodal staging, margin status, and tumor regression in cases where neo-adjuvant therapy has been given. Make close-up photographs of the specimen slices.

NOTE: The block selection includes the transection margin, (splenic) vascular resection margins, gastric wall margins, blocks of tumor demonstrating maximum size and distance to margins, background pancreas, representative section of spleen, and lymph nodes.

6.2. Take tissue samples from the tumor en-bloc with the peripancreatic fat from the superior and inferior borders that harbors lymph nodes from stations 11 and 18. Dissect out individual lymph nodes from the peripancreatic fat in parts of the specimen that are well away from the tumor site.

NOTE: Especially take additional samples from what macroscopically seems to be the periphery of the tumor and the adjacent tissues that appear tumor-free. It is in the latter that microscopically invasive tumor is most often found to be present. The same rationale for thorough block taking applies to the assessment of the margins. In contrast to colorectal carcinomas that typically have a more well-circumscribed tumor front, pancreatic ductal adenocarcinomas have an infiltrative, discontinuous pattern of growth. The interface of tumor with normal pancreas is often obscured by the presence of chronic pancreatitis, placing further onus on margin sampling[3]. It is strongly recommended not to remove the posterior peripancreatic fat, as this will compromise assessment of the posterior margin.

6.3. For tumors 20 mm or less, sample the entire tumor to allow for accurate pT staging. For larger tumors, sample sufficient tissue. When sampling tumor, include the peripancreatic fat, lymph nodes and the relationship with margins and adjacent structures.

NOTE: In the accompanying video, the relationship between tumor and gastric wall is highlighted. Regional lymph nodes for the distal pancreas are station 11 along the superior border of the pancreas, station 18 along the inferior border, and station 10 within the splenic hilar fat[13]. For tumors located in the pancreatic body the surgeon will additionally resect lymph node stations 8a and 9, which are submitted in separate pots.

6.4. Take perpendicular sections of the spleen at 3−5 mm intervals, followed by sampling of splenic hilum (station 10) lymph nodes.

NOTE: There is no recommendation on whether to remove the spleen from the specimen or not. In case the tumor is located in the tip of the pancreas, do not remove the spleen and extent the sagittal slicing into the spleen.

6.4. Take blocks from additional organs.

REPRESENTATIVE RESULTS:

Microscopic assessment showed a 28 x 25 x 30 mm, moderately differentiated, pancreatic ductal adenocarcinoma as shown in **Table 3**. There was perineural and lymphovascular invasion without splenic artery or vein involvement. In total, 17 lymph nodes were found, of which 3 were involved (1 superior border, 2 inferior border). Distant lymph nodes (station 8 and hepatic artery) showed no evidence of metastatic malignancy. All resection margins were clear of tumor: transection margin >40 mm and posterior margin 2 mm. Tumor was over 40 mm from the splenic vessel margins. The minimal margin to the anterior surface was 1 mm. Tumor invaded the muscularis propria of the gastric wall, but was clear of the stapled margin

by at least 3 mm. Using the AJCC/UICC 8th edition staging system tumor was classified as pT2, N1, M0, stage IIB.

TABLE LEGENDS:

Table 1: Nomenclature of distal pancreatectomy specimen margins/surfaces.

Table 2: Steps for pathological assessment distal pancreatectomy specimen.

Table 3: Representative results of microscopic assessment.

DISCUSSION:

Adequate histopathological assessment of a resected specimen is essential for the stratification of disease prognosis and guidance of further treatment strategies. Standardized protocols for the assessment of specimens resulting from distal pancreatectomy for PDAC are lacking. This potentially creates a considerable variability among the reported histopathological findings[14]. Differences in definitions and practice between centers limit the comparability of studies[15]. Furthermore , the R1 (tumor <1 mm of margin) resection rates vary from 16–85%[15]. The discrepancies in R1 rates relate to divergence in the extent of tissue sampling, differences in microscopic assessment and the lack of international consensus regarding an appropriate R1 definition[8,16].

The current protocol provides a standardized protocol for the macroscopic assessment of distal pancreatectomy specimens with PDAC. This includes improved specimen orientation by using sutures of the posterior margin marked by the surgeons and different colors to distinguish the relevant margins, including those of additional organs.

According to AJCC/UICC TNM8 staging, tumors are staged according to size and tumor extent. Macroscopic assessment of tumor size can be challenging owing to the presence of fibrosis and chronic pancreatitis that can mimic tumor macroscopically. It is for this reason that extensive tumor sampling, supported by a detailed block description and close-up photographs of the specimen slices, are required to ensure accurate assessment of tumor size and extent. It should be noted that in case of intraoperative frozen assessment the accurate distance between tumor and transection margin is the sum of the distance between tumor and transection margin in the specimen and the thickness of the intraoperative sample of the transection margin. The standardized methods of tumor size assessment will increase appropriate staging of the tumor according to the 8th edition of the TNM system.

Finally, for tumors of the pancreatic body or tail the minimum number of lymph nodes sampling for sufficient nodal staging has not yet been established[17]. However, understanding the pattern of lymph node metastasis is of potential importance for further improvement of treatment and prognosis. Therefore, inking the superior and inferior border of the pancreas will help the identification of lymph node stations 11 and 18 and adequate lymph node sampling. Separate reporting on the regional lymph node stations is not common practice. In general, only the total number of lymph nodes, number of involved lymph nodes, and lymph node ratio are reported[8].

A limitation of the methods used is the removal of the stapler line of the transection margin. Although staplers are increasingly used for the transection of the pancreas and closure of the pancreatic stump[18], staples cannot be cut by microtome blades during further processing. It is therefore advised to remove the staplers with as little tissue as possible to minimize the loss of tissue at the true margin and report when tumor is macroscopically close to the staple line.

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DISCLOSURES:
The authors have nothing to disclose.

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Figure

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Table 1

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Table 2

Specimen orientation	1	Formalin fixation, at room temperature, for 24−48 h
	2	Identification posterior dissection plane by orientation suture (p
	3	Peripancreatic fat should be left intact and attached to the spec
Measurements	1	Dimensions are measured in millimeters.
	2	Pancreas from medial to lateral, cranial to caudal (height) and a
	3	Spleen cranial to caudal (length), anterior to posterior, medial t
	4	Additional measurements for additional organs resected, includ
Inking	1	Inking of transection margin, posterior dissection margin and an
	2	Additional margins (superior and inferior border) can be inked f
	3	Inking of additional resected organs, including adrenal gland
Specimen dissection	1	'En face' dissection of the transection margin and removal of th
	2	'En face' dissection of splenic artery and vein
	3	Remove any additional margin
	4	Pancreatic specimen from medial to lateral in 3−5 mm intervals
	5	Number and record number of the transverse sections laid out
Tumor assessment	1	Macroscopic assessment of tumor involvement of margins and/
	2	Assessment of tumor size by measuring height, width and lengtl
	3	Perpendicular sections of the spleen can be taken at 3−5 mm int
Block taking	1	Sample tumor in sufficient number of blocks, detailed block des
	2	Blocks of tumor are selected in continuity with sections of the p
	3	For tumors 20 mm or less, whole tumor sampling is reasonable
	4	Sampling of splenic hilum (station 10) lymph nodes
	5	Additional blocks for additional organ(s)

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eripancreatic fat from the superior and inferior borders in order to capture lymph node station

ly identify the spleen.

11 and 18

Table 3

Click here to access/download;Table;60343_Table 3 Representative results.xlsx

Table of Materials

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Name of Material/Equipment	Company	Catalog Number
Formalin	Genta	BFNC50
Gloves	Healthline	FTG182, FTG183, FTG184 (depending on size)
Blade Handles	Swann Morton	
Blades	Swann Morton	FSF440
Scales	Ohaus	
Long Knives	Cellpath	KMY811
Ruler	Solmedia	RUL003
Scissors	Weiss	FGP8939

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1. Please note that all information in the video should be in the written protocol. For this reason, I have added some details to the written protocol based on the video (see red text and comments in the attached manuscript). Please review for accuracy and make changes if necessary.

> We have accepted all changes in the manuscript as proposed by the editor and have added the word ‘splenic’. This can be found as track changes.

2. Video 10:28-10:38: The conclusion section should have both video and audio. If audio cannot be added here, please remove the conclusion section from the video.

> Conclusion section is removed and newest video is uploaded to dropbox.

3. Video 10:39: Please remove the "Thank you" card from the video.

> Thank you card is removed.