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ORIGINAL ARTICLE

Axial slicing versus bivalving in the pathological examination of pancreatoduodenectomy specimens (APOLLO): a multicentre randomized controlled trial

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Abstract

Background: In pancreatoduodenectomy specimens, dissection method may affect the assessment of primary tumour origin (i.e. pancreatic, distal bile duct or ampullary adenocarcinoma), which is primarily determined macroscopically. This is the first study to prospectively compare the two commonly used techniques, i.e. axial slicing and bivalving.

Methods: In four centres, a randomized controlled trial was performed in specimens of patients with a suspected (pre)malignant tumour in the pancreatic head. Primary outcome measure was the level of certainty (scale 0–100) regarding tumour origin by four independent gastrointestinal pathologists based on macroscopic assessment. Secondary outcomes were inter-observer agreement and R1 rate.

Results: In total, 128 pancreatoduodenectomy specimens were randomized. The level of certainty in determining the primary tumour origin did not differ between axial slicing and bivalving (mean score 72 [sd 13] vs. 68 [sd 16], p = 0.21), nor did inter-observer agreement, both being moderate (kappa 0.45 vs. 0.47). In pancreatic cancer specimens, R1 rate (60% vs. 55%, p = 0.71) and the number of harvested lymph nodes (median 16 vs. 17, p = 0.58) were similar.

Conclusion: This study demonstrated no differences in determining the tumour origin between axial slicing and bivalving. Both techniques performed similarly regarding inter-observer agreement, R1 rate, and lymph node harvest.

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Introduction

Pancreatoduodenectomy (PD) is mostly performed for malignant and premalignant lesions in the pancreatic head. Tumours in this region include pancreatic adenocarcinoma, distal cholangiocarcinoma, ampullary adenocarcinoma and duodenal adenocarcinoma, each with a distinctly different prognosis and different indications for adjuvant systemic treatment.[1,2] Periampullary malignancies often cannot be reliably differentiated by means of tumour morphology, presence of precursor lesions or immunohistochemistry. Therefore, macroscopic assessment plays a crucial role in determining the primary tumour origin.[3] As such, the method of specimen dissection and macroscopic assessment can directly influence how the primary tumour origin is determined.

Several approaches for the dissection of PD specimens have been described.[4–9] International consensus regarding the best method is lacking, potentially due to a lack of comparative studies and personal preference of pathologists.[10] The two most commonly used grossing techniques for PD specimens are axial slicing as described by Verbeke et al., and bivalving of the pancreatic head as described by Adsay et al.[8,9] Axial slicing is performed by serially slicing the specimen perpendicular to the long axis of the duodenum. It is relatively straightforward and offers the advantage of concordance with axial imaging. It is also said to increase accuracy in detecting margin involvement in pancreatic ductal adenocarcinoma (PDAC).[8,11–13] Bivalving is performed by slicing the pancreatic head over the plane constructed by probing the common bile duct (CBD) and pancreatic duct. By opening the pancreatic and bile ducts longitudinally, visualization of the (peri)ampullary region may be improved, facilitating the identification of the tumour origin.[9,14] There is a pressing need for a consistent grossing method, as exemplified by a current survey from the Pancreatobiliary Pathology Society (PBPS), which evaluates the practice patterns regarding the grossing and reporting of PD specimens, with the ultimate goal of establishing a standardized grossing protocol.[15] However, there is no convincing evidence on which technique is superior in terms of determining the tumour origin, margin-positive resection (R1) rate, or lymph node harvest.[10]

Although both techniques are well established and widely used, a prospective comparison has never been performed. As bivalving provides a direct view of the relevant structures in relation to each other, we believe it may aid the pathologist when establishing the location of the tumour bulk, particularly in the periampullary area. The hypothesis of the current APOLLO multicentre trial was that in the pathology assessment of PD specimens, bivalving of the pancreatic head provides more certainty in determining the tumour origin, as compared with axial slicing, and results in a higher inter-observer agreement within a panel of expert pathologists.

Materials and methods

Study design and patients

This multicentre randomized controlled superiority trial was performed in four centres of the Dutch Pancreatic Cancer Group (DPCG) and followed the CONSORT guidelines for reporting of clinical trials.[16] All adult patients with an indication for elective PD for (suspected) malignant or neoplastic disease in the pancreatic and periampullary region were screened for eligibility. Patients with PD performed for one of the following indications were excluded: chronic pancreatitis, metastatic lesion(s) in the pancreas, duodenal tumours not involving the periampullary region, high suspicion of mesenchymal neoplasms or of pancreatic neoplasms other than ductal adenocarcinoma (i.e. pancreatic neuro-endocrine, solid-pseudopapillary or acinar cell neoplasms).

Participating centres and quality assurance

All participating centres were high-volume centres, performing and processing over 40 PDs annually over the past three years. Prior to inclusion at every new participating centre, an implementation phase was instituted to monitor and maintain quality standards for APOLLO. Following approval of the local Medical Ethics Review Committee, a site visit took place to inform the pathologists, surgeons, pathology residents and other involved personnel. Clear instructions, including a standard operating procedure (SOP) and a video for each grossing technique, were shared among the participating centres. Before a new participating centre was allowed to start accrual, test rounds were held to assess technical skills and the quality of the macroscopic photos. Both techniques were closely observed and transformed in a SOP, including step-by-step instructions, schematic pictures of the slicing method and a set of example photos (Fig. 1A and B). As stated in the Dutch guidelines, the grossing technique is left to the discretion of the pathologist, with both axial slicing and bivalving being recommended.[17] Before the start of the APOLLO trial, most centres routinely performed axial slicing, as a result of a previous recommendation of the DPCG.

Randomization

Eligible patients were recruited from the operating schedule of the participating hospitals when scheduled for PD. The Medical Research Involving Human Subjects Act did not apply as judged by the Medical Ethics Review Committee of the Amsterdam UMC, location Academic Medical Center, as the national guideline allows both techniques. Participating patients were therefore only required to provide written consent for using non-anonymized data for research purposes.

The specimen was randomized after PD was performed using an online randomization module (Castor Electronic Data Capture, Amsterdam, the Netherlands) in a 1:1 ratio between axial slicing and bivalving of the pancreatic head. The sequence of

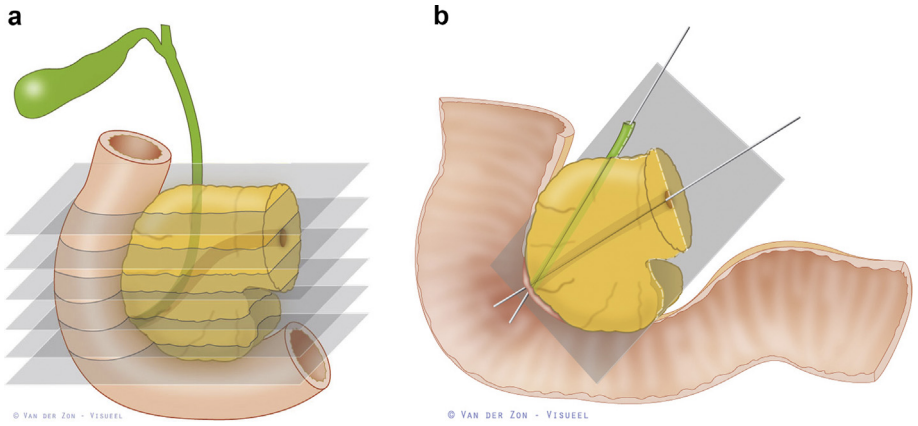

Figure 1 **a.** Standardized approach of the upfront axial slicing technique. Legend: Step-by-step instructions for the axial slicing technique according to Verbeke,[8] 1. Ink the different surfaces/margins according to color code, 2. Open the duodenum antimesenterically and take a high-quality macroscopic close-up photograph of the papilla of Vater, 3. Take parallel margins (en face) from the pancreatic neck margin, proximal distal bile duct margin and enteric proximal and distal margin, 4. Fix of the specimen in formalin over night, 5. Serially slice the specimen in the axial plane in slices of 3–5 mm thick, 6. Take high-quality macroscopic close-up photographs of the specimen slices, 7. Sample the tumor and lymph nodes extensively for microscopic evaluation. **b.** Standardized approach of the bivalving technique. Legend: Step-by-step instructions for the bivalving technique according to Adsay,[9] 1. Ink the different surfaces/margins according to color code, 2. Open the duodenum anti-mesenterically and take a high-quality macroscopic close-up photograph of the papilla of Vater, 3. Take parallel margins (en face) from the pancreatic neck margin, proximal distal bile duct margin and enteric proximal and distal margin, 4. Probe the main pancreatic duct and common bile duct and slice the specimen along the plane defined by both probes, thereby longitudinally opening both ducts, i.e. bivalving the pancreatic head, 5. Take high-quality macroscopic close-up photographs of the bivalved head, which show the ampullary region and other potential relevant regions, 6. Fix the specimen in formalin over night, 7. Serially slice the remaining two halves of the specimen in the axial plane, followed by taking macroscopic photographs of the axial slices. 8. Sample the tumor and lymph nodes extensively for microscopic evaluation

allocation remained concealed by randomizing centrally using the online module and by waiting until PD was completed. Randomization was stratified for centre and neoadjuvant therapy. Randomization was performed centrally in blocks for each stratum, with concealed block sizes varying between four, six and eight to safeguard the unpredictability of the next randomization. As the grossing technique is readily visible, blinding of the assessors was not possible.

Specimen handling prior to grossing

Grossing was performed by a GI consultant pathologist, or a dedicated pathology resident supervised by a consultant GI pathologist. First, the specimen was orientated by identification of anatomical landmarks in the resection specimen, which was aided by marks provided by the surgeon. The following pancreatic margins and surfaces were inked according to local color-codes: the anterior surface, the superior mesenteric vein (SMV) margin, superior mesenteric artery (SMA) margin, and posterior margin. The stomach and duodenum were opened anti-mesenterically and rinsed. Surgical sutures and clips were carefully removed from the specimen and the transection margins (proximal [gastric or duodenal], distal [duodenal or jejunal], pancreatic neck and common bile duct) were submitted en face. Any other important structures (e.g., venous patch in case of venous resection) were also inked. If a stent was present, it was removed.

Grossing technique: axial slicing

The specimen was left in 4 per cent buffered formalin for at least 24 h. After fixation, the specimen was serially sliced perpendicular to the long axis of the duodenum over its entire craniocaudal length with slices of 3–5 mm thick, as described by Verbeke et al.[8,11] The sections were systematically positioned in sequential order with the caudal side up (as on CT scan) and the tumour size was measured.

Grossing technique: bivalving

The main pancreatic duct and CBD were probed and the pancreatic head was bivalved along the plane defined by both probes. By longitudinally slicing the ducts, the (peri)ampullary region becomes visible as described by Adsay et al.[9] If the pancreatic duct could not (entirely) be probed, the pancreatic head was bivalved along the CBD probe in the direction of the pancreatic duct to visualize at least the lumen of the CBD and the ampullary region. After bivalving, the specimen was left in 4 per cent buffered formalin for at least 24 h. After fixing, the

ampullary region was sampled and each half of the pancreatic head was serially sliced perpendicular to the long axis of the duodenum in 3-5-mm-thick slices.

Macroscopic photographs and expert surveys

High-definition photos of the macroscopic sections from each specimen were taken in a standardized way according to the study protocol. All photos were centrally uploaded in the online data manager (Castor EDC). Each specimen was then randomly assigned to four dedicated consultant GI pathologists from the APOLLO expert panel, who performed macroscopic assessment based on the macroscopic photos through a digital survey. The APOLLO expert panel consisted in total of nine dedicated GI pathologists (JV, LAAB, MD, MLFV, AFS, CMB, MGR, KCK, CAS). Pathologists did not assess specimens from their own centre. Assessment involved identification of the tumour origin (pancreatic, distal bile duct, ampullary or duodenal) and the level of certainty in determining this on a visual analogue scale of 0–100. The visual analogue scale was chosen to prevent loss of information from categorizing the pathologists' responses and to substantiate the potential uncertainty when assigning a category. For every specimen, four pathologists performed assessment of the macroscopy photos independently from each other and without further clinical information. Only the study coordinators (SvR and ECS) had access to both patient information and the expert surveys.

Pathology reporting

Parameters were reported in accordance with the Dutch Guideline Database (Dutch Pathological-Anatomy National Automated Archive [PALGA].[17,18] Tumour cells growing less than 1 mm from a resection or dissection margin (pancreatic neck margin, proximal/distal enteric margins, CBD margin, SMV margin [including venous patch], SMA margin, posterior margin), or breaching the anterior surface was considered as R1. Final pathology was established in routine diagnostic practice and was based on macroscopic assessment (inspection, palpation), microscopy (morphology, location, precursor lesions, and ancillary tests such as immunohistochemistry) and at times included consultation of preoperative imaging or additional information from the clinical record.

Primary and secondary outcome measures

The primary outcome measure was the level of certainty (scale of 0–100) for the primary tumour origin based on macroscopic assessment by four independent GI pathologists. The level of certainty was calculated as the average score (mean) of four independent pathologists, with scores being compared between the two arms.

The most important secondary outcome was the interobserver agreement among the four pathologists in the macroscopic assessment of the tumour origin. Other secondary outcomes included the incidence rate of different cancers and correlation of the macroscopic assessment by the APOLLO expert panel with the final diagnosis. For malignant specimens, secondary cancer-specific outcomes, including tumour size, T-stage, lymph node yield, number of positive lymph nodes, lymph node ratio, R1 rate and tumour grade were compared between the two arms.

Microscopic assessment was not regarded in this trial, as the macroscopic assessment of tumor bulk is leading in establishing tumor origin. Whilst in practice the presence of a microscopic precursor lesion may guide the pathologist, inclusion of microscopy was deemed too complicated, as it is unclear how precursor lesions should be taken into account and interpretation can differ between pathologists.

International review and validation

All participating pathologists attended two meetings during the design phase of the APOLLO trial to discuss both techniques and reach consensus on the exact steps of each technique. Also, pathologists Prof. Dr. C.S. Verbeke, for the axial slicing technique, and Prof. Dr. V. Adsay, for the bivalving technique, were visited by the study coordinators (SvR and ECS) at Oslo University Hospital, Oslo, Norway and Koç University Hospital, Istanbul, Turkey, respectively. During the work visits, the APOLLO study protocol was reviewed and multiple specimens were observed to acquire more insight in technical skills of each grossing technique.

Sample size calculation

The APOLLO trial was designed as a superiority trial, hypothesizing that the tumour origin could be determined with a higher level of certainty using the bivalving technique, as compared with axial slicing without compromising the assessment of other oncologic outcomes. The sample size was calculated to detect an increase in level of certainty of 10 points -the smallest increase we deemed clinically significant- and with a standard deviation of 20 points (scale of 0–100), corresponding to a Cohen's d value of 0,5, i.e. an expected medium effect size. Significance level α was set at 0.05 for a two-sided test and power (1-β) at 80 per cent. These parameters resulted in a sample size of 128 patients; 64 for each arm.

Statistical analysis

The two techniques were analysed according to an intention-to-treat principle. Normally distributed continuous variables were expressed by means and standard deviations and compared using the independent-samples t-test. Continuous non-normally distributed variables were expressed by medians and interquartile ranges and compared using the Mann–Whitney U test. Categorical parameters were presented as frequencies and percentages and were compared using Chi-square (or Fisher's exact in case frequencies lower than 5). Clustered assessments (4 per specimen) were adjusted for by generalized estimation equation (*GEE* package in R for repeated measures). Interobserver agreement was expressed by Fleiss' kappa (similar as Cohen's

kappa but for more than 2 assessors). The kappa value was interpreted as follows: values ≤ 0 as indicating no agreement, 0.01–0.20 as none to slight, 0.21–0.40 as fair, 0.41–0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement. Also, the tumour origin on macroscopic assessment by the APOLLO expert panel was cross-tabulated with the origin on final pathology (incorporating both microscopic and macroscopic findings). Two-tailed P-values of less than 0.05 were considered statistically significant. All analyses were performed using R version 3.4.3 (cran.r-project.org).

Results

Baseline characteristics

Between 7 August 2018 and 4 November 2019, 128 specimens of patients who underwent PD for a malignant and premalignant lesion in the periampullary or pancreatic head region were randomized in four centres, 64 were allocated to each arm (Fig. 2). The median age at time of surgery was 70 years; 71 patients (55 per cent) were male. The preoperative indication for surgery (based on imaging/endoscopy) was equally distributed among groups, with 55 patients (43 per cent) undergoing surgery for preoperative suspected PDAC, 21 patients (16 per cent) for distal bile duct adenocarcinoma and 19 (15 per cent) for ampullary adenocarcinoma. Twenty-two per cent of patients had other preoperative diagnoses, mainly cystic lesions and suspected duodenal carcinoma with involvement of the ampulla and/or pancreas. In a small number of patients (4 per cent), no certain primary origin could be ascertained. There were no differences in baseline characteristics (Table 1). Thirty-one patients (24 per cent) received neoadjuvant therapy.

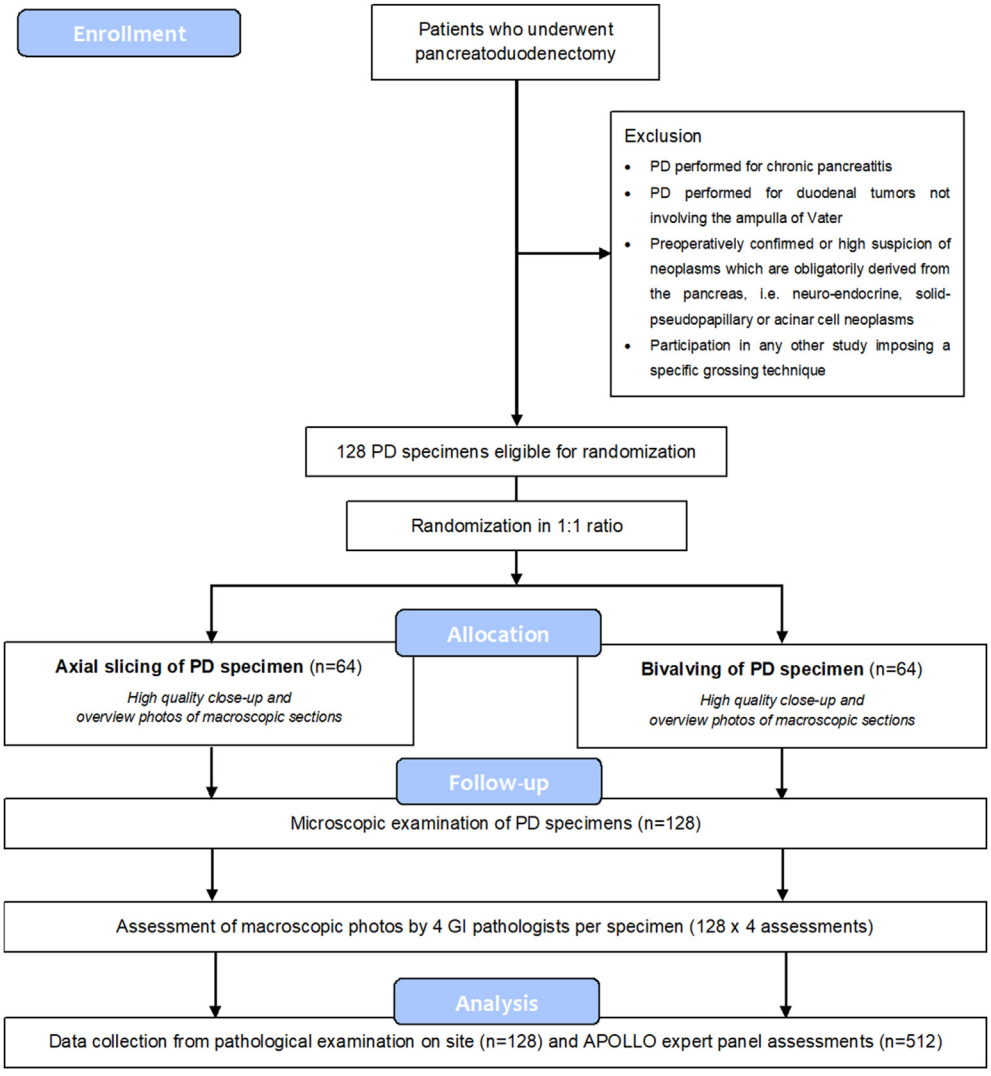

Figure 2 CONSORT 2010 Flow diagram of APOLLO trial

Table 1 Baseline characteristics

	Total cohort (n = 128)	Axial slicing (n = 64)	Bivalving (n = 64)
Age, median (IQR)	70 (63–75)	70 (62–75)	70 (64–76)
Male, n (%)	71 (55%)	36 (56%)	35 (55%)
Type of surgery, n (%)			
PPPD	48 (38%)	22 (34%)	26 (41%)
Whipple	80 (63%)	42 (66%)	38 (59%)
VMS resection, n (%)	14 (11%)	9 (14%)	5 (8%)
Preoperative suspected diagnosis*, n (%)			
Pancreatic cancer	55 (43%)	29 (45%)	26 (41%)
Distal bile duct cancer	21 (16%)	12 (19%)	9 (14%)
Ampullary cancer	19 (15%)	9 (14%)	10 (16%)
Unsure of primary origin	5 (4%)	2 (3%)	3 (5%)
Other (duodenal, cystic lesions, etc.)	28 (22%)	12 (19%)	16 (25%)
Neoadjuvant therapy, n (%)	31 (24%)	14 (22%)	17 (27%)

* Conclusion of multidisciplinary tumor board meeting, typically derived from radiologic and endoscopic imaging.

Macroscopic assessment by expert panel

The median number of photos for macroscopic assessment was 9 (IQR 7–11) per specimen. Examples of these photos are shown in the supplementary material. Macroscopic assessment of each specimen was performed by 4 independent expert panel pathologists, resulting in a total of 512 (128 x 4) macroscopic assessments. The primary outcome, i.e. the mean level of certainty in determining the tumour origin on a scale of 0–100, was 68 (sd 16) for the bivalving technique and 72 (sd 13) for the axial slicing technique (p = 0.21). There were no differences in the incidence of the different pancreatic/periampullary tumours: 48 versus 52 per cent pancreatic tumours (p = 0.66), 21 versus 17 per cent distal bile duct tumours (p = 0.47) and 18 versus 16 per cent ampullary tumours (p = 0.76), when comparing axial slicing versus bivalving, respectively. With bivalving, more often no abnormalities were found on macroscopy (7 vs. 0 per cent, p = 0.007) compared to axial slicing (Table 2). The level of certainty in determining the tumour origin did not differ between the specimens of patients who underwent upfront surgery compared to those who received neoadjuvant treatment (respectively 71 sd 15 vs. 66 sd 15, p = 0.11).

In the axial slicing group, at least three out of four expert pathologists agreed on primary tumour origin in 48 specimens (75 per cent) as compared with 49 specimens (77 per cent) in the bivalving group, p = 0.84. The interobserver agreement kappa was 0.45 in the axial slicing arm and 0.47 in the bivalving group, both being moderate.

Final pathology

On final pathology (macroscopy and microscopy incorporated), distal bile duct cancer was more frequently encountered with axial slicing, as compared with bivalving (16 vs. 8 per cent, p = 0.17), whereas ampullary cancer was less frequently encountered (13 vs. 19 per cent, p = 0.33), although both differences were not statistically significant. The incidence of other tumours did not differ between groups (Table 3). In neoadjuvantly treated specimens with the preoperative diagnosis of PDAC, final pathology showed a different site of origin in 5 cases (3 with axial slicing and 2 with bivalving).

In the subgroup of specimens with an invasive malignancy, the number of harvested lymph nodes did not differ between groups (median of 16 lymph nodes in both groups, p = 0.14). The R1 rate was also similar, both within the subgroup of patients who underwent PD for a malignancy (41 per cent [axial slicing] vs. 44 per cent [bivalving], p = 0.86) as well as within the subgroup who underwent PD for PDAC (60 per cent [axial slicing] vs. 55 per cent [bivalving], p = 0.71). Overall, the R0 rate was higher after neoadjuvant therapy than after upfront surgery (50 vs. 36 per cent), which was seen in both arms.

Comparison of macroscopic assessment and final pathology

Table 4A (axial slicing) and Table 4B (bivalving) present the distribution of tumour origin according to the macroscopic assessment of the APOLLO expert panel (columns), stratified by the tumour origin on final pathology report (rows). Axial slicing resulted in slightly higher concordance percentages, as compared with bivalving for tumours from pancreatic origin (74 vs. 71 per cent), distal bile duct origin (43 vs. 30 per cent), ampullary origin (59 vs. 52 per cent) and duodenal origin (50 vs. 38 per cent).

Discussion

This first multicentre randomized controlled trial on the pathological examination of pancreatoduodenectomy specimens assessed whether bivalving increases the level of certainty in the

Table 2 Macroscopic assessment by the study expert panel

	Both techniques (n = 512 surveys)	Axial slicing (n = 256 surveys)	Bivalving (n = 256 surveys)	P value
Expert panel outcome (4 assessors per specimen)				
Level of certainty in determining the primary tumor origin on a scale of 0–100, mean (sd)				
All pancreatoduodenectomy specimens (n = 128)	70 (15)	72 (13)	68 (16)	0.21
Preoperative diagnosis pancreatic cancer (n = 55)	70 (13)	70 (12)	70 (13)	0.91
Preoperative diagnosis CBD cancer (n = 21)	69 (14)	66 (15)	73 (13)	0.27
Preoperative diagnosis ampullary cancer (n = 19)	73 (14)	78 (11)	69 (15)	0.16
Preoperative diagnosis unsure (n = 5)	73 (15)	86 (5)	64 (12)	0.09
Preoperative diagnosis other (n = 28)	69 (20)	75 (12)	64 (23)	0.13
Primary tumor origin according to expert panel, (%)				
Pancreas	256 (50%)	124 (48%)	132 (52%)	0.66
Distal bile duct	97 (19%)	53 (21%)	44 (17%)	0.47
Ampulla of Vater	88 (17%)	46 (18%)	42 (16%)	0.76
Duodenum	31 (6%)	22 (9%)	9 (4%)	0.13
Other	11 (2%)	7 (3%)	4 (2%)	0.55
No abnormalities on macroscopy	12 (2%)	1 (0%)	18 (7%)	0.007
Unable to assess	17 (3%)	3 (1%)	7 (3%)	0.31
Specimens with agreement of at least 3 out of 4 assessors with final pathology[a], n (%)	73 (57%)	36 (56%)	37 (58%)	0.86
Specimens with consensus among at least 3 out of 4 assessors among panel itself, n (%)	97 (76%)	48 (75%)	49 (77%)	0.84
Interobserver agreement, kappa[b]	0.46	0.45	0.47	

MDT = multidisciplinary team.
< 0 No agreement.
0.0–0.20 Slight agreement.
0.21–0.40 Fair agreement.
0.41–0.60 Moderate agreement.
0.61–0.80 Substantial agreement.
0.81–1.00 Almost perfect agreement.
[a] Incorporating macroscopic and microscopic findings.
[b] General interpretation of kappa.

determination of primary tumour origin compared to axial slicing. In contrast to our hypothesis, we did not find any difference in the level of certainty or in other relevant outcomes such as lymph node retrieval and R1 detection rate between both techniques. Overall, interobserver agreement for the macroscopic assessment of tumour origin was moderate for both bivalving and axial slicing. Although these results might not be entirely surprising, we feel that this study is original, and the results are foremost valuable within the field of pancreas pathology.

The variation in incidence of the different periampullary cancers indicate that the histopathological distinction between these cancers is difficult.[11] The prevalence of PDAC, ampullary cancer and bile duct cancer reported in this study corresponds to the incidence rate as reported by other groups in Western Europe and North America.[19] Typically, specimens in which a primary origin cannot reliably be established are regarded as PDAC due to its higher incidence and better-defined treatment strategies. We therefore hypothesized that a higher incidence of non-PDAC periampullary malignancies would indicate a better display of the periampullary region as a result of improved macroscopic assessment. However, the bivalving technique was not associated with higher incidence rates of distal bile duct, ampullary or duodenal tumours, based on the macroscopic assessment by the study expert panel. In bivalved specimens, the expert panel more frequently encountered a specimen without abnormalities on macroscopy (p = 0.007). When looking closer at these cases, there were 4 specimens in which the majority of the expert panel scored 'no abnormality on macroscopy'. The lack of an abnormality or tumour in these specimens did not seem to be related to a poor dissection, as all specimens were properly bivalved (both ducts were successfully probed). One specimen appeared to be a ypT0 duodenal tumour after immunotherapy and the other three specimens were non-invasive lesions on final pathology (two side-branch IPMNs with low-grade dysplasia [tumour size 13 and 50 mm] and one ampullary adenoma with

Table 3 Data from the final pathology reports of all specimens and subgroups

All pancreatoduodenectomy specimens	Total cohort	Axial slicing	Bivalving	P value
	n = 128	n = 64	n = 64	
Probing of ducts, n (%)				-
Both ducts successfully probed	40 (31%)	n.a.	40 (63%)	
Only common bile duct probed	21 (16%)	n.a.	21 (33%)	
Only pancreatic duct probed	1 (1%)	n.a.	1 (2%)	
Failed to probe both ducts, other technique performed	2 (2%)	n.a.	2 (3%)	
Primary tumor origin on final pathology, n (%)				
Pancreatic cancer	59 (46%)	30 (47%)	29 (45%)	0.86
Distal bile duct cancer	15 (12%)	10 (16%)	5 (8%)	0.17
Ampullary cancer	20 (16%)	8 (13%)	12 (19%)	0.33
Duodenal cancer	6 (5%)	3 (5%)	3 (5%)	0.99
Premalignant/benign	28 (22%)	13 (20%)	15 (23%)	0.67
All specimens with a malignancy	**n = 100**	**n = 51**	**n = 49**	
Tumor size, median (IQR)	30 (20–40)	32 (24–40)	28 (19–35)	0.11
Harvested lymph nodes, median (IQR)	16 (12–20)	16 (14–22)	16 (11–18)	0.14
Positive lymph nodes, median (IQR)	2 (0–4)	1 (0–4)	2 (0–4)	0.59
Positive resection margin (1 mm definition), n (%)	43 (43%)	21 (41%)	22 (44%)	0.86
Total number of blocks sampled, median (IQR)	16 (11–23)	16 (11–22)	16 (12–23)	0.47
All PDAC specimens	**n = 59**	**n = 30**	**n = 29**	
Tumor size, median (IQR)	30 (25–39)	30 (25–40)	29 (20–36)	0.43
Pathological T stage (8th ed.), n (%)				0.45
T1	9 (15%)	3 (10%)	6 (21%)	
T2	35 (59%)	18 (60%)	17 (59%)	
T3	15 (25%)	9 (30%)	6 (21%)	
Pathological N stage (8th ed.), n (%)				0.47
N0	13 (22%)	8 (27%)	5 (17%)	
N1	24 (41%)	10 (33%)	14 (48%)	
N2	22 (37%)	12 (40%)	10 (34%)	
Harvested lymph nodes, median (IQR)	17 (14–21)	16 (14–23)	17 (14–19)	0.58
Positive lymph nodes, median (IQR)	2 (1–5)	2 (0–4)	2 (1–5)	0.80
Positive resection margin (1 mm definition), n (%)	34 (58%)	18 (60%)	16 (55%)	0.71
R1 in upfront surgery group, n/total (%)	21/32 (64%)	13/19 (68%)	8/14 (57%)	0.51
R1 in neoadjuvant group, n/total (%)	13/26 (50%)	5/11 (45%)	8/15 (53%)	0.69
Total number of blocks sampled, median (IQR)	17 (13–24)	16 (12–21)	18 (13–26)	0.27

n.a. = not applicable, PDAC = pancreatic ductal adenocarcinoma
* 2 patients had an unknown size of the lesion.

low-grade dysplasia [tumour size 10 mm]). In the axial slicing group, tumours were slightly larger, although not statistically significant (32 vs. 28 mm, p = 0.11), which also might have helped in identifying an origin on macroscopy.

Several studies have suggested that in PDAC the R1 rate and lymph node yield may also depend on the method of specimen dissection.[20–23] We found a similar lymph node yield and R1 rate with both techniques. A specific method of peripancreatic adipose tissue dissection, i.e. orange peeling, is often performed in conjunction with bivalving for lymph node harvest.[23] It was shown to increase lymph node yield in a retrospective study, and it is thought to reduce double counting of lymph nodes.[21] We did not perform orange peeling, as it would interfere with margin assessment (as defined as the presence of tumour cells within 1 mm of the surgical margins or breaching the anterior surface). We found an R1 rate of 58 per cent, which is somewhat lower than the rates reported by others.[8,20,22] Possibly this is the result of

Table 4A Cross-tabulation of final pathology and macroscopic assessments for axial slicing

Origin on final pathology (macroscopy and microscopy)	Primary tumor origin according to APOLLO expert panel on macroscopy Based on macroscopic assessment (4 assessors per specimen)						
	Pancreas	Bile duct	Ampulla	Duodenum	Other	No abnormality	Unable to assess
Pancreas (n = 36 specimens)	106 74%	28 19%	9 6%	0 0%	0 0%	0 0%	1 1%
Bile duct (n = 11 specimens)	15 34%	19 43%	5 11%	3 7%	2 5%	0 0%	0 0%
Ampulla (n = 11 specimens)	0 0%	6 14%	26 59%	11 25%	0 0%	1 2%	0 0%
Duodenum (n = 4 specimens)	3 19%	0 0%	4 25%	8 50%	1 6%	0 0%	0 0%
Other origin (n = 1 specimen)	0 0%	0 0%	0 0%	0 0%	4 100%	0 0%	0 0%
No abnormality (n = 1 specimens)	0 0%	0 0%	2 50%	0 0%	0 0%	0 0%	2 50%

All shown percentages are row percentages.

limited sampling for microscopy, as it has been previously shown that R1 rate increases with more extensive sampling.[12] The extent of sampling was left to the discretion of the macroscopic assessor, as standardization of sampling was not deemed possible. However, the number of blocks submitted for microscopy did not differ between the two groups (a median of 16 blocks in axial slicing vs. 18 in bivalving, p = 0.27), demonstrating that sampling was performed to a similar extent in both arms.

This study has some limitations. First, when interpreting the results of the APOLLO study, one should take into account that the primary outcome and related parameters were assessed in an artificial setting. Only macroscopic photographs were available to the expert panel; clinical information was not provided and palpation was not possible. On occasion, these macroscopy photos may have lacked in detail, which would have been available during physical assessment. The inability to incorporate palpatory changes also limits the amount of information. Normally, the firmness of the tumour aids in its localization, which makes palpation an important instrument in the assessment of primary origin. Second, some of the pathologists and residents who performed grossing had limited experience with the bivalving technique. Although we attempted to increase familiarity with both techniques via a clear SOP, on-site visits and training, the lack of experience may have affected our results. Third, due to small numbers in some of the subgroups (e.g. only 15 specimens had a final diagnosis of distal bile duct adenocarcinoma), the probability of a type II error was increased and the study was underpowered to find differences in secondary outcomes. Finally, in the absence of a reference standard to determine tumour origin, the assessment by four independent GI pathologists was considered to approach a reference standard. Previous studies have used R1 rate and/or lymph node yield as primary endpoint, which can be established with more certainty.[21,22,24] However, we deemed tumour origin the most important parameter in comparing the different techniques, as

Table 4B Cross-tabulation of final pathology and macroscopic assessments for bivalving

Origin on final pathology (macroscopy and microscopy)	Primary tumor origin according to APOLLO expert panel on macroscopy Based on macroscopic assessment (4 assessors per specimen)						
	Pancreas	Bile duct	Ampulla	Duodenum	Other	No abnormality	Unable to assess
Pancreas (n = 40 specimens)	114 71%	20 13%	8 5%	1 1%	4 3%	10 6%	3 2%
Bile duct (n = 5 specimens)	9 45%	6 30%	3 15%	1 5%	0 0%	0 0%	1 5%
Ampulla (n = 14 specimens)	7 13%	12 21%	29 52%	1 2%	0 0%	5 9%	2 4%
Duodenum (n = 4 specimens)	2 13%	2 13%	2 13%	6 38%	0 0%	3 19%	1 6%
Other origin (n = 1 specimen)	0 0%	4 100%	0 0%	0 0%	0 0%	0 0%	0 0%

All shown percentages are row percentages.

primary tumour origin impacts prognosis and adjuvant treatment.

In our cohort, 24 per cent of patients underwent neoadjuvant therapy. In the future, these numbers will increase, especially for PDAC. The assessment of specimens from these patients comes with its own challenges. Fibro-inflammatory changes are typically more pronounced in these patients, further obscuring tumour borders and complicating the accurate measurement of tumour size. Often, it is necessary to map the microscopically identified viable tumour locations onto the macroscopic photos. As axial slicing increases standardization and enables straightforward reconstruction, it may be better suited for tumour mapping.

Summary

We found that bivalving and axial slicing performed equally with regard to the pathologist's certainty in determining tumour origin and therefore conclude that the decision should be left to the discretion of the pathologist, which is in compliance with current guidelines. The moderate agreement between pathologists in the determination of primary origin underlines the difficulty in the macroscopic assessment of primary origin.

CRediT authorship contribution statement

Stijn van Roessel: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Eline C. Soer:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **Susan van Dieren:** Conceptualization, Methodology, Formal analysis, Writing - review & editing. **Lianne Koens:** Conceptualization, Methodology, Investigation, Writing - review & editing. **Marie Louise F. van Velthuysen:** Conceptualization, Investigation, Investigation, Writing - review & editing. **Michael Doukas:** Conceptualization, Investigation, Writing - review & editing. **Bas Groot Koerkamp:** Conceptualization, Resources, Writing - review & editing. **Arantza Fariña Sarasqueta:** Conceptualization, Investigation, Investigation, Writing - review & editing. **Carolien M. Bronkhorst:** Conceptualization, Data curation, Investigation, Writing - review & editing. **G. Mihaela Raicu:** Conceptualization, Data curation, Investigation, Writing - review & editing. **Karel C. Kuijpers:** Conceptualization, Data curation, Resources, Investigation, Writing - review & editing. **Cornelis A. Seldenrijk:** Conceptualization, Data curation, Investigation, Writing - review & editing. **Hjalmar C. van Santvoort:** Conceptualization, Writing - review & editing. **I. Quintus Molenaar:** Conceptualization, Writing - review & editing. **Rachel S. van der Post:** Conceptualization, Data curation, Investigation, Writing - review & editing. **Martijn W.J. Stommel:** Conceptualization, Resources, Writing - review & editing. **Olivier R. Busch:** Conceptualization, Resources, Writing - review & editing. **Marc G. Besselink:** Conceptualization, Supervision, Resources, Writing - review & editing. **Lodewijk A.A. Brosens:** Conceptualization, Methodology, Investigation, Investigation, Writing - review & editing. **Joanne Verheij:** Conceptualization, Methodology, Supervision, Resources, Investigation, Writing - review & editing.

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Trial registration

ISRCTN registry, ISRCTN12141624. Registered on 25 April 2019.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Tol JA, Brosens LA, van Dieren S, van Gulik TM, Busch OR, Besselink MG *et al*. (2015) Impact of lymph node ratio on survival in patients with pancreatic and periampullary cancer. *Br J Surg* 102: 237–245.
2. Xue Y, Vanoli A, Balci S, Reid MM, Saka B, Bagci P *et al*. (2017) Non-ampullary-duodenal carcinomas: clinicopathologic analysis of 47 cases and comparison with ampullary and pancreatic adenocarcinomas. *Mod Pathol: Off J USA Can Acas]d Pathol* 30:255–266.
3. Bledsoe JR, Shinagare SA, Deshpande V. (2015) Difficult diagnostic problems in pancreatobiliary neoplasia. *Arch Pathol Lab Med* 139: 848–857.
4. Staley CA, Cleary KR, Abbruzzese JL, Lee JE, Ames FC, Fenoglio CJ *et al*. (1996) The need for standardized pathologic staging of pancreaticoduodenectomy specimens. *Pancreas* 12:373–380.
5. Luttges J, Zamboni G, Kloppel G. (1999) Recommendation for the examination of pancreaticoduodenectomy specimens removed from patients with carcinoma of the exocrine pancreas. A proposal for a standardized pathological staging of pancreaticoduodenectomy specimens including a checklist. *Dig Surg* 16:291–296.
6. Chatelain D, Flejou JF. (2002) [Pancreatectomy for adenocarcinoma: prognostic factors, recommendations for pathological reports]. *Ann Pathol* 22:422–431.
7. Maksymov V, Hogan M, Khalifa MA. (2013) An anatomical-based mapping analysis of the pancreaticoduodenectomy retroperitoneal margin highlights the urgent need for standardized assessment. *HPB: Off J Int Hepato pancre Biliary Assoc* 15:218–223.
8. Verbeke CS, Leitch D, Menon KV, McMahon MJ, Guillou PJ, Anthoney A. (2006) Redefining the R1 resection in pancreatic cancer. *Br J Surg* 93:1232–1237.

9. Adsay NV, Basturk O, Saka B, Bagci P, Ozdemir D, Balci S *et al*. (2014) Whipple made simple for surgical pathologists: orientation, dissection, and sampling of pancreaticoduodenectomy specimens for a more practical and accurate evaluation of pancreatic, distal common bile duct, and ampullary tumors. *Am J Surg Pathol* 38:480–493.
10. Soer E, Brosens L, van de Vijver M, Dijk F, van Velthuysen ML, Farina-Sarasqueta A *et al*. (2018 Apr) Dilemmas for the pathologist in the oncologic assessment of pancreatoduodenectomy specimens : an overview of different grossing approaches and the relevance of the histopathological characteristics in the oncologic assessment of pancreatoduodenectomy specimens. *Virchows Arch: Int J Pathol* 472: 533–543. https://doi.org/10.1007/s00428-018-2321-5.
11. Verbeke CS, Gladhaug IP. (2012) Resection margin involvement and tumour origin in pancreatic head cancer. *Br J Surg* 99: 1036–1049.
12. Verbeke CS. (2013) Resection margins in pancreatic cancer. *Patholoqe* 34(Suppl 2):241–247.
13. Verbeke CS. (2008) Resection margins and R1 rates in pancreatic cancer–are we there yet? *Histopathology* 52:787–796.
14. Esposito I, Born D. (2010) *Pathological reporting and staging following pancreatic cancer resection*. New York, NY: Springer New York, pp. 1015–1034. Pancreatic Cancer.
15. Shi J, Basturk O. (2019) Whipple grossing in the era of new staging: should we standardize? *Diagnostics* 9.
16. Schulz KF, Altman DG, Moher D. (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.
17. Pathologie bij pancreascarcinoom. Available from: https://richtlijnendatabase.nl/richtlijn/pancreascarcinoom/pathologie.html, (2019).
18. Casparie M, Tiebosch AT, Burger G, Blauwgeers H, van de Pol A, van Krieken JH *et al*. (2007) Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. *Cell Oncol: Off J Int Soc Cell Oncol* 29:19–24.
19. He J, Ahuja N, Makary MA, Cameron JL, Eckhauser FE, Choti MA *et al*. (2014) 2564 resected periampullary adenocarcinomas at a single institution: trends over three decades. *HPB: Off J Int Hepato pancre Biliary Assoc* 16:83–90.
20. Verbeke CS. (2013) Resection margins in pancreatic cancer. *Surg Clin* 93:647–662.
21. Lino-Silva LS, Salcedo-Hernandez RA, Segales-Rojas P, Zepeda-Najar C. (2018) Comparison of 3 ways of dissecting the pancreatoduodenectomy specimen and their impact in the lymph node count and the lymph node metastatic ratio. *Int J Surg Pathol* 26:707–713.
22. Esposito I, Kleeff J, Bergmann F, Reiser C, Herpel E, Friess H *et al*. (2008) Most pancreatic cancer resections are R1 resections. *Ann Surg Oncol* 15:1651–1660.
23. Adsay NV, Basturk O, Altinel D, Khanani F, Coban I, Weaver DW *et al*. (2009) The number of lymph nodes identified in a simple pancreatoduodenectomy specimen: comparison of conventional vs orange-peeling approach in pathologic assessment. *Mod Pathol: Off J USA Can Acas]d Pathol* 22:107–112.
24. Chandrasegaram MD, Goldstein D, Simes J, Gebski V, Kench JG, Gill AJ *et al*. (2015) Meta-analysis of radical resection rates and margin assessment in pancreatic cancer. *Br J Surg* 102:1459–1472.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hpb.2021.01.005.

