Axial Slicing versus Bivalving of the Pancreatic Head in the Pathological Examination of Pancreatoduodenectomy Specimens (APOLLO): study protocol for a randomized controlled trial

Stijn van Roessel, MD MSc[1]*, Eline C. Soer, MD[2]*, Susan van Dieren, MD[1], Lianne Koens MD PhD[2], Marie Louise F. van Velthuysen, MD, PhD[3], Michael Doukas, MD PhD[3], Bas Groot Koerkamp, MD PhD[4], Arantza Fariña Sarasqueta, MD PhD[2,5], Carolien M. Bronkhorst, MD[6], Mihaela G. Raicu, MD PhD[7], Karel C. Kuijpers, MD PhD[7], Cornelis A. Seldenrijk, MD PhD[7], Hjalmar van Santvoort MD PhD[8,9], I. Quintus Molenaar MD PhD[8,9], Rachel S. van der Post MD PhD[10], Martijn W.J. Stommel MD PhD[11], Olivier R. Busch, MD PhD[1], Marc G. Besselink, MD MSc PhD[1]**, Lodewijk A.A. Brosens, MD PhD[10,12]**, Joanne Verheij, MD PhD[2]** for the Dutch Pancreatic Cancer Group

* Shared first authorship

** Shared senior authorship

Affiliations:

[1]Department of Surgery, Cancer Center Amsterdam, Amsterdam UMC, University of Amsterdam, the Netherlands

[2]Department of Pathology, Cancer Center Amsterdam, Amsterdam UMC, University of Amsterdam, the Netherlands

[3]Department of Pathology, Erasmus MC University Medical Center, Rotterdam, the Netherlands

[4]Department of Surgery, Erasmus MC University Medical Center, Rotterdam, the Netherlands

[5]Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands

[6]Department of Pathology, Pathology-DNA, Jeroen Bosch Hospital, 's Hertogenbosch, the Netherlands

[7]Department of Pathology, Pathology-DNA, St. Antonius Hospital, Regional Academic Cancer Center Utrecht (RAKU), Nieuwegein, Utrecht, the Netherlands

[8]Department of Surgery, St. Antonius Hospital, Regional Academic Cancer Center Utrecht (RAKU), Nieuwegein, Utrecht, the Netherlands

[9]Department of Surgery, University Medical Center Utrecht, Regional Academic Cancer Center Utrecht (RAKU), Nieuwegein, Utrecht, the Netherlands

[10]Department of Pathology, Radboud University Medical Center, Nijmegen, the Netherlands

[11]Department of Surgery, Radboud University Medical Center, Nijmegen, the Netherlands

[12]Department of Pathology, University Medical Center Utrecht, Regional Academic Cancer Center Utrecht (RAKU), Nieuwegein, Utrecht, the Netherlands

Corresponding author:

J. Verheij, MD, PhD

Amsterdam UMC, University of Amsterdam

Department of Pathology

P.O. Box 22660, 1105 AZ Amsterdam

Phone: +31 20 566 2558

E-mail: j.verheij@amsterdamumc.nl

ABSTRACT

Background: The primary tumor origin in pancreatoduodenectomy (PD) specimens (e.g. pancreatic ductal adenocarcinoma, distal cholangiocarcinoma, ampullary adenocarcinoma) may be challenging to determine clinically and microscopically. It is therefore typically determined by macroscopic assessment of the location of the tumor bulk during pathological examination. This diagnosis of the primary tumor determines prognosis, the indication of adjuvant treatment and eligibility for clinical trials. However, there is no international consensus on how to best perform grossing of PD specimens and the macroscopic assessment. Two commonly used grossing techniques for PD specimens are axial slicing and bivalving of the pancreatic head. Data regarding these techniques are contradicting in terms of determination of primary tumor origin and margin-positive resection (R1) rate. Prospective comparative studies are lacking.

Methods/design: APOLLO is a randomized controlled, parallel-group, multicenter superiority trial, comparing two grossing techniques in the pathological assessment of a PD specimen. A total of 128 PD specimens, performed for a suspected (pre)malignant tumor in the pancreatic head region, will be randomly assigned to either axial slicing or bivalving of the pancreatic head. Stratification is performed for participating center and neoadjuvant therapy. Photographs are taken of the macroscopic sections of the specimen. The primary outcome measure is the level of certainty (scale of 0 to 100) for the primary tumor origin based on macroscopic assessment by four independent pancreatic pathologists. Secondary outcomes are inter-observer agreement among the four pathologists (kappa) and margin-positive resection (R1) rate for the subgroup with proven malignancy, and pancreatic cancer in particular.

Discussion: The APOLLO trial is designed to compare bivalving of the pancreatic head to axial slicing in the pathological assessment of a PD specimen for the level of certainty in determining the primary tumor origin, inter-observer agreement among pathologists and other pathological parameters. High-level evidence for the superiority of one grossing technique will contribute to more uniform application of this technique and eventually to more accurate assessment of prognosis, indication for adjuvant treatment and therefore treatment outcome.

Trial registration: ISRCTN registry, ISRCTN12141624. Registered on 25 April 2019.

Keywords: Pancreatoduodenectomy, Specimen, Pathology assessment, Axial slicing, Bivalving

BACKGROUND

Pancreatoduodenectomy (PD) is typically performed for (pre)malignant lesions arising from the pancreatic head, common bile duct, ampulla of Vater, and duodenum, which all congregate in the periampullary region. Pancreatic adenocarcinoma, distal cholangiocarcinoma, ampullary adenocarcinoma and duodenal adenocarcinoma each have a distinctly different prognosis and different guidelines regarding adjuvant systemic treatment and follow-up.[1,2] These periampullary malignancies often cannot reliably be distinguished from each other using tumor cell morphology or immunohistochemistry. Therefore, the primary tumor origin is typically determined on the basis of macroscopic assessment of the location of the bulk of the tumor.[3] Several approaches for the pathological assessment of PD specimens have been described.[4-9] International consensus on how to best perform gross pathological examination of a PD specimen is however lacking.[10]

The two most commonly used grossing techniques for PD specimens are axial slicing as propagated by Verbeke et al., and bivalving of the pancreatic head as propagated 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 cancer.[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 better, which facilitates accurate identification of the origin of the tumor.[9,14] However, there is no convincing evidence on which technique shows superiority in terms of determining the tumor origin, margin-positive resection (R1) rate, or lymph node harvest.[10] The frequent reclassification of tumor origin following case review, and the wide variation in incidence of pancreatic and periampullary cancers indicate that the histopathological distinction between those cancers is less accurate than generally believed.[11]

Although both techniques are well established and widely advocated, a prospective comparison has never been performed. The hypothesis of the APOLLO multicenter trial is that in the pathological assessment of PD specimens, bivalving of the pancreatic head provides more certainty in determining the tumor origin compared to axial slicing.

METHODS

Study design and trial population

The APOLLO trial is a multicenter, randomized, controlled study investigating whether bivalving of the pancreatic head of PD specimens provides more certainty than axial slicing in macroscopically determining the primary tumor origin. PD specimens from eligible patients will be randomized in a 1:1 ratio to either axial slicing or bivalving of the pancreatic head. All adult patients with an indication for elective PD for (suspected) malignant or neoplastic disease in the pancreatic and periampullary region will be screened for eligibility. The APOLLO trial was initiated by the Dutch Pancreatic Cancer Group (DPCG), a national collaboration of surgeons, gastroenterologists, medical oncologists, pathologists, (interventional) radiologists, dieticians, and nurses. Reporting of the protocol is in accordance with the SPIRIT statement.[15] Reporting of the trial will follow the CONSORT guidelines for reporting of clinical trials.[16]

Inclusion criteria

- Age at least 18 years
- Electively performed PD (pylorus-preserving or Whipple), including both open and minimally-invasive procedures

Exclusion criteria

- PD performed for the preoperative indication of chronic pancreatitis
- PD performed for pathologically proven metastatic lesion(s) in the pancreas
- PD performed for duodenal tumors not involving the ampulla of Vater
- Preoperatively confirmed or high suspicion of neoplasms which are non-epithelial or obligatorily derived from the pancreas, i.e. gastro-intestinal stromal tumor, neuro-endocrine, solid-pseudopapillary or acinar cell neoplasms
- Participation in any other study imposing a specific grossing technique

Participating centers and quality assurance/control

All participating centers are high-volume centers, performing and processing over 40 PDs annually over the past three years. Prior to inclusion at every new participating center, an

implementation phase will be followed to monitor and maintain quality standards for APOLLO. Following approval of the local Medical Ethics Review Committee, a site visit is planned 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, was shared among the APOLLO expert panel. Before a new participating center is allowed to start accrual, test rounds will be held to assess technical skills and the quality of the macroscopy photos, as the quality is of paramount importance for accurate assessment of the primary endpoint. 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 (Figure 2A and 2B). These SOPs were dispersed among the participating centers. After approximately 50% accrual, a meeting was held with all participating centers to discuss progress, quality control and challenging cases.

Experience with both grossing techniques

Before the start of the APOLLO trial, most centers routinely performed axial slicing. However, in all involved centers there was a hepatopancreatobiliary (HPB) consultant pathologists present who had experience with bivalving. In academic centers in the Netherlands, grossing is typically performed by pathology residents under supervision of an HPB consultant pathologist. Among the pathologists of the APOLLO expert panel a sense of clinical equipoise was present regarding both grossing techniques. Clear instructions, a SOP and a video of each grossing technique was shared to increase familiarity with both techniques and to ensure the pathology assessment was being performed in a standardized fashion. As stated in the Dutch guidelines, the grossing technique is left at the discretion of the pathologist, with both axial slicing and bivalving being recommended in the guideline.[17]

Randomization

Eligible patients are recruited from the operating room schedule of the participating hospitals when scheduled for PD (Figure 1, CONSORT flow diagram). The APOLLO trial is considered a non-interventional study by the Medical Ethics Review Committee of the Amsterdam UMC, location AMC, and it was judged that the Medical Research Involving Human Subjects Act does not apply. Patients are only required to provide written consent for using non-anonymized data for research purposes. The specimen of included patients will be randomized after PD is performed using an online randomization

module (Castor Electronic Data Capture, Amsterdam, the Netherlands) on a 1:1 ratio between axial slicing and bivalving of the pancreatic head. By randomizing centrally using the online module and to wait until PD is completed, the sequence of allocation remains concealed. Randomization will be stratified for center and neoadjuvant therapy (yes/no) to ensure equal allocation of subgroups to each arm. Randomization is 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. Blinding of the assessors is unfortunately not possible as the grossing technique is readily visible in assessing the primary outcome.

Specimen handling prior to grossing

Upon receiving the surgical specimen at the pathology department, the specimen of the patient is screened for eligibility and randomized in case it meets the inclusion criteria. Grossing is performed by an HPB consultant pathologist or a dedicated pathology resident supervised by the consultant HPB pathologist. First, orientation of the specimen is done by identifying the different resected structures provided. Usually the anatomical landmarks are marked with sutures by the surgeon. Measurements are taken from the stomach (if partially resected), duodenum, head of pancreas (in three dimensions: craniocaudal, anterioposterior, mediolateral), gallbladder, and any other structures or organs included in the specimen (e.g. venous resection). The following pancreatic margins and surfaces are inked according to local color-codes: the anterior surface, the portal and superior mesenteric vein (SMV) margin, superior mesenteric artery (SMA) margin and posterior margin. The stomach and duodenum are opened antimesenterially and rinsed. Surgical sutures and clips are carefully removed from the specimen and the transection margins (proximal [gastric or duodenal], distal [duodenal], pancreatic neck and common bile duct) are shaved and submitted en face. Any other important structures (e.g. venous patch in case of venous resection) are also inked.

Grossing technique: Axial slicing

One or two incisions are made in the axial plane to obtain tumor tissue for biobanking. After that, the specimen is fixed in formalin for at least 24 hours. When the specimen is properly fixed, it is serially sliced perpendicular to the long axis of the duodenum over its entire craniocaudal length with

slices of 3-5 millimeter thick, as described by Verbeke et al.[8,11] The sections are systematically positioned in sequential order with the caudal side up (as on CT scan) and the tumor size is measured.

Grossing technique: Bivalving of the pancreatic head

The main pancreatic duct and CBD are identified and probed. The CBD is virtually always probe-patent even in case of constrictive tumors. The pancreatic duct may be difficult to probe entirely due to the presence of a kink or due to an obstructing tumor. Hereafter, the pancreatic head is bivalved along the plane defined by both probes so that both ducts are longitudinally opened and the (peri)ampullary region becomes visible as described by Adsay et al.[9] In case the pancreatic duct is not (entirely) probe-able, the pancreatic head is bivalved along the CBD probe in the direction of the pancreatic duct in an attempt to visualize at least the lumen of the CBD and the ampullary region. After bivalving, the tumor can be appreciated in its relation to the different structures in this area. The lumen of the CBD is inked yellow and the lumen of the pancreatic duct black for orientation during microscopy. Tumor tissue is obtained for biobanking and the specimen is fixed for at least 24 hours. After fixation, the ampullary region is sampled and each halve of the pancreatic head is serially sliced perpendicular to the long axis of the duodenum in 3-5 millimeter thick slices and the tumor size is measured.

Margin assessment and lymph node yield

The smallest distance to each margin and surface is measured and sampled. After this, the peripancreatic fat is searched for lymph nodes. Due to serially slicing the specimen, some lymph nodes may have been halved which can lead to double counting of lymph nodes. Whenever possible, these halved lymph nodes are submitted together to minimize double counting.

Macroscopic photographs and expert surveys

High-definition photos, either with a separate high-quality camera or built-in camera for macroscopy purposes, are taken of the macroscopic sections from each specimen in a standardized way: at least one photo of the ampulla after opening the duodenum, a photo of the bivalved pancreatic head (in case randomized for bivalving), and an overview and close-up photos from the sections. All photos are centrally uploaded in the online data manager (Castor EDC). Hereafter, each PD-specimen is randomly assigned to four dedicated hepatopancreatobiliary (HPB) consultant pathologists from the

APOLLO expert panel, who are asked to assess the macroscopic photos of the specimen and fill out a digital survey based on these photos. Pathologists are excluded from the assessment of cases which were grossed in their own center. The survey consists of questions regarding the tumor origin (pancreatic, distal bile duct, ampullary, duodenal, other) and the level of certainty in determining this based on the photos (visual analogue scale of 0 to 100). The four pathologists fill out the online survey independently from each other and without further clinical information regarding the case. A total of nine dedicated HPB pathologists (JV, LAAB, MD, MLFV, AFS, CMB, MGR, KCK, CAS) will assess the macroscopic photos, functioning as the APOLLO expert panel.

Pathology reporting

Both standardized and narrative reporting is used for PD specimens. In all cases, the following parameters are reported in accordance with the Dutch Guideline Database (Dutch Pathological-Anatomy National Automated Archive [PALGA][18]): primary origin, histological subtype, grade of differentiation, tumor diameter, tumor distance to the surgical margins and anterior free surface, tumor growth into adjacent structures (as indicated by the TNM, 8th edition) number of total and positive lymph nodes, vascular and perineural growth, and response to neoadjuvant therapy (when applicable).[17] Tumor cells growing less than 1 millimeter from a resection or dissection margin (pancreatic neck margin, proximal/distal enteric margins, CBD margin, SMV margin [including venous patch], SMA margin, posterior margin) is considered an R1 resection. The anterior free surface is not considered a surgical margin.

Primary outcome measure

The primary outcome measure is the level of certainty (scale of 0 to 100) for the primary tumor origin based on macroscopic assessment by four independent pancreatic pathologists. The level of certainty is calculated as the average score (mean) of four independent pathologists (i.e. linear outcome) per case. Mean scores will eventually be compared between the two arms, axial slicing versus bivalving of the pancreatic head.

Secondary outcome measures

The most important secondary outcome is the inter-observer agreement among the four pathologists in determining the tumor origin on the macroscopic photos. Other secondary outcomes include the distribution of the cancers, correlation with preoperative imaging and correlation of the expert surveys with microscopy and final pathology. For the specimens randomized for bivalving, the number of cases in which both ducts were probe-patent is noted. For all cases, the number of sampled blocks is noted. For malignant cases, secondary cancer-specific outcomes, including tumor size, T-stage, lymph node yield, number of positive lymph nodes, lymph node ratio, R1 rate and tumor grade will be reported and compared between the two arms separated according to primary tumor origin.

Data collection and follow-up

Patient demographics, operative information and pathology parameters will be collected using standardized electronic case record forms (e-CRF) and stored in an online data manager (Castor Electronic Data Capture, Amsterdam, the Netherlands). Baseline characteristics will be entered prior to randomization, and stratification will be performed for center and the receipt of neoadjuvant treatment. Pathology variables can be entered in the e-CRF as soon as all microscopy parameters from the final pathology are determined. The macroscopic photos will be uploaded centrally in the online data manager and digital surveys will be distributed using Castor EDC. Only the study coordinators (SvR and ECS) have access to both patient information and the primary outcome from the expert surveys.

International review and validation

The participating pathologists had two meetings during the design phase of the APOLLO trial during which both techniques were discussed and consensus was reached on the exact steps of each technique. During the study, the pathologists Dr. C.S. Verbeke, for the axial slicing technique, and 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 cases were observed to acquire more insight in technical skills of each grossing technique.

Sample size calculation

The APOLLO trial is designed as a superiority trial, hypothesizing that the tumor origin could be determined with a higher level of certainty using the bivalving technique as compared to axial slicing without compromising other outcomes. The sample size was calculated to detect an increase in level of certainty of 10 points (average score of four independent pathologists of 0 to 100, i.e. linear outcome) with a standard deviation of 20 points. Significance level (α) was set at 0.05 for a two-sided test and power (1-β) at 80%. These parameters resulted in a sample size of 64 for each arm, i.e. 128 in total. Assuming a drop-out rate of 2% due to unavailable macroscopic photos, the APOLLO trial will randomize 131 specimens in total.

Statistical aspects

The two grossing techniques will be analyzed according to an intention-to-treat principle. Missing data will be imputed if more than 10% of the data is missing per variable, except for missing data on the primary and secondary outcomes. The primary endpoint, the average level of certainty among four pathologists in determining the tumor origin, will be compared between the two groups. For comparison of normally distributed continuous variables the independent-samples t-test will be used and values will be expressed as means with standard deviations. Continuous non-normally distributed variables will be compared using the Mann–Whitney U test and values will be expressed as medians with interquartile ranges. The distribution of continuous variables will be assessed using visual inspection of the histogram and the Shapiro Wilk-test. Categorical parameters will be compared using Chi-square tests. Inter-observer agreement will be determined for each case with a Fleiss' kappa (>2 assessors) and the mean kappa of each technique will be compared. A difference with a two-tailed P-value < 0.05 will be considered statistically significant. Separate analyses are planned for the subgroup of patients who underwent surgery for a malignancy (pancreatic, ampullary, distal bile duct or duodenal cancer) and for those with pancreatic cancer specifically. R1 rate will be compared between the two arms; if the number of submitted tumor blocks differ significantly between the two arms, stratification for the number of tumor blocks will be performed according to the median and R1 rate will be compared within each subgroup separately.

DISCUSSION

The APOLLO trial is a multicenter randomized controlled trial designed to assess whether bivalving of the pancreatic head increases the level of certainty in determining the primary tumor origin, as compared to axial slicing, in the pathological assessment of a PD specimen. The APOLLO trial follows a comprehensive review of our group, discussing all advantages and disadvantages encountered during the different pathological approaches of a PD specimen.[10]

Comparative studies of these two grossing techniques in the pathological assessment of a PD specimen are lacking. When initiating a prospective comparative study regarding pathological assessment, selecting the appropriate study design is challenging. Ideally, both techniques would be performed on each specimen to create comparable (i.e. identical) groups to ensure fair comparison. For obvious reasons, it would not be possible to sequentially perform both slicing techniques on the same specimen. An alternative would be a case-series of consecutive specimens sliced by each grossing technique, either in series or with each center being assigned one grossing technique. Although this would potentially increase familiarity with the technique, this would also introduce time-dependent and/or site-dependent bias. Moreover, many case-series have been published already of both techniques, with most of them lacking any comparison at all.[19] In our opinion, a randomized controlled trial offers a valid solution to ensure, or at least approach, an equal distribution of different specimens allocated to both techniques.

Another point of discussion is the primary endpoint. Some retrospective studies report on R1 rates as a reflection of quality of pathological examination, whereas other studies rather focus on lymph node yield.[19-21] Many of those studies include only pancreatic cancer and disregard the fact that firstly, the diagnosis of pancreatic cancer has to be reliably established which requires distinction from other (peri)ampullary cancers and may be highly challenging in itself, especially in advanced cancers or after neoadjuvant therapy.[2,3] Since indication of adjuvant treatment and eligibility for trials mostly depend on tumor origin, a primary endpoint related to determining the tumor origin was chosen for the APOLLO trial. All PD specimens were included in the trial, as the preoperative diagnosis derived from imaging, lab and preoperatively obtained pathology is essentially a clinical diagnosis and not infrequently changed during final pathology assessment. In the absence of a reference standard for determining the tumor origin, the assessment of four independent HPB pathologists was considered to approach a reference standard. Level of certainty was thought to reflect the pathologists' ability to determine the

tumor origin based on macroscopy, which, in our opinion, is the most important parameter in comparing the two grossing techniques. Also, the inter-observer agreement between the four pathologists is assessed in the APOLLO trial and will be reported in the final publication.

The two compared slicing techniques in the APOLLO trial are derived from the original publications by Verbeke et al. and Adsay et al., respectively.[8,9] Both techniques were transformed in a SOP to achieve quality output and uniformity of performance. Whenever possible, the SOPs adhere to the original publications, in terms of inking, grossing and the assessment of margins. For this trial, we adhere to the authentic axial slicing technique.[8,11] In case of bivalving, we believed certain parts of the described protocol were difficult to reconcile with our current practice, i.e. orange peeling and bread loafing of the specimen after bivalving. We were unsure how orange peeling would affect our microscopic margin assessment; for this reason, we decided to forego this step. After bivalving, we serially sliced our specimen in the axial plane. We performed this modification because we have most experience with slicing in the axial plane, which allows good visualization of the vascular margins and the relation between the duodenum and pancreas. The number of blocks submitted for each specimen was left at the discretion of the grosser, but will be taken into account for the analyses and adjusted for if necessary. Results regarding lymph node parameters and margin status should therefore be interpreted with these decisions in mind.

In conclusion, the APOLLO trial is a multicenter randomized superiority trial investigating the level of certainty in determining the tumor origin in the pathological assessment of a PD specimen using axial slicing and bivalving of the pancreatic head. This trial aims to identify which grossing technique offers a more accurate determination of the tumor origin for patients who underwent PD for a malignant or premalignant lesion of the pancreatic or periampullary region.

Trial status

The specimen of the first patient was randomized on 7 August 2018. At the time of protocol submission (August 2019), four centers were actively recruiting patients for the trial and 117 of 128 (92%) specimens have been randomized and the primary outcome was assessed for 81 (63%) specimens. Accrual is according to schedule.

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Node Count and the Lymph Node Metastatic Ratio. *International journal of surgical pathology.* 2018;26(8):707-713.

FIGURES

Figure 1. CONSORT 2010 Flow diagram of APOLLO trial

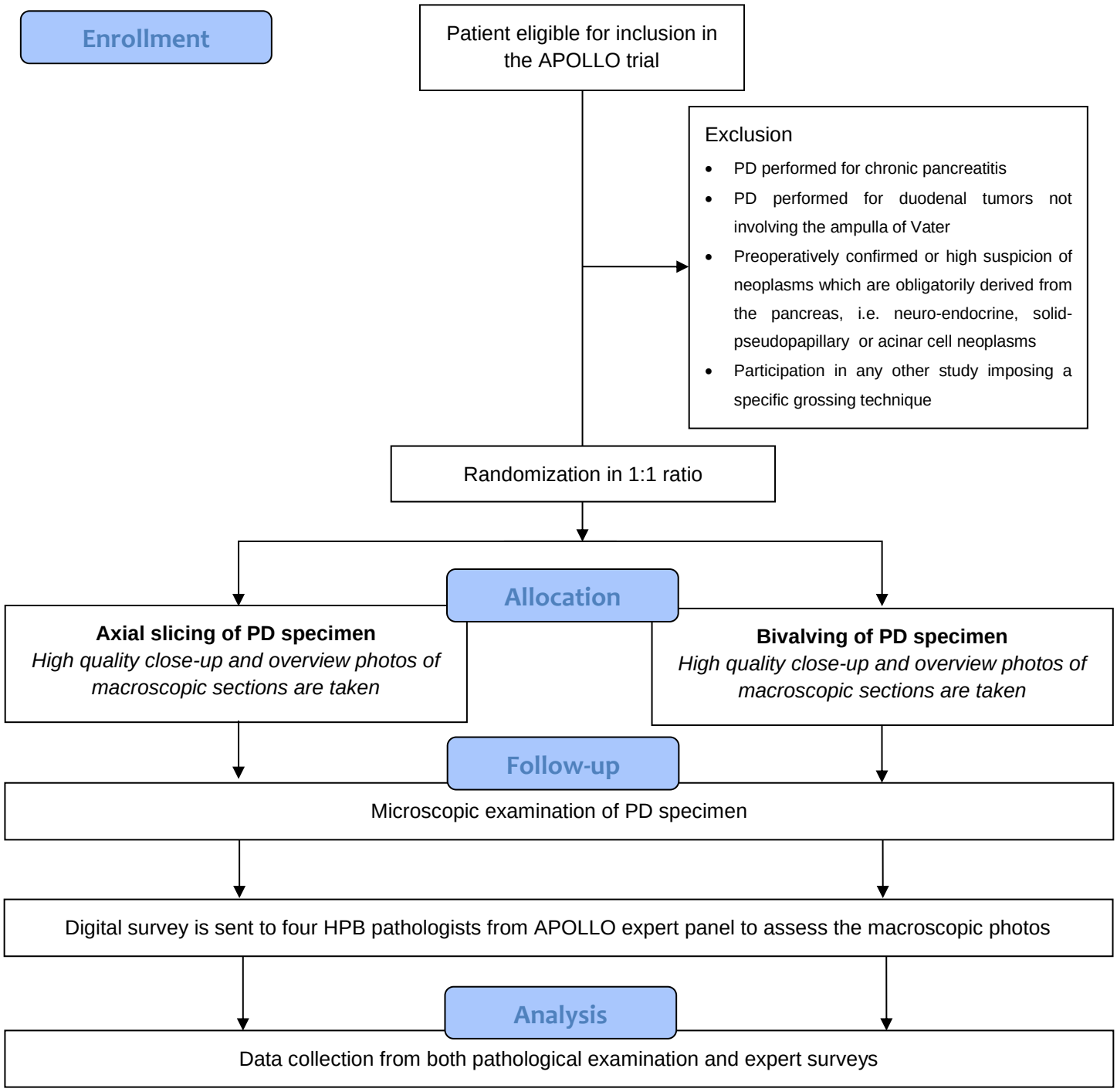

Figure 2A. Standardized approach of the upfront axial slicing technique[8]

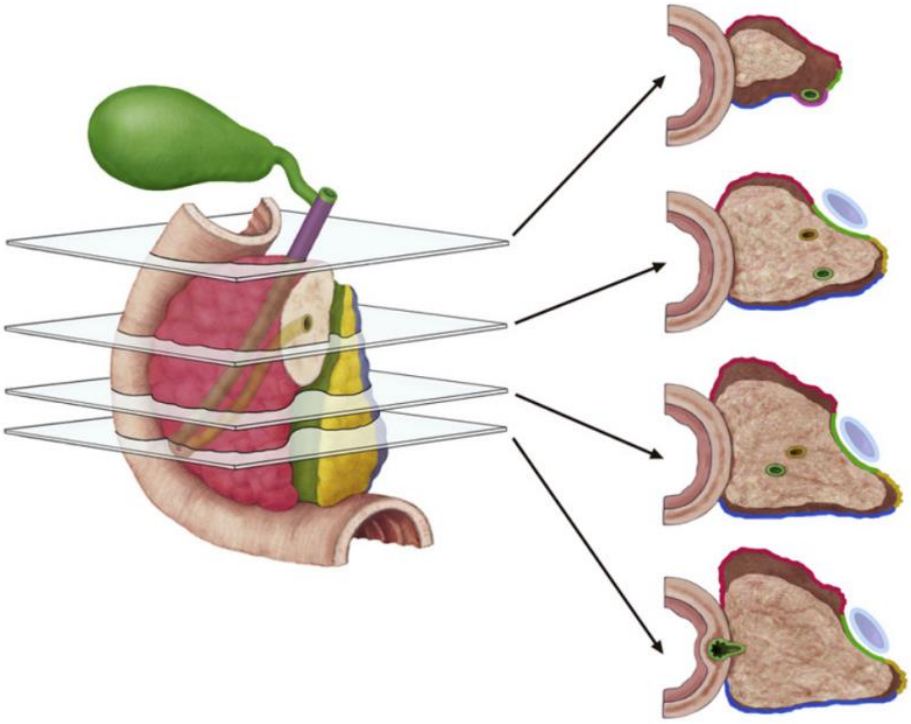

Figure legend 2A. Step-by-step instructions for the axial slicing technique according to Verbeke[8]

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 millimeter thick
6. **Take high-quality macroscopic close-up photographs** of the specimen slices
7. Sample the tumor and lymph nodes extensively for microscopic evaluation

Figure 2B. Standardized approach of the bivalving technique[9]

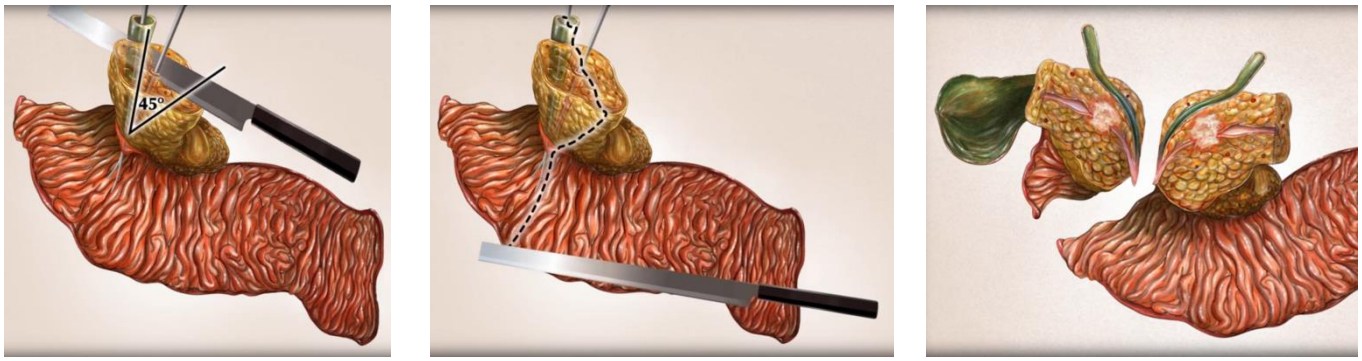

Figure legend 2B. Step-by-step instructions for the bivalving technique according to Adsay[9]

1. Ink the different surfaces / margins according to color code
2. Open the duodenum 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. 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 of 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