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From Cell Biology to Tissue Engineering

Standardized grossing protocol is useful for the pathology reporting of malignant neoplasms other than adenocarcinomas treated with pancreaticoduodenectomy

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Summary: Background: There is no universally accepted protocol for gross examination of pancreaticoduodenectomy specimens. Standardized protocol (SP), known as Leeds Pathology Protocol, was previously validated in pancreatic adenocarcinoma. In this study we aimed to assess usefulness of SP in a series of specimens with pancreatic, ampullary, and duodenal malignant neoplasms other than adenocarcinomas. Materials and methods: SP was based on multi-colour inking and serial slicing of the specimens in a plane perpendicular to the duodenal axis. SP was used in a prospective cohort of 35 neoplasms of neuroendocrine, acinar, and solid-pseudopapillary lineage (SP cohort). Surgical margin status, primary tumour stage, and lymph node yield in SP group were compared with corresponding data of a historical cohort of 19 cases examined using non-standardized protocol (NSP). Samples examined in NSP and SP cohorts were comparable in terms of basic clinical characteristics, median tumour diameter, and distribution of histopathological diagnostic categories. Results: In SP cohort we noticed: (1) higher rate of detection of tumour tissue at surgical margins, (2) more frequent peripancreatic fat tissue invasion, (3) higher percentage of perineural invasion, (4) larger number of lymph nodes retrieved from the specimen, in comparison to NSP group. Application of SP was associated with significantly higher number of tissue blocks taken for histology. Conclusions: SP can be successfully applied for macroscopical examination of pancreaticoduodenectomy specimens with malignant pancreatic, ampullary, and duodenal neoplasms other than adenocarcinomas. SP with proper microscopical diagnosis enables an appropriate schedule of patients with these neoplasms to adjuvant therapy and surveillance programmes.

Key words: (MeSH): Dissection, Pancreas, Pancreatectomy, Pancreatic neoplasms, Pancreaticoduodenectomy

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Introduction

Pancreatic neoplasms of non-ductal differentiation, although less frequent than ductal adenocarcinomas (DAC), may also pose diagnostic and therapeutic difficulties. Neuroendocrine neoplasms (NEN) are the most common non-ductal pancreatic tumours. They are

Abbreviations. ACC, acinar cell carcinoma; ACRS, Anterior circumferential radial surface; AJCC, American Joint Committee on Cancer; DAC, ductal adenocarcinoma; ENETS, European Neuroendocrine Tumor Society; LN, lymph node; LNR, lymph node ratio; NEC, neuroendocrine carcinoma; NEN, neuroendocrine neoplasm; NET, neuroendocrine tumour; NS, non-significant; NSP, Non-standardized protocol; PB, pancreatoblastoma; PCRM, pancreatic circumferential resection margin; RCP, Royal College of Pathologists; SM, surgical margin; SMAM, superior mesenteric artery margin; SMVGM, superior mesenteric vein groove margin; SP, standardized protocol; SPN, solid pseudopapillary neoplasm; WHO, World Health Organization

well-known for a wide range of clinical presentations ranging from an incidental finding of a virtually benign tumour up to an aggressive neoplasm with a high metastatic potential and a dismal prognosis (Klimstra et al., 2010; Verbeke, 2010; Basturk et al., 2014). Other pancreatic tumours of (usually or predominantly) non-ductal differentiation (acinar cell carcinomas (ACC) (Schmidt et al., 2008; La Rosa et al., 2012) and pancreatoblastomas (PB) (Salman et al., 2013) as well as peculiar tumours of uncertain cellular lineage (solid pseudopapillary neoplasms (SPN) (Estrella et al., 2014; Kang et al., 2014)) may also be associated with unfavourable prognosis. Malignant neoplasms other than adenocarcinomas, in particular NEN, may also develop in structures adjacent to the head of the pancreas ('peripancreatic'), i.e. duodenum, ampulla of Vater, and distal portion of common bile duct (Randle et al., 2014; Untch et al., 2014).

Similarly to DAC, surgery is the only potentially curative treatment option for patients with neoplasms of non-ductal differentiation originating from pancreas (La Rosa et al., 2012; Basturk et al., 2014; Estrella et al., 2014), ampulla, and duodenum (Randle et al., 2014; Untch et al., 2014). Although a pivotal role of histopathological examination of pancreatectomy specimens in assessment of local tumour stage is fully accepted (Ramage et al., 2012), the clinical significance of examination of the surgical margins (SM) in these specimens remains controversial (Raut and Evans, 2008; Verbeke, 2008a; Verbeke and Menon, 2008; Warren, 2008; Buchler et al., 2010; Chua and Saxena; 2010; Hernandez and Rosemurgy, 2010; Verbeke and Smith; 2010).

The gross examination of surgical specimens is of vital importance for adequate documentation of SM status (Kloppel et al., 2009). Unfortunately, at the moment there is no universally accepted protocol for gross examination of surgical specimens obtained following pancreatic resections, in particular pancreaticoduodenectomies (PD) (Raut and Evans, 2008; Verbeke, 2008b; Verbeke and Menon, 2008; Gomez-Mateo Mdel et al., 2014). Several protocols and checklists useful during both macroscopical and microscopical reporting of pancreatic neoplasms were proposed and successfully implemented (Verbeke et al., 2006; Khalifa, 2007; Rowsell et al., 2007; Esposito et al., 2008; Verbeke, 2008b, Westgaard et al., 2008; Adsay et al., 2009; Campbell et al., 2009; Dillhoff et al., 2009; Gill et al., 2009; Khalifa et al., 2009; Luttges et al., 2009; Menon et al., 2009; Verbeke and Menon, 2009; Campbell et al., 2010; Jamieson et al., 2010; Hartwig et al., 2011; Janot et al., 2012; Ramage et al., 2012; Stephenson et al., 2012; Zhang et al., 2012; Tang et al., 2013; Adsay et al., 2014; Elebro and Jirström, 2014; Sabater et al., 2014; Gebauer et al., 2015). Although in all of these protocols attention to documentation of SM status has been paid, they differ significantly in the applied nomenclature of SM, techniques of specimen dissection, extensiveness of tissue sampling for microscopy, and costs.

Recently, several teams described their experience in the implementation of standardized protocols (SP) of gross examination of PD specimens in patients with DAC, biliary, and ampullary adenocarcinomas (Verbeke et al., 2006; Esposito et al., 2008; Verbeke, 2008b; Campbell et al., 2009; Menon et al., 2009; Jamieson et al., 2010; Hartwig et al., 2011; Janot et al., 2012; Zhang et al., 2012; Sabater et al., 2014; Gebauer et al., 2015). Despite some minor technical differences, all these grossing protocols share their aim (i.e. optimal documentation of tumour stage and SM status) and general methodology. In principle, SP is based on recognition of several SM on the surface of PD specimen (as detailed below), marking of these SM with different colour inks (on fresh or on fixed specimen), serial slicing of the specimen in parallel planes perpendicular to the duodenal axis, taking multiple tissue sections for histology, and subsequent microscopical documentation of the distance between tumoural tissue and inked SM. These multi-colour-inking protocols are very sensitive in the identification of patients with microscopically residual (R1) cancer who are at particular risk of regional recurrence (Verbeke, 2008b) and reduced survival (Verbeke et al., 2006; Esposito et al., 2008; Campbell et al., 2009; Menon et al., 2009; Jamieson et al., 2010; Hartwig et al., 2011; Zhang et al., 2012). We also reported our preliminary data on application of similar protocol in patients with pT3 DAC (Liszka et al., 2010).

At the moment it is not clear whether SP is also suitable for documentation of SM status in patients treated with PD for the pancreatic and peripancreatic neoplasms other than adenocarcinomas. Although usage of this protocol in patients with NEN of the pancreas was recommended (Verbeke, 2010; Ramage et al., 2012; Stephenson et al., 2012), to our knowledge there are no reports describing original data on that issue.

In this study, we described our institutional experience with implementation of SP of examination of PD specimens obtained from patients with malignant neoplasms of the pancreas, duodenum and ampulla other than adenocarcinomas. Moreover, we compared results of documentation of stage and SM status obtained using SP with historical data taken from a cohort of specimens examined before implementation of SP in our laboratory.

Materials and methods

Ethics

This study was performed in accordance with regulations on research in medicine binding in Poland. All the patients provided an informed consent for surgery. Ethics Review Board at our institution agreed on performing the present study with an annotation that it did not need a detailed investigation by Board which is

mandatory for experimental studies.

Study design

In this study we compared results of histopathological examinations of PD specimens performed using two grossing protocols: (1) non-standardized protocol (NSP), and (2) SP, applied in our institution in consecutive periods of time. Specimens examined with SP were collected prospectively.

We specifically aimed to check if application of SP resulted in a change of rate of detection of 'positive' SM (R1 resections, as defined further) and extrapancreatic invasion (in particular peripancreatic fat tissue invasion) in comparison to NSP. We also compared the rates of detection of perineural invasion, lymph-vascular invasion, lymph node (LN) metastasis, as well as the numbers of examined and metastatic LN, and lymph node ratio (LNR) between the SP and NSP cohorts.

Study cases

We included PD specimens obtained from patients with primary malignant non-ductal neoplasms of pancreas and malignant non-glandular/non-adenocarcinomatous neoplasms of ampulla and duodenum, which were examined in our laboratory between January 1989 and June 2015. Specifically, NSP was applied from

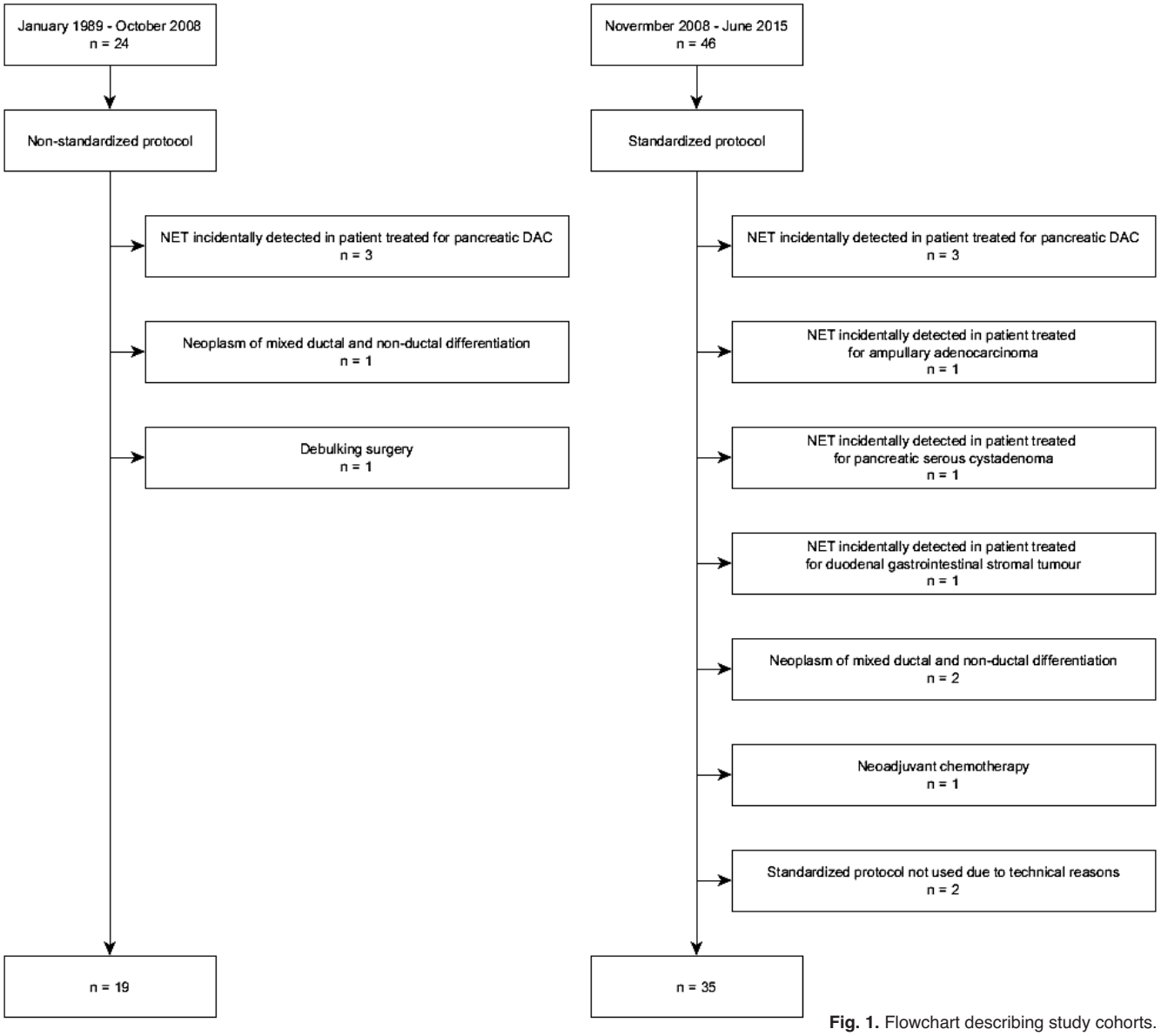

Fig. 1. Flowchart describing study cohorts.

January 1989 to October 2008, and SP was applied from November 2008 to June 2015. We did not include patients scheduled for total pancreatectomy. None of the cases included was associated with familial/hereditary cancer syndrome as known during pathological examination. Resections of superior mesenteric vein or portal vein were not performed in any of the included cases. A minor portion of samples were excluded from the study for different reasons, as depicted in a flowchart (Fig. 1).

For each case, we recorded patient's age and sex, grossing protocol (SP *versus* NSP), and type of PD (classical Whipple procedure *versus* pylorus-preserving Traverso procedure). We also recorded presence of synchronous distant metastases and whether they were resected or biopsied during or before the PD.

SP

All macroscopical examinations using SP were performed by a dedicated investigator (Ł.L.). SP applied here was virtually identical to the protocol proposed and illustrated by Verbeke et al. and later named "Leeds Pathology Protocol" (Verbeke et al., 2006; Verbeke, 2008b; Menon et al., 2009; Verbeke and Menon, 2009; Verbeke and Gladhaug, 2012). In rare cases, frozen sections of pancreatic transection margin (PTM) were requested by a surgeon. In these cases *en face* section of this SM was shaved and examined intraoperatively. Duodenum was opened longitudinally on the surface opposite to the pancreatic head and then the entire surgical specimen was fixed in buffered formalin for 24-48 hours. Following fixation, PTM was shaved as a thin slice (unless examined as a frozen section). Before further dissection, the entire surface of pancreatic specimen was inked with tissue dyes using standardized colour codes for recognized SM: superior mesenteric vein groove margin (SMVGM), superior mesenteric artery margin (SMAM), posterior circumferential radial margin (PCRM) and anterior circumferential radial surface (ACRS). The surface of previously shaved PTM was also marked with a dye for orientation purposes. The bile duct margin was sampled as a shave section. Then the specimen was manually sliced in a consecutive series of thin (whenever possible up to 3 mm thick) slices perpendicular to the duodenal axis, containing tissues of the pancreatic head, duodenum, common bile duct, and peripancreatic fat tissue with LN. Importantly, the common bile duct and the main pancreatic ducts were not opened longitudinally in our SP. Then, we recorded the site of tumour origin and its diameter, as well as presence of gross tumour invasion (into peripancreatic fat tissue, the duodenal wall, the ampulla, or the common bile duct). Then, slices were trimmed to fit to the size of standard plastic cassettes, aiming to show the relationship between the tumour and SM (two or more pieces of tissue were allowed in a single cassette). We aimed to sample the entire pancreatic surface, the entire fat tissue (irrespective of presence of LN during macroscopical inspection) and the entire tumour circumference. In a proportion of cases some minor portions of grossly uninvolved pancreatic parenchyma and/or duodenum as well as some inner portions of tumour tissue were not sampled. LN were not specifically dissected from fat tissue. Tissue slices were put into the cassettes and then routinely embedded in paraffin. The effort was made to put slices of fat tissue with LN into cassettes in an ordered fashion, aiming to prevent counting 2 or more sections of the same large LN as 2 separate LN.

NSP

Gross examinations using NSP were performed by several general pathologists or experienced pathology residents. Specimens were examined following formalin fixation. PTM and bile duct margin were identified and taken as shave sections. A single shave section was also taken from an area corresponding to SMAM. PCRM and ACRS were not sampled and examined. Specimens were then dissected aiming to document tumour size and its relationship to adjacent structures. Planes of dissection of specimens were applied as preferred by grossing pathologists (longitudinal opening of the bile duct or the pancreatic duct was allowed). LN were searched using visual inspection and palpation, retrieved from the adipose tissue, and submitted entirely for histopathology. Fat tissue without grossly detected LN was not taken for microscopical examination. For the purpose of this study, data on tumour diameter, and presence of gross tumour invasion were retrieved from the original pathology reports.

Microscopical examination

Slides were stained routinely with hematoxylin and eosin. All the slides were re-examined for this study by the main author (Ł.L.). For all the cases we confirmed the tumour origin (as recorded during gross dissection) and established histopathological diagnoses, which were based on World Health Organization (WHO) source (Klimstra et al., 2010), and whenever necessary or advised (Kloppel et al., 2009; La Rosa et al., 2012; Basturk et al., 2014), confirmed with immunohisto-chemical stains. For NEN, we described WHO 2010 grade, and additionally for pancreatic NEN, WHO 2004 diagnostic category (Heitz et al., 2004). Tumour stage (pT and overall) was assessed using both American Joint Committee on Cancer (AJCC) staging system (7^{th} edition) (Edge et al., 2010) and European Neuroendocrine Tumor Society (ENETS) staging system (Rindi et al., 2006). Other variables recorded were: AJCC/ENETS pT stage discrepancy (Liszka et al., 2011), nodal status (pN stage), invasion of the duodenal wall (assessed microscopically), invasion of peripancreatic fat tissue (assessed microscopically), presence of extrapancreatic invasion (overall, assessed microscopically), perineural invasion, lymph-vascular

invasion (assessed routinely in hematoxylin and eosin slides; in rare cases confirmatory immunohistochemical stainings with vascular markers were performed) (Tang et al., 2013), number of metastatic and examined LN, LNR and SM status (as detailed further). There are controversies related to the definition of "tumour extension beyond pancreas" (in particular peripancreatic fat invasion), which is important for the assessment of pT3 stage according to AJCC staging system (Adsay et al., 2012). In this study, peripancreatic fat invasion was defined as microscopically evident infiltrative tumour growth into peripancreatic fat tissue (i.e. beyond most peripheral acinar/ductal/neuroendocrine parenchymal elements). Protrusion of bulging tumoral mass into peripancreatic soft tissues with microscopically confirmed pushing tumour border, usually with separation of tumour cells from adipocytes by a (pseudo) capsule of any thickness was not sufficient for pT3 diagnosis.

LN were counted in two ways: (a) LN count in PD specimen (anterior and posterior pancreatoduodenal, superior and inferior pancreatic, LN around the distal portion of the common bile duct, LN next to the SMAM, but not peripyloric LN in classical PD), (b) total LN count (LN in the PD specimen listed above, but also peripyloric LN in classical PD, LN in hepatoduodenal ligament, LN next to the common hepatic artery, vena cava and other LN submitted by a surgeon in separate containers). LNR was defined as the number of metastatic LN divided by number of all examined LN - in all cases and separately in N1 cases.

Since there are controversies regarding the optimal classification of residual (R) tumour status following pancreatic resection (Verbeke, 2008b; Stephenson et al., 2012), we applied two approaches of reporting the SM status:

(a) approach recommended by AJCC (Wittekind et al., 2002; Tang et al., 2013), according to which the presence of tumoral tissue in microscopically examined SM constitutes R1 resection ("0 mm clearance", "AJCC R1 definition"),

(b) approach recommended for DAC by Royal College of Pathologists (RCP) (Campbell et al., 2010), according to which R1 resection is defined as the presence of tumoral tissue within 1 mm of tissue adjacent to SM (SMAM, SMVGM and PCRM, but not ACRS, "1 mm clearance") and/or at inked ACRS ("RCP R1 definition").

Additionally, the extensiveness of SM involvement was recorded in a simple semi-quantitative way: R1 status was considered 'focal' if involvement of a particular SM using above listed AJCC/RCP criteria was detected just in a single slide/tissue block - otherwise R1 status was considered 'nonfocal'. For clarity of presentation, distant metastases and their SM were not considered while establishing R0/R1 status.

Additionally, the number of examined tissue blocks in PD specimen was recorded for each case. Peripyloric LN as well as portions of tissue provided in separate containers (e.g. fat tissue with hepato-duodenal LN) were excluded from these counts.

Statistical analysis

Fisher exact tests or χ^2 tests were used for comparisons of frequencies in 2x2 and 2xn tables, respectively. Mann-Whitney U tests were used for comparison of continuous variables. All tests were two-sided, and p value less than 0.05 was considered statistically significant. Statistica 10 (Statsoft, Tulsa, USA) and WinPepi (Abramson, 2011) were utilized for statistical calculations.

Results

Fifty-four PD specimens were evaluated in this study: 19 using NSP and 35 using SP. Fourteen patients were included in our previous NEN paper (Liszka et al., 2011), not directly related to grossing techniques.

Baseline clinical and pathological data of included patients are presented in Table 1. Specifically, patients treated with PD whose specimens were examined using SP and NSP did not differ significantly in respect of age, sex, tumour origin, histopathological diagnoses, WHO 2010 grade, median tumour diameter, presence of grossly detectable tumour invasion and distal metastases. For that reason, cases in NSP and SP cohorts were comparable. The only significant change during the study period was a recent decrease in rate of classical Whipple PD (with pylorus resection), in accordance with the universal trend (Barugola et al., 2013).

The median number of blocks/slides examined in SP cohort was almost 2.5 times larger than in NSP group (median 32 and 13 slides, respectively, $p<0.001$). Number of blocks did not correlate with tumour diameter (not shown).

As shown in Table 2, the distribution of primary tumour stage assessed using ENETS criteria was similar in NSP and SP groups. This was expected, since tumour diameter is a core component of ENETS staging system (Rindi et al., 2006). On the contrary, cases examined with SP (both in the entire study group and in a subgroup of pancreatic neuroendocrine tumours (NET) were more frequently diagnosed with AJCC pT3 stage than cases in NSP cohort. This was related to an increase of detection of invasion of peripancreatic fat tissue (74.3% in the entire SP group *versus* 21.05% in entire NSP group, $p<0.001$). This finding suggested clearly that NSP underestimated the prevalence of peripancreatic fat tissue invasion in the present series. SP was also associated with approximately two-fold higher rate of detection of perineural invasion in the whole study population and in patients with pancreatic NET (Table 2), but in the latter subgroup this comparison was not statistically significant ($p=0.083$), most likely due to a relatively small number of examined samples. Interestingly, the prevalence of lymph-vascular invasion did not differ significantly between SP and NSP cohorts.

Application of SP resulted in some increase in detection of presence of LN metastases (i.e. N1 rate) - 48.6% using SP *versus* 26.3% using NSP, but this difference did not reach statistical significance (p=0.151). Importantly, LN yield in PD specimen using SP was two times larger than using NSP (median 13 and 6 LN, respectively, p<0.001). However, the median number of metastatic LN in PD specimens did not increase in SP cohort in comparison with NSP cohort (median 3 in each group). LNR was lower in SP group in comparison to NSP group in about 30%, but again this was a statistically non-significant finding.

Application of SP resulted in almost three-fold increase of detection of AJCC R1 rate (45.7% using SP *versus* 15.8% using NSP, p=0.038) (Table 2). A similar trend was observed in a subgroup of pancreatic NET, but it did not reach statistical significance. As expected, an increase in R1 rate was caused by the examination of circumferential radial surface of PD specimens (SMAM, SMVGM, PCRM, and ACRS) in SP cohort, as the percentage of cases with PTM involvement was low, whereas biliary/gastric/duodenal SM were uniformly negative (Table 3). Application of RCP R1 definition in SP group resulted in a further increase of R1 detection rate – almost 83% of cases were recognized as R1. In the majority of cases a single SM was involved when AJCC

Table 1. Baseline clinical and pathological data of the study cases.

Variable	Entire study group (n=54)			Patients with NET of the pancreas (n=36)		
	NSP (n=19)	SP (n=35)	p	NSP (n=12)	SP (n=24)	p
Sex (M : F)	9 : 10	13 : 22	NS	6 : 6	12 : 12	NS
Age (median, range)	54.0 (35-68)	55.0 (23-75)	NS	54.0 (35-65)	56.0 (23-75)	NS
Pylorus resection:			<0.001			<0.001
Yes (Whipple resection)	11 (57.9%)	3 (8.6%)		8 (66.7%)	1 (4.2%)	
No (Traverso resection)	‘8 (42.1%)	32 (91.4%)		4 (33.3%)	23 (95.8%)	
Tumour origin:			NS			-
Pancreas	19 (100%)	32 (91.4%)		12 (100%)	24 (100%)	
Ampulla of Vater	0 (0%)	1 (2.9%)				
Duodenum	0 (0%)	2 (5.7%)				
Histopathological diagnosis:			NS			-
Neuroendocrine tumour	12 (63.2%)	26 (74.3%)		12 (100%)	24 (100%)	
Neuroendocrine carcinoma	1 (5.3%)	4 (11.4%)				
Acinar cell carcinoma	2 (10.5%)	1 (2.9%)				
Solid pseudopapillary neoplasm	3 (15.8%)	2 (5.7%)				
Pancreatoblastoma	0 (0%)	1 (2.9%)				
Mixed acinar-neuroendocrine carcinoma	1 (5.3%)	1 (2.9%)				
WHO 2010 grade*:	(n=13)	(n=30)	NS			NS
G1	6 (46.1%)	10 (33.3%)		6 (50%)	9 (37.5%)	
G2	6 (46.1%)	16 (53.3%)		6 (50%)	15 (62.5%)	
G3	1 (7.7%)	4 (13.3%)		-	-	
WHO 2004 diagnostic category**:	(n=13)	(n=27)	NS			NS
1A	1 (7.7%)	3 (11.1%)		1 (8.3%)	3 (12.5%)	
1B	6 (46.1%)	5 (18.5%)		5 (41.7%)	5 (20.8%)	
2	5 (38.5%)	16 (59.3%)		6 (50%)	16 (66.7%)	
3	1 (7.7%)	3 (11.1%)		-	-	
Tumour diameter (median, range, in mm)	40 (6-100)	33 (9-85)	NS	32.5 (6-90)	27.5 (9-85)	NS
Tumour diameter:			NS			NS
Up to 20 mm	3 (15.7%)	5 (14.3%)		2 (16.7%)	4 (16.7%)	
21-40 mm	10 (52.6%)	17 (48.6%)		7 (58.3%)	13 (54.2%)	
Above 40 mm	6 (31.6%)	13 (37.1%)		3 (25%)	7 (29.1%)	
Gross tumour invasion***:	8 (42.1%)	21 (60%)	NS	3 (25%)	13 (54.2%)	NS
M stage:			NS			NS
cM0	18 (94.7%)	30 (85.7%)		11 (91.7%)	21 (87.5%)	
pM1	1 (5.3%)	5 (14.3%)		1 (8.3%)	3 (12.5%)	
Resection of liver metastasis	1 (5.3%)	3 (8.6%)	NS	1 (8.3%)	1 (4.2%)	NS
Surgical biopsy of liver metastasis	0 (0%)	2 (5.7%)	NS	0 (0%)	2 (8.3%)	NS
No of examined tissue blocks in pancreatico-duodenectomy specimen (median, range)	13 (7-20)	32 (19-57)	<0.001	12 (7-20)	32 (19-57)	<0.001

* for NET/NEC only; ** for pancreatic NET/NEC only; *** in cases of pancreatic tumours - invasion into peripancreatic fat tissue, duodenal wall, ampulla, or common bile duct, or – in cases of ampullary/duodenal tumours – invasion into pancreas or fat tissue.

Table 2. Tumour stage, status of surgical margins, and other microscopical characteristics of the study cases.

Variable	Entire study group (n=54)			Patients with NET of the pancreas (n=36)		
	NSP (n=19)	SP (n=35)	p	NSP (n=12)	SP (n=24)	p
pT stage (ENETS):			0.135			0.145
T1	2 (10.5%)	5 (14.3%)		1 (8.3%)	4 (16.7%)	
T2	8 (42.1%)	6 (17.1%)		7 (58.3%)	6 (25.0%)	
T3	9 (47.4%)	24 (68.6%)		4 (33.3%)	14 (58.3%)	
pT stage (AJCC):			0.022			0.025
T1	2 (10.5%)	3 (8.6%)		1 (8.3%)	3 (12.5%)	
T2	9 (47.4%)	5 (14.3%)		8 (66.7%)	5 (20.8%)	
T3	8 (42.1%)	27 (77.1%)		3 (25%)	16 (66.7%)	
AJCC/ENETS pT stage discrepancy	3 (15.8%)	5 (14.3%)	1	1 (8.3%)	4 (16.7%)	0.646
pN stage:			0.151			0.468
N0	14 (73.7%)	18 (51.4%)		9 (75%)	14 (58.3%)	
N1	5 (26.3%)	17 (48.6%)		3 (25%)	10 (41.7%)	
ENETS stage:			0.198			0.331
1	2 (10.5%)	5 (14.3%)		1 (8.3%)	4 (16.7%)	
2A	7 (36.8%)	4 (11.4%)		6 (50%)	4 (16.7%)	
2B	5 (26.3%)	8 (22.9%)		2 (16.7%)	5 (20.8%)	
3A	0	0		0	0	
3B	4 (21.1%)	13 (37.1%)		2 (16.7%)	8 (33.3%)	
4	1 (5.3%)	5 (14.3%)		1 (8.3%)	3 (12.5%)	
AJCC stage:			0.292			0.343
1a	2 (10.5%)	3 (8.6%)		1 (8.3%)	3 (12.5%)	
1b	7 (36.8%)	4 (11.4%)		6 (50%)	4 (16.7%)	
2a	5 (26.3%)	10 (28.6%)		2 (16.7%)	6 (25.0%)	
2b	4 (21.1%)	12 (34.3%)		2 (16.7%)	8 (33.3%)	
3	0	1 (2.9%)*		0	0	
4	1 (5.3%)	5 (14.3%)		1 (8.3%)	3 (12.5%)	
Extrapancreatic invasion (overall)**	8 (42.1%)	27 (77.1%)	0.017	3 (25%)	16 (66.7%)	0.033
Invasion of duodenal wall***	7 (36.8%)	17 (48.6%)	0.567	3 (25%)	10 (41.7%)	0.468
Invasion of peripancreatic fat tissue	4 (21.05%)	26 (74.3%)	<0.001	1 (8.3%)	15 (62.5%)	0.004
Perineural invasion	7 (36.8%)	24 (68.6%)	0.043	3 (25%)	14 (58.3%)	0.083
Lymph-vascular invasion	11 (57.9%)	27 (77.1%)	0.212	7 (58.3%)	18 (75%)	0.446
No of examined LN in PD specimen (median, range)	6 (1-16)	13 (2-35)	<0.001	7 (3-16)	14 (2-35)	0.013
No of metastatic LN in PD specimen (in N1 cases) (median, range)	3 (1-6)	3 (0-18)	0.904	3 (1-6)	3 (0-18)	1
No of examined LN in total specimen (median, range)	6 (1-19)	15 (2-50)	0.002	8 (3-19)	15.5 (2-50)	0.025
No of metastatic LN in total specimen (in N1 cases) (median, range)	3 (1-6)	3 (1-18)	0.842	3 (1-6)	3 (2-18)	0.727
Lymph node ratio (in all cases) (median, range)	0 (0-1)	0 (0-0.692)	0.214	0 (0-1)	0 (0-0.692)	0.585
Lymph node ratio (in N1 cases) (median, range)	0.308 (0.167-1)	0.2 (0.06-0.692)	0.347	0.333 (0.25-1)	0.188 (0.06-0.692)	0.128
Resection margin status (AJCC definition):			0.038			0.115
R0	16 (84.2%)	19 (54.3%)		11 (91.7%)	15 (62.5%)	
R1	3 (15.8%)	16 (45.7%)		1 (8.3%)	9 (37.5%)	
Resection margin status (RCP definition):	-		-	-		-
R0		6 (17.1%)			4 (16.7%)	
R1		29 (82.9%)			20 (83.3%)	
Status of pancreatic transection margin:			0.280			1
negative	17 (89.5%)	34 (97.1%)		11 (91.7%)	23 (95.8%)	
positive	2 (10.5%)	1 (2.9%)		1 (8.3%)	1 (4.2%)	
Status of superior mesenteric artery margin:			0.468			0.646
negative	17 (89.5%)****	28 (80%)*****		11 (91.7%)****	20 (83.3%)*****	
positive	2 (10.5%)****	7 (20%)*****		1 (8.3%)****	4 (16.7%)*****	
Status of bile duct margin, gastric and duodenal margins:			-			-
negative	19 (100%)	35 (100%)		12 (100%)	24 (100%)	

* NET of the duodenum; ** extraduodenal/extraampullary invasion in cases of duodenal/ampullary tumours, respectively; *** invasion of pancreas in cases of duodenal/ampullary tumours; **** *en face* sections; ***** perpendicular sections, tumour at the inked margin.

R1 definition was applied (9/16), but usage of RCP R1 definition resulted in a frequent detection of involvement of 2 or more SM (16/29). SMAM, SMVGM, PCRM, and ACRS were positive in comparable frequencies. Importantly, focal SM involvement (for all positive margins in a particular case) was rare when RCP definition was considered (1/29), but relatively frequent using AJCC definition (5/16). A single case of duodenal NET was recognized as R1 resection using RCP criteria solely due to a proximity (less than 1 mm) of metastatic deposits in LN from SMAM and PCRM – in any other case R1 status (using both AJCC and RCP criteria) was established solely due to metastases in LN.

Additionally, we aimed to check which clinical and pathological factors were associated with the detection of AJCC R1 status and invasion of peripancreatic fat tissue. The results of these calculations are shown in Table 4. In brief, AJCC R1 was more probable if: (1) SP was used, (2) gross invasion into adjacent tissues was detected, (3) pT stage according to AJCC increased (particularly to pT3), (4) microscopically confirmed invasion into adjacent organs (overall) and into fat tissue, or perineural invasion was found, (5) the number of examined tissue blocks increased (as a continuous variable). Peripancreatic fat tissue invasion was more frequently found, if: (1) SP was used, (2) WHO 2010 grade was G2 or G3 (3) WHO 2004 diagnostic category was more advanced (especially well-differentiated or poorly differentiated endocrine carcinoma), (4) a gross invasion into the adjacent tissues was detected, (5) ENETS primary tumour stage was pT3, (6) LN metastases were found, (7) invasion of the duodenal wall, or perineural, or lymph-vascular invasion was detected, (8) SM were positive (irrespective of whether AJCC or RCP criteria were applied), (9) number of examined tissue blocks increased (as a continuous variable and using median cut-off value). A multivariate analysis was not attempted due to interrelationship of many variables.

Discussion

SP for gross examination of PD specimens in cases of pancreatic or peripancreatic adenocarcinoma offers many advantages over NSP (summarized in Table 5). In this study we showed that SP may also be useful for examination of PD specimens with malignant neoplasms other than adenocarcinomas. The advantages and disadvantages of application of SP in these cases are listed in Table 6.

SM status

The main advantage of SP of grossing PD specimens with cancers other than adenocarcinoma was more effective recognition of R1 status, in comparison to NSP. Documentation of the SM status is mandatory during examination of specimens with NEN (Kloppel et al., 2009; Ramage et al., 2012). Surprisingly, it is not clear whether R1 status is (Ballain et al., 2009; Hashim et al., 2014) or is not (Bilimoria et al., 2008) a prognostic factor in pancreatic NEN. According to the ENETS guidelines (Falconi et al., 2012), surgeons should avoid leaving macroscopical residual pancreatic NEN (R2 status) during surgery. However, little attention to clinical significance of R0/R1 distinction was paid in these guidelines. R1 rate in recent series of NEN varied from nil (Tsutsumi et al., 2014) to 18% (Ballain et al., 2009). In this series (SP cohort) AJCC R1 rate was 37.5% (Table 2). This high value could be caused in part by referral bias, proactive surgical strategy in our center, and underestimation of R1 in other reports. We speculate that underestimation of R1 status may partially explain lack of prognostic value of R1 in many NEN series, as it was found in DAC (Verbeke, 2008b; Verbeke and Menon, 2009). In DAC high frequency of R1 resections is a marker of high quality of pathological evaluation of the specimen but not of a low quality of surgical technique (Esposito et al., 2008).

Another related issue is optimal R1 definition in non-ductal pancreatic cancers. It is not clear what SM clearance should define R1 in pancreatic NEN (Verbeke, 2010). At this moment all proposed SM clearance values seem to be arbitrary. In almost all studies on pancreatic NEN (with rare exceptions (Hashim et al., 2014)) AJCC R1 ("0 mm clearance") definition was used, in full concordance with a statement that even a "very close" clearance is enough for complete resection (Ramage et al., 2012). However, alternative RCP R1 ("1 mm clearance") definition may be taken into account during planning adjuvant radiotherapy (Arvold et al., 2012). In this study we reported SM status using RCP R1 definition just for investigative purposes rather than in the belief that it is a clinically meaningful variable. In

Table 3. Distribution of surgical margins' involvement in SP cohort (n=35).

Variable	AJCC R1 definition (n=16)	RCP R1 definition (n=29)
Number of involved margins:		
1	9	13
2	5	9
3	2	4
4	0	3
Involved margins:		
SMAM	7	13
SMVGM	6	17
PCRM	4	17
ACRS	7	7
PTM	1	1
Other*	0	0
Mode of margins involvement:		
Focal (for all positive margins)	5	1
Nonfocal (for at least 1 positive margin)	11	28

* Bile duct margin, proximal (gastric or duodenal) margin, distal duodenal margin.

this series RCP R1 rate had a very high value (83%), almost the same as reported previously in DAC (Verbeke et al., 2006).

In many cases pancreatic NET are well defined grossly against adjacent non-neoplastic tissues (Verbeke, 2010). However, sharp tumour border is not an universal finding in these neoplasms. In this study some non-ductal neoplasms extensively replaced pancreatic parenchyma and this resulted in RCP R1 status for several SM (Table 3). In our experience, gross recognition of tumour border in NET may be difficult even for experienced prosectors, especially when dealing

Table 4. Factors associated with R1 status (AJCC definition) and presence of invasion of peripancreatic fat tissue.

Variable	R1 status (AJCC definition)		Invasion of peripancreatic fat tissue	
	n (%)	p (Fisher exact test)	n (%)	p (Fisher exact test)
Grossing protocol:		0.038		<0.001
NSP	3/19 (15.8%)	OR 4.49	4/19 (21.05%)	OR 10.83
SP	16/35 (45.7%)	(95% CI 1.1-21.8)	26/35 (74.3%)	(95% CI 2.8-44.6)
Sex:		NS		NS
Male	7/22 (31.8%)		12/22 (54.55%)	
Female	12/32 (37.5%)		18/32 (56.25%)	
Age:		NS		NS
Up to median (54.0 y)	11/28 (39.3%)		18/28 (64.3%)	
Above median (54.0 y)	8/26 (30.8%)		12/26 (46.15%)	
Age (median, range):		NS*	-	-
in R1 resections (n=19)	49.0 (23-72)			
in R0 resections (n=35)	55.0 (23-75)			
Age (median, range):	-	-		NS*
in cases with invasion of peripancreatic fat tissue (n=30)			47.5 (23-68)	
in cases without invasion of peripancreatic fat tissue(n=24)			55.5 (35-75)	
Pylorus resection:		NS		NS
Yes (Whipple resection)	3/15 (20%)		6/15 (40%)	
No (Traverso resection)	16/39 (41%)		24/39 (61.5%)	
Tumour origin:		NS		NS
Pancreas	19/51 (37.25%)		27/51 (52.9%)	
Other	0/3 (0%)		3/3 (100%)	
Histopathological diagnosis:		NS		NS
NET	10/38 (26.3%)	(0.060)	18/38 (47.4%)	
Other	9/16 (56.25%)		12/16 (75%)	
WHO 2010 grade**:		NS		<0.001****
1	2/16 (12.5%)	****	2/16 (12.5%)	(reference)
2	8/22 (36.4%)		16/22 (72.7%)	OR 18.6
3	3/5 (60%)		5/5 (100%)	OR ∞
WHO 2004 category***:		NS		<0.001****
1A	0/4 (0%)	****	0/4 (0%)	(reference)
1B	2/11 (18.2%)		1/11 (9.1%)	OR 4.0E+6
2	8/21 (38.1%)		15/21 (71.4%)	OR 1.0E+8
3	3/4 (75%)		4/4 (100%)	OR ∞
Tumour diameter (median, range, in mm):		NS*	-	-
in R1 resections (n=19)	40 (9-80)			
in R0 resections (n=35)	30 (6-100)			
Tumour diameter (median, range, in mm):	-	-		NS*
in cases with invasion of peripancreatic fat tissue (n=30)			40 (9-85)	
in cases without invasion of peripancreatic fat tissue (n=24)			30 (6-100)	
Tumour diameter:		NS****		NS****
Up to 20 mm	2/8 (25%)		2/8 (25%)	(p=0.058)
21-40 mm	8/27 (29.6%)		14/27 (51.85%)	
Above 40 mm	9/19 (47.4%)		14/19 (73.7%)	
Gross tumour invasion*****:		0.046		<0.001
Absent	5/25 (20%)	OR 3.73	6/25 (24%)	OR 15.20
Present	14/29 (48.3%)	(95% CI 1.1-13.5)	24/29 (82.8%)	(95% CI 3.9-59.7)
M stage:				NS
cM0	17/48 (35.4%)		25/48 (52.1%)	
cM1/pM1	2/6 (33.3%)		5/6 (83.3%)	

with tumours which infiltrate fat or duodenum, in cases with coexistent chronic pancreatitis, and in rare NET with extensive fibrosis. Microscopical infiltration of adjacent pancreatic parenchyma is also a typical picture in a subset of SPN and in pancreatic NEC. Intraductal spread of ACC may also extend far to pancreatic lobules and complicate gross recognition of tumour borders, in our experience. Another related finding in this study was the observation that 'focal' SM involvement using AJCC definition was in fact not rare (5 out of 16 AJCC R1 cases). This indicated that taking just "representative" tissue sections for histology may be insufficient for a reliable R0 report. This result again addresses the usefulness of SP for examination of specimens with non-

Table 4. (continued).

Variable	R1 status (AJCC definition)		Invasion of peripancreatic fat tissue	
	n (%)	p (Fisher exact test)	n (%)	p (Fisher exact test)
pT (ENETS):		NS		<0.001****
1	2/7 (28.6%)	****	2/7 (28.6%)	(reference)
2	2/14 (14.3%)		3/14 (21.4%)	OR 0.68
3	15/33 (45.45%)		25/33 (75.8%)	OR 7.81
pT (AJCC):		0.003****		NA
1	0/5 (0%)	(reference)	0/5 (0%)	
2	1/14 (7.1%)	OR 3.8E+6	0/14 (0%)	
3	18/35 (51.4%)	OR 5.3E+7	30/35 (85.7%)	
AJCC/ENETS pT stage discrepancy:		NS		NS
Absent	14/46 (30.4%)		25/46 (54.35%)	
Present	5/8 (62.5%)		5/8 (62.5%)	
pN:		NS		0.012
0	11/32 (34.4%)		13/32 (40.6%)	OR 4.97
1	8/22 (36.4%)		17/22 (77.3%)	(95% CI 1.4-18.0)
Extrapancreatic invasion (overall)******:		<0.001		-
Absent	1/19 (5.3%)	OR 19.06	0/19 (0%)	
Present	18/35 (51.4%)	(95% CI 2.8-421.4)	30/35 (85.7%)	
Invasion of duodenal wall:		NS		0.012
Absent	9/32 (28.1%)		13/32 (40.6%)	OR 4.97
Present	10/22 (45.45%)		17/22 (77.3%)	(95% CI 1.4-18.0)
Invasion of peripancreatic fat tissue:		<0.001	-	-
Absent	1/24 (4.2%)	OR 34.50		
Present	18/30 (60%)	(95% CI 4.9-753.7)		
Perineural invasion:		0.023		<0.001
Absent	4/23 (17.4%)	OR 4.45	4/23 (17.4%)	OR 24.70
Present	15/31 (48.4%)	(95% CI 1.2-17.9)	26/31 (83.9%)	(95% CI 5.7-109.6)
Lymph-vascular invasion:		NS		<0.001
Absent:	4/16 (25%)		3/16 (18.75%)	OR 10.64
Present:	15/38 (39.5%)		27/38 (71.05%)	(95% CI 2.5-52.0)
Surgical margins' status (AJCC definition):	-	-		<0.001
R0			12/35 (34.3%)	OR 34.50
R1			18/19 (94.7%)	(95% CI 4.9-753.7)
Surgical margins' status (RCP definition):		0.022		0.027
R0	0/6 (0%)	OR ∞	2/6 (33.3%)	OR 9.60
R1	16/29 (55.2%)	(95% CI 1.6-∞)	24/29 (82.8%)	(95% CI 1.2-84.8)
No. of examined tissue blocks in PD specimen:		NS		0.013
Up to median (24.5)	6/27 (22.2%)		10/27 (37.0%)	OR 4.86
Above median (24.5)	13/27 (48.15%)		20/27 (74.1%)	(95% CI 1.5-16.0)
No. of examined tissue blocks in PD specimen (median, range):		0.008*	-	-
in R1 resections	34 (8-52)			
in R0 resections	21 (7-57)			
No. of examined tissue blocks in PD specimen (median, range):	-	-		<0.001*
in cases with invasion of peripancreatic fat tissue (n=30)			30.5 (8-57)	
in cases without invasion of peripancreatic fat tissue (n=24)			18.5 (7-40)	

* Mann-Whitney U test; ** for NET/NEC only; *** for pancreatic NET/NEC only, **** χ^2 test, ***** in cases of pancreatic tumours - invasion into peripancreatic fat tissue, duodenal wall, ampulla, or common bile duct, or – in cases of ampullary/duodenal tumours – invasion into pancreas or fat tissue; ****** extraduodenal/extraampullary invasion in cases of duodenal/ampullary tumours, respectively; OR – odds ratio; CI – confidence interval.

ductal neoplasms. The issue of representativeness of sampling of PD specimens with DAC was previously discussed (Verbeke et al., 2006).

In contrast to NET, there are few data on prognostic value of SM status in neuroendocrine carcinoma (NEC), ACC, and PB. More than half (56.7%, 17/30) of patients with strictly defined pancreatic NEC amenable for resection show positive SM, but SM status is not a prognostic factor in NEC (Basturk et al., 2014), possibly due to the almost uniformly fatal disease course. SM status (R0 *versus* R1/R2) is an independent prognostic factor in ACC (Schmidt et al., 2008). In children with PB, curative surgery (R0 status) is a favourable prognostic factor in univariate analysis (Bien et al., 2011). In adults with PB, tumour resectability is critically important for disease eradication (Salman et al., 2013), but no data on clinical significance of SM involvement are available. Local or distal recurrence is rare, but still possible in patients with surgically resected SPN (Estrella et al., 2014; Kang et al., 2014). Although not statistically proven, metachronous disease recurrence or metastasis seem to be more frequent in patients with SPN who underwent R1 resection in comparison to patients who underwent R0 resections (1 out of 8 (11.1%) *versus* 9/308 (2.9%), p=0.184 (Kang et al., 2014), and 1 out of 10 (10%) *versus* 1/34 (2.9%), p=0.41 (Estrella et al., 2014)). Considering ampullary and duodenal NEN, Untch et al. (2014) reported that SM status was not far from statistical significance (p=0.06) in univariate analysis as a predictor of recurrence.

Table 5. Advantages and disadvantages of SP during examination of pancreaticoduodenectomy specimens with adenocarcinoma.

Advantages:
1. Identification of a small but significant group of patients with 'curatively' resected DAC ('true' R0 resections), whose survival rates are among the best reported so far in DAC (Esposito et al., 2008; Jamieson et al., 2010). 2. Better correlation between percentages of R1 resection and risk of subsequent local recurrence rates in DAC (Esposito et al., 2008; Campbell et al., 2009; Menon et al., 2009; Verbeke and Menon, 2009; Jamieson et al., 2010; Hartwig et al., 2011). 3. Reduction of the risk of underestimation of R1 status leading to improper prognostication and qualification to adjuvant therapy (Esposito et al., 2008; Campbell et al., 2009; Menon et al., 2009). 4. Possibility of gathering the data on optimal R1 definition (in the context of the distance of the tumour from surgical margin (Campbell et al., 2009). 5. Possibility of documentation of prognostic significance of tumoral involvement of each SM separately, toward better prognostic information and recognition of the biology of DAC (Esposito et al., 2008; Campbell et al., 2009; Khalifa et al., 2009; Jamieson et al., 2010; Zhang et al., 2012). 6. Detection of R1 status in higher percentage of cases (R1 AJCC definition: 44-55% (Esposito et al., 2008; Campbell et al., 2009; Jamieson et al., 2010), R1 RCP definition: 52-85% (Verbeke et al., 2006; Esposito et al., 2008; Campbell et al., 2009; Menon et al., 2009; Jamieson et al., 2010; Zhang et al., 2012; Sabater et al., 2014; Gebauer et al., 2015), in comparison to techniques of less extensive sampling of specimens (Esposito et al., 2008; Sabater et al., 2014; Gebauer et al., 2015), in which R1 rates (AJCC or RCP definition) were below 30%. 7. Higher rates of detection of vascular invasion (Sabater et al., 2014), lymphatic vessel invasion (Gebauer et al., 2015), pN1 stage (Verbeke et al., 2006), and larger numbers of resected (Sabater et al., 2014) and metastatic (Sabater et al., 2014) LN in comparison with NSP.
Disadvantages:
1. Larger number of tissue blocks in comparison with NSP (Verbeke et al., 2006; Elebro and Jirström, 2014). 2. The same lymph nodes may be seen in consecutive slices of the specimen – this must be carefully taken into account during histopathological reporting.

Table 6. Advantages and disadvantages of SP during examination of pancreatioduodenectomy specimens with malignant neoplasms other than adenocarcinomas.

Advantages:
1. Adequate reporting of R1 status. 2. Adequate recognition of peripancreatic fat invasion and AJCC pT3 stage. 3. Adequate identification of perineural invasion. 3. Large number of retrieved lymph nodes. 4. Simple procedure, which may be applied in virtually all specimens irrespective of gross tumour configuration and preoperative or final microscopical diagnosis. 5. Useful for individuals with little experience in gross pathology of pancreatic–duodenal area.
Disadvantages:
1. Large number of tissue blocks. 2. The same lymph nodes may be seen in consecutive slices of the specimen – this must be carefully taken into account during histopathological reporting.

AJCC pT3 stage

The second advantage of SP over NSP is its better ability to recognize AJCC pT3 stage related to peripancreatic soft tissue invasion. This is probably a clinically important finding, since disease-specific survival in patients with pancreatic NEN differs significantly between patients with AJCC pT2N0 and pT3N0 tumours (p<0.001) (Rindi et al., 2012). In contrast, AJCC pT3 is almost a rule (32/34, 94.1%) among resected NEC (Basturk et al., 2014). AJCC pT stage is also a prognostic factor in ACC in univariate, but not in multivariate analysis (Schmidt et al., 2008). Prognostic utility of pT stage in PB was not confirmed

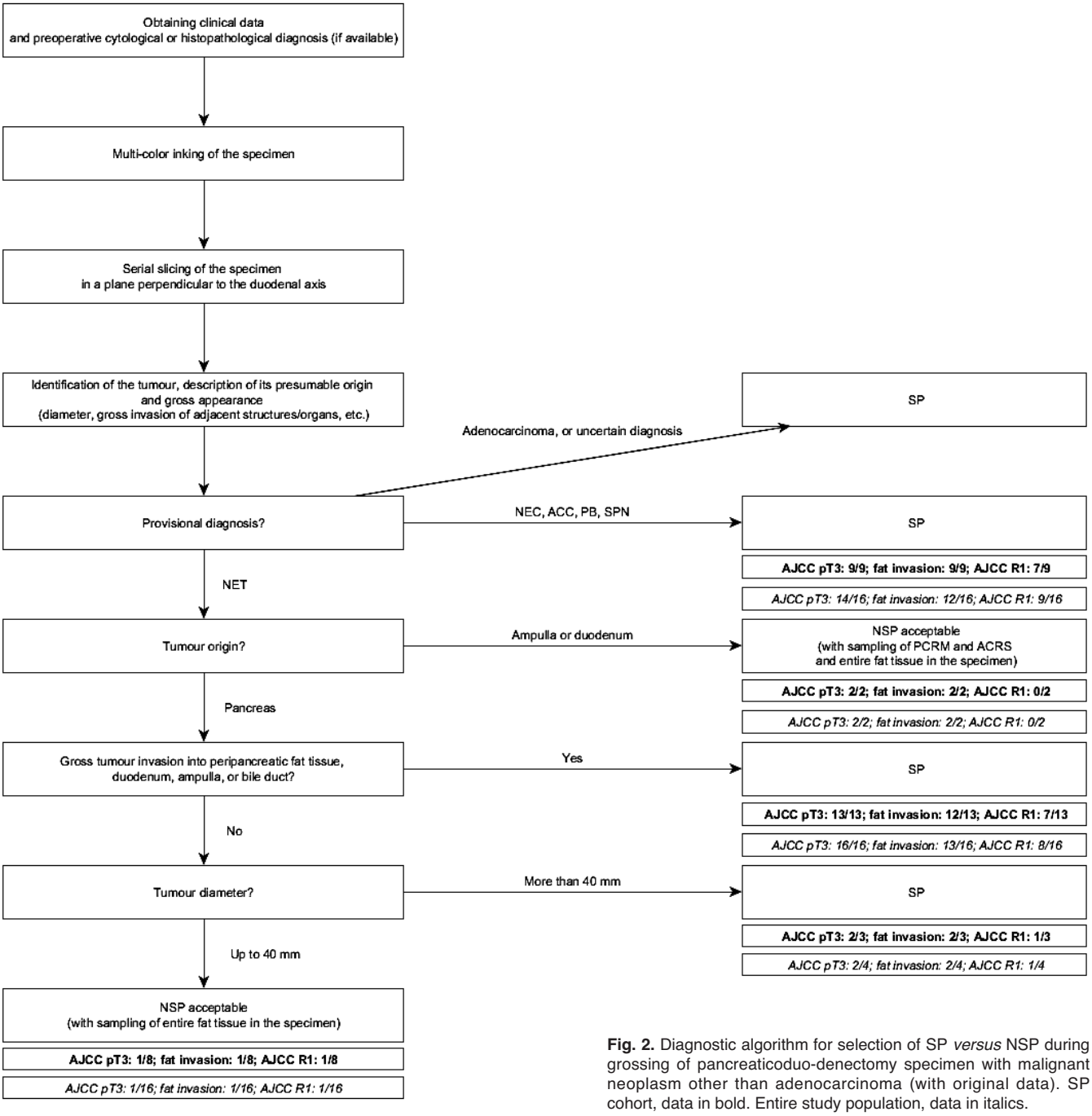

Fig. 2. Diagnostic algorithm for selection of SP *versus* NSP during grossing of pancreaticoduo-denectomy specimen with malignant neoplasm other than adenocarcinoma (with original data). SP cohort, data in bold. Entire study population, data in italics.

(Bien et al., 2011; Salman et al., 2013). AJCC pT3 (almost always due to peripancreatic fat tissue invasion) is a very frequent finding in SPN (75%), but it does not seem to be a predictor of disease recurrence or metastasis (Estrella et al., 2014). A simplified tumour stage is a prognostic factor in patients with ampullary and duodenal NEN (Randle et al., 2014).

Perineural invasion

In this study, SP usage was associated with significantly higher rate of detection of perineural invasion. Perineural invasion seems to be a powerful risk factor for recurrence in pancreatic NET (Tsutsumi et al., 2014), but is not a prognostic factor in NEC (Basturk et al., 2014) and SPN (Estrella et al., 2014). In ACC perineural invasion was not far from statistical significance (p=0.066) as a predictor of overall survival (La Rosa et al., 2012).

LN status

No data on optimal sampling technique and number of LN which should be retrieved from the specimen with pancreatic NEN for accurate staging are on record (Verbeke, 2010). As expected, SP gave higher LN yield than NSP among pancreatic NET and in the entire study population. pN1 rate in SP cohort was higher (48.6% *versus* 26.3% in NSP cohort), albeit not significantly (p=0.151). This indicated that using SP one may find and examine some additional, presumably small LN, not detectable in a routine visual and manual inspection of fat tissue. We suspect that they are less likely to have metastases, but measurement of LN diameters was beyond the scope of our study. It is not obvious whether pN1 stage is a prognostic factor in pancreatic NEN (Krampitz et al., 2012; Rindi et al., 2012) and in duodenal/ampullary NEN (Randle et al., 2014). The prognostic significance of LN metastases in ACC is well documented (Schmidt et al., 2008; La Rosa et al., 2012). In SPN LN involvement is very rare (Estrella et al., 2014).

Application of SP resulted in non-significant decrease of LNR in this series. It was previously shown that LNR above 0.07 (Ricci et al., 2013) or above 0.20 (Boninsegna et al., 2012) is an independent risk factor for recurrence in pancreatic NEN/NET. In this study SP and NSP cohorts did not differ in regard to LNR calculated as dichotomous variable using mentioned pre-defined cut-off points (data not shown).

Simplicity

In our opinion, an important advantage of SP is its simplicity. The technique of inking and dissecting the specimen is always the same irrespective of baseline pathology. This is particularly relevant as many patients with resectable pancreatic mass are treated with potentially curative surgery without an attempt to obtain preoperative microscopical diagnosis. Moreover, SP may be utilized by individuals with little experience in gross pathology of the pancreatic–duodenal area with none to minimal risk of submission of suboptimal tissue sections for histology. For that reason we recommend using SP during grossing of all PD specimens, whenever possible.

Disadvantages

The main disadvantage of SP is the large number of tissue blocks which are taken for histology (Table 1), larger than using NSP. This was concordant with reports on SP of examination of specimens with adenocarcinomas (Verbeke et al., 2006; Elebro and Jirström, 2014). Another weakness of SP is a need for careful counting of LN during reporting, since parallel sections through large LN may be visualized in 2 or more slides. Submission of tissue slices in a strictly consecutive manner minimizes the risk of double counting of a single LN.

Diagnostic algorithm

Based on our observations we propose an algorithm which may be utilized for selection of SP *versus* NSP during grossing of PD specimens with malignant neoplasms other than adenocarcinomas (Fig. 2). This algorithm may be useful especially in those pathology departments in which cost of routine usage of SP (related directly to the number of examined tissue blocks) may be prohibitive. It was not based on a formal decision-tree construction, but it is still supported by the original data. In our experience NSP may be safely applied during grossing of (previously diagnosed microscopically) pancreatic NET which are less than 40 mm in diameter, and without gross invasion into the adjacent organs or peripancreatic fat tissue. For ampullary/duodenal NET, we recommend submission of PCRM and ACRS, as postulated in ampullary adenocarcinoma (Verbeke and Gladhaug, 2012). During grossing of NET using NSP we still advocate sampling of the whole peripancreatic fat tissue found in the specimen – this will allow examination of virtually all LN.

Limitations

Our study has several limitations. (1) The number of cases was not very large and the neoplasms examined here differed in terms of their biological aggressiveness. However, malignant non-ductal neoplasms are less common than adenocarcinomas in PD specimens, and their diversity in this series reflected our diagnostic practice. Importantly, SP and NSP cohorts were comparable in terms of baseline clinical and pathological characteristics. (2) This was an observational study, not a randomized one. We compared NSP and SP in 2 consecutive cohorts of patients. The same approach was successfully used in studies on SP in DAC (Verbeke et al., 2006; Esposito et al., 2008; Sabater et al., 2014;

Gebauer et al., 2015). (3) We did not include the follow-up data. Because of biological and clinical variety of the cases included, this study did not aim to examine a long-term prognosis. (4) We did not report the functional status of tumours. Our data on this issue in NSP cohort were incomplete. (5) The data on gross tumour invasion in NSP group were taken from original pathology reports and they could have been incomplete. However, median tumour diameter in NSP and SP groups was similar. (6) We did not report data on gross measurements of SM clearance. In our opinion it is not particularly important if SM are sampled entirely. Non-ductal neoplasms may still have infiltrative borders and gross measurement may underestimate SM clearance.

Conclusions

In this study we showed that standardized grossing protocol may be successfully applied in the examination of pancreaticoduodenectomy specimens with malignant neoplasms other than adenocarcinomas. It allows accurate documentation of the status of surgical margins, as well as of a presence of peripancreatic fat invasion and perineural invasion in these tumours. It is also sensitive in detection of lymph nodes in the specimen. The number of tissue blocks taken for histology in adherence with the standardized protocol is significantly higher in comparison to non-standardized approach. Standardized protocol may be applied irrespective of baseline pathology and with a subsequent proper microscopical diagnosis it enables an appropriate schedule of patients with non-ductal neoplasms to adjuvant therapy and surveillance programmes.

Conflicts of Interest and Financial sponsorship and support. None to declare

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