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Research Article

Morphological Validation of Locoregional Recurrence in Patients with Pancreatic Ductal Adenocarcinoma

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Abstract

Introduction: Pancreatic ductal adenocarcinoma (PDAC) is associated with a very poor prognosis, early locoregional recurrence and rapid development of distant metastases. One of the reasons for this is a high rate of perineural invasion.

Aim: To demonstrate the role of extrapancreatic perineural invasion in development of locoregional recurrence of PDAC.

Results: In 14.5% of cases (22/152), the tumor was localized within the pancreas, which corresponded to pT1-2, pN0, extrapancreatic perineural invasion (EPn) 0, R0. R1 was identified in 32.1% of cases (49/152). Extrapancreatic perineural invasion was detected in 36.8% of cases (56/152). Metastases in the regional lymph nodes were revealed in 62.5% of cases (95/152) of patients. The analysis of the autopsy material demonstrated that the main morphological parameters of tumor development include locoregional progression and/or distant progression. Locoregional recurrence was identified in 85% of cases (29/34) and manifested as extrapancreatic perineural invasion in the bed of the removed tumor. The average length of time from surgery to locoregional recurrence without EPn was 14 ± 3 months, with EPn - 9 ± 2,5 months.

Conclusion: Locoregional recurrence in patients is directly related to the presence of extrapancreatic perineural invasion. The identified subpopulation of patients without distant progression but only with locoregional recurrence dictates the need for a combined treatment approach that leads to an increase of overall survival in patients with PDAC.

Keywords: *Pancreatic Ductal Adenocarcinoma; Locoregional Recurrence; Perineural Invasion*

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the top five causes of cancer deaths worldwide. In most cases, PDAC is inoperable at the time of diagnosis, which leads to a low 5-year survival rate - less than 5 - 7% [1]. At the time of the PDAC diagnosis, the absolute majority of patients have local tumor spread (perineural invasion and regional lymph node involvement, etc.) and remote metastases [2]. Even a radical nature of the surgery according to the data of the pathological examination does not eliminate the possibility of progression of the disease in the near future [3]. The criteria for the radical nature of surgery (R status) include: R0 - histologically radical resection in the absence of tumorous clusters within 1 mm from the examined edge of resection, R1 - in the presence of tumorous clusters within 1 mm from the examined edge of resection, R2 - macroscopically positive edge of resection.

The key significance of this parameter for the outcome of PDAC resection was universally recognized as early as at the beginning of 1990 [4]. According to modern protocols for dissection of PDAC, the edges of resection include: surgical section of the pancreas, radial

periductal edge (section of the common bile duct), posterior surface of the pancreas, medial surface of the pancreas (with compulsory separation into the edge of the superior mesenteric vein and artery - SMV and SMA), upper surface of the pancreas.

A distinctive characteristic of PDAC is the spread of tumor growth along the ducts, lymphatic pathways and perineural spaces. Perineural invasion (Pn) is explained by the anatomical location of the pancreas and was first described by J. R. Drapiewski., *et al.* in 1940 [5]. A detailed study of perineural invasion in patients with PDAC was conducted in 1970 in Japan through the introduction of an aggressive approach to the surgical treatment of pancreatic cancer, including the removal of retroperitoneal tissue [6-8]. Perineural invasion in PDAC should be divided into three subtypes: Intrapancreatic (IPn), intrapancreatic perineural extra-tumoral, and extrapancreatic invasion [9]. As a rule, intrapancreatic perineural invasion is found in 90 - 100% of cases and has no clinical significance [9]. Meanwhile, extraPn is detected in 15 - 30% of cases and is an independent prognostic factor. According to the Japanese classification, extraPn is divided as follows [7]: (I) pancreatic head plexus, (II) plexus of the abdominal cavity, (III) superior mesenteric plexus, (IV) plexus within the hepatoduodenal ligament, (V) aortic plexus and (VI) splenic plexus. It should be noted that the correlation between the size of the tumor and the presence of extrapancreatic perineural invasion was not observed. There is also a microscopic gradation of perineural infestation in PDAC depending on the number of affected nerve trunks: Pn0 - no involvement; Pn1 (not expressed) - involvement of 1-5 nerve trunks; Pn2 (moderate) - 6-10 nerve trunks; and Pn3 (expressed) - with involvement of more than 10 nerve trunks [8]. According to the TNM classification of malignant tumors, perineural invasion is classified as: [9] PnX - perineural invasion cannot be evaluated; Pn0 - no perineural invasion; Pn1 - perineural invasion.

Lymphogenous metastasising is one of the main routes by which malignant tumors of the gastrointestinal tract spread including PDAC. A large number of clinicopathologic studies have demonstrated that the presence of lymph node metastasis is an unfavorable prognostic factor in PDAC [10-14]. According to the 2010 WHO classification, the morphological status of lymph nodes of patients with PDAC can be determined by studying at least 10 lymph nodes (LN) and includes: pN0 - no involvement of regional lymph nodes; pN1 - positive regional lymph nodes are present [9]; however, a number of articles published recently show that it is not the absolute number of positive lymph nodes that has prognostic value, but the ratio of positive LN to the total number of studied LN (LN_+/LN_t) [15-17]. This is analogous to gastric cancer [18], esophageal cancer [19], ampullary carcinoma [20]. It is believed that the prognosis in PDAC patients is unfavorable with a ratio of $LN_+/LN_T > 0.3$ [20]. The groups of lymph nodes according to the Japanese classification are shown in table 1 [21].

Group	Head	Body and tail
I	13a, 13b, 17a, 17b	8a, 8p, 10, 11p, 11d, 1 8
II	6, 8a, 8p, 12a, 12b, 12p14p14d	7, 9, 14p, 14d, 15
III	1, 2, 3, 4, 5, 7, 9, 10, 11p, 11d, 15, 16a2, 16b1, 18	5, 6, 12a, 12b, 12p, 13a, 13b, 17a, 17b, 16a2, 16b1

Table 1: *The groups of lymph nodes according to the Japanese classification.*

According to our research, PDAC spreads in three different ways [22]: direct (R1 only) - positive resection edges, locoregional type (pN1 and/or extraPn) - regional lymph node involvement and/or extrapancreatic perineural invasion, mixed type (R1 and pN1 and/or extraPn) - a combination of direct and locoregional spread.

Thus, taking into account the high rate of perineural invasion in PDAC and consequently, the high frequency of locoregional tumor recurrence in patients, the problem of preventing rapid PDAC progression remains relevant and requires a search for combined treatment methods.

In our study we tried to evaluate association of different features of locoregional recurrence with findings of tumor spreading from primary pathologic evaluation of specimen of the patients with PDAC who underwent surgical treatment.

Materials and Methods

The study is based on surgical material obtained from 152 patients with PDAC treated at medical institutions in Moscow from 2005 to 2012. In 65,8% (100/152) tumor localization was in the head of the pancreas and in 34,2% (52/152) - in the body and tail of the pancreas. The PDAC patients were predominantly male (1.2:1). The age of the patients ranged from 40 to 80 years, median - 65,3. The median age of men was 62.3 years, the median age of women was 54 years. Pancreatoduodenectomy was performed with D2 lymphadenectomy, in two odifications, either with pyloric valve preservation or with antrectomy.

In the course of the subsequent standardized morphological examination, special attention was paid to the surfaces of the removed pancreas. For convenience of microscopic identification of R0 and R1, during the macroscopic examination the surfaces of the pancreatic tissue were marked with a special Thermo Scientific™ Richard-Allan Scientific™ Mark-It™ Tissue Marking Dye, as recommended [23].

Tumor progression was assessed 8 - 12 weeks after chemotherapy. Clinical, radiological and biochemical criteria were used to assess locoregional recurrence of PDA in patients. The following was interpreted as clinical signs: pain, weight loss, body mass index decrease, duodenal obstruction. Multi-slice helical abdominal CT was routinely used as the basic radiological diagnostic method. The CT criterion of locoregional recurrence was the appearance of hypovascular formations in the projection of the bed of the removed tumor, and signs of perivascular hypovascular masses. In cases when helical CT data was not sufficient, we used MRI with intravenous contrast, and PET-CT when indicated. Elevation of the level of tumor markers (CA 19-9 and CEA) was used as a sign of tumor recurrence.

The study also included 34 cases of autopsy material of patients who had undergone radical surgical treatment for PDAC. We studied only the retroperitoneal peripancreatic tissue, which corresponded to the bed of the removed cluster. According to the histological examination records for the primary tumor, the standardized specimen dissection protocol for PDAC was not used in all cases (~14,7%), therefore it is not possible to reliably estimate the effect of the R1 status rate on the development of locoregional recurrence in this group.

Associations between variables were examined using Fisher's exact test, chi-square test. Unadjusted survival analysis was performed using the Kaplan-Meier method, comparing curves using log-rank test. Multivariable Cox regression analysis was used for adjusted survival analysis. The proportional hazard assumption was tested graphically. For all tests, 2-sided $p < 0.05$ was considered statistically significant. Statistical analyses were performed in PASW Statistics 6,1 for Windows (SPSS).

Results

In the macroscopic study of the tumor section, the tumor was matte whitish-gray or whitish-yellow in color, with rocky density. Tumor destruction loci with the formation of cavities of various shapes could often be seen at the center of the tumor.

Microscopically, in 76.9% of cases (117/152) the tumor was presented by glandular structures of irregular form, consisting of tumor cells with different mucin content, pronounced desmoplastic stromal reaction, usually occupying a large portion of the area of the tumor mass (from 50 - 80%). In 35/152 cases, in addition to glandular structures, an anaplastic (sarcoma-like) component was detected, represented by large polymorphous or spindle-shaped cells with hyperchromatic nuclei, sometimes multinucleated, with a minimal stromal component. The volume of the anaplastic component varied from 5 to 20% of the total tumor mass, but it was never predominant.

In most cases, the tumor had spread beyond the pancreas (Figure 1): invasion of the duodenal wall was found in 68% of cases (68/100) (Figure 1A), invasion of the spleen in 48% (25/52), invasion of peripancreatic soft tissue - in 50% (76/152) (Figure 1B); extrapancreatic perineural invasion - in 36.8% (56/152) (Figure 1C).

In 24.3% (37/152) of cases, the tumor remained within the pancreas - pT1 and pT2 stages. The size of the tumor did not influence the presence or absence of tumor spread, since small carcinomas also showed dissemination beyond the pancreas, in the form of lesions of

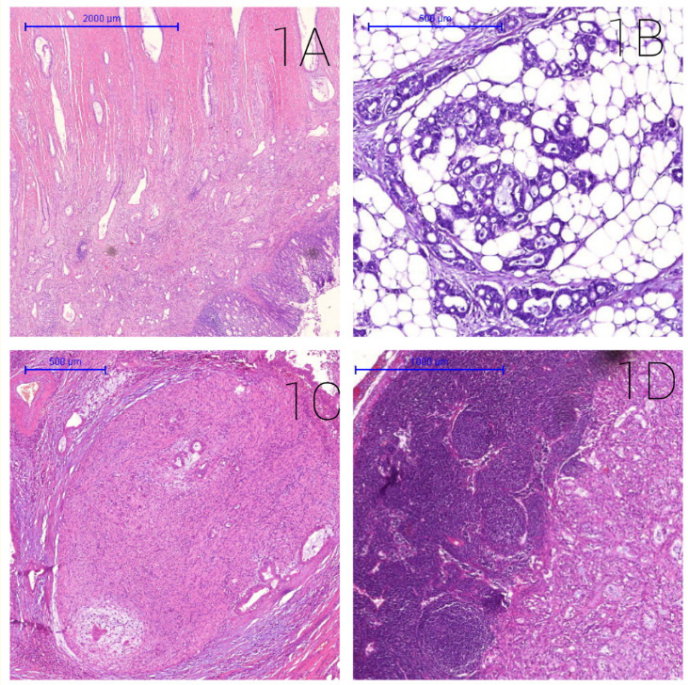

Figure 1: *Microscopic characteristics of PDA: 1A: Invasion of the duodenal wall. Hematoxylin and eosin stain. Magnification x200. 1B: Invasion of peripancreatic soft tissue. Hematoxylin and eosin stain. Magnification x200. 1C: Extrapancreatic perineural invasion. Hematoxylin and eosin stain. Magnification x400. 1D: Metastasis in a regional lymph node. Hematoxylin and eosin stain. Magnification x200*

the regional lymph nodes or extrapancreatic perineural invasion. Therefore, in 14.5% of cases (22/152) the tumor was localized within the pancreas, which corresponds to pT1-2, pN0, extraPn 0, R0 (Table 2).

Type of spread (n = 152)		pT1	pT2	pT3	pT4
None		7	15	15	0
Direct (R1)		0	0	14	1
Locoregional	pN1	2	12	25	0
	extraPn1	0	2	9	0
	pN1+ extraPn1	0	0	14	0
Mixed	R1 + pN1	0	0	12	0
	R1+ extraPn1	0	0	4	2
	R1+ pN1+ extraPn1	0	0	18	2

Table 2: *Spread of pancreatic ductal carcinoma.*

Note: Pn: Perineural Invasion; extraPn: Extrapancreatic Perineural Invasion; pN1: Positive Lymph Nodes.

R1-resection was identified in 32.1% of cases (49/152), with the medial surface (SMA) affected most often - 17/49 (34,7%); then, in descending order, the posterior surface - 17/49(34,7%), and in 1/49(2,0%) cases - the upper surface and radial periductal edge. By the number of positive edges: one edge - 57.1% (28/49), simultaneous lesions of two edges - 38.7% (19/49) and three edges - 4.1% (2/49).

Extrapancreatic perineural invasion was identified in most (31/49) cases in the region of the superior mesenteric artery (SMA edge) with the tumor in the head of the pancreas. In cases when the tumor was located in the body/tail of the pancreas, extraPn was more often detected in the region of the splenic plexus.

According to our data, extraPn can be considered as an independent prognostic factor. An inverse correlation was revealed between the life expectancy of patients after surgical treatment and the presence of extrapancreatic perineural invasion ($r = -0.52$ $p = 0.0002$). Thus, 6 months after the surgical treatment, the survival rate was equal to 0.85 both with and without extrapancreatic perineural invasion. During later periods the data diverged, and 12 months after the surgery the survival rate was 0.58 without and 0.38 with extrapancreatic perineural invasion, respectively. We discovered a statistically significant relationship between extrapancreatic perineural invasion and the presence of hematogenous metastasis ($r = 0.71$ $p = 0.0002$), as well as the stage of the disease ($r = 0.30$ $p = 0.01$). We did not identify a statistically significant correlation between the R1 status (a positive edge of resection) and the presence of extrapancreatic perineural invasion ($p > 0.05$).

Metastases in the regional lymph nodes were revealed in 62.5% of cases (95/152) of pancreatic ductal adenocarcinoma (Figure 1D). A statistically significant correlation between lymph node involvement and extraPn ($r = 0.36$ $p = 0.002$) was identified, in contrast to cases with intraPn, which is explained by the progression of the disease and the synchronization of metastatic pathways.

The analysis of the autopsy material demonstrated that the main morphological parameters of tumor development include locoregional progression and/or distant progression (Table 3). Locoregional recurrence was identified in 85% of cases (29/34) and manifested as extrapancreatic perineural invasion in the bed of the removed tumor. There was no statistically significant correlation between the presence of locoregional recurrence and R1 status ($p > 0.05$), which could be caused to a lack of a standardized morphological study of the primary tumor. It is evident that a performed pancreatoduodenectomy does not affect the frequency of locoregional recurrence, but rather is its temporal characteristic. In addition, no metastases were detected in level 3 lymph nodes during the study of autopsy material. Distant progression was detected in 71% (24/34) of cases. A subpopulation of patients was identified (10/24 cases) in whom only locoregional progression was detected without distant progression. The average length of time from surgery to locoregional recurrence without extraPn was 14 months, with extraPn - 9 months (Table 4).

Discussion

Pancreatic ductal adenocarcinoma is characterized with high frequency of perineural invasion. Several large morphological studies have shown that the presence of perineural invasion is the cause of locoregional recurrence after surgical treatment [6,7]. However, the prognostic role of perineural invasion is still controversial.

	Group 1 (n = 34)
Local progression	Yes 29/34
Distant progression	Liver 17/34 (HEP (C22)) Lungs 12/34 (PUL (C34)) Peritoneum 15/34 (PER (C48.1, 2))

Table 3: *Morphological indices of tumor progression in the autopsy examination.*

Parameter	Surgical material n = 152	Autopsy material n = 34 Initial data
Localization		
Head	100	20
Body/tail	52	14
Sex		
M:F	1.2:1	1.5:1
Age (years)	40-80	54-81
Average	54	61.5
pT1	9	-
pT2	29	6
pT3	109	28
pT4	5	-
Pn		
intraPn	112	N/A
extraPn	56	29/34
G1	2	N/A
G2	71	
G3	17	
+AC	35	
R0	103	N/A
R1	49	
pN1	95	21
Adjuvant chemotherapy		
None	56	23
Monotherapy (GCB)	54	9
Combined	42	2

Table 4: *Generalized data of the PDA.*

Note: M: Males; F: Females; Pn: Perineural Invasion; intraPn: Intrapancreatic Perineural Invasion; AC: Anaplastic Component; extraPn: Extrapancreatic Perineural Invasion; pN1: Positive Lymph Nodes; GCB: Gemcitabine

One of the main and actual interdisciplinary goals is to increase both relapse-free and overall survival in patients with PDAC. According to our study, locoregional recurrence in patients is directly related to the presence extraperineural invasion.

Lymphogenous metastasizing is also one of the main ways of spreading of malignant tumors of the gastrointestinal tract. We found the relationship between regional lymph node involvement and the life expectancy of patients was found ($r = -0,24$ $p = 0,04$). Median survival of patients without regional lymph nodes metastases was 16 months, in contrast to 11 months in patients with metastases that corresponds with the literature data ($p = 0,03$) [24].

In addition, the revealed subpopulation of patients without distant progression, but only with the presence of locoregional recurrence, dictates the need for a searching for new strategies of treatment of pancreatic cancer.

Conclusion

Locoregional recurrence in patients is directly related to the presence of extrapancreatic perineural invasion. The identified subpopulation of patients without distant progression but only with locoregional recurrence dictates the need for a combined treatment approach that leads to an increase of overall survival in patients with PDAC.

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