Prognostic Factors after Curative Resection of Pancreatic Ductal Adenocarcinoma: a Retrospective Study

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Abstract

Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive tumours with dismal prognosis. Although curative resection with adjuvant chemoradiotherapy is the most effective treatment, the surgery is frequently limited by cancer spread or poor general status of the patient. Relatively few studies have analysed the outcomes of surgically treated pancreatic cancer. Controversies exist about several biological markers that could be important in the era of personalized medicine. Thus, the **aim** of our study was to determine frequency and extent of expression of biological markers (Ki-67, p53, Bcl-2, vimentin, CD44) in relation to survival after potentially curative radical surgical treatment of PDAC.

Methods: The study was designed as retrospective, protocol-based evaluation of 63 consecutive pancreatic ductal adenocarcinoma cases. Ki-67, p53, Bcl-2, vimentin and CD44 expression was evaluated by immunohistochemistry. The survival was assessed by Kaplan-Meier method. Spearman's rank correlation, t-test and Kruskal-Wallis test, log-rank and Cox regression analysis were used for appropriate statistical analysis. The study was approved by Committee of Ethics.

Results: Patients' mean age was 63.2 years. The mean survival was 19.5 month (95% confidence interval (CI) = 11.3-27.8). The tumours were mostly T3-4 (95.2%; 95% CI = 86.7-98.2) and larger than 2 cm (90.9%; 95% CI = 78.8-96.3). Metastases in lymph nodes were identified in 67.2% (95% CI = 54.7-77.7) cases. The most frequent tumour stage was IIB (62.3%; 95% CI = 49.7-73.4). Resection margins were involved in 51.7% (95% CI = 39.1-64.1) cases. Perineural and intraneural invasion was found in 84.1% (95% CI = 73.1-91.1) of patients. Correlation was found between survival and high histologic grade ($p = 0.017$), resection margin involvement ($p = 0.039$), high Ki-67 ($p = 0.022$) and vimentin positivity ($p = 0.023$), as well as between CD44 expression and N stage ($p = 0.022$) and Ki-67 ($p = 0.010$). There was a trend to correlation between CD44 and vimentin expression ($p = 0.058$) as well as between p53 expression and peri- and intraneural invasion ($p = 0.053$).

Conclusions: The survival after surgical resection of pancreatic carcinoma remains poor. The tumour grade, positive resection margins, proliferation fraction and epithelial-mesenchymal transition affect patients' survival. CD44 expression correlates with Ki-67 and N stage. Trend to correlation was observed between p53 expression and peri- and intraneural growth.

Keywords: pancreatic cancer, survival, immunohistochemistry, CD44, Ki-67, vimentin, p53

Background

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with a grave prognosis. In 2008 there were 277 000 new cases of pancreatic carcinoma, ranking it in the 13th place among all cancers excluding non-melanoma skin cancer. Pancreatic carcinoma was the 9th leading cause of cancer-related death worldwide [1]. Five-year survival rate of inoperable patients is 0.6-3.8% across all the PDAC stages, but in surgically treated patients it reaches around 5% [2,3]. Better five year survival rates for surgically treated patients are represented in Bilimoria *et al* research (2.8-31.4%), where all types of pancreatic

adenocarcinomas were included [2]. Early diagnostics and surgery are the best options to improve patients' survival. However, lack of early specific symptoms and diagnostic markers result in frequent occurrence of widespread tumour invasion and presence of metastases at the time of PDAC diagnostics [4-8]. As surgical treatment is feasible only if distant metastases are absent, major blood vessels are not affected and the patients' general health status is acceptable, only 15-20% patients undergo curative surgical resection [9,10]. Chemotherapy alone cannot ensure survival improvement. Adjuvant chemoradiotherapy improves postoperative survival results [11]. In comparison

to other malignant tumours, PDAC is characterised by almost the worst constellation of characteristics describing tumour spread and patients' survival. Active scientific research is devoted to the analysis of prognostic and predictive factors. Findings that are associated with significantly reduced survival, include large tumour size (above 3 cm), metastasis in lymph nodes, positive resection margins, high histologic grade, intravascular invasion and marked tumour cell proliferation [5,6,12,13]. Expression of several biological markers in tumour tissue is described as significant due to correlation with the progression of tumour and resistance to chemoradiotherapy. Relevant examples include S100 calcium-binding protein A4, human equilibrative nucleoside transporter 1, vascular endothelial growth factor and low expression of insulin-like growth factor binding protein 7 [6,14,15]. Studies of several other markers have yielded contradictory results. These markers include tumour suppressor protein p53, mesenchymal cell intermediate filament vimentin, apoptosis regulator protein Bcl-2 as well as CD44 – cell surface glycoprotein, which is involved in cell adhesion, migration, proliferation and apoptosis [14,16-20]. Thus, the aim of our study was to determine frequency and extent of expression of biological markers (Ki-67, p53, Bcl-2, vimentin, CD44) in relation to survival of patients after potentially radical surgical treatment of pancreatic carcinoma.

Methods

Patients and Tissue Samples

The study was performed as a retrospective investigation. Sixty three consecutive cases of potentially radically operated pancreatic ductal adenocarcinoma in the time interval from January 2004 to June 2012 were consecutively selected from the archive of single university hospital. No patients had received preoperative chemotherapy or radiotherapy.

The analysed factors were: patient's age, gender, type of surgery, tumour localization, tumour size by the largest diameter, tumour characteristics by TNM (T: size and local

spread of primary tumour, N: status of regional lymph nodes regarding metastasis, M: presence or absence of distant metastasis) and stage, histological grade (high, moderate or low), status of resection margins, vascular and perivascular, perineural and intraneural invasion, as well as survival rate (SR). Tumours were classified according to the World Health Organization [21]. The TNM staging was performed in accordance with the Seventh Edition of the AJCC Cancer Staging Handbook [22]. In order to reach data consistency, the diagnostic pathology slides were reviewed by three independent pathologists (ZS, IS, AA). The clinical data were reviewed by an experienced surgeon (AV). The survival was defined as an interval between the date of surgery and the last follow-up or death due to pancreatic cancer. Censoring occurred if patients were alive at last follow-up or died due to other reasons.

Immunohistochemistry

Immunohistochemical (IHC) staining of formalin-fixed paraffin-embedded tumour tissue was performed using panel of primary antibodies (Table 1). In brief, 3-micrometre-thick sections were cut on electrostatically charged glass slides (Histobond, Marienfeld, Germany) and subjected to deparaffinisation in graded alcohols and xylene. Heat-induced antigen retrieval was performed in microwave oven (3x5 min.) using basic TEG buffer (pH 9.0). After blocking of endogenous peroxidase, the sections were incubated with primary antibodies at room temperature for 60 min. The bound primary antibodies were detected by enzyme-conjugated polymeric visualisation system EnVision linked with horseradish peroxidase. 3,3'-diaminobenzidine was used as chromogen, followed by counterstaining implying Meyer's hematoxylin. All IHC reagents were produced by Dako (Glostrup, Denmark). Positive and negative controls were performed and reacted appropriately.

Evaluation of immunohistochemical staining

The expression of all markers was evaluated in the appropriate subcellular compartment of the neoplastic cells.

Table 1. The applied panel of primary antibodies: characteristics of antibody and applied dilution

Antigen	Antibody	Clone	Producer	Code	Dilution
Ki-67	Monoclonal mouse anti-human Ki-67 antigen	MIB-1	Dako, Glostrup, Denmark	M7240	1:100
p53	Monoclonal mouse anti-human p53 protein	DO-7	Dako, Glostrup, Denmark	M7001	1:400
Bcl-2	Monoclonal mouse anti-human Bcl-2 oncoprotein	124	Dako, Glostrup, Denmark	M0887	1:800
Vimentin	Monoclonal mouse anti-vimentin	V9	Dako, Glostrup, Denmark	M0725	1:200
CD44	Monoclonal mouse anti-human CD44, phagocytic glycoprotein-1	DF1485	Dako, Glostrup, Denmark	M7082	1:50

Ki-67 and p53 expression were counted as positive if only nuclei of tumour cells were stained. Bcl-2 and vimentin expression was evaluated in the cytoplasm and CD44 in the membrane of neoplastic cells. The expression was graded by intensity as 0, negative; 1, weak; 2, moderate and 3, intense. Only moderate or intense reactivity was considered positive. After intensity evaluation, positive tumour cells were counted among 500 malignant cells and expressed as the percentage.

Statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS, version 20). Data were expressed as mean $\pm$ standard deviation (SD) or percentage. The 95% confidence interval (CI) was invariably calculated by Confidence Interval Analysis (CIA) software [23]. For survival analysis Kaplan – Meier method was used. Survival for positive versus negative IHC staining was compared with log-rank test. In multivariate analysis Cox regression analysis was used. Cox regression analysis was used to determine the relative impact of tumour stage, T (size and local invasion of primary tumour), G (histological grade), N (regional lymph nodes regarding metastasis), R (status of resection margins) parameters and positive versus negative IHC staining on survival. Correlation between SR and expression of the studied IHC markers (count of positive cells), tumour size and the mutual correlation of these parameters were analysed by Spearman's rank correlation. T-test was used to determine the correlation between morphological factors (tumour size, N stage, surgical resection margins, vascular and perivascular invasion, perineural and intraneural invasion) and SR as well as expression of Ki-67, p53, Bcl-2, vimentin and CD44. Correlation between histologic grade and SR was evaluated by Kruskal-Wallis test. ANOVA was applied to determine correlation between pathologic stage and SR. The p-values of ≤ 0.05 were considered statistically significant for all analyses.

Ethical considerations

The study was carried out in accordance with the Declaration of Helsinki and was approved by the Committee of Ethics, Riga Stradins University.

Results

Clinical and morphological characteristics of the study group

During the evaluated period (2004-2012), 63 patients underwent potentially curative resection of pancreatic carcinoma. The main clinical and morphological findings are summarized in Table 2. Patients' mean age was 63.2 ± 10.8 years (range 38-81; 95% CI = 60.5-65.9). Pancreatoduodenectomy was performed in 54/63 (85.71%; 95% CI = 75.0-92.2) cases, distal pancreatectomy in 5/63

(7.9%; 95% CI = 3.5-17.3) cases and total pancreatectomy in 4/63 (6.4%; 95% CI = 2.6-15.2) cases. In addition, splenectomy was carried out in 5/63 (7.9%; 95% CI = 3.5-17.3) cases. The mean tumour size was 3.7 ± 1.4 cm (range 1.5-9; 95% CI = 3.2-4.1). After evaluation of TNM parameters, tumour stage was following: IA in 1.6% (95% CI = 0.4-8.7) cases, IB in 3.3% (95% CI = 1.0-11.1) cases, IIA in 26.2% (95% CI = 16.8-38.5) cases, IIB in 62.8% (95% CI = 49.7-73.4) cases, III in 1.6% (95% CI = 0.4-8.7) cases. In 4.9% (95% CI = 1.8-13.5) cases, the tumour stage was IV due to the presence of liver metastases. As

Table 2. Clinical and morphological characteristics of the study group

Parameters	No. of patients	Percentage of patients (%)	95% Confidence Interval
Age (years)			
≤60	26	41.3	29.9 – 53.6
>60	37	58.7	46.4 – 70.1
Gender			
Male	30	47.6	35.8 – 59.8
Female	33	52.4	40.2 – 64.3
Tumour localization			
Pancreatic head	54	85.7	75.0 – 92.2
Other	9	14.3	7.8 – 25.0
Tumour size (cm)			
≤2	4	9.1	3.7 – 21.2
>2	40	90.9	78.8 – 96.3
≥3	30	68.2	53.4 – 80.0
T stage			
T1 and T2	3	4.8	1.8 – 13.3
T3 and T4	59	95.2	86.7 – 98.2
N stage			
N0	20	32.8	22.3 – 45.3
N1	41	67.2	54.7 – 77.7
Tumour grade			
Low	8	12.7	6.6 – 23.2
Moderate	39	61.9	49.5 – 72.9
High	16	25.4	16.3 – 37.4
Resection margins			
Negative	28	48.3	35.9 – 60.9
Positive	30	51.7	39.1 – 64.1
Vascular and perivascular invasion			
Absent	41	65.1	52.7 – 75.7
Present	22	34.9	24.3 – 47.3
Intraneural and perineural invasion			
Absent	10	15.9	8.9 – 26.9
Present	53	84.1	73.1 – 91.1

Abbreviations. T1, tumour limited to the pancreas, 2cm or less in greatest dimension; T2, tumour limited to the pancreas, more than 2 cm in greatest dimension; T3, tumour extends beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery; T4, tumour involves the celiac axis or the superior mesenteric artery; N0, no regional lymph node metastasis; N1, regional lymph node metastasis [22].

Figure 1. Pancreatic ductal adenocarcinoma. Note the perineural invasion and the subsequent spread to the inked surgical resection margin (→). Haematoxylin–eosin (HE), original magnification (OM) 100x.

Figure 2. Immunohistochemical visualisation of Ki-67. (A) Nuclear expression of Ki-67 in few ductal epithelial cells of normal pancreas. Immunoperoxidase (IP), OM 100x. (B) Nuclear expression of Ki-67 in pancreatic ductal adenocarcinoma (PDAC). IP, OM 400x.

Figure 3. Immunohistochemical visualisation of p53. (A) Note the absence of p53 expression in the normal pancreatic duct. IP, OM 400x. (B) Weak nuclear expression of p53 in PDAC. IP, OM 400x. (C) Moderate nuclear expression of p53 in PDAC. IP, OM 400x. (D) Intense nuclear expression of p53 in PDAC. IP, OM 400x.

shown in [Table 2](#), the tumours were mostly larger than 2 cm and localised in the pancreatic head. Perineural and intraneural invasion ([Figure 1](#)) was frequent as well as resection margin involvement by the tumour ([Figure 1](#)) and neoplastic invasion in or around blood vessels.

Immunohistochemical findings

The staining of Ki-67 was localized to the nucleus of tumour cells ([Figure 2](#)). It was found on average in 20.2% (range 1-55%; 95% CI = 17.3-25.0) of malignant cells. By t-test, no correlation was found between Ki-67 and clinical and morphological parameters ($p > 0.05$).

Nuclear staining with p53 was detected in 35/50 (70.0%; 95% CI = 56.2-80.9) of all cases. Aberrant p53 expression was present in 37.5% (range 2-97%; 95% CI = 30.4-44.6) of malignant cells. By independent samples t-test, trend to correlation ($p = 0.053$) was found between p53 expression and peri- and intraneural invasion ([Figure 3](#)).

Bcl-2 expression was found in the cytoplasm of pancreatic ductal epithelium. There was no positive staining in any malignant cell (0/44 cases; 0%; 95% CI = 0-7.9) in contrast to positive expression in lymphocytes ([Figure 4](#)).

The staining results with vimentin were positive in 10/49 (20.4%; 95% CI = 11.5-33.7) cases. Vimentin expression was found in 35.3% (range 3-98%; 95% CI = 24.1-46.5) of malignant cells ([Figure 5](#)). No significant correlation was found between vimentin expression and clinical and or morphological characteristics ($p > 0.05$).

CD44 expression was observed in 42/49 (85.7%; 95% CI =

Figure 4. Immunohistochemical visualisation of Bcl-2 protein. (A) Cytoplasmic expression of Bcl-2 in pancreatic ductal epithelium and lymphocytes. IP, OM 400x. (B) Note the loss of Bcl-2 expression in PDAC, but positive expression in lymphocytes. IP, OM 400x.

Figure 5. Immunohistochemical visualisation of vimentin. (A) Note the absence of vimentin expression in benign pancreatic ductal epithelium. IP, OM 400x. (B) Weak cytoplasmic expression of vimentin in PDAC. IP, OM 400x. (C) Moderate cytoplasmic expression of vimentin in PDAC. IP, OM 400x. (D) Intense cytoplasmic expression of vimentin in PDAC. IP, OM 400x.

Figure 6. Immunohistochemical visualisation of CD44. (A) Weak basal membranous expression of CD44 in benign pancreatic ductal epithelium. IP, OM 400x. (B) Weak membranous expression of CD44 in PDAC. IP, OM 400x. (C) Moderate membranous expression of CD44 in PDAC. IP, OM 400x. (D) Intense membranous expression of CD44 in PDAC. IP, OM 400x.

73.3-92.8) cases. CD44 positivity was found in 28.2% (range 1-97%; 95% CI = 20.5-35.9) of malignant cells (Figure 6). By using independent sample t-test, there was statistically significant association between CD44 expression and presence of metastases in regional lymph nodes ($p = 0.022$). Positive correlation was found between CD44 and Ki-67 ($p = 0.010$) as well as a trend to correlation between CD44 and vimentin expression ($p = 0.058$) by Spearman's rank correlation.

Survival analysis

The mean patients' survival was 19.5 month (95% CI = 11.3-27.8) with 1- and 3-year survival rates of 41.8% (95% CI = 29.7-55.0) and 7.3% (95% CI = 3.0-17.3). Survival curve by Kaplan-Meier analysis is shown in Figure 7. Survival for one patient was 99 months (till June 2012). It was a female patient age 68 years. The greatest diameter of tumour was 2.5 cm. Cancer invaded peripancreatic fat tissue, but resection margins were free of tumour at extensive sampling. The tumour was characterised by moderate histologic grade, presence of mitoses, lymph node metastasis, and perineural invasion (Figure 8).

Tumour size, lymph node status, pathologic stage, tumour grade, surgical resection margin status, vascular and perivascular invasion, peri- and intraneural invasion, Ki-67, p53, Bcl-2, vimentin and CD44 expression were evaluated as possible factors affecting survival. When analyzing

Figure 7. Survival for patients of pancreatic ductal adenocarcinoma after potentially radical surgical treatment. Kaplan-Meier survival analysis.

patients' survival with Spearman's rank correlation, positive correlation between poor prognosis and high proliferative activity by Ki-67 ($p = 0.022$) as well as vimentin expression ($p = 0.023$) was found. By independent samples t-test, the outcome correlated with tumour grade ($p = 0.017$) and positive surgical resection margins ($p = 0.039$). Log-rank test did not show correlation between SR and positive versus negative IHC staining. Cox regression analysis also yielded no association between SR and tumour stage, T, G, N, R parameters and positive versus negative IHC staining. The other analyzed factors did not show any significant correlation with survival.

Discussion

Extensive studies have been made to explore prognostic and predictive factors of pancreatic carcinoma. As a result, several factors have been identified that convincingly correlate with a progression of the carcinoma, resistance to chemo- and radiotherapy and survival rate of patients. However, contradictory findings have been published regarding several parameters [6,14,17]. The aim of this study was to acquire comprehensive data of characteristic features of pancreatic carcinoma in the Latvian population, frequency of the known unfavourable prognostic factors, the extent of the immunohistochemical expression of several biologic markers and the impact of all the acquired parameters on the survival rate of the patients.

In our study the mean age of patients at the time of diagnosis was 63.2 years (95% CI = 60.5-65.9). This is in

Figure 8. Pancreatic ductal adenocarcinoma of patient with 99 months survival. (A) PDAC with moderate histologic grade. Note the mitosis. HE, OM 400x. (B) PDAC showing perineural invasion. HE, OM 100x. (C) Lymph node metastasis of PDAC. HE, OM 100x.

accordance with several other studies that have described the mean age of PDAC patients as 60.5 to 65 years [15,24,25]. Considering the CI, our study shows slight but statistically significant difference with the publication of Yang *et al.*, where the mean age is 67 years [11]. The mean patients' survival was 19.5 month (95% CI = 11.3-27.8) with 1- and 3-year survival rates of 41.8% (95% CI = 29.7-55.0) and 7.3% (95% CI = 3.0-17.3). In the study of An *et al.*, the survival characteristics of surgically treated patients are similar: 12.2 month, 38% and 10%, respectively [15]. However, at least one study has detected statistically significantly different three year survival [12] that could be attributed to smaller tumour size and lower rate of metastases in regional lymph nodes. The differences could also be caused by genetic structure of the population as well as environmental and healthcare factors but in general our group reflects the problems in the treatment of pancreatic cancer and thus the results are applicable for the characteristics of this tumour in general.

Successful radical surgery is one of the most important prognostic factors in case of pancreatic carcinoma. The extent and type of surgery depends on the localization and spread of the cancer. Most frequently the tumour is localized in the head of pancreas therefore pancreatoduodenectomy is mostly necessary [12,13,26]. In our study it was performed in 85.7% (95% CI = 75.0-92.2) cases, and total pancreatectomy was done in 6.4% (95% CI = 2.6-15.2) cases. In another study pancreatoduodenectomy was performed in 335/356 (94.1%) and a total pancreatectomy in 21/356 (5.9%) of cases [13]. Thus, the surgical approach in our study also is comparable to the accepted practice. Significant correlation between the total pancreatectomy and shorter survival ($p = 0.04$) has also been described [13]. It should be taken into account that a total pancreatectomy is performed in cases when the tumour is multifocal or widely invasive and it is impossible to perform an anastomosis of pancreas due to risk of fistula. All these features are unfavourable characteristics of carcinoma. The general health status and thus the prognosis worsens after total pancreatectomy also due to exocrine and endocrine insufficiency, malabsorption, unmanageable level of glycemia and possible liver failure [27,28].

The most frequently noted prognostic factors are tumour size, metastasis in lymph nodes, distal metastasis, high tumour grade and positive resection margins. Regarding tumour size by the largest diameter, two values have been described as prognostic threshold: 2 cm ($p = 0.02$) and 3 cm ($p = 0.02$) [12,13,26]. In our study there was no correlation between the tumour size and survival. The size of tumour was larger than 2 cm in 90.9% (95% CI = 78.8-96.3) cases exceeding the frequency described by Lim *et al.* – 239/309 (77.3%) cases as well as by Allema *et al.* – 98/176 (55%) cases [12,26]. In our group, the tumour size exceeded 3 cm in 68.2% (95% CI = 53.4-80.0) cases

that also is statistically significantly more than in other studies, where it was 42% and 139/356 (39.1%), respectively [13,25]. There is a statistically significant difference in the N1 parameter between our data, namely, 67.2% (95% CI = 54.7-77.7) and other three studies – 37.0%, 193/396 (48.7%) and 80.0% [12,24,25]. Although in our study we found no correlation between N stage and survival rate, in other studies presence of lymph node metastases is described as a significant factor ($p = 0.01$ or 0.05) that affects the survival [12,13]. It is likely that the generally large tumour size and high rate of regional lymph node metastases in our group embarrass the prognostic evaluation of these findings. Regarding the postsurgical survival correlation with tumour grade and resection margins, it was found that survival rate is worse in the group of patients with a high grade tumours ($p = 0.017$) and positive resection margins ($p = 0.039$). Similar results ($p = 0.01$; $p = 0.01$) are described by Handra - Luca *et al.* [13].

The progression of pancreatic carcinoma and resistance to chemotherapy is thought to be associated with lack of apoptosis and adhesion, higher proliferation and epithelial-mesenchymal transition in neoplastic cells. To acknowledge the frequency and significance of each factor, the immunohistochemical staining was performed, detecting Ki-67, p53, Bcl-2, vimentin and CD44.

Ki-67 is one of the most important markers that indicate active cellular proliferation. The highest expression of this protein is in G_2 phase of proliferative cells. Expression is noted also in phases G_1 , S and M, but no expression is found in phase G_0 [29]. Ki-67 expression correlates with the tumour grade and lymph node metastasis [29]. It has also been stated that the expression of Ki-67 becomes prognostically significant if it exceeds 5% [6,7]. Our results show that the level of the Ki-67 expression is statistically significantly related to postoperative survival ($p = 0.022$), although there are some other studies that have not been able to demonstrate this correlation [30,31].

p53 is a tumour suppressor protein. The corresponding gene is located in chromosome 17p. p53 protein participates in DNA reparation, as well as in the regulation of the cell cycle and apoptosis. P53 gene mutation is the most frequent known mutation in malignant tumours, found in about 50-75% cases of PDAC [6,10]. Expression of aberrant p53 has been noted also in 10% of cases of normal pancreatic tissue [32]. A rapid elevation of p53 positive cell count has been detected in the 3rd stage of pancreatic intraepithelial neoplasia, known also as carcinoma in situ. It follows that p53 is an early and important biological marker in the development and diagnosis of pancreatic carcinoma [33]. In our study p53 was found in 70% (95% CI = 56.2-80.9) of cases. The results about the expression of p53 in the tumour cells and its correlation with a survival are contradictory [6,14,34]. In our study p53 expression showed trend to correlation with peri- and intraneural invasion ($p = 0.053$)

but not with survival.

Bcl-2 is an anti-apoptotic protein that regulates apoptosis by mediating cytosolic release of cytochrome C from mitochondria in response to cellular stress [35]. Although expression of Bcl-2 is described in 12-67% of PDAC cases [14], no expression of Bcl-2 in malignant cells was identified in our study. The differences can be attributed to technological factors as incubation time and dilution of primary antibody. In our study, appropriate positive controls were used to ensure the quality of investigation. The presence of reactivity in lymphocytes and normal ductal epithelia is likely to exclude technological problems that theoretically could be related to prolonged storage of paraffin blocks. Expression of Bcl-2 in tumour cells has been noted as independent favourable prognostic factor and unfavourable predictive factor [14,36-38]. Resistance to chemotherapy-induced apoptosis is associated with over-expression of Bcl-2 in pancreatic cancer [16,17]. A positive correlation has been found between the Bcl-2 expression and tumour invasion and development of metastases [16,38].

Vimentin is an intermediate-size filament polypeptide that is expressed in normal mesenchymal tissues, but normally is absent in epithelium of pancreatic ducts. Expression of vimentin in cancer cells is one of the manifestations of epithelial-mesenchymal transition [13,18,24]. Expression of vimentin in PDAC cells is mentioned in about 45% of cases, that statistically significantly differs from our study, where the expression of vimentin was found in 20.4% (95% CI = 11.5-33.2) pancreatic carcinoma cases. In the study of Handra-Luca *et al.*, a correlation between vimentin expression in tumour cells and worse survival ($p = 0.02$) was found similarly to our results ($p = 0.023$). A correlation of vimentin with a high grade, marked invasive growth and metastatic ability has also been described. However, in our study there was no significant relation between these factors. Similarly to Bcl-2, vimentin expression is related to the resistance to chemotherapy [13,24,25].

CD44 is an integral cell surface glycoprotein and a type I transmembrane adhesion molecule. It mediates cell differentiation, migration and development of tumour metastases [20]. In our study CD44 was expressed in 85.7% (95% CI = 73.3-92.8) cases and these results statistically significantly differ from the study of Immervol *et al.* [19] describing lower expression rate of 67%. Expression of CD44 is related to worse prognosis, but the published results are not unequivocal [19]. In our study no correlation was found between CD44 and survival, but there was a correlation between CD44 and N stage ($p = 0.022$), proliferative activity ($p = 0.010$). Trend to correlation between CD44 and vimentin expression ($p = 0.058$) was found. The latter two parameters also correlated with the survival of patients. Other studies show that CD44 expression correlates also with tumour invasion and epithelial mesenchymal transition [20,39].

In general, the survival analysis discloses significant

problems in the treatment of pancreatic carcinoma. The identified biologic markers could become an important target for future therapeutic intervention if expressed with significant frequency as CD44 and p53 and associated with important characteristics of neoplastic process as proliferation and development of metastases. However, the constellation of the observed proliferative activity level and high frequency of markers known for the association with resistance to chemotherapy also point towards significant role of surgery in the present treatment of pancreatic carcinoma.

Conclusions

1. In case of pancreatic ductal adenocarcinoma, the survival after potentially curative resection is poor, with the mean value of 19.5 month. However, the constellation of tumour biological properties still points towards the crucial role of surgery in the treatment of pancreatic ductal adenocarcinoma.

2. High tumour grade, positive resection margins, high proliferation fraction and epithelial-mesenchymal transition negatively affect patients' survival.

3. CD44 expression is frequent finding in pancreatic ductal adenocarcinoma, occurring in 85.7% of cases. It correlates with basic features of neoplastic process as proliferation (by Ki-67) and metastatic spread to regional lymph nodes. This biological marker could be considered as a therapeutic target in the future.

4. The frequently observed expression of aberrant p53 protein trends to correlate with perineural and intraneural invasion.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

All authors participated in the design of the study. ZS carried out the archive search. ZS, IS, AA performed the re-evaluation of surgical slides to reach consensus estimate of morphological data. AV re-evaluated the clinical data. ZS performed immunohistochemical stains. ZS, IS and AA evaluated the immunohistochemical data in order to reach consensus statement. AA assisted with survival data obtaining and analysis. ZS and AV were responsible for statistical analysis. ZS, IS and AV performed the literature studies. ZS wrote the draft; IS and JG improved the manuscript. All authors read and approved the final manuscript.

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