UNIVERSITATEA DE MEDICINĂ ȘI FARMACIE
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OPTIMIZATION OF DIAGNOSTIC AND TREATMENT METHODS IN PANCREATIC MALIGNANT TUMORS

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The doctoral thesis includes:

- A total of 169 pages of which the general overwiew takes 49 pages
- The iconography consists of 115 figures and 33 tables
- Bibliographic references include 391 items from national and international literature
- List of articles published during the PhD research period: 1 BDI article and 2 ISI articles as first author

Note: The abstract selectively renders the iconography and bibliography in the text.

Keywords: pancreatic cancer, inhumhistochemistry, E-cadherin, EGFR, HER2-neu

1. Introduction

The pancreas was one of the last described organs of the abdominal cavity, on which anatomists, pathophysiologists and surgeons turned their attention. The earliest known description of pancreatic cancer was provided in 1761 by Giovanni Battista Morgagni, an Italian pathologist who spent most of his career at the University of Padua, who reported the presence of pancreatic lesions but lack of a microscope and a histological report at that time only made it possible to raise the assumption of a diagnosis of pancreatic cancer (1, 2).

In 1912, Walther Carl Eduard Kausch, a German surgeon, performed the first successful pancreatic duodenectomy in two stages and, two years later, Georg Hirschel described the first successful pancreatic duodenectomy performed in a single procedure (3). In 1937, Alexander Brunschwig extended the indication for pancreatico-duodenectomy to include pancreatic head cancer (4).

In the 1980s and 1990s, a dramatic decline in hospital mortality was achieved in several centers, and in recent years it has been shown that the mortality rate could be reduced to below 5% by concentrating experience in specialized centers (4).

After the 1990s, there was a development of diagnostic imaging technology that provides an accuracy in staging pancreatic cancer so necessary to establish appropriate therapeutic behavior. In all this type, the

surgical techniques have been improved, there are specialized centers for pancreatic surgery, which lead to the shortening of the operating time, to the decrease of the complication rate and to the reduction of the hospitalization period (5).

As we gather more genomic, proteomic, and immune information from pancreatic neoplastic cells and accumulate evidence of this type of cancer, we expend molecular treatment approaches. The efforts of cancer associations, and cancer research centers will play an important role in providing patients access to personalized treatment, so that hypotheses about the predictive value of tumor markers can be tested (6).

Many studies are planing to investigate immunotherapy for the treatment of pancreatic adenocarcinoma. This neoplasm is known for its immunosuppressive impact on the body, which makes immunotherapeutic approaches that have been used successfully in other cancers ineffective as single agents in the therapeutic plan to pancreatic lesions. Immune therapy is changing the current treatment paradigm for malignancy, especially through the recent development of antibodies that can modulate immune control pathways (10).

Preliminary results suggest that these tactics may be successful in combating immunosuppression, but do not lead to cancer cell death. Therefore, the focus shifted to the discovery of a combination of checkpoint inhibitors with a therapeutic vaccine strategy or other agents, such as chemotherapy or radiation (5,6).

2. Purpose and objectives of the study

Pancreatic malignancies are a public health problem and remain among one of the fastest rates of progression despite decades of research conducted to better understand molecular biology and cell signaling pathways. The limited therapeutic possibilities depend on the advanced stage at the time of the first diagnosis, on the anatomy of the pancreas which limits the diagnostic possibilities and delays the first diagnosis.

The specific objectives in carrying out this thesis were:

I. Study of serum biomarkers for resectable pancreatic cancer to detect prognostic factors for resectable pancreatic cancer

II. Classification of clinical and histopathological risk factors for pancreatic cancer

III. Characterization of the morphological structure of pancreatic ductal adenocarcinoma (PDAC), pTNM analysis, degree, manifestations of invasive growth

IV. Evaluation of the results of pancreatic resections in relation to the duration of surgery, local factors (anatomical variants of the hepatic arteries in duodenopancreatectomy and their implications in choosing the surgical approach), complications, length of hospitalization and mortality;

V. Evaluation of the molecular profile of PDAC - immunohistochemical evaluation, analyzing the frequency and extent of expression and the degree of

concordance in terms of tissue expression of EGFR, HER 2 -neu and E-cadherin in primary pancreatic cancer, to elucidate the importance of these molecules for treatment disseminated disease;

VI. Detection of the survival of patients affected by surgically treated PDAC and the factors that influence it;

The secondary objectives consisted of:

I. Detection of risk groups in pancreatic cancer

II. Optimization of the complementary treatment algorithm (chemotherapy and / or radiotherapy) in patients with pancreatic neoplasm

III. Detection of factors with impact on the occurrence of recurrences (biological, immunohistochemical factors) and ways of influencing them

IV. Optimization of oncological follow-up, as a factor to improve the vital and functional prognosis of the patient with pancreatic malignancies

3. Material and method

The present research represents a study carried out on a group of 349 patients diagnosed with pancreatic cancer in the I and II Surgery Clinics of the County Emergency Clinical Hospital „St. Spiridon ”from Iași in an interval of 11 years between January 2005 and December 2015.

The study was performed retrospectively and the data were centralized using the hospital's computerized

system (INFOWORD), analysis of patients sheets, operating protocols and histopathological reports analysis .

A number of 81 patients received curative surgery related to the location of the tumor process, and the remaining 268 patients underwent surgery for diagnosis or palliative treatment.

In the group of patients with curative pancreatic resections, we performed a battery of immunohistochemical tests on the paraffin blocks existing in the archive of the pathological anatomy service with the evaluation of the expression of E-cadherin, EGFR and HER 2-neu.

The clinical - biological and imaging follow-up of these patients was performed until June 2018, when we completed the statistical processing of the data. The introduction of the subjects in the study group was made based on the inclusion and exclusion criteria.

Inclusion criteria:

• patients with pancreatic neoplasm diagnosed on the basis of imaging and biological examinations or hystopathological examination,

• staging of pancreatic tumors with the help of clinical, biological and imaging explorations,

• signing the informed consent according to the legislation in force, consent attached to this scientific report

Exclusion criteria:

• refusal of patients to participate in clinical-paraclinical investigations or recommended therapeutic conduct

• loss of patients' records from the time of diagnosis until the completion of doctoral studies or death

• patients under 18 years of age

•patients who die of a cause other than pancreatic neoplasm

Statistical analysis was performed using the IBM SPSS Statistics Version 20.0 statistical software package (International Business Machines Corp., Armonk, New York, USA). The confidence interval (CI) was invariably calculated using the confidence interval analysis software. For the survival analysis, the Kaplan-Meier method was used with the log-rank test and the Cox proportional risk model. $P \leq 0.05$ values were considered statistically significant for all analyzes.

4. Results

4.1. Statistical analysis of clinical-paraclinical and perioperative parameters

The doctoral study was conducted retrospectively over a period of 11 years, namely between 2005 and 2015, which included patients diagnosed with pancreatic malignancies in the Emergency County Clinical Hospital St. Spiridon, Clinic I and II Surgery and who benefited of surgical treatment with palliative and curative purpose. During this period, 349 patients were identified,

aged between 31 and 91 years, with a mean age of 69.33 years (Tab 4.1.I).

Table 4.1.I. Distribution of patients by years of study according to age

Years of study	Age							TOTAL
	30-39	40-49	50-59	60-69	70-79	80-89	>90	
2005	1	0	3	5	1	0	1	**11**
2006	0	2	9	8	5	3	1	**28**
2007	0	0	6	13	15	8	2	**44**
2008	1	2	10	9	10	6	0	**38**
2009	0	1	8	18	10	9	1	**47**
2010	0	0	7	14	17	3	1	**42**
2011	0	0	9	14	11	4	0	**38**
2012	1	4	6	3	6	5	0	**25**
2013	0	0	2	5	9	5	0	**21**
2014	0	1	1	10	9	5	0	**26**
2015	0	2	2	15	6	2	1	**28**
Total	**3**	**12**	**63**	**115**	**99**	**50**	**7**	**349**

Sclerotegumentary jaundice was the dominant symptom that determined addressability to the doctor (65.90%), being a direct consequence of local tumor status in 94.6% of cases and in 5.4% of cases due to secondary hepatic dissemination. Pain was also an important factor in the onset symptoms, being found in a proportion of 43.26%, more commonly associated with distal pancreatic lesions, representing the motivation of these patients for addressability to the doctor.

Other nonspecific symptoms such as physical asthenia or marked weight loss (> 10 kg in the last 3 months) were reported with a lower weight, but with a

significant impact on the quality of life and performance status of patients. In 4 cases, the onset symptomatology was represented by upper digestive hemorrhage (HDS) secondary to intragastric (1 case) or intraduodenal (3 cases) tumor extension (Tab 4.1.II).

Table 4.1.II Statistical analysis of signs and symptoms associated with pancreatic neoplasm

DESCRIPTIVE ANALYSIS OF SYMPTOMS						
		Statistic	Bias	Std. Error	95% Confidence Interval	
					Lower	Upper
Fatigue	N	349	0	0	349	349
	Sum (%)	108 (28.02)				
	Mean	.08	.00	.01	.05	.11
	Std. Deviation	.272	-.001	.023	.222	.312
Jaundice	N	349	0	0	349	349
	Sum (%)	230 (65.90)				
	Mean	.66	.00	.02	.63	.70
	Std. Deviation	.474	.000	.006	.460	.484
Pain	N	349	0	0	349	349
	Sum (%)	151 (43.26)				
	Mean	.43	.00	.03	.39	.49
	Std. Deviation	.496	-.001	.004	.487	.501
Digestive stenosis	N	349	0	0	349	349
	Sum (%)	43 (12.32)				
	Mean	.12	.00	.02	.09	.16

	Std. Deviation	.330	-.001	.020	.289	.365
Weight loss	N	349	0	0	349	349
	Sum (%)	52 (9.16)				
	Mean	.09	.00	.01	.07	.12
	Std. Deviation	.289	-.001	.021	.249	.326
HDS	N	349	0	0	349	349
	Sum (%)	4 (1.14)				
	Mean	.01	.00	.01	.00	.02
	Std. Deviation	.107	-.004	.029	.054	.150
Valid N	N	349	0	0	349	349

Statistical analysis of the onset symptoms in relation to the location of the lesions reveals statistically significant strong correlations regarding jaundice, pain and weaker in terms of weight loss (tab. 4.1.III).

Table 4.1.III. Analysis of symptoms in relation to the location of the primary lesion

		Loss of appetite	Jaundice	Pain	Digestive stenosis	Weight loss
Tumor Location	Pearson Correlation	-.076	-.413**	.185**	.105	.145**
	Sig. (2-tailed)	.157	.000	.001	.050	.007
	N	349	349	349	348	348
** Correlation is significant at the 0.01 level (2-tailed).						

Jaundice was the dominant symptom in cephalic pancreatic lesions, showing a slow onset over several

days, weeks, accompanied by hyperchromic urine and alcoholic stools at values greater than 3 mg / dl. The presence of abdominal pain syndrome was found mainly in cephalic and bodily locations as well as in large lesions accompanied by celiac plexus syndrome.

The staging of the pancreatic neoplasm was performed on clinical, lab results and imaging criteria, corroborated with intraoperative findings. Staging of pancreatic tumors was performed according to AJCC (American Joint Committee on Cancer) 8th edition of 2018.

Most patients introduced in this study had large tumors in the T3 or T4 tumor stage, representing about 90% of cases . T2 tumors accounted for only 6.88%. The diagnosis of pancreatic tumors in the T1 stage was incidental, they were detected by computed tomography in the context of an associated pathology or a sudden symptomatology.

Lymph node involvement was detected in 92.84% of patients, the highest share showing stage N1 (60.46%), and in 32.38% of cases stage N2 was detected at the time of the first diagnosis.

The presence of metastases was highlighted in 45% of cases, the most common being hepatic (25%), followed by peritoneal (7%).

The clinical, blood testing and imaging evaluation of the patients also revealed secondary bone and lung lesions and in 1 patient the presence of multiple brain metastases was found (fig. 4.1.1).

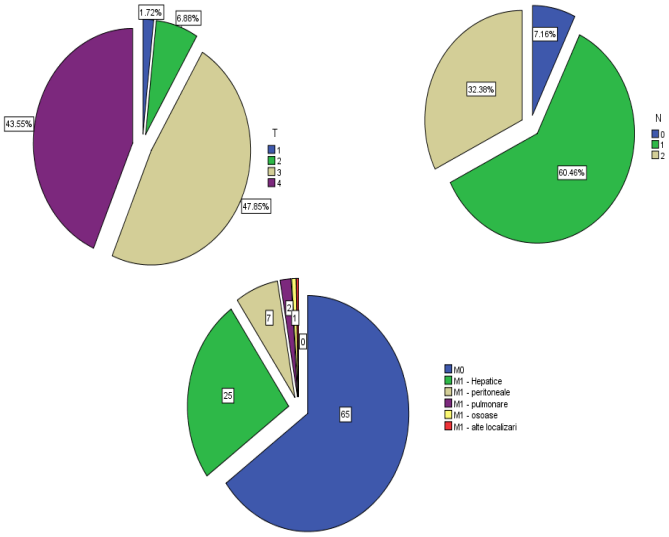


Fig. 4.1.1 Distribution of cases according to TMN staging

Statistical analysis of tumor stage in relation to lymph node involvement revealed lymph node metastases associated with locally advanced tumor stages (T3 and T4), with a higher rate of N2 stage in patients with T3 tumors, without finding statistically significant correlations between them (tab 4.1.IV).

Tabel 4.1.IV. Correlations between the stage of the primary tumor and the lymph node

		TUMOR STAGE	LYMPH NODE STAGE
TUMOR STAGE	Pearson Correlation	1	.137*
	Sig. (2-tailed)		.010
	N	349	349
*. Correlation is significant at the 0.05 level (2-tailed).			

The presence of distant metastases was highlighted in cases with T3 or T4 tumors (fig 3.1.4.2.3), being reported a statistically significant correlation between tumor stage and distant secondary lesions (tab. 4.1.V).

Tabel 4.1.V. Correlations between primary tumor stage and metastases

		TUMOR STAGE	METASTASES
TUMOR STAGE	Pearson Correlation	1	.348**
	Sig. (2-tailed)		.000
	N	349	349
**. Correlation is significant at the 0.01 level (2-tailed).			

All patients in the study group benefited from surgery either for diagnostic purposes (laparoscopy or exploratory laparotomy allowing the assessment of lesions, evaluation of local extension and biopsy), or for therapeutic purposes. palliative purpose (tab.4.1.VI).

Table 4.1.VI Distribution of patients according to surgery

INTERVENȚIA CHIRURGICALĂ	NR. CAZURI	%
Diagnostic laparoscopy	13	3.72
Diagnostitc laparotomy	24	6.87
Bilioenteric anastomosyi	139	39.82
Gastroeneterinc anastomosis	25	7.16
Double digestive anastomosis	42	12.03
2 steps double digestive anastomosis	11	3.15
Pancreatic resection	81	23.20
Colecistostomy	8	2.29
Splahnicectomiy	6	1.71

Locoregional status, the presence of ganglion and distant metastases are the decisive factors in establishing therapeutic behavior.

In the studied group were identified 81 patients (23.20%) who received surgical treatment with curative visa. Their distribution by year maintained the same characteristics as the general group.

The age of patients who benefited from pancreatic resections was between 31 and 79 years, with an average age of 61.54,.

The evaluation of the tumor marker CA 19-9 in relation to the characteristics of the primary tumor showed statistically significant correlations between the level of CA19-9 and lymphatic invasion, by associating values> 200 IU / ml with the constant presence of intratumoral lymphatic invasion (tab. 4.1.VII).

Tabel 4.1.VII. Table 4.1.VII. Analysis of correlations between CA19-9 and tumor characteristics

		CA 19-9	Clasif. G	Clasif. T	Clasif. N	Invazie vasculara	invazie perineurala	Invazie limfatica
CA 19-9	Pearson Correlation	1	.032	-.120	.060	.367**	-.039	.389**
	Sig. (2-tailed)		.796	.358	.908	.089	.804	.012
	N	81	81	81	81	81	81	81
*. Correlation is significant at the 0.05 level (2-tailed).								

Pancreatic resections such as duodeno-pancreatectomies were associated with a higher number

of days of postoperative hospitalization, this being a surgery with a much higher degree of complexity compared to distal splenopancreatectomies and being associated with a higher rate of postoperative complications. (fig. 4.1.2)

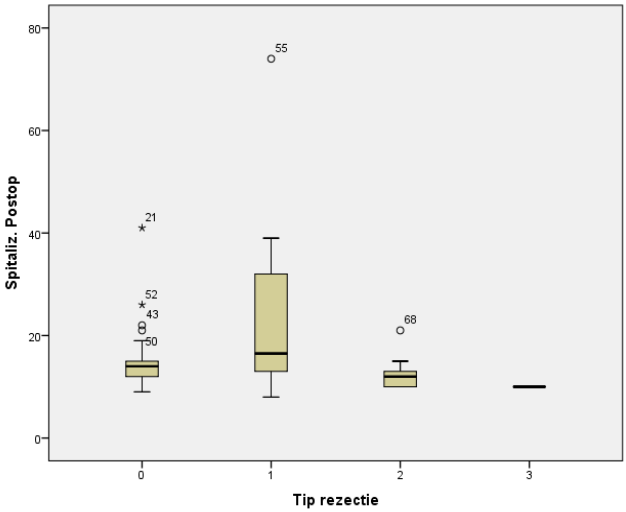


Fig. 4.1.2. Analysis of hospitalization days in relation to the type of surgery (0- DPC type Whipple, 1- DPC with pyloric preservation, 2- caudal splenopancreatectomy, 3 - quasi-total splenopancreatectomy)

Pancreatic resections are large-scale interventions, burdened by multiple complications, these being much more common in the case of cephalopancreatic resections compared to distal ones (Fig. 4.1.3).

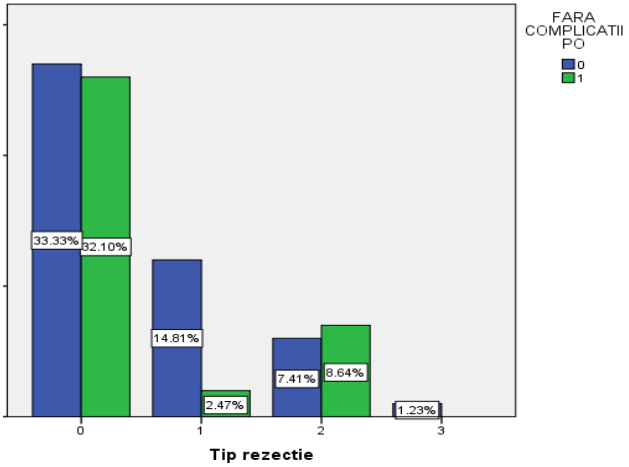


Fig. 4.1.3 Distribution of complications in report with the type of intervention0- Whipple type CPD, 1- Pile preservation CPD, 2- caudal splenopancreatectomy, 3 - quasi-total splenopancreatectomy

The total incidence of complications was 46.92%, the most common being pancreatic fistula and delayed gastric emptying (tab. 4.1.VIII).

Tabel 4.1.VIII. Postoperative complications

		N (%)	Std. Deviation
No complications	81	43 (53.08)	.498
Pancreatic fistula	81	11(13.58)	.418
Delayed gastric emptying	81	11(13.58)	.316
Pancreatititis	81	10(12.34)	.300
Biliary fistula	81	5 (6.17)	.218
Digestive fistula	81	2 (2.46)	.156
Haemoperitoneum	81	5 (6.17)	.300
Intraabdominal abscess	81	6 (7.40)	.242
Wound infections	81	7 (8.64)	.418

Evisceration	81	1 (1.23)	.111
Perioperative mortality	81	14(17.28)	.347

In the studied group, the pancreatic fistula was more frequently found in patients with duodenopancreatectomies, compared to those with distal pancreatectomies in which their number was reduced and also the size of the fistula was self-limiting (tab. 3.2.5.II), without registering differences with statistical impact.

Factors that contributed to these complications were local (soft structure of the pancreas, associated inflammatory lesions, obesity) or technical (poor hemostasis, lack of tightness of the anastomosis, inadequate exposure of structures).

4.2.Statistical analysis of the morphopathological and immunohistochemical characteristics of pancreatic adenocarcinoma

Histological analysis of resection pieces provided information on the macroscopic and microscopic characteristics of pancreatic malignancies (Fig. 4.2.1).

In our study we established the definitive diagnosis by anatomopathological examination performed on the primary tumor, sent to the specialized laboratory as soon as possible after extraction from the peritoneal cavity to achieve proper preservation and fixation in optimal conditions

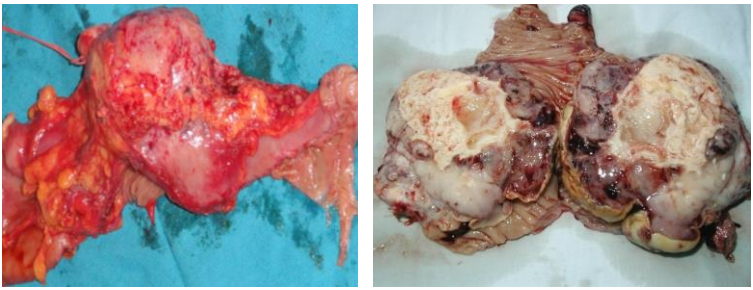
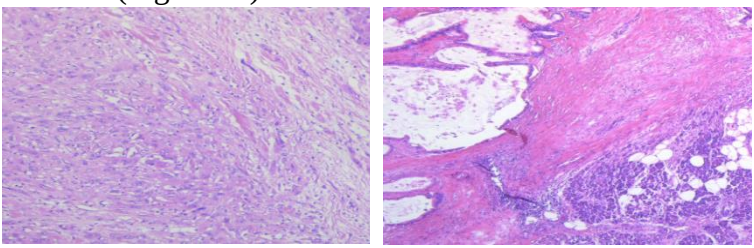


Fig. 4.2.1. Collection of I-II Surgery Clinics. Macroscopic appearance of the pancreatic tumor at the time of extraction of the resection piece from the peritoneal cavity and after fixation in formalin

In 78 patients, adenocarcinoma was identified and in 3 cases cystadenocarcinoma was detected, but all were invasive, and no cases of in situ carcinoma were detected (Fig. 4.2.2).



He 10x HE 40x

Fig.4.2.2 Microscopic appearance of pancreatic adenocarcinomas after staining with hematoxylin eosin (He)

Statistical analysis of the primary tumor stage in relation to tumor grading, lymph node status and the presence of resection margins revealed statistically significant correlations between tumor stage and grading, without detecting in the studied group an association between local primary tumor stage and the

presence lymph node metastases, which are also detected in the T1 stages or with microscopic detection of positive edges at the level of the resection probe (tab. 4.2.I)

Tabel 4.2.I Statistical correlations of tumor grading				
		Tumor grade	Rezection margins	Lymph node staage
Tumor stage	Pearson Correlation	.265*	.000	.193
	Sig. (2-tailed)	.017	1.000	.084
	N	81	81	81
*. Correlation is significant at the 0.05 level (2-tailed).				

The association of perineural and lymph-vascular invasion did not show statistically significant correlations in relation to tumor staging or garding, but strong correlations were found between the presence of perineural invasion and lymphatic invasion (p = 0.00238) and especially with vascular invasion (p = 0 , 000385), but also with resection R0 (tab. 4.2.II).

Tabel 4.2.II Correlations of morphopathological parameters

		Vascular invasion	Perine-ural Invasion	Stage T	Stage N	Rezection R0
Vascular invasion	Pearson Correlation	1	.385**	-.030	181	-.289**
	Sig. (2-tailed)		.000	.795	.107	.009
	N	81	81	81	81	81

Perineural invasion	Pearson Correlation	.385**	1	.111	.311**	-.230*
	Sig. (2-tailed)	.000		.322	..005	.039
	N	81	81	81	81	81
	**. Correlation is significant at the 0.01 level (2-tailed).					
	*. Correlation is significant at the 0.05 level (2-tailed).					

Perineural invasion was a negative prognostic factor being statistically significantly correlated with the presence of lymph node metastases, this invasion being one of the ways of distant metastasis.

Immunohistochemical determination of E-cadherin

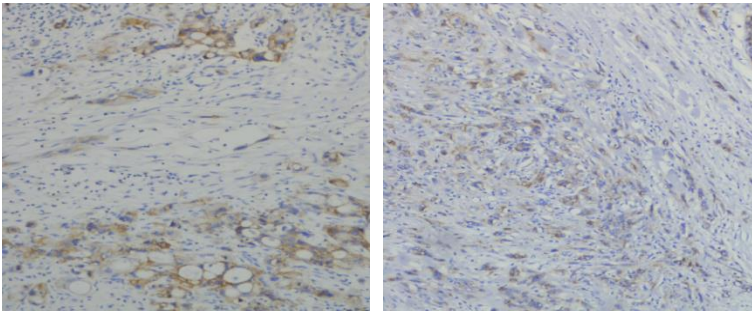
Evaluation of the e-cadherin label was performed by Envision overnight technique after incubation of the antibody (Clone EP700Y). The results were determined by conventional visual microscopy and computerized image analysis methods.

Immunogenic labeling of E-cadherin was considered:

- absent when the label was absent or present in <5% of cancer cells.
- present if the intensity of fixation was strong (2+) or weak (1+) and if the magnitude was greater than or equal to 5% of the cancer cells.

Poorly differentiated pancreatic adenocarcinoma was more susceptible to loss of e-cadherin expression compared to well and moderately differentiated, finding

that cancers with partial loss of e-cadherin were more likely to be poorly differentiated (Fig. 4.2.3).



E –caderina x4 - intensitate 1 E-caderina x10 - pierdere difuză

Fig. 4.2.3 Quantitative immunohistochemical determination of E-cadherin with different intensities in tumor tissue

The score of E-cadherin showed a statistically strong association with the degree of tumor differentiation, but although there was a significant association between stage T of the tumor and the size of e-cadherin loss (tab.4.2.III).

Tabel 4.2.III Statistical analysis of E-cadherin in relation to tumor morphology

		Grad	Stage tumor	Lymph node stage	Lymphatic invasion	Vascular Invasion	Perineural invazie
Scor E-cadherină	Pearson Correlation	-.514**	-.220*	-.054	-.217	-.088	-.120
	Sig. (2-tailed)	.000	.049	.633	.054	.432	.284
	N	81	81	81	81	81	81

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

We compared patient survival with e-cadherin expression classified as either intact or any loss of expression. The median survival in patients with intact e-cadherin-labeled cancer was 13.6 months, while for those with complete loss of e-cadherin tumor label, it was only 3 months (tab. 3.4.2.IV). There was a statistically significant difference in survival between patients whose cancers had intact e-cadherin labeling compared to those with partial loss of e-cadherin and those with total e-cadherin loss (p = 0.007) (Fig. 4.2.4).

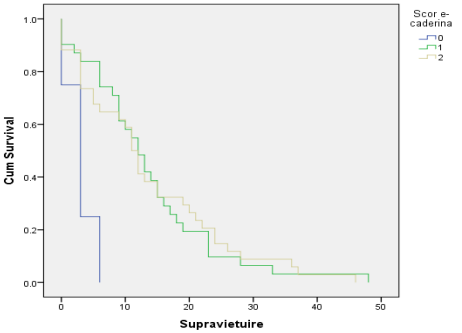


Fig. 3.4.2.15 Survival curve relative to E-cadherin

Immunohistochemical determination of HER2-neu

Evaluation of the IHC results of Her 2-neu labeling was possible in all resected pancreatic adenocarcinomas.

The immunohistochemical examination protocol used in the identification of Her 2-neu consisted of antigenic unmasking at pH6, 1/100 dilution and

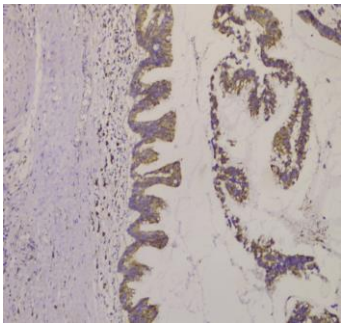
incubation of the antibody (Clone CB11) overnight, which allowed the assessment with the technical support of the Hysto-Pathological Laboratory of the presence of the marker in tumor tissue at the level of paraffin blocks.

According to the scoring guidelines, the cases examined were classified as follows:

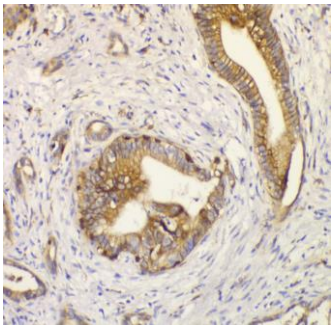
- Score 0: no staining or staining in less than 10% of tumor cells;
- Score 1+: weak, incomplete staining of the membrane in more than 10% of tumor cells;
- Score 2+: complete weak or moderate complete membrane staining in more than 10% of tumor cells
- Score 3+: complete strong staining at the membrane level in more than 10% of tumor cells.

Scores 0 and 1+ were considered negative for HER2 / neu expression while 2+ and 3+ were considered positive (overexpression).

The evaluation of the markings allowed the evaluation of 58% of the cases as 0 and 9% with a weak coloration (score 1+). Overexpression was evident in 33% of cases (score 2+ and 3+) (Fig. 4.2.5).



Scor 2 x10



Scor 3x10

Fig. 4.2.5 Immunohistochemical determination of HER 2-neu

Statistical analysis of correlations of HER 2-neu labeling with tumor morphological aspects and peritumoral parenchymal changes identified a strong association with tumor grading and a less strong one in the presence of pancreatitis lesions (tab. 4.2.IV).

Tabel 4.2.IV. Analiza relației HER 2-neu și caracteristicile tumorale

		Grading	Pancreatita	Lez PAN-IN	Displazie intraductala	IMPN	Invazie vasculara	Invazie perineurala	Invazie limfatica
HER 2-neu	Pearson Correlation	.308**	.321*	.038	-.245	-.163	-.082	.020	.055
	Sig. (2-tailed)	.005	.012	.759	.059	.216	.465	.858	.630
	N	81	81	81	81	81	81	81	81

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

The impact of intense HER 2-neu labeling was not statistically significant on survival curves (p = 0.489) (Fig. 4.2.6).

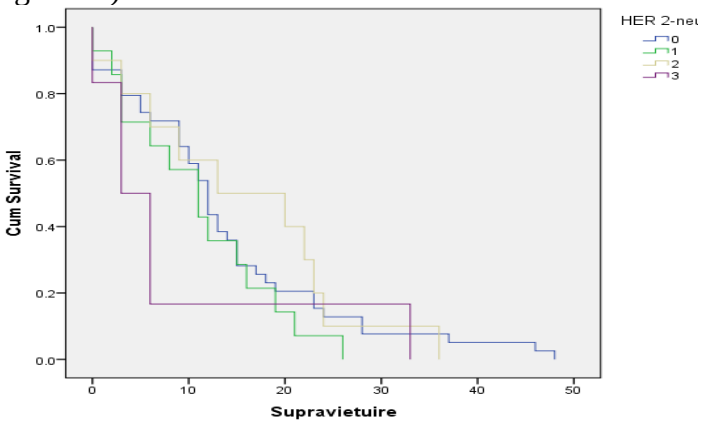


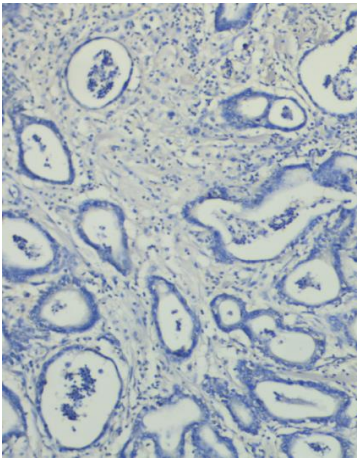
Fig. 4.2.6 Survival curve according to HER 2-neu expression

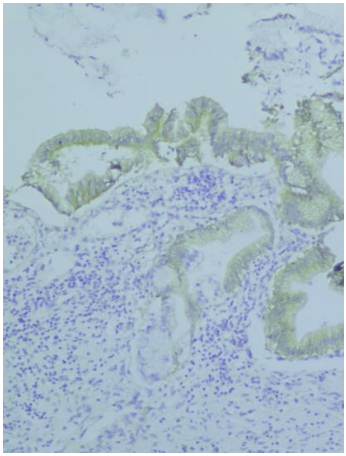
Immunohistochemical determination of EGFR

The evaluation of the EGFR staining was performed in all patients and the scale used for color subclassification is as follows, in accordance with the manufacturer's instructions:

- Score 0: membrane staining absent or less than 10% of tumor cells;
- Score 1+: weak and incomplete membrane staining in more than 10% of tumor cells;
- Score 2+: complete weak or moderate complete membrane staining in more than 10% of tumor cells

- Score 3+: strong complete membrane staining in more than 10% of tumor cells.
-





Scor 0 x 10 Scor 1 x 10

Fig. 4.2.7. Immunohistochemical determination of EGFR and evaluation of labeling scores in various microscopic magnification scales

Score 0 and 1+ represented the normal expression of EGFR and score 2+ and 3+ overexpression of EGFR (fig. 4.2.7).

EGFR overexpression in pancreatic adenocarcinomas was detected in 35% of cases. Of the 81 adenocarcinomas, 11% had a score of 3+ or more labels, and 24% had a score of 2+.

EGFR overexpression was more frequently associated in poorly differentiated tumors, being associated with over 50% of these tumors. The evaluation of the correlations of EGFR overexpression with the morphopathological aspects of the tumor reveals a

statistically significant association with the tumor grading and also with the presence of the HER 2-neu hypermark (tab. 4.2.V).

Tabel 4.2.V Analysis of the EGFR relationship and tumor characteristics							
		HER 2-neu	Grading	Invazie limfatica	Invazie perineurala	Invazie vasculara	Clasif. T
EGFR	Pearson Correlation	.228*	.248*	-.156	-.093	.059	-.103
	Sig. (2-tailed)	.041	.026	.166	.410	.603	.410
	N	81	81	81	81	81	81
*. Correlation is significant at the 0.05 level (2-tailed).							

Patients' age, sex, tumor stage, perineural or lymphovascular invasion were not significantly correlated with EGFR overexpression.

Evaluation of EGFR marking in tumor tissue relative to survival of patients with pancreatic neoplasm identified a mean survival of 15.1 months in those with no or minimal marking for EGFR, while its overexpression was associated with a reduction in survival rate to 11.7 months and 10.3, respectively. months for patients with hypermark 2+ and 3+, respectively.

Statistical analysis did not detect a statistical difference between mean survival and increased EGFR expression at the tumor level (p = 0.274) (tab 4.2.VI).

Tabel 4.2.VI Survival analysis in relation to EGFR expression

EGFR	Mean[a]				Median			
	Estimate	Std. Error	95% Confidence Interval		Estimate	Std. Error	95% Confidence Interval	
			Lower	Upper			Lower	Upper
0	15.152	2.302	10.640	19.663	11.000	1.148	8.749	13.251
1	9.250	2.323	4.697	13.803	6.000	3.000	.120	11.880
2	11.786	2.039	7.790	15.781	12.000	3.118	5.889	18.111
3	10.389	2.193	6.090	14.688	6.000	2.828	.456	11.544
Overall	12.884	1.325	10.287	15.481	11.000	.923	9.191	12.809

The average survival of the patients selected in the current study has a downward curve, influenced by the aggressiveness characteristic of pancreatic neoplasm, which increases from 7.3 months in the general group to 13.5 months in those with curative surgery with limits between 3 months and 48 months.

5. Discussions

Pancreatic cancer is not among the tumors with the highest incidence, but, nevertheless, is the seventh leading cause of cancer mortality in industrialized countries, being known for it's aggression (7, 8). The overall 5-year survival rate is about 6% and an average survival rate ranging from about 2 years for resectable disease to several months in the case of advanced or metastatic local disease (9,10).

In this study there was a higher incidence in males compared to females (50.43: 49.57%), although the addressability in operable stages was higher in females. We also reported an increase in the incidence of pancreatic cancer in the age range 60-79 years, these findings being found in the literature previously presented (7,9,11).

The value of preoperative plasma levels of CA 19-9 to predict pancreatic cancer status and establish resectability has been extensively studied (12). Kim et al. evaluated CA 19-9 serum levels in 114 patients with pancreatic cancer who underwent pancreatic resection (n = 72) or palliative bypass surgery (n = 42). These authors reported a positive aspect of the correlation between the stage of pancreatic cancer and the preoperative mean serum CA 19-9 (12). In our research we did not find a correlation between the serum level of CA 19-9 and the tumor stage, but this level was closely correlated with lymphovascular tumor invasiveness ($p < 0.05$) with a threshold of> 200 IU / ml.

Due to the continuous decrease in the mortality rate, denopancreatectomy has today become a routine procedure performed for tumors of the periampullary region and the pancreatic head, with or without invasion on the mesenteric-portal axis (13). Hemorrhage and complications of the pancreatic abutment remain major concerns in assessing postoperative morbidity.

Slight differences were observed in the frequency of selection between types of operations, the most common being Whipple-type duodenopancreatectomies. For example, in this study pancreatoduodenectomy was performed in 79.5% of cases of pancreatic adenocarcinoma, but in other studies it was performed either less frequently (64.6%) or, conversely, more often, ie 91.1%.

The incidence of complications in our study group was 46.92%, with a perioperative mortality of 17.28%, overlapping with the results obtained in other centers.

Other studies have concluded that the overall survival for these patients is similar compared to the overall survival in patients with resection without lymph nodes involved (11, 14). However, in the current recommendation of the TMN system, all these patients are classified as N1 (15, 16). The other possible explanation for the similar long-term survival of patients without metastases or with a single lymph node metastasis could be attributed to the aggressive biology of pancreatic adenocarcinoma per se (17,18).

The T stage of pancreatic adenocarcinomas was similar to other studies (19). Tumor size is a prognostic factor for extrapancreatic extension (20). However, a number of studies have shown that patients with larger tumors have a shorter survival (21). In this study, the size of the tumor, characterized by the largest diameter, varied between 2.1 and 6 cm. The average tumor size was 3.4 +/- 1.2, with tumors larger than 2 cm being identified in 76 of the cases (93.82%). Cases of pancreatic adenocarcinoma were most often detected and treated surgically as pT3, meaning that the cancer invaded the extrapancreatic tissues (343). Tumor stage pT3 accounted for 94% of cases in the study by Lim et al. but in the present study there were 98.7% cases that are also confirmed in our findings (21).

The present study provided several explanations based on pathogenesis, which reveals a significant association between pT and tumor grade ($p < 0.05$). Thus, one reason for the increase in pT parameters is that the cancer cells become more anaplastic, ie they increase the degree of the tumor and also develop the ability to invade the surrounding tissue and blood vessels ($p = 0.005$). In the present study and in most other studies, the occurrence of N1 was observed in more than half of the PDAC cases.

The association of perineural and lymphovascular invasion did not show statistically significant correlations in relation to tumor staging or grading, but strong correlations were found between the presence of perineural invasion and lymphatic invasion (p =

0.00238) and especially with vascular invasion (p = 0.000385). , these aspects supporting the invasive and aggressive character of pancreatic carcinomas.

Focal loss of e-cadherin is common and has a prognostic significance indicating that the mechanisms responsible for focal loss in a subset of infiltrated pancreatic adenocarcinoma cells are likely to be biologically significant. Consistent with previous studies, e-cadherin loss was found to be more common in poorly differentiated pancreatic adenocarcinomas (22). However, we also found a partial loss of e-cadherin in pancreatic cancers that were not classified as poorly differentiated and often found loss of expression in both gland-forming and non-glandular cells of invasive pancreatic adenocarcinomas.

Regarding the expression of HER2 / neu protein, there are numerous antibodies (monoclonal or polyclonal) that recognize different domains of the molecule. For example, clones, such as CB11 or Pab1, detect the intracellular part, HercepTestTM detects the intracellular membrane, while CBE1 or TAB 250 reacts with the extracellular domain of the receptor (22). Therefore, the interpretation of the IHC assay depends on the selection of the specific antigen epitope targeted by the corresponding antibody. Co-evaluation of gene status with protein overexpression provides better knowledge and meaningful information than protein overexpression alone (23).

In our study, an increased concordance was observed between the expression of the 2-neu HER

marking and the tumor grading, the scores 2+ and 3+ being more frequently associated with poorly differentiated tumors.

Statistical analysis of correlations of HER 2-neu labeling with tumor morphological aspects and peritumoral parenchymal changes identified a strong association with tumor grading ($p < 0.05$).

The EGFR family has a central role in the pathogenesis and progression of variable carcinomas (24). They ultimately affect cell proliferation, survival, motility and adhesion to various carcinomas, including pancreatic cancer (25). Gene amplification, overexpression and EGFR activation mutations have been reported in various human cancers. Previous studies have shown that EGFR overexpression was correlated with shorter postoperative survival in pancreatic carcinomas (26). In this research, overexpression of EGFR in pancreatic adenocarcinomas was detected in 35% of cases. Of the 81 pancreatic carcinomas, in 11% of cases a score of 3+ was identified, and in 24% a score of 2+.

EGFR overexpression was more frequently associated in poorly differentiated tumors, being associated with over 50% of these tumors. Evaluation of EGFR overexpression in relation to the morphopathological characteristics of pancreatic tumors reveals statistically significant correlations with tumor grading and also with the presence of the HER 2-neu hyperlabel.

6.Conclusions

1. Pancreatic carcinoma is an aggressive neoplasia with an incidence that increases with age and is usually diagnosed in advanced stages.

2. The socio-economic situation may be a prognostic factor due to comorbidities and associated risk factors. Pancreatic resection remains the only therapeutic option and is not influenced by socio-economic standards.

3. The clinical and morphological characteristics of pancreatic ductal adenocarcinoma in the local population revealed high rates of diagnosis in advanced stages, with frequent involvement of extrapancreatic organs and tissues and the presence of metastases.

4. The mechanism involved in the association of pancreatic cancer and diabetes is not fully elucidated, but may provide new avenues for therapeutic opportunities. Diabetes was associated with low survival in pancreatic cancer patients and revealed a link between chronic glucose intolerance and pancreatic cancer survival. Reducing the risk of pancreatic cancer could also be achieved by preventing and treating diabetes and early detection of tumors in resectable stages.

5. The average survival time of the patients studied after the potentially radical surgical treatment of the pancreatic neoplasm was 13.5 months. Survival data, including perioperative mortality, correspond to global experiences. The demographic and surgical background was in line with global findings and practices.

6. In pancreatic carcinoma, median survival showed significant correlations with tumor grade, invasion of large blood vessels and resection margins. There was a trend for survival impact depending on lymph node status, tumor stage, resection margins, and invasive cancer in the perineural and lymphovascular.

7. Perineural invasion was a negative prognostic factor being statistically significantly correlated with the presence of lymph node metastases and long-term survival, which is lower in cases with perineural invasion.

8. The histopathological study demonstrated the predominant distribution of cases of ductal pancreatic adenocarcinoma in patients over 60 years of age and showed the predominance of this neoplasm among the male population, however with a higher rate of operability in females. In most cases the tumor formation was located at the head of the pancreas, the stage of tumor progression was stage III with vascular and perineural invasion but without distant metastases. Histologically, most cases of ductal pancreatic adenocarcinoma were moderately differentiated.

9. Tumor biology studies could provide the key to developing new therapeutic strategies. The current study performed a detailed analysis of morphopathological and immune-histological features in a comparative manner using representative samples for cases of ductal pancreatic adenocarcinoma (intratumoral area and peritumoral area). In addition, a correlation was made between the immunochemical parameters and the

main clinical and histopathological characteristics of the studied cases.

10. In pancreatic cancer, E-cadherin, EGFR and HER 2-neu were important molecular mechanisms involved in multiple molecular loops, significantly related to the cardinal features of carcinogenesis including cell proliferation and invasiveness and major clinical features representing tumor stage, degree of differentiation.

11. E-cadherin is a negative prognostic factor in the evolution and prognosis of pancreatic cancer, its absence being correlated with a much lower survival of patients with pancreatic neoplasm regardless of its location.

12. The immunohystological study of EGFR and HER 2-neu highlighted their correlation with tumor grading and implicitly a negative impact on patient prognosis and the introduction of these markers in the routine evaluation of pancreatic resection pieces will identify the population that can benefit from new lines. targeted with HER 2 -neu inhibitors and EGFR

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