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Does the Overall Survival of the Resectable Periampullary Carcinomas Correlate with High Expression of p53 and ki67?

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Rezumat

Supraviețuirea globală a carcinoamelor periampulare rezecabile poate fi corelată cu expresiile ridicate ale markerilor p53 și ki 67?

Introducere: Carcinoamele periampulare reprezintă un grup de tumori ce se dezvoltă într-o regiune complexă, formată din diferite structuri anatomice. Cel mai frecvent tip histologic al carcinoamelor periampulare este adenocarcinomul. Adenocarcinoamele periampulare de tip pancreatic au cel mai sumbru prognostic. Merkerii imunohistochimici, precum ki-67 şi p53, pot fi utilizați în predicția supraviețuirii.
Material şi metodă: au fost selectați pacienții cu adenocarcinoame periampulare de tip intestinal sau biliopancreatic, cu tumori rezecabile şi au fost realizate marcaje imunohistochimice pentru markerii ki-67 şi p53. Supraviețuirea a fost analizată în funcție de expresia markerilor imunohistochimici, stadializarea TNM, diferențierea tumorală şi invazia perineurală.
Rezultate: 67 de pacienți au fost incluşi în studiu. Mediana supraviețuirii pe întreg lotul de studiu a fost de 12 luni, cu o rată de supraviețuire la 2 ani de 25%. Rata de proliferare tumorală crescută (ki-67 peste 80%) a avut o asociere semnificativă cu scăderea supraviețuirii (o mediană a supraviețuirii de 3 luni versus 17 luni pentru grupul de pacienți cu ki-67 mai mic de 80%). Creşterea expresiei proteinei p53 s-a asociat cu o scădere a supraviețuirii. Supraiețuirea scăzută s-a asociat cu tumorile slab diferențiate şi statusul limfoganglionar.

Concluzii: expresiile markerilor imunohistochimici ki-67 şi p53 pot fi utilizați ca factori de prognostic şi predictivi ai supraviețuirii pentru pacienții cu adenocarcinoame periampulare rezecabile.

Cuvinte cheie: adenocarcinoame periampulare, p53, ki 67, supraviețuire, prognostic

Abstract

Introduction: Periampullary carcinomas represent a group of tumors that develop in a complex area, implying different anatomical structures. The most common histological type of periampullary carcinomas is the adenocarcinoma. The pancreatic type of periampullary adenocarcinomas has the worst prognosis. Immunohistochemical markers, such as ki-67 and p53, can be used in predicting survival.

Material and method: we selected the patients with periampullary adenocarcinomas, intestinal or biliopancreatic type, with resectable tumors, and we performed immunohistochemical stains for ki-67 and p53 markers. The overall survival was analyzed according to the expression of immunohistochemical markers, TNM staging, tumor grade and perineural invasion.

Results: Sixty-seven patients were included in the study. The median overall survival for the whole cohort was 12 months, with a 2-year survival rate of 25%. High rate of tumor proliferation (ki67 more than 80%) was significantly associated with shorter overall survival (median survival 3 months compared with 17 months for the group with ki67 index less than 80%). A high expression of p53 protein has been associated with low overall survival. The low survival was associated with poorly differentiated tumor grade and lymph node status.

Conclusion: Both immunohistochemical expression of ki67 and p53 can be used as prognostic and predictive factors for overall survival of patients with resectable periampullary adenocarcinomas.

Key words: periampullary adenocarcinomas, ki-67, p53, survival, prognosis

Introduction

Periampullary carcinomas consist of a group of tumors developed in the area delimited by a radius of 2 cm around the Vater ampulla (1-4). This complex area is composed by different anatomical structures, such as ampulla of Vater, duodenal wall, head of the pancreas, distal main bile duct, pancreatic duct (2,3,5). The histological and immunohistochemical examination have an essential role in establishing the starting point. The most common histological type of periampullary carcinomas is the adenocarcinoma, either intestinal or biliopancreatic (6). Pancreatic adenocarcinoma is the most frequent and has the worst prognosis with a 5-year survival rate of 7%, unlike duodenal adenocarcinoma, the 5-year survival of which can reach up to 66% (4,7-9).

The TP53 gene is a tumor suppression gene that plays a major role in maintaining genomic stability (10,11). When DNA alterations occur, the TP53 gene induces apoptosis (10,11). Most human cancers have mutations in the p53 oncogene. The ki67 antigen (12-15) is an indicator of the proliferation rate of a tumor. Our study aims to assess the correlation between p53 protein, an expression of p53 oncogene mutations (11,12,16), and ki67 antigen and overall survival in resectable periampullary adenocarcinomas in order to validate their use as survival markers.

Material and Method

We searched our Surgical Department database for patients admitted between 2014 and 2019 for periampullary tumors. We conducted

a retrospective study and patients with resectable tumors, treated by pancreatoduodenectomy (PD) and pathological reports of adenocarcinoma, either intestinal, biliary or pancreatic type, including pancreatic ductal adenocarcinomas developed 2 cm around ampulla of Vater, were included in this study. Exclusion criteria were as follows: (1) other malignancies, such as neuroendocrine tumors; (2) distant metastases; (3) patients with major vascular invasion; (4) patients with palliative therapy.

Medical records were reviewed and data about age, gender, other associated conditions, surgical procedure, pathology reports (tumor size, TNM stage, lympho-vascular invasion, perineural invasion), follow-up and overall survival were recorded in an Excel database sheet.

Hematoxylin-eosin-stained slides of the resection specimens were reviewed by a pathologist, who was blinded to all clinical data. For immunohistochemical analysis of ki67 and p53, the microarray tissue sections of 2 μm were displayed on slides SuperFrostPlus and placed on the thermostat at 60 degrees for one hour to ensure adhesion. Reactivation of the epitrope was performed by Heat Induced Epitope Retrieval, pH=6. We used the following antibodies: for Ki-67 (Leica Biosystems Newcastle, UK, diluted 1:200, clone MM1) and for p53 (Thermo Scientific, diluted 1:800; clone DO-7). Individualized scoring systems were used for semiquantitative analysis. The number of positive tumor cells was quantified by counting a minimum of 300 tumor cells in the areas with the highest expression (hot-spot) and reported as a percentage value (values between 1 and 99% for ki67, and for p53: value "0" for absence of TP53 mutation, and value between 1 and 99% for those with positive immunohistochemistry) (*Fig. 1, 2*).

High rate of tumor proliferation was defined as ki67 expression in more than 80% of tumor cells and high expression of p53 was defined as p53 expression in more than 40% of tumoral cells.

Statistical Analysis

To perform statistical analysis, the SPSS software was used for, with statistical significance set at $p<0.05$. Results were expressed as either mean ± standard deviation or median (range). For categorical variables, the comparisons between variables were analyzed using the Chi-square test. Comparisons between continuous variables were analyzed using the Mann-Whitney test. The Kaplan-Meier method with log-rank test was used for survival analysis.

Results

Sixty-seven patients were included in this study, 36 males and 31 females. The mean age of patients was 59.73 years (range 40-79 years).

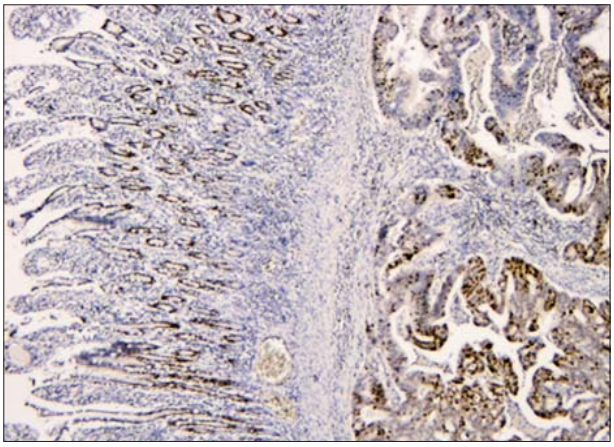

Figure 1. PDAC - immunostaining with ki67 (brown staining)

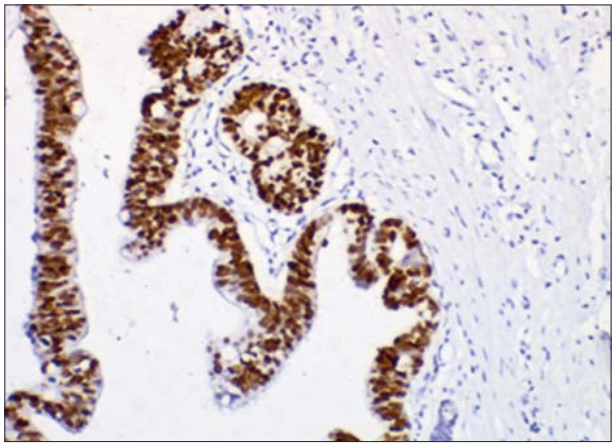

Figure 2. PDAC-immunostaining with p53 (brown staining)

Table 1. TNM staging and univariate analysis

	Percentage	Median survival (months)	95% CI (months)	p-value
Tumor (T)				0.916
T1	13.56%	12	0.22-23.78	
T2	61%	12	6.64-17.35	
T3	25.43%	11	1.94-20.05	
Node status (8th edition)				0.059
No	38,60%	19	16.15-21.84	
N1	52.63%	12	10.83-13.16	
N2	8.77%	12	0.2-31.32	
Node status				0.021
Negative	38.60%	19	16.15-21.84	
Positive	61.40%	12	10.97-13.02	
Tumor grade				0.044
Well differentiated	31.57%	18	14.08-21.92	
Moderate differentiated	57.90%	12	10.80-13.20	
Poorly differentiated	10.53%	3	0.2-19.20	
Perineural invasion				0.662
No	51.9.%	11	6.151-15.48	
Yes	48.1%	17	10.27-28.73	

CI: Confidence Interval

In terms of histological diagnosis, 44 patients had pancreatic-type adenocarcinoma, and 23 periampullary adenocarcinomas intestinal and biliary type.

The mean size of the primary tumor was 31.18 mm, with a minimum of 5 mm and maximum of 56 mm.

TNM staging, according with the 8th edition, the degree of tumor differentiation and perineural invasion are presented in *Table 1.*

The median overall survival for the whole cohort was 12 months, with a 2-year survival rate of 25%. The patients with pancreatic ductal adenocarcinomas had a median overall survival of 12 months (95% CI: 11.63- 20.34 months), and those with periampullary adenocarcinomas had a median overall survival of 12.25 months (95% CI: 7.11 – 25.85 months).

Univariate analysis for TNM staging variable associated with overall survival is also presented in *Table 1.* Decreased overall survival was noted in poorly differentiated tumors.

Distribution of patients according to the rate of tumor proliferation and expression of p53, as well as their correlations with overall survival are presented in *Table 2*, *Fig. 3*, and *Fig. 4.* High rate of tumor proliferation (ki67 more than 80%) was significantly associated with shorter overall survival (median survival 3 months compared with 17 months for the group with ki67 index less than 80%).

Furthermore, the correlation between p53 protein and pT staging, histological grade G, perineuronal invasion and lymph node status was assessed, with a significant association (p= 0.021) with the last parameter (*Table 3*). All patients with more than four positive lymph nodes had a percentage of p53 protein

Table 2. Immunohistochemistry and univariate analysis of overall survival

	N(%)	Median survival (months)	CI (months)	P value
Ki67				0.001
<80	50 (74,63%)	17	11.11-22.88	
≥80	17 (25.37%)	3	0.2-10.63	
P53				0.046
<40	19 (28,36%)	20	16.08-23.92	
≥40	35 (52.23%	12	8.96-15.03	
No p53 expression	13 (19,4%).			

CI: Confidence Interval

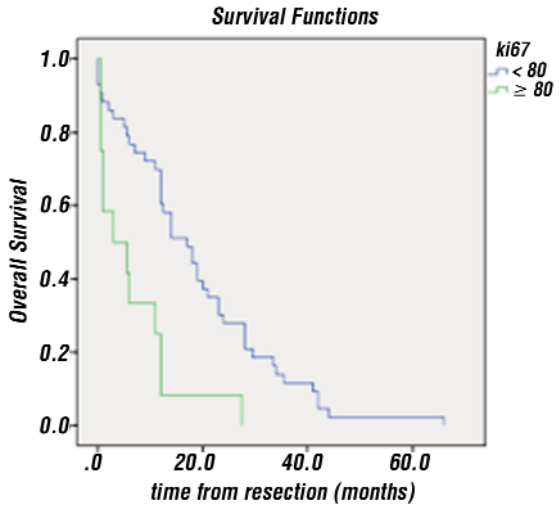

Figure 3. Kaplan-Meier curve, overall survival in dependence on ki67 index

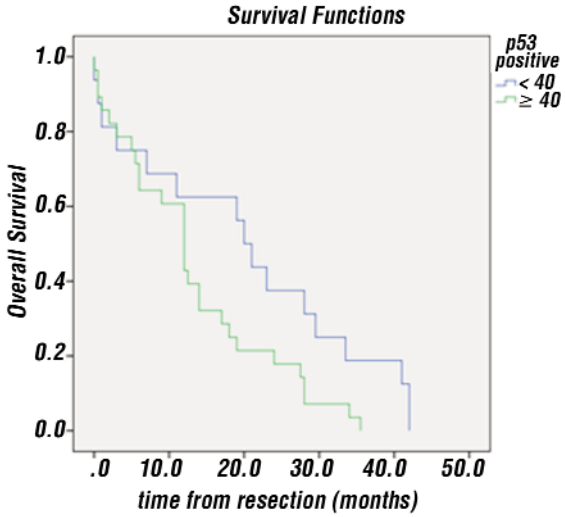

Figure 4. Kaplan-Meier curve, overall survival in dependence on p53 expression

higher than 40. The correlation of the p53 expression and pT staging, histological grade G, and perineural invasion were not statistically significant (*Table 3*).

The ki67 index was also correlated with pT staging, lymph node status, histological grade G and perineuronal invasion (*Table 4*).

Discussion

Despite constant progress of surgical techniques and large panel of oncological treatment, the overall survival of periampullary adenocarcinoma is still low. The only curative therapy associated with an increasing survival rate is the surgical resection such as radical pancreatoduodenectomy, but it can be performed in only 20% of cases (2,4,8,17-19, 20). Many published studies have focused on predictive factors for survival of resectable and unresectable periampullary adenocarcinomas (21-26).

The present study focused on two markers p53 protein expression and ki67 index,

Table 3. Association between p53 and pT, pN, histological grade, perineuronal invasion

Parameter analyzed		P53 IHC marker		Total	95% Confidence Interval
		<40	≥ 40	%	ML-χ^2
pT	T1	28.6%	71.4%	100	χ^2=3.775
	T2	27.6%	72.4%	100	
	T3	57.1%	42.9%	100	r=-.229 P=.151
pN	N0	52.4%	47.6%	100	χ^2=5.426
	N1	25.0%	75.0%	100	r=0.336
	N2	0%	100%	100	P=.021*
G	G1	28.6%	71.4%	100	χ^2=5.263
	G2	28.6%	71.4%	100	r=-.227
	G3	80%	20%	100	P=.072
Perineuronal invasion	negative	30%	70%	100	χ^2=.016
	positive	31.8%%	68.2%%	100	r=-.020 P=.582
					(*) Marcks significant differences P<.05

Table 4. Association between ki67 and pT, pN, histological grade, perineuronal invasion

Parameter analyzed		Ki67 IHC marker		Total	95% Confidence Interval
		<80	≥ 80	%	ML-χ^2
pT	T1	75%	25%	100	χ^2=.225
	T2	72.2%	27.8%	100	r=-.060
	T3	66.7%	33.3%	100	P=.652
pN	N0	63.6%	36.4%	100	χ^2=1.244
	N1	76.7%	23.3%	100	r=-.140
	N2	80%	20%	100	P=.537
G	G1	88.9%	11.1%	100	χ^2=4.491
	G2	60.6%	39.4%	100	r=-.224
	G3	66.7%	33.3%	100	P=.095
Perineuronal invasion	negative	63%	37%	100	χ^2=1.036
	positive	76%	24%	100	r=-.141
					P=.237
					(*) Marcks significant differences P<.05

analyzing their correlation with TNM status, the degree of tumor differentiation, perineural invasion, and overall survival of patients with resected periampullary adenocarcinomas.

Studies involving patients with pancreatic ductal adenocarcinomas have shown an association between increased expression of these markers and decreased survival and high risk of early local recurrence (13,15,27, 28). To our knowledge, no studies have reported the correlation between the survival and the immunohistochemical markers ki-67 and p53 for patients with periampullary adenocarcinomas including the biliary and the intestinal type.

The present study confirmed a strong correlation between ki67 index and the overall survival, a high ki67 index being associated with a poor survival (median overall survival of 3 months for patients with ki67 index more than 80%). Ki67 was an independent prognostic marker, regardless of the TNM status, the perineural invasion or the degree of differentiation.

A high expression of p53 protein has been associated with low overall survival and disease-free survival in patients with pancreatic adenocarcinoma and is considered a negative prognostic factor (12). Even if, in our study, there is no significant difference in overall survival between the group of patients with positive expression of p53 more than 40% and the whole cohort, the difference is significant when comparing the group with positive expression of p53 more than 40% and the group with less than 40%.

There are in vitro studies that have shown that gemcitabine has a stabilizing effect on the mutant p53 protein in cell nuclei, which induces chemoresistance (12,14). Temraz et al. observed that patients with pancreatic ductal adenocarcinoma and expressions of TP53 over 15% have a shorter disease-free survival, and the protein p53 can be used as a prognostic biomarker for survival (14). This can also explain the low survival observed in our study group and the inappropriate response to adjuvant treatment.

The degree of tumor differentiation is a marker of tumor aggressiveness, associated with the progression of disease and it is considered a prognostic factor for overall survival (17,29). Well-differentiated tumors, small size, with no invasion of the extra pancreatic nerve plexus are associated with long term survival (23,28). In our study, poorly differentiated tumors have a drop of the overall survival 6 times lower than that of well-differentiated tumors (median survival of 3 months vs 18 months). However, neither p53 expression nor ki67 index were correlated with the degree of differentiation.

Also, no correlation was found in our study between the size of the primary tumor and the

overall survival, even if in other studies a diameter larger than 3 cm is a significant independent risk factor for poor survival (29).

Lymph node positivity is considered an important marker of poor prognosis after pancreatic resections for malignancies, and it has also been reported as a marker for local recurrence (22,23).

Long term survival was observed in patients with negative lymph node status, R0 resections, without postoperative complications (24). In accordance with these data, Sessa et al. showed, in univariate analysis, that lymph node metastasis with advanced clinical stages and positive margins of resection are associated with shorter survival (21).

In our study, lymph node involvement has a significant influence on the overall survival considering their status as negative or positive and not their status according to the 8th edition of TNM staging (N0, N1 and N2). Similar results were found by other studies, showing that the number of invaded lymph nodes has no predictive value, but only the presence or absence of lymph node invasion (30) or that staging in N0, N1, and N2 does not bring any benefit, on the contrary, it leads to the loss of independent predictive value (27).

Despite these considerations, Sakata et al. demonstrated that for ampullary carcinomas, the N2 predicted the outcome better than lymph node ratio (31).

We have also found a strong correlation between p53 expression and lymph node status with high expression both in N1 and N2 stages. No correlation at all was found between the high rate of tumor proliferation expressed by ki67 index and lymph node invasion.

Finally, the expression of p53 protein and ki67 index were not significantly correlated with the presence of perineural invasion, a factor of tumor aggressiveness, though some studies have found correlations between high rate of perineural invasion and pancreatobiliary subtype of ampullary adenocarcinoma (25).

Conclusions

Both immunohistochemical expression of ki67 and p53 can be used as prognostic and predictive factors for overall survival of patients with resectable periampullary adenocarcinomas. Ki67 is an independent prognostic marker, regardless of the TNM status, the perineural invasion or the degree of differentiation. P53 expression correlates rather with lymph node involvement, but not with T stage, perineural invasion or degree of tumor differentiation.

Author's Contributions

All authors had equal contributions.

Conflicts of Interests

Authors have no conflict of interest related to this article.

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