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Is the non-metastatic, locally advanced colon adenocarinoma a distinct biological tumor variant? A study based on pathological evaluation, immunohistochemical panel and survival.

É o carcinoma colônico localmente avançado não metastático uma variante biológica distinta? Estudo baseado na avaliação histológica, painel imuno-histoquímico e sobrevida.

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A B S T R A C T

Objective: to evaluate the clinical and pathological differences between locally advanced colonic adenocarcinomas (LACA) with adhesions between adjacent organs or structures, and colonic adenocarcinomas with other clinical presentations. **Methods:** we conducted a retrospective study from a convenience sample of patients with colonic adenocarcinoma, pathological stage pT3, distributed according to clinical and pathological characteristics in three groups: locally advanced tumors (LACA), pT3 tumors without adhesions or distant metastases (SF) and tumors with metastatic disease (M1). We evaluated clinical and pathological characteristics and the expression of seven immunohistochemical markers related to proliferation/apoptosis, cell invasion/migration and metastasis. **Results:** we studied 101 patients: 30 LACA, 44 SF and 27 M1. Locally advanced tumors presented larger dimensions and were associated with increased lymphocyte infiltration rates, lower levels of bax expression, and CD 44v6 when compared with SF and M1 groups. We observed significant differences between LACA and M1 in relation to colonic location, histology, lymph node status and bax and CD44v6 expression. We found differences were observed between the three groups for tumor size and lymphocytic infiltrate. Survival was similar in the LACA and SF groups (p=0.66) and was lower in the M1 group (p<0.001). **Conclusion:** the data suggest that locally advanced colonic adenocarcinomas with adhesions between adjacent organs or structures represent a distinct entity.

Keywords: Surgical Oncology. Cell Biology. Immunohistochemistry. Colorectal Neoplasms.

INTRODUCTION

Colorectal adenocarcinoma represents 98.6% of primary cancers of the colon and rectum. A total of 61.9% of colorectal adenocarcinoma lesions are tumors without adhesion to organs and/or other structures and without metastatic disease, suitable for traditional surgery; 9.1% of lesions are tumors without adhesion to organs and/or structures but with metastatic disease; and 29% of lesions are locally advanced (LA) tumors, i.e., adhering to or infiltrating adjacent organs and structures[1], 18.2% of which have no metastasis and are suitable for resection, 5.4% are unresectable and 5.4% are LA and have metastases[2].

LA colorectal tumors are found in patients who have very large tumors, with adhesions forming in adjacent organs and/or structures. Histopathological evaluation shows that neoplastic cells can invade surrounding organs and structures or the peritoneal membrane of the colon (pT4 lesions) or can adhere and fuse to the peritoneal membrane, resulting in thickened fibrous tissue interposed between the structures, delimited by the respective elastic laminae (pT3 tumors)[3]. When these lesions are resected through extended surgeries or multivisceral resection, contrary to the expectations, patients present reasonable survival rates[4], as high as 80.7% over five years for patients with R0 (no residual tumor) resection[5,6].

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Thus, the current recommendation for tumors that adhere to adjacent structures is extended resection of organs or structures, but this treatment only occurs in 33.3% of patients due to tumor location, patient gender and age[7], preoperative planning and surgeon training. These lesions are associated with high perioperative mortality[8], but a high rate of survival was achieved in the presence of R0 resections[1,6,8,9], explained by suspected tumor biology[4,10,11].

The hallmarks of cancer comprise six biological capabilities, two of which are invasion and metastasis. Conceptual progress has added the reprogramming of energy metabolism machinery and evasion of immune destruction, creating a "tumor microenvironment"[12]. Despite advances in the assessment of tumor biology by immunohistochemistry and microarray studies, little progress has been made in understanding the biology of LA colorectal tumors. They have distinct characteristics related to the tumor microenvironment because they are associated with local inflammatory reactions, the formation of thickened fibrous tissue interposed between structures and a low capacity of lymph node or distant metastasis. Under light microscopy, most of the adhesions are inflammatory, lymph node impairment is low, and lymphocytic infiltration is extensive[2,5,13-15]. TNM individualizes this condition and is clinically considered stage pT4[16,17].

Thus, there is a lack of well-designed studies to evaluate whether these lesions actually have different biological characteristics. The evaluation of rectal tumors is generally difficult because patients often receive preoperative radiation therapy, which modifies the biological expression profiles of such lesions. The exclusive evaluation of colonic tumors that have not been subjected to previous treatment, with comparisons among different clinical and pathological forms, is a good model for the evaluation of the anatomopathological characteristics of these tumors, which motivated this study.

METHODS

This retrospective, longitudinal, convenience-sampling study was conducted among patients with previously untreated colonic adenocarcinomas who were admitted to the A.C. Camargo Cancer Center. The local Ethics Committee approved this study (nº 242/2000). Were selected the patients according to the clinical characteristics observed during surgery combined with pathological findings of the resected tumor specimens. In this study, all patients were at pathological stage pT3-TNM, separated into three different groups based on the macroscopic status of tumors at laparotomy: non-metastatic pT3 tumors with no invasion of adjacent organs (standard form, SF), pT3 tumors with metastatic disease (M1), and locally advanced, non-metastatic tumors (LACA) that were subjected to one-stage *en bloc* resection.

Extended resection was defined as *en bloc* extended to any organ or structure to which the primary tumor was adhered. Organs that were not removed en bloc, such as cholecystectomy for lithiasis or spleen removal due to iatrogenic injury, were not included[18]. The study cases were selected from a database of previous publications[1].

Initially, we evaluated all colorectal tumors treated at the Institution (1960-2000), and subsequently separated them into groups depending on clinical and pathological characteristics (adenocarcinoma, pT3-TNM) and the availability of specimens in paraffin blocks for analysis. We excluded from the analysis patients with familial or non-familial colon polyposis, synchronous tumors and second primary tumors or those with no pathological material for evaluation. We evaluated the clinical data and, reviewed the pathological material and immunohistochemical evaluation of the selected paraffin blocks.

Pathological evaluation

Two pathologists reviewed the cases. The following histopathological variables were analyzed: histological subtype; the degree of cell differentiation; pathologic clinical stage (pTNM 7th edition); tumor invasion (pT-TNM); lymph node status (pN-TNM); blood, lymph node and perineural invasion; and the presence of lymphocytic, eosinophilic and neutrophilic infiltrates.

In the presence of mucin, tumors were considered to have mucinous differentiation. Adenocarcinomas were divided according to the degree of differentiation: well, moderately or poorly differentiated or undifferentiated. In undifferentiated tumors, the markers were investigated by immunohistochemistry to exclude other cancers, such as neuroendocrine carcinomas (NSE, chromogranin and synaptophysin), lymphomas and melanoma (S-100 and HMB45). To confirm the diagnosis of adenocarcinoma, an immunohistochemistry study with cytokeratin (AE1/AE3, CK20) was performed, along with staining for mucin, to distinguish poorly differentiated adenocarcinomas from undifferentiated carcinomas.

Lymphocytic, neutrophilic and eosinophilic infiltration was categorized as absent, mild, moderate and severe. Severe infiltration was characterized by a large focal or diffuse accumulation of cells around the tumor. In the mild form, there were few and sparse cells around the tumor. Moderate infiltration represented an intermediate presentation between severe and mild.

Immunohistochemistry

The immunohistochemistry markers were selected because they were associated with proliferation (p53, PCNA), apoptosis (bax, bcl-2)[19] and cell invasion/migration (cathepsin B, galectin-3 and CD44 isoform v6 [CD44v6])[20].

Antigen retrieval was performed in a pressurized vial in all cases. The processed histological samples then underwent blocking of endogenous peroxidase activity, followed by overnight reactions at 4°C with the indicated primary antibodies. Table 1 shows the primary and secondary antibodies and dilutions.

Table 1. *Markers used for the immunohistochemistry technique.*

Tissue protein	Primary antibody	Codes markers	Dilution	Secondary antibody	Positive control	Cutoff
p53	Monolconal DO-7	Dako - M7001 Denmark	1:100	Dako, K0492 Strept ABC/HRP	Breast tumor	10%
PCNA	Monoclonal PC10	Dako - 0879 - Denmark	1:6000	Dako, K0492 Strept ABC/HRP	Tonsil palatine	72.5%
CD44 isoform v6	Monoclonal VFF-7	Novocastra NCL-CD44v6 United Kingdom	1:100	Dako, K0492 Strept ABC/HRP	Lung tumor	10%
Cathepsin B	Policlonal Cathepsin B**	Binding site PC049 - EUA	1:5000	Sheep Ig	Liver	10%
Galectin-3	Policlonal M338*	ATCC+	1:16	Mouse Ig	Papillary thyroid tumor	10%
Bax	Policlonal B9	Santa Cruz - sc7480 - EUA	1:100	Dako, K0492 Strept ABC/HRP	Prostate tumor	25%
bcl2	Monoclonal 124	Dako - M0887	1:50	Dako, K0492 Strept ABC/HRP	Tonsil palatine	25%

** Anti-mouse biotinylated - Vector BA4000; ** Anti-sheep biotinylated - Vector BA6000;+ATCC= american tissue culture collection; Grown in the RPMI 1640 plus 20% fetal bovine serum. Provided by LIM Laboratory. School of Medicine, USP.*

The reactions were developed with diaminobenzidine substrate chromogen (DAB, Sigma[â]) and hydrogen peroxide and were counter-stained with Harris haematoxylin. Corresponding positive and negative controls were included for all reactions. Negative controls were performed by excluding the primary antibody. Immunohistochemical analysis of marker expression utilized the quantitative method: cell counts of 1,000 cells were performed in several fields that were considered to be the most representative to determine the percentage of stained cells. The nuclear expression levels of p53 protein and proliferating cell nuclear antigen (PCNA) were evaluated; for the other antigens, cytoplasmic expression was used for evaluation. The cut-off for p53, cathepsin B, CD44v6, and galectin-3 expression was 10%. The cut-off for bax and bcl-2 was =25%.

Statistical analysis

To compare categorical variables, we used the chi-square test (X^2), and in the presence of variables with an expected value equal to or below 5, we used the Fisher's exact test. When the association was statistically significant, we performed logistic regression, and calculated a hazard ratio between the LACA and the other groups to verify where the differences occurred.

For the calculation of survival indicators, we used the Kaplan-Meier method. We compared the survival probability curves with the log-rank test. The significance level chosen for statistical differences was 5% for all analyses. We performed data analysis using the SPSS® software, version 12 (SPPS Inc., Chicago, IL, USA).

RESULTS

The segregation of pT3 colonic adenocarcinomas was the following: 30 patients with LA colonic adenocarcinomas (LACA) who underwent extended resection, 44 with no adhesions to organs and/or adjacent structures, subjected to classical colectomy (SF) and 27 with no adherence to organs and/or adjacent structures and only liver metastases (M1).

Tables 2 to 4 show the clinical and pathological characteristics and the expression of immunohistochemistry markes. In patients with LACA we observed similar rates between genders, 56.7% were older than 60 years, 62.1% had sympthoms over six months, associated with weight loss (79.3%) and rectal bleeding (60.7%). Upon physical examination, the deteriorated general condition was significant (53.3% with Karnofsky index below 70), and a palpable mass was present (70%). The presence of palpable tumors during physical examination was related to tumor size, with a median value of 9cm (95% confidence interval: 8.7-14.1cm), and patients were impaired, with low albumin levels (75.9%) and red blood cell counts (40%).

Table 2. *Comparison of the clinical and pathological data between different groups.*

Variable	Category	pT3 LA n(%)	pT3 SF n(%)	pT3 M1 n(%)	p(X^2)
Clinical findings					
Palpable mass	Present	21 (70.0)	17 (39.5)	6 (22.2)	0.001
	Absent	9 (30.0)	26 (60.5)	21 (77.8)	
Localization	Left colon	13 (43.3)	23 (52.3)	20 (74.1)	0.05
	Other	17 (56.7)	21 (47.7)	7 (25.9)	
Tumor size	=9cm	15 (50.0)	37 (84.1)	26 (96.3)	0.001
	>9cm	15 (50.0)	7 (15.9)	1 (3.7)	

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Variable	Category	pT3 LA n(%)	pT3 SF n(%)	pT3 M1 n(%)	p(X^2)
Pathologic findings					
Macroscopy	Infiltrative	10 (34.5)	15 (34.1)	18 (66.7)	0.01
	Expansive	19 (65.5)	29 (65.9)	9 (33.3)	
Histology	Pure	19 (63.3)	28 (63.6)	25 (92.6)	0.003
	Mucinous*	6 (20.0)	15 (34.1)	2 (7.4)	
	Other	5 (16.7)	1 (2.3)	0	
Histologic grade	G1	7 (23.3)	9 (20.4)	5 (18.5)	0.41
	G2	16 (53.4)	27 (61.4)	18 (66.7)	
	G3 e G4	7 (23.3)	8 (18.2)	4 (14.8)	
pN (TNM)	N0	19 (63.3)	24 (54.5)	4 (16.0)	0.002
	N1	9 (30.0)	9 (20.5)	11 (44.0)	
	N2	2 (6.7)	11 (25.0)	10 (40.0)	
Invasion venous	Absent	29 (96.7)	43 (97.7)	25 (92.6)	0.55
	Present	1 (3.3)	1 (2.3)	2 (7.4)	
Invasion perineural	Absent	28 (93.3)	41 (93.2)	23 (85.2)	0.45
	Present	2 (6.7)	3 (6.8)	4 (14.8)	
Invasion lymphatic	Absent	22 (73.3)	28 (63.6)	13 (48.1)	0.14
	Present	8 (26.7)	16 (36.4)	14 (51.9)	
Lymphocytic infiltration	Absent/Slight	16 (53.3)	33 (75.0)	26 (96.3)	0.001
	Moderate/High	14 (46.7)	11 (25.0)	1 (3.7)	
Eosinophilic infiltration	Absent/Slight	18 (60.0)	20 (45.5)	13 (48.1)	0.18
	Moderate/High	12 (40.0)	24 (54.5)	14 (51.9)	
Neutrophilic infiltration	Absent/Slight	18 (60.0)	32 (72.7)	24 (88.9)	0.007
	Moderate/High	12 (40.0)	12 (27.3)	3 (11.1)	
Desmoplasia	Slight	19 (63.3)	31 (70.5)	8 (29.6)	0.002
	Moderate/High	11 (36.7)	13 (29.5)	19 (70.4)	
Immunohistochemistry markers					
p53 nuclear	Negative	10 (34.5)	11 (25.0)	11 (40.7)	0.36
	Positive	19 (65.5)	33 (75.0)	16 (59.3)	
Bcl2	Negative	15 (50.0)	15 (36.6)	10 (41.7)	0.53
	Positive	15 (50.0)	26 (63.4)	14 (58.3)	
Bax	Negative	11 (37.9)	8 (19.5)	3 (11.5)	0.05
	Positive	18 (62.1)	33 (80.5)	23 (88.5)	
Cathepsin B	Negative	20 (66.7)	26 (61.9)	14 (51.8)	0.51
	Positive	10 (33.3)	16 (38.1)	13 (48.2)	
CD 44 v6	Negative	19 (65.5)	18 (42.8)	8 (29.6)	0.02
	Positive	10 (34.5)	24 (57.2)	19 (70.4)	
Galectin 3	Negative	2 (6.7)	-	2 (7.6)	0.20
	Positive	28 (93.3)	42 (100.0)	24 (92.4)	
PCNA	=72.5	11 (37.9)	17 (38.6)	13 (48.1)	0.67
	>72.5	18 (62.1)	27 (61.4)	14 (51.9)	
Total		30	44	27	-

** Association between mucinous adenocarcinomas and adenocarcinoma with mucinous differentiation.*

Table 3. *Odds ratio between clinical and pathologic variables.*

Variable	Category	Group	Odds Ratio	CI
Clinical variables				
Palpable mass	Present	LA	1.00	-
		SF	0.28	0.09-0.84*
		M1	0.12	0.03-0.47*
Localization	Left colon	LA	1.00	-
		SF	1.43	0.51-4.05
		M1	3.74	1.07-13.54*
Tumor size	>9cm	LA	1.00	-
		SF	0.19	0.06-0.63*
		M1	0.04	0.00-0.67*
Pathologic variables				
Macroscopy	Infiltrative	LA	1.00	-
		SF	0.98	0.37-2.64
		M1	3.80	1.25-11.50*
Histology	Pure	LA	1.00	-
		SF	1.01	0.35-2.96
		M1	7.24	1.26-53.91*
pN TNM	Positive	LA	1.00	-
		SF	1.44	0.50-4.16
		M1	9.07	2.14-41.88*
Lymphocytic infiltration	Moderate/High	LA	1.00	-
		SF	0.38	0.13-1.14*
		M1	0.04	0.00-0.36*
Neutrophilic infiltration	Moderate/High	LA	1.00	-
		SF	0.56	0.19-1.69
		M1	0.19	0.04-0.88*
Desmoplasia	Moderate/High	LA	1.00	-
		SF	0.72	0.37-2.34
		M1	4.10	1.25-4.50*
Immunohistochemistry expression				
CD44 v6	Positive	LA	1.00	-
		SF	2.53	0.86-7.62
		M1	4.51	1.28-16.50*
Bax	Positive	LA	1.00	-
		SF	2.52	0.76-8.51
		M1	4.69	0.98-25.15*

*CI= confidence interval 95%; * p<0.05.*

Table 4. *Comparison between different histological groups variables with CD44 isoform v6 and bax.*

Variable	Category	Negative	Positive	p
CD44 isoform v6				
Macroscopy	Infiltrative	15 (34.1)	27 (50.9)	0.09
	Expansive	29 (65.9)	26 (49.1)	
Histology	Pure	28 (62.2)	42 (79.2)	0.06
	Other	17 (37.8)	11 (20.8)	
pN (TNM)	Negative	24 (53.3)	21 (41.2)	0.23
	Positive	21 (46.7)	30 (58.8)	
Lymphocytic infiltration	Absent/Slight	29 (64.4)	43 (81.1)	0.06
	Moderate/High	16 (35.6)	10 (18.9)	
Neutrophilic infiltration	Absent/Slight	28 (62.2)	43 (81.1)	0.04
	Moderate/High	17 (37.8)	10 (18.9)	
Desmoplasia	Slight	34 (75.6)	22 (41.5)	0.01
	Moderate/High	11 (24.4)	31 (58.5)	
Bax expression	Negative	12 (27.9)	9 (17.3)	0.21
	Positive	31 (72.1)	43 (82.7)	
Bax				
Macroscopy	Infiltrative	7 (31.8)	24 (46.6)	0.22
	Expansive	15 (68.2)	39 (54.4)	
Histology	Pure	10 (45.5)	59 (79.7)	0.002
	Other	12 (54.5)	15 (20.3)	
pN (TNM)	Negative	13 (59.1)	31 (43.1)	0.19
	Positive	9 (40.9)	41 (56.9)	
Lymphocytic infiltration	Absent/Slight	14 (63.5)	56 (75.7)	0.26
	Moderate/High	8 (36.4)	18 (24.3)	
Neutrophilic infiltration	Absent/Slight	12 (54.5)	58 (78.4)	0.03
	Moderate/High	10 (45.5)	16 (21.6)	
Desmoplasia	Slight	15 (68.2)	40 (54.1)	0.24
	Moderate/High	7 (31.8)	34 (45.9)	

When comparing groups macroscopically LA tumors were larger in size, and from the histological point of view, they presented histological subtypes different from pure adenocarcinomas, lower rates of lymph node metastases, higher rates of lymphocytic infiltrates and moderate and severe neutrophilic infiltrates (Table 2). Immunohistochemical analyses revealed that the tumors had lower expression levels of bax and CD44 isoform v6 (Table 2; Figure 1). When assessing the odds ratio between the groups, we observed linear relationships between the groups (Table 3) for most of the variables.

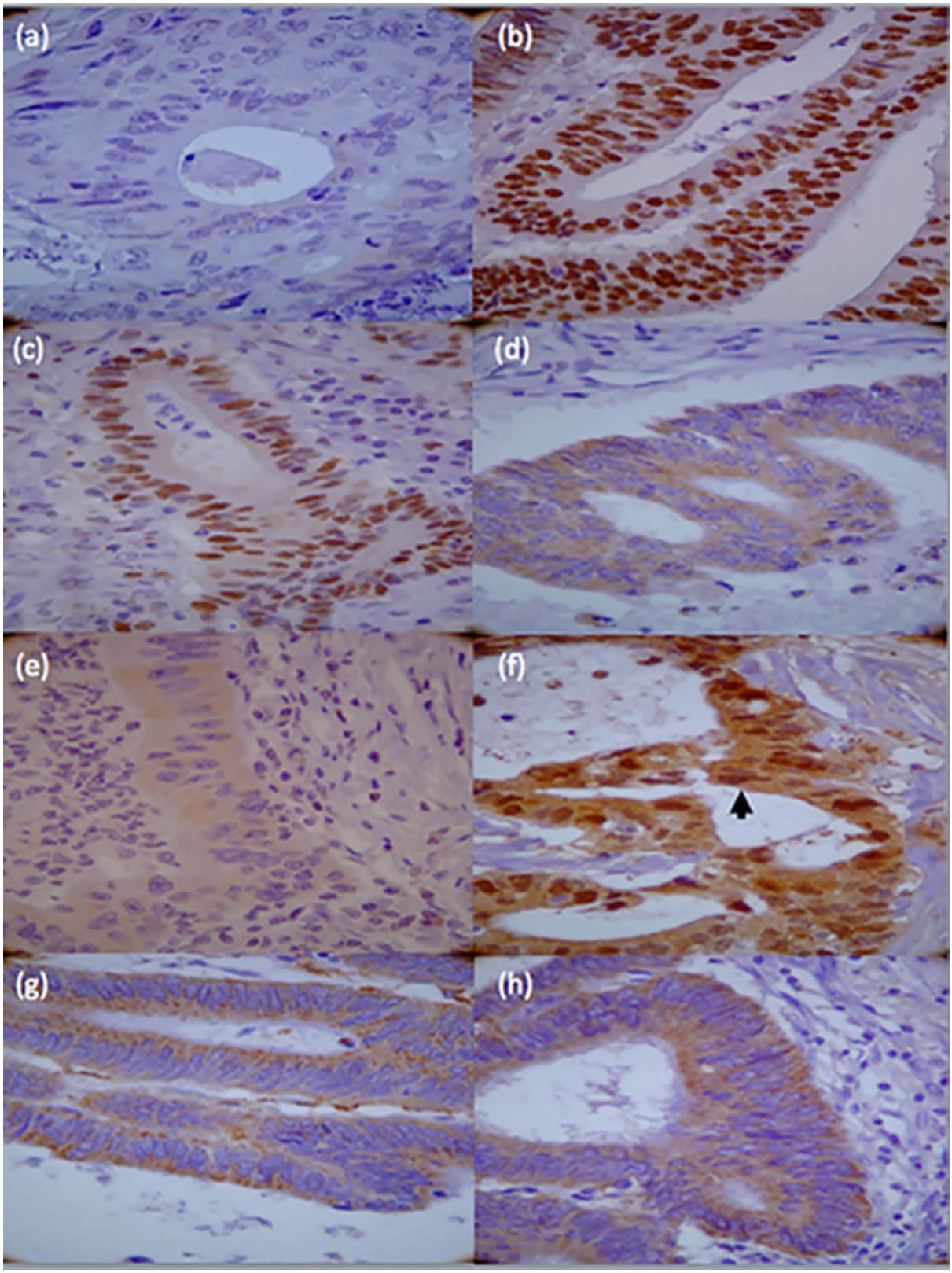

Figure 1. *Figure 1. Immunohistochemistry photomicrographs (400x) of colonic adenocarcinoma showing marker expression profiles: (a) lack of expression; (b) expression of p53 in virtually 100% of nuclei; (c) high proliferative activity shown by PCNA; (d) expression of the bax protein; (e) expression of the bcl-2 protein; (f) cytoplasmic and nuclear expression of galectin-3; (g) significant expression of the CD44 v6 protein on the luminal edge; and (h) significant cytoplasmic expression of cathepsin B.*

In spite of the linear ratio between the variables, the only significant differences were the ones between LA and M1 tumors with regards to the location of the tumor in the left colon, pure adenocarcinoma, compromised lymph node status and expression of CD44 isoform v6 and bax; additionally, we observed decreased risks with regards to red blood cell count and neutrophilic infiltrates. We found significant differences between the three groups only for the variables related to complaints about weight loss, presence of palpable tumor during physical examination, tumor size and the presence of lymphocytic infiltrates (in order of decreasing occurrence). The Karnofsky index was lower in LA patients, followed by M1 and SF patients.

When evaluating the association between the main differential pathologic variables observed between the groups and the expression of CD44 isoform v6, we observed that although not significant the lower expresion of CD44 isoform v6 had a near association with non-pure adenocarcinoma histology (p=0.06), moderate/high lymphocytic infiltration (p=0.06), but a significant association with moderate/high neutrophilic infiltration (p=0.04) and slight desmoplasia (p=0.01) (Table 4). Bax expression was associated with pure adenocarcinoma histology (p=0.002) and absent/slight neutrophilic infiltration (p=0.03) (Table 4).

Cancer-specific survival

The average follow-up was 61.1 months [range 0 (postoperative mortality) to 290 months], and the rate of loss to follow-up was 7.9%. When evaluating the groups' evolution, the postoperative death rates in the pT3 LA, pT3 SF and pT3 M1 groups were 10%, 15.9% and 14.8%, respectively (p=0.30). Follow-up time was longer for pT3 LA patients (mean 89.1 months) when compared with pT3 SF (75.4 months) and pT3 M1 (average 13.1 months) patients (p<0.001).

The cancer-specific survival rates at 60 and 120 months were 79.9% and 67%, respectively, in patients with pT3 LA tumors; in the SF group, the respective rates were 75.6 and 75.6%. No patients with metastatic disease were still alive at 60 months (p<0.001) (Figure 2). When survival in patients with pT3 LA tumors was compared exclusively with pT3 standard patients (SF), there was no significant difference with (Figure 2a; p=0.66) and without (Figure 2b) postoperative death evaluation.

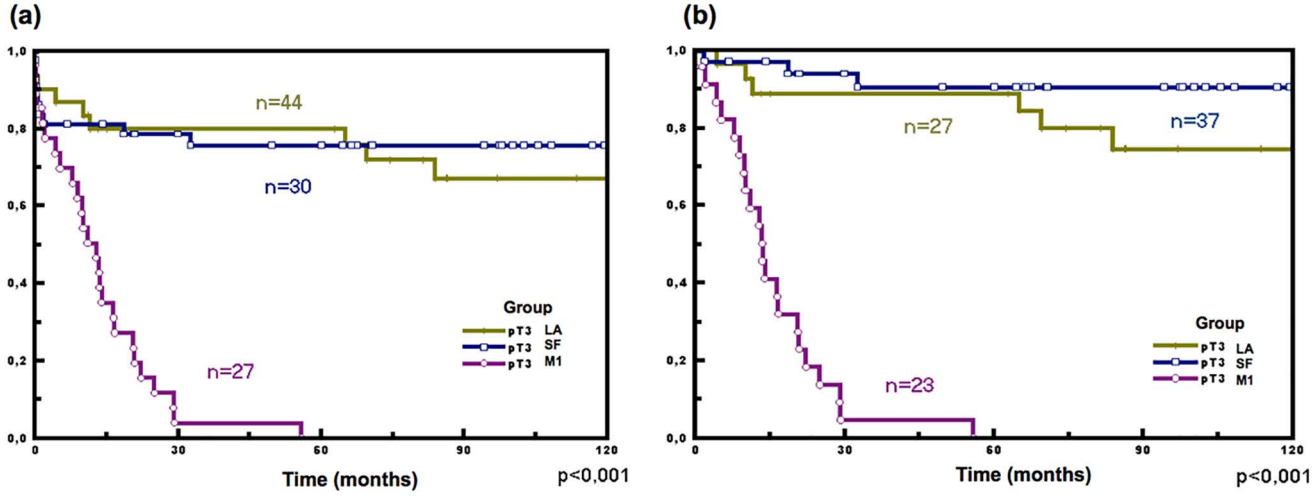

Figure 2. *Cancer-specific survival in patients with pT3 colonic adenocarcinoma.*

DISCUSSION

LA colorectal tumors comprise 5.5 to 22% of all cases[1]. Despite progress in chemotherapy and radiation therapy, surgery is still the best treatment for LA colorectal lesions[1,7,8]. Resections including structures and/or organs that have macroscopic adhesions are considered to be expanded or one-stage en bloc resections, which is a curative treatment for this type of tumor, with survival rates of 39.2% to 80.7% at five years[1,2,5,6,8,13-15,21], in contrast to the survival prediction of months in the presence of unresectable disease[2]. Multivisceral resection provides the best possibility of long-term survival in R0 resection[8], but patients with incomplete resection (R1 and R2 resection) or metastatic disease have limited survival[2,6,9,18,22-24]. Laparoscopic surgery does not alter survival[24,25].

The characteristics that determine clinical masses, limited disease and low lymph nodes commitment[10] are considered to be caused by the different biological profiles of these tumors[4]. Most case-control studies assessing the importance of extended surgery are limited to characteristics related to survival[7,18,22,23]. When the pathological characteristics of patients who were subjected to classical and multiorgan resection were evaluated, LA lesions were larger and associated with a significant increase in perineural infiltration[17]; however, selection bias was observed in these studies.

TNM staging classifies pT4 tumors as those that are macroscopically adherent to other organs or structures, but no tumor is present in the adhesion. Microscopically, the classification should be pT3 in the 6th edition and is based on the depth of wall invasion in the 7th edition. Therefore, we excluded patients with pT2 tumors, and based on this condition, we tried to associate clinical and pathological conditions to better understand LA tumors by comparing distinct clinical and pathological forms of pT3. Considering that pT3 and pT4 LA lesions have similar characteristics, with the exception of invasion to organs or structures and possible biases related to pT4 tumors not submitted to extended resections, we excluded pT4 colonic lesions. Clinical pT4 rectal carcinoma submitted to neoadjuvant treatment may impose a bias on pathologic evaluation associated with alterations in primary immunohistochemistry characteristics, a fact that led us to exclude rectal adenocarcinomas. On the other hand, up to 4.5% of the LA tumors are pT2 tumors[1,6,18,23,24], which confirms the presence of distinct clinical and morphological characteristics. Some tumors never adhere to adjacent structures but are associated with distant metastasis, a fact that reinforces different LA tumor characteristics and made us evaluate three distinct clinical forms of pT3 lesions.

When evaluating clinical and microscopic characteristics LACA tumors, were identified several features that have been described in the literature (Table 2)[2,4,5,13-15]. LA colonic tumors have a mild tendency to occur in males, which was not observed in rectal tumors, where pelvic conditions make extended resections easier in women[4]. The time between the onset of symptoms and the start of treatment was higher in the LACA group, which indicates both slow growth and a low rate of metastasis. It is clinically associated with complaints about weight loss, the presence of palpable mass, low Karnofsky indices and low albumin and red blood cell levels. All of these findings are the results of large tumor sizes[1] and high degrees of chronic impairment.

Most LACA tumors were moderately and well-differentiated adenocarcinomas (76.7%), characterized by absence of lymph node metastasis (63.3%), absence of blood vessel invasion (96.7%), absence of perineural invasion (93.3%), and lack of lymphatic invasion (73.3%), with a good rate of mucinous tumors (20%). These findings are in agreement with several authors who observed few lymph node metastases[1,2,4,5,10,11,13-15,23], peritumoral inflammatory reactions and the absence of perineural, lymphatic or vascular invasion[1,10] in LA lesions. Another important finding is the presence of moderate and severe lymphocytic infiltrates (46.7%), mainly diffuse (72.5%). The role of lymphocytic and neutrophilic infiltrates in local tumor control remains unclear, although some studies suggest that these characteristics lead to improved immune control, resulting in better prognosis[1,10,26].

When comparing groups statistically, if the difference is very small, a large number of patients are usually necessary. Large differences can be observed with a small number of patients, as observed in the present study. Patients with metastatic disease, whose prognosis is known as unfavorable, and patients in the different clinics are thought to have a good prognosis, so we use a similar model to compare morphological differences between groups, observing large morphological differences with a limited number of patients. We observed a set of different characteristics in LACA tumors compared with M1 lesions, and the SF group is an intermediate group between LACA and M1 tumors. M1 lesions are usually pure adenocarcinomas and have higher rates of blood and perineural invasion, lymph node metastasis and lymphatic invasion and lower rates of lymphocyte infiltration. Table 3 shows the relationship between these variables in the form of risk ratios.

There are no previous studies assessing the expression of markers by immunohistochemistry in LACA tumors. In the current study, we aimed to evaluate potential markers related to proliferation/apoptosis (p53, PCNA, bax, bcl-2)[19] and cell invasion/migration (cathepsin B, galectin-3 and CD44v6)[20]. In the first group, PCNA is a marker of cell proliferation, p53 is related to cell division and is associated with poor prognosis, bcl-2 has anti-apoptotic activity and bax has pro-apoptotic characteristics[19]. There was no previous correlation of p53 or PCNA with the clinical or morphological form of the tumor[27], which was confirmed in the present study. In this study, pT3 LA had higher PCNA expression, but the difference between groups was not significant. pT3 LA had lower bax expression (p=0.05) in contrast to pT3 M1 tumors, in which high bax expression may be correlated with smaller tumor size (Table 3) and pure histology (Table 4). The expression of bcl-2 was not associated with the clinical and morphological forms of colorectal adenocarcinomas. This finding is in agreement with those of other authors who consider tumor size to be a direct consequence of the balance between proliferation and apoptosis, while apoptosis inhibition may contribute to tumor growth[28,29].

During the process of invasion/migration, malignant cells interact with the extracellular matrix, move, destroy the extracellular membrane, enter the circulation and adhere to distant capillary beds, where they invade the endothelium and proliferate as metastases. In this context, cathepsin B, galectin-3 and CD44v6 have significant roles. Galectin-3 is a transmembrane protein associated with cell adhesion and apoptosis regulation. Cathepsin B is a lysosomal protease that acts in the degradation of the basement membrane.

In colorectal cancer, the expression of cathepsin B is related to a poor prognosis[30], and no association with staging has been observed[30], although its expression has been associated with the presence of liver metastasis[31]. Those findings are in agreement with our results, but without a statistically significant difference. Higher[32] and lower[33] expression levels have been observed, which can be attributed to the limited number of cases in the study or the selection criteria utilized. Low CD44 expression is associated with lymphocyte activation, and high expression is associated with a metastatic potential[34]. CD44v6 is a cell membrane glycoprotein involved in cell-cell and cell-matrix interactions that affect lymphocyte activation and metastasis processes. Lower expression of CD44v6 was associated with largest tumors[35], lower lymph node metastasis and better survival[36]. The relationship between CD44v6 expression and the presence of liver metastases has shown conflicting results: an association[37], an inverse relationship[38], and a lack of association[35,36]. The differences reported in the literature may be explained by its presence only affecting some clones[39], but more studies are necessary to better evaluate this condition. Lower expression of CD44v6 was associated with LACA tumors, and higher expression was associated with the M1 form (p=0.02). In our series, lower CD44v6 expression was associated with many characteristics present in LACA tumors (Table 4), a fact that reinforces distinct biologic characteristics of LACA.

Several authors who compared LA colorectal tumors that underwent complete resection (R0) with tumors with no adhesion to organs and/or structures that were subjected to classical resection at the same stage observed similar survival rates between the two groups[18,23]. The high postoperative mortality observed through a historical series negatively influenced the results of extended resections[1,18], but this finding was homogeneous between groups. In the current study, survival rates in patients with pT3 LA and SF were similar, and a lower survival rate was expected in the M1 group.

Multivisceral resection is associated with increased postoperative mortality, but the survival is similar to that of a standard resection[23,40]. Considering multivisceral resection as a case group and conventional surgery as a control group, we found no matched case control study, and in general, the groups are not homogeneous[17,40]. Gezen *et al*. compared the survival of colorectal cancer in patients submitted to a single organ resection (n=264) with multivisceral resection (n=90), observing no difference in survival, but there were differences related to clinical T, N, and M, stages and R1 resections were included in the analysis[17]. Leijssen *et al*. compared pT3/pT4 patients (n=725) submitted to non-multivisceral resection and to multivisceral resection. pT4 patients submitted to multivisceral resection had the same survival as that of pT3 patients with and without multivisceral resection[41]. Nakafusa *et al*. compared pT3 patients (n=270) with pT3/pT4 patients (n=53) who underwent multivisceral resection, and the survival was similar between the groups[42]. Gebhartdt *et al*. compared multivisceral resection (n=140) with non-multivisceral resection (n=828), observing similar survival related to clinical stages II and III[23]. Lehnert *et al*. presented the best model, comparing patients submitted to R0 resection, conventional and multivisceral resection for pT3 and pT4 tumors, including patients with clinical stages II, III and IV, but observed no differences in survival as for the type of surgery[18]. As previous studies did not match the groups, we evaluated only pT3 patients with the intent to compare pathological characteristics. Our study evaluated pT3 R0 tumors, and when comparing SF and LA, survival was not different between these groups, but it was lower in patients with pT3 M1 tumors.

We included and evaluated pT3 M1 patients with an expected lower survival, but this group gave us conditions to compare biological differences among the three forms, considering pT3 M1 characteristics associated with worse prognosis.

One limitation of the study is the long-term enrolment of patients, but we tried to minimize these differences through the systematic review of samples by two pathologists. Immunohistochemical reactions were performed in one stage, the quantitative method was used, and each pathologist performed the counts for specific markers to minimize any possible bias in the study.

The bax and CD44v6 isoform hypoexpression may contribute to local control, to determination of large tumors without metastasis and to lymphocytic and neutrophilic infiltration, which influences local immune control. Further studies with more patients are necessary to evaluate the type of local immune response in LA tumors. There is currently a lack of studies investigating these possibilities. Similarly, studies using array technology based on macroscopic aspects will also help to clarify the characteristics of these tumors.

Based on clinical, pathological and immunohistochemical expression, LA tumors have many "biological characteristics" that differ from those of M1 lesions, and SF adenocarcinomas have intermediate characteristics. The three main clinical and morphological presentations of colonic adenocarcinoma thus represent different entities. These findings highlight the importance of extended surgeries for LA tumors.

R E S U M O

Objetivo: *avaliar diferenças clínicas e patológicas entre os adenocarcinomas colônicos localmente avançados com aderências entre órgãos ou estruturas adjacentes (LACA) e adenocarcinomas colônicos com outras apresentações clínicas.* **Métodos:** *estudo retrospectivo a partir de amostra de conveniência de pacientes com adenocarcinoma colônico, estádio patológico pT3, distribuídos de acordo com características clínicas e patológicas em três grupos: tumores localmente avançados (LACA), tumores pT3 sem aderências ou metástases à distância (SF), e tumores com doença metastática (M1). Foram avaliadas as características clínicas e patológicas, e a expressão de sete marcadores imuno-histoquímicos relacionados à proliferação/apoptose, invasão celular/migração e metástase.* **Resultados:** *foram avaliados 101 pacientes: 30 LACA, 44 SF e 27 M1. Tumores localmente avançados apresentaram dimensões maiores e estiveram associados a aumento das taxas de infiltração linfocitária, menores níveis de expressão de bax e de CD 44v6 quando comparados aos grupos SF e M1. Diferenças significantes foram observadas em relação aos LACA e M1 em relação à localização colônica, histologia, estado linfonodal e expressão bax e CD44v6. Diferenças foram observadas em relação aos três grupos frente ao tamanho do tumor e infiltrado linfocítico. A sobrevida foi similar entre os grupos LACA e SF (p=0,66) e foi inferior no grupo M1 (p<0,001).* **Conclusão:** *os dados sugerem que os adenocarcinomas colônicos localmente avançados com aderências entre órgãos ou estruturas adjacentes representam uma entidade distinta.*

Descritores: *Cirurgia Oncológica. Biologia Celular. Imuno-Histoquímica. Neoplasias Colorretais.*

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