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Tumor budding: a prognostic factor in rectal cancer

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Abstract: Tumor budding (TB) is the presence of isolated tumor cells located in the infiltrating front of the tumor. Objective: to determine if the presence of TB is associated with other histopathological factors of poor RC prognosis such as lymph node involvement, tumor size greater than 5 cm, configuration of the infiltrative tumor margin, vasculolymphatic and perineural invasion, as well as determining whether the presence of TB influences the disease-free period. Results: 89 patients were evaluated, only 25 met inclusion and exclusion criteria, the average age was 56.52 years, men were more affected (56%), the RAB (72%), was the surgery, as used followed by RAP (24 %), and EP (4%). The distribution according to TNM: stage I (32%), 7 IIIB (28%), 4 IIIC (16%), 3 IIA (12%), 2 IIB (8%) and 1 IIIA (4%). 56% of patients N0, 44% N1. Tumor size: 36% tumors between 1 and 2 cm, 32% between 2.5 and 3.5 cm, 28% between 4 and 5 cm, 4% greater than 5 cm. Tumor invasion pattern: 64% had an infiltrating pattern, while 36% had an expansive pattern. Distribution of TB: Bd1 44%, Bd2 28% and Bd3 28%. 28% of the patients presented relapses, more frequent in the first 24 months. Disease-Free Survival (DFS) was evidenced in terms of irrigation groups TB, Bd1 of 76 months, Bd2 of 60 months, Bd3 of 20 months. TB is a prognostic factor associated with decreased disease-free survival in rectal cancer.

Key words: tumor budding; rectal carcinoma; tumor invasion pattern.

1 Introduction

Rectal cancer (RC) is one of the most common cancers, yet its incidence and mortality rates vary greatly worldwide. Globally, according to figures from the American Cancer Society, colorectal cancer is the third most common cancer in both men and women, with an estimated rate of new cases for 2018 of 7,560 and 63,640, respectively [1].

In recent decades, various lines of research have been opened with the aim of identifying factors that can predict the outcome of patients with colorectal cancer and help select groups at high risk of tumor recurrence. Numerous studies have sought to determine the prognostic value of clinical and histopathological factors of the tumor, but the results obtained have often been contradictory [2].

Prognosis and treatment decisions are based primarily on the extent of the disease, as coded by the TNM staging system. There is consensus that patients with RC with affected lymph nodes will benefit from chemotherapy, and rectal carcinomas that infiltrate beyond the intestinal wall are eligible for chemoradiation to reduce the risk of local failure,

regardless of lymph node status. However, this stratification excludes the small but significant proportion of patients (10-15% of the total) who, despite being classified as low risk, follow an adverse clinical course with metachronous metastatic disease. Therefore, prognostic factors in addition to TNM are important elements for medical oncologists, surgical pathologists and surgical oncologists [3].

For this reason, the search for these additional prognostic factors in the evaluation of RC has been an important focus of research. Of the histopathological factors, lymph node status is the most important prognostic indicator in RC, but there is growing evidence that this is insufficient to predict the clinical outcome of these patients, particularly those in stage II according to the TNM classification (T3, T4 N0 M0). Similarly, other more promising histopathological factors include extramural venous invasion, tumor growth pattern (expansive vs. infiltrative), inflammatory infiltrate, microsatellite instability, and tumor budding. There is now overwhelming evidence that tumor budding is an independent prognostic factor in RC, particularly [4,5].

The histological finding of tumor budding or focal budding, defined as the presence of isolated tumor cells or small groups of more than 4 cells located at the infiltrating front of the tumor, is an independent poor prognostic factor in RC, as these cells act as advance guards, preparing the ground for the invasive front to progress. Its high-grade finding is associated with other characteristics of aggressive tumors such as advanced T level of tumor invasion, lymph node metastasis, low grade of differentiation, infiltrative growth pattern, and lymphatic invasion [6].

Regarding the characteristics of the tumor invasion pattern, two models of tumor margin growth have been described: infiltrative and non-infiltrative (displacing or expansive) [7].

The objective of this study is to determine whether the presence of tumor budding is associated with other anatomopathological factors of poor prognosis in RC, such as lymph node involvement, tumor size greater than 5 cm, configuration of the infiltrative tumor margin, and vasculolymphatic and perineural invasion. A secondary objective is to determine whether the presence of tumor budding influences the disease-free period (from the time of surgery to the last follow-up).

2 Method

Retrospective study conducted at the Hospital Oncology Service of the Venezuelan Social Security Institute (SOH-IVSS). Patients diagnosed with rectal adenocarcinoma in stages I, II, and III according to the 2010 TNM classification who underwent curative surgery between 2008 and 2015 were included, with surgical specimens (slides) available for review, operated on and followed up at our institution. Variables such as age, sex, surgical procedure performed (low anterior resection (LAR), abdominoperineal resection (APR), or pelvic exenteration (PE)), stage (according to the 2010 TNM classification of the American Joint Committee on Cancer - AJCC), tumor invasion pattern, tumor budding, tumor size (according to the largest diameter of the tumor in centimeters), vascular and lymphatic invasion, number of positive lymph nodes, recurrence, and disease-free survival (DFS) were recorded. These data were entered into a database designed for this purpose.

For the Tumor Budding (TB) report, the standards established in Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016 were used [8].

For the evaluation of tumor budding in RC, the "hotspot" method is recommended. In most studies, tumor budding counts have been performed in a single field with the highest density of tumor buds ("hotspot" method), while others have used multiple fields (e.g., high- power fields). Counting across multiple fields has the advantage of being more representative of the entire invasive front, and there is also some evidence of better inter-observer agreement using this approach. Therefore, the "hotspot" method better reflects the maximum extent of tumor budding at the invasive front. The

ITBCC group recommends the use of the "hotspot" method, as this is the method used in the vast majority of outcome-based studies, and inter-observer agreement with this method is quite acceptable [8].

For the present study, the diagnostic criteria for infiltrative configuration of the tumor invasion pattern described by Jass et al. and subsequently adopted by the AJCC, were used: tumor dissection through the thickness of the muscularis propria, dissection of mesenteric adipose tissue by irregular groups or cords of cells, and perineural invasion. The presence of any of these three patterns in a tumor is sufficient to consider it an "infiltrative tumor invasion pattern" [7].

We determined prognostic utility through multivariate statistical analysis, correlating tumor budding, tumor invasion pattern, tumor size, TNM stage, presence of vascular and lymphatic invasion, number of positive lymph nodes, recurrence, disease-free interval, and survival. Patients were excluded if slides were unavailable for review, if they underwent surgery at external centers, if the histological diagnosis was other than adenocarcinoma, if they did not attend postoperative follow-up, and if they underwent transanal resections.

The ITBCC group establishes a series of steps to be followed during the study and reporting of TB:

1. Define the field area (sample) for the 20x objective of your microscope according to the field diameter of the eyepiece.
2. Select the Hematoxylin-Eosin slide with the highest degree of sprouting at the invasive front.
3. Scan 10 individual fields at medium power (10x objective) to identify the "hotspot" on the invasive front.
4. Count the tumor buds at the selected "hotspot" (20x objective).
5. Divide the bud count by the normalization factor to determine the tumor bud count per 0.785 mm^2.

For risk stratification based on TB counts, most studies have used numerical cut-offs (including two-tier and three-tier systems), while some studies have used a continuous scale to predict the probability of recurrence. However, cut-offs are more practical in the clinical setting, and there is insufficient evidence to support the use of a continuous scale for tumor budding in clinical decision-making.

The ITBCC group recommends the use of a three-level system such as that used by the Japanese Society for Colon and Rectal Cancer:

0-4 buds - Low budding (Bd 1).

5-9 buds - Intermediate budding (Bd 2).

10 or more buds - High budding (Bd 3).

This system allows for the risk stratification of pT1 colorectal cancer and stage II colorectal cancer. In pT1 colorectal cancer, Bd 2 and Bd 3 are associated with a higher risk of lymph node metastasis, whereas in stage II colorectal cancer, Bd 3 is associated with a higher risk of recurrence and mortality.

The ITBCC group recommends that, in addition to the Bd category, the absolute bud count be provided (e.g., Bd 3 (count 17)). This avoids the loss of information that may occur when applying a cutoff to borderline cases. For example, a bud count of 9 (Bd 2) may be biologically similar to a bud count of 10 (Bd 3), but they fall into different risk categories.

The results were processed using the Statistical Program for the Social Sciences (SPSS version 15.0). Initially, a descriptive analysis of the different variables under study was performed to determine their behavior in each of the evaluations carried out. The mean and mode were calculated for continuous variables; for nominal variables, their frequency and percentage were calculated. Subsequently, different tests were performed to study the independence of TB with the other variables. The significance level α used for each of these tests was 0.05. In each case, the critical region corresponding to the significance level α was constructed, and the test statistic defined as Chi-square ($\chi 2$) was obtained.

3 Results

Eighty-nine patients diagnosed with RC were evaluated during the study period, of whom only 25 met the inclusion and exclusion criteria. The average age was 56.52 years (range 40-75) (Table 1). In terms of gender distribution, the sample consisted of 11 women (44%) and 14 men (56%) (Table 2). The most frequent surgical intervention was RAB, which was performed in 18 patients (72%), followed by RAP in 6 patients (24%) and EP in 1 patient (4%) (Table 3).

Table 1. Age frequency

	N	Minimum	Maximu	Mean
Age (years)	25	40	75	56.52

Table 2. Distribution by gender

	FREQUENCY	PERCENTAGE
FEMALE	11	44%
MALE	14	56%
Total	25	100%

Table 3. Surgical Intervention

	FREQUENCY	PERCENTAGE
RAB	18	72%
RAP	6	24%
EP	1	4%
TOTAL	25	100%

In terms of distribution according to the postoperative stage of rectal tumors based on the 2010 AJCC TNM classification, we have: 8 patients in stage I (32%), 7 patients in stage IIIB (28%), 4 patients in stage IIIC (16%), 3 in stage IIA (12%), 2 in Stage IIB (8%) and 1 patient in stage IIIA (4%) (Table 4).

Table 4. Distribution by stage

		FREQUENCY	PERCENTAGE
Valid	I	8	32%
	IIA	3	12%
	IIB	2	8%
	IIIA	1	4%
	IIIB	7	28%
	IIIC	4	16%
	TOTAL	25	100%

In 14 patients (56%), no lymph nodes positive for metastasis were found, and they were classified as N0; the remaining 11 patients (44%) were classified as N1. No N2 patients were found in this study (Table 5).

Table 5. Positive lymph nodes

	FREQUENCY	PERCENTAGE
0	14	56%
1	6	24%
2	3	12%
3	2	8%
Total	25	100%

On the other hand, 9 patients (36%) had tumors between 1-2 cm, 8 patients (32%) between 2.5–3.5 cm, 7 patients (28%) between 4–5 cm, and only 1 patient (4%) had a tumor larger than 5 cm (Table 6).

Table 6. Tumor size

	FREQUENCY	PERCENTAGE
1.0	4	16%
1.2	1	4%
1.5	2	8%
2.0	2	8%
2.5	1	4%
2.7	1	4%
3.0	4	16%
3.5	2	8%
4.0	3	12%
4.5	1	4%
5.0	3	12%
6.0	1	4%
Total	25	100%

Regarding the pattern of tumor invasion, 16 patients (64%) presented an infiltrative pattern, while only 9 patients (36%) presented an expansive pattern (Table 7).

Table 7. Tumor Invasion Pattern

	FREQUENCY	PERCENTAGE
INFILTRATIVE	16	640%
EXPANSIVE	9	360%
TOTAL	25	100%

Similarly, the distribution of TB according to the scale established by the ITBCC group was as follows: 11 Bd1

patients (44%), 7 patients Bd2 (28%), and 7 patients Bd3 (28%) (Table 7).

Table 8. Distribution of TB

		FREQUENCY	PERCENTAGE
Valid	0	4	16%
	1	3	12%
	2	3	12%
	3	1	4%
	5	2	8%
	6	2	8%
	7	1	4%
	8	1	4%
	9	1	4%
	10	1	4%
	14	2	8%
	15	1	4%
	16	2	8%
	17	1	4%
	Total	25	100%

Of the patients studied, only 7 patients (28%) experienced recurrence, while the remaining 18 patients (72%) did not. Relapses were more frequent in the first 24 months of follow-up, with 4 patients (57.14%) experiencing a relapse; 2 patients (28.57%) experiencing a relapse in the first 12 months, and only 1 patient (14.29%) relapsed in the first 2 months of follow-up (Table 9).

Table 9. Relapse

	FREQUENCY	PERCENTAGE
YES	7	28%
NO	18	72%
TOTAL	25	100%

Regarding disease-free survival (DFS), the data across tumor budding (TB) risk groups were as follows: Bd1 at 76 months (range 36-108 months), Bd2 at 60 months (range 24-108 months), and Bd3 at 20 months (range 6-36 months).

4 Discussion

To date, multiple clinical, histopathological, and molecular prognostic factors have been identified in RC; however, recent evidence has emerged of other factors that may influence the clinical behavior of RC, such as TB.

TB has been stratified into three risk groups according to the number of cells or clusters greater than 4 cells (buds), which have been associated with a worse prognosis in terms of disease-free survival (DFS) and overall survival (OS), as they have been shown to be directly related to other poor prognostic factors in colorectal cancer (CRC), such as Lymphovascular Invasion (LVI), Perineural Invasion (PNI), lymph node metastasis, and tumor size.

Recent studies have demonstrated the utility and feasibility of routinely measuring TB and reporting it in the pathological examination report of surgical specimens in RC.

Regarding the presence of lymph node metastasis, 11 patients (44%) in the study sample were reported as positive, similar to the data obtained in the study by El-Gendi et al.[3], who reported lymph node positivity in 40% of cases.

Horcic [9] and Labalde [6] reported in their studies that the most frequent pattern of tumor invasion was infiltrative (68.6% and 61%, respectively). The results were very similar to those found in this study, where it was represented by 16 patients (64%).

With regard to disease-free survival (DFS), a progressive decrease was observed with increasing TB, Bd1 of 76 months, Bd2 of 60 months, and Bd3 of 20 months. These findings are consistent with data reported in the international literature [3,6,9,10].

In this study, this was demonstrated with a significance level of 5%, with degrees of freedom of 13 and according to the Chi-square table, obtaining a limit value of 22.362. Based on the analysis, the Chi-square values for each variable were as follows: 17.989 for positive lymph nodes, 13.788 for the infiltrative invasion pattern, 20.994 for perineural invasion, and 22.197 for lymphovascular invasion, where it can be concluded that within a range of 0-22.362 (acceptance region) and with the values described above for each variable, these variables are related in a dependent manner.

Similarly, we analyzed whether there is a relationship between the degree of TB and other factors of poor prognosis of RC. We demonstrated that there is a statistically significant association between high degree of TB and other factors of poor prognosis of CRC included in the TNM staging of the AJCC (seventh edition, 2010), such as: the presence of lymph node metastasis ($p = 0.004$), larger tumor size ($p=0.006$). Because the variables that are related to each other (TB, LVI, PNI,, tumor invasion pattern) are qualitative variables but are not dichotomous or binomial, the logistic regression model cannot be applied.

Our results show that there is a statistically significant relationship between high-grade tumor budding and other poor prognostic factors for colorectal cancer, such as the presence of metastasis in tumor-invaded lymph nodes, lymphatic and perineural invasion, and infiltrative growth pattern. These findings are consistent with the study by Labalde [6].

The lack of consensus on the histopathological method used to assess the degree of TB and its classification, as well as the use of different techniques to identify bud cells, such as hematoxylin-eosin staining or immunohistochemical techniques with cytokeratin, make it difficult to compare results across different studies. However, despite the fact that the method for assessing the degree of tumor budding is not standardized, all authors agree that the categories with the higher degree of TB are associated with a worse prognosis in RC.

We recommend continuing this study and working with a larger sample size over a longer period of time in order to corroborate the statistical trends found here.

It is also advisable to conduct similar studies in other oncology hospitals across the country, enabling standardized reporting and management of patients based on similar criteria, and to obtain more homogeneous populations for multicenter studies.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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