Tumor Budding in Rectal Cancer. Relationship Between the Bud Density of Tumor and Other Prognostic Factors

Javier Chinelli[1], Viviana Escobar[2], Valentina Porro[2], Emilia Moreira[3], Gustavo Rodríguez[4], Elisa Laca[5]

From Department of Pathology and Surgery, Hospital Maciel, Montevideo, Uruguay

[1] Surgeon, Clinica Quirurgica 2.

[2] Pathologist.

[3] Resident General Surgery, Clinica Quirurgica 2.

[4] Surgeon, Full Prof., Clinica Quirurgica 2.

[5] Pathologist, Chief Department of Pathology.

ABSTRACT

Introduction: *Tumor budding (TB) is defined as isolated or small groups of neoplastic cells located at the invasive front of the tumor. High-grade TB is an independent poor prognostic factor in colorectal cancer.*

Objective: *To determine if the degree of TB is associated with other prognostic factors in rectal cancer.*

Materials and methods: *Rectal oncological resections during the period 2013-2017 were included. Cases were stratified according to the density in the formation of TB in 3 groups: low, intermediate and high. The calculation of the odds ratio (OR) was used as statistical value.*

Results: *The resection specimens of 27 patients (15 women and 12 men) with a mean age of 68.4 years (40-86) were analyzed. The OR was calculated for positive lymph nodes, vascular invasion and recurrence depending on the degree of tumor budding.*

Discussion: *A tendency to the presence of poor prognosis histological factors was observed in relation to high grade budding, although the low number of cases did not allow demonstrate it in this study.*

Conclusions: *Analysis of the grade of tumor budding is reproducible and could help to identify rectal cancer patients with worse prognosis.*

Keywords: *Tumor Budding; Rectal Cancer; Prognostic Factor*

INTRODUCTION

In Uruguay, colon and rectal cancer ranks second in incidence, with more than 920 cases per year, being also the second leading cause of cancer death in both genders.[1] With regard to rectal cancer, significant therapeutic advances have been achieved in recent years. Among them, we can highlight neoadjuvant therapy, adopting total mesorectal excision (TME) as the gold standard surgical treatment[2,3] as well as the best understanding of histopathological factors determinant of the final oncologic prognosis.[4]

According to the International Union Against Cancer (UICC), and the American Joint Committee on Cancer (AJCC), the prognosis of rectal cancer is mainly based on the stage or anatomical extension of the disease, where tumor penetration, lymph node involvement, and the presence of metastasis are considered (TNM).[5] Efforts have been made to improve the prognostic prediction of this system due to the observation of different clinical results among patients with the same stage. This ambiguity can be explained by he fact that this standardized pathological staging system does not reflect exactly the biological behavior, which makes it necessary to consider other morphological parameters that can correlate with tumor aggressiveness, and risk of recurrence.[6]

Tumor budding (TB) reflects the loss of adhesion of neoplastic cells to the tumor, and is presumed to be the first step in the metastatic process.

In colorectal carcinomas it has been observed that TB, defined as the presence of individual tumor cells or groups of up to five small cells in the invasive margin of the tumor, represent an indicative sign of neoplastic progression and is an independent factor of adverse prognosis, with more probability of lymph node metastasis, distant metastases, local recurrence, and overall and disease-free survival.[7] TB represents in histology the epithelial-mesenchymal transition defined by Jass[8] in 1987, which subsequently gained wide acceptance for its application among surgical pathologists.

The objective of the present study is to determine if there exists correlation between the density of tumor buds with the depth of invasion, lymph node metastasis, and vascular invasion, as well as with the development of lo-

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Dr. Javier Chinelli

jchinelli01@gmail.com

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Javier Chinelli - https://orcid.org/0000-0003-2381-697X, Viviana Escobar - https://orcid.org/0000-0003-2381-697X, Valentina Porro - https://orcid.org/0000-0001-6803-6120, Emilia Moreira - https://orcid.org/0000-0002-9300-9981, Gustavo Rodríguez - https://orcid.org/0000-0003-3465-8364, Elisa Laca - https://orcid.org/0000-0001-6215-2059

coregional and distant recurrence, in a series of 27 patients operated on for invasive adenocarcinoma of the rectum.

MATERIAL AND METHODS

Retrospective, observational and analytical study

Includes cases of adenocarcinoma of the rectum (define as those tumors located up to 18 cm from the anal margin) operated on at Surgical Service 2 of Maciel Hospital in the period 2013-2017. Patients who received neoadjuvant therapy were excluded. Data were obtained from patient´s medical records.

The original histological reports from the archives of the Pathology Department were reviewed by two independent observers searching for TB intensity, following the recommendations made by the American College of Pathologists (CAP 2018).[9] Data of prognostic significance: histological grade, depth of parietal invasion, lymph node metastasis, vascular invasion, or development of local and/or distant recurrence were collected. The histological method used to determine the presence of TB was that of the International Tumor Budding Consensus Conference (ITBCC) 2016,[10] that recommends staining with hematoxylin and eosin (H&E), evaluating the invasion front in all plates, and selecting the area named "hot spot". The count of buds is performed using a 20X objective with a 0.785 mm2 field in a Nikon Eclipse Ci microscope. When other microscope model was used adjustment was performed depending on the size of the field. For the count of TB areas without limitations for the evaluation, such as peritumoral inflammation, glandular artifactual fragmentation, necrosis and or hemorrhage, were considered.

Following the consensus of the ITBCC group, TB definition considered a limit of up to 4 tumoral cells. The degree of budding was established for each case as follows:

a. Low budding: 0-4 buds (Bd1).
b. Intermediate budding: 5-9 buds (Bd2).
c. High budding: ≥10 buds (Bd3).

Statistical analysis

The relationship between the density of the tumor buds on the front of invasion with three histopathological parameters (vascular invasion, lymph node metastasis, and depth of invasion), as well as with the appearance of locoregional and/or systemic recurrence was evaluated.

As a measure of association, the odds ratio (OR) with a 95% confidence interval was used. The data was processed using the statistical software SPSS IBM (version 22.0). A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

The plates corresponding to 27 patients (15 female, 12 male), with a mean age of 68.4 (40-86) years were analyzed. None of the cases corresponded to synchronous tumors or hereditary colorectal cancer syndrome. The mean follow-up time was 47 (17-77) months. Regarding the location of the tumors,

they were below the peritoneal reflection in 3 cases, while the remaining 24 were above. The demographic data, histological grade, lymph node involvement, vascular invasion, parietal invasion, and TB grade are summarized in Table 1. Figures 1, 2 and 3 illustrate the different degrees of TB found, corresponding to Bd1, Bd2, and Bd3, respectively.

Association between degree of budding and prognostic factors

The correlation between the degree of TB and the different prognostic factors: lymph node involvement, vascular and parietal invasion, as well as development of local and/or distant recurrence were determined. For the latter, the risk of Bd1 or low grade against that of Bd2 and Bd3 together or high grade was compared. These results are shown in Tables 2 and 3.

Regarding the depth of invasion (T) (Table 2), OR was not calculated given the absence of observations in some of the categories.

DISCUSSION

The resection specimens of 27 adenocarcinoma cases were reviewed. They were mostly intraperitoneal, locally advanced (pT3) and moderately differentiated, highlighting that in all of them the presence and degree of tumor budding (TB) could be established.

The distribution of patients in relation to presence or absence of lymph node and vascular invasion was homogeneous with respect to other variables of prognostic value (i. ex. parietal invasion, tumor grade), which allows to affirm that the groups are comparable to define the role of TB as a prognostic factor. Those cases that had received treatment were excluded from the study. According to current recommendations and the available evidence, it is suggested not to report the TB in these cases, given the scarcity of sufficient data about its prognostic importance.[11] However, excluding those who received neoadjuvant therapy introduces an important bias in this study since it leaves a high-risk group (locally advanced subperitoneal adenocarcinomas) with chance to present poor histopathological prognostic factors, or recurrence, out of the analysis.

TABLE 1: DEMOGRAPHICS, PROGNOSTIC FACTORS, AND TUMOR BUDDING GRADE

Total cases	27
Mean age, years (range)	68.4 (40-86)
Grade of differentiation	n
Well differentiated	0
Moderate	25
Poor	2
Lymph node invasion	n
Present	13
Absent	14
Vascular invasion	n
Present	11
Absent	16
Parietal invasion	n
pT0	1
pT1	1
pT2	5
pT3	18
pT4	2
Budding grade	n
Bd1	13
Bd2	9
Bd3	5

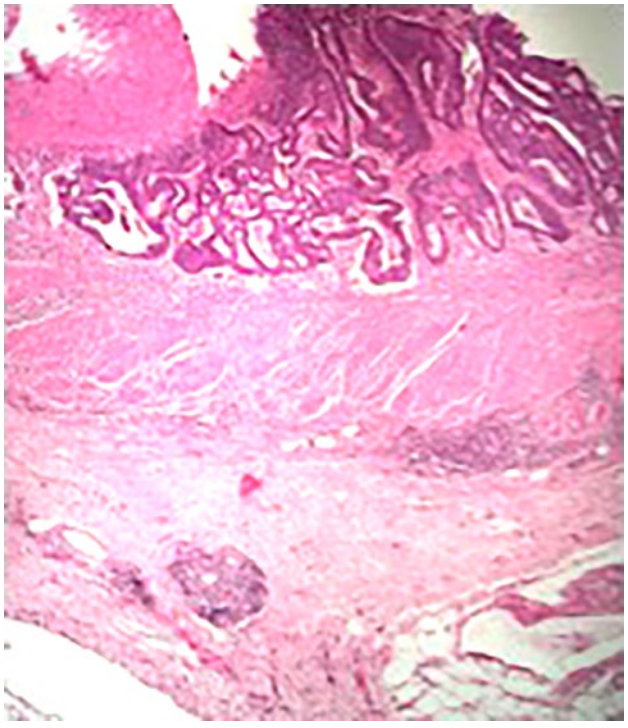

Figure 1: Low-grade budding Bd1. 0-4 buds. (H&E 10x and 20x).

Staging based on the TNM system remains insufficient, as the increase of the stage does not reflect necessarily a progressive increase in the disease, nor the risk of recurrence or death. It has been shown that survival of Stage IIIa (T1/2, N1) colon cancer patients is higher than that of patients with stage IIb disease (T4, N0).[12] Given that the TNM criteria seem to be insufficient, it is necessary to consider evaluating the local and systemic tumor environment, that includes the interface between the tumor and the guest. In this environment the loss of anti-tumoral immune response, the stromal expansion associated with the tumor, and the presence of tumor buds, all poor prognosis markers, are assessed.[13,14] These characteristics can be easily evaluated in samples prepared with conventional techniques of formalin-fixed and paraffin-embedded tissues.

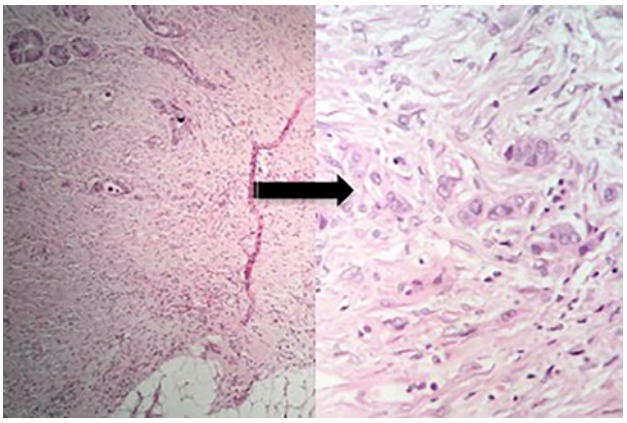

Figure 2: Intermediate budding Bd2. (Arrow). 5-9 buds. (H&E 10x and 40x)

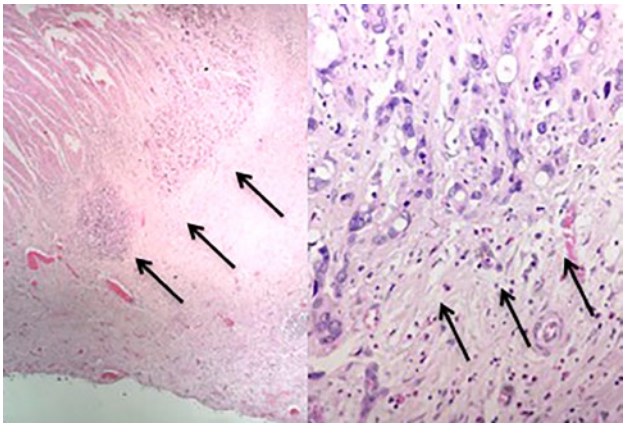

Figure 3: High-grade budding Bd3 (Arrows). More than 10 buds (H&E 10x and 40x)

In recent years it has been observed that the TB in the front of tumor advancement is related to the presence of lymph node metastasis, distant metastatic disease, local recurrence, worse overall survival, and shortening of disease-free periodsurvival.[15,16] In experimental studies, the dedifferentiation and dissociation of cancer cells constitute the first events in the process of invasion and metastasis, and TB would be the morphological expression of these events.[17] It is argued that tumor sprouting reflects the process of epithelial-mesenchymal transition,

TABLE 2: RELATIONSHIP PROGNOSTIC FACTORS, AND BUDDING GRADE

Pronostic factor	Bd1 (n)	Bd2 (n)	Bd3 (n)
Vascular invasion			
Absent (n=16)	9	5	2
Present (n=11)	4	4	3
Metástasis ganglionar			
Absent (n=14)	9	4	1
Present (n=13)	4	5	4
Depth of invasion			
pTis	1	0	0
pT1	1	0	0
pT2	3	2	0
pT3	8	5	4
pT4	0	2	1
	No	Yes	
Bd1	12	1	13
Bd2/3	11	3	14

TABLE 3: RISK OF LYMPH NODE METASTASIS, VASCULAR INVASION, AND RECURRENCE ACCORDING TO TUMOR BUDDING

LYMPH NODE INVASION			
Budding grade	OR	95% CI	P
Bd1	0.444	0.137-1.443	0.177
Bd2	1.25	0.366-4.655	0.739
VASCULAR INVASION			
Budding grade	OR	95% CI	P
Bd1	0.444	0.137-0.443	0.177
Bd2	0.8	0.215	2.979
RECURRENCE			
Bd (High vs. Low)	OR	95% CI	
	3.273	0.295-36.311	

through which the highly differentiated epithelial mucosal cells become invasive phenotypes.[18] The tumor buds show the loss of the adhesion E-Catherin molecule, and express signaling markers such as nuclear beta-catenin and APC.[19,20]

One of the main reasons why TB is not routinely reported is the lack of a standardized scoring system, simple and reproducible.[21]

TB is defined as a single tumor cell, or a group of no more than 4 cells, which we adopt for the present investigation. It is an independent predictor of lymph node metastases in pT1 tumors, and survival in stage II patients. In this stage, the presence of high-grade TB is an indicator of shorter disease free survival compared to low-grade TB or no sprouting.[22,23] Therefore, patients with stage II colorectal cancer with high-grade TB could be considered for adjuvant treatment.[24]

The TB is evaluated with H&E whenever there are not characteristics that limit its evaluation (peritumoral inflammation, glandular fragmentation) at the so-called "hot spot" of the invasive front of the tumor (where there is a higher concentration of buds), in a field that measures 0.785 mm2. Immunohistochemistry is applied in cases where evaluation with H&E is limited.

In immunohistochemical stained samples, the software provides an objective bud count. Immunohistochemistry assessment was not performed in our study, since there are studies that demonstrate it does not influence levels of agreement between pathologists compared to slides stained with H&E.[27]

A three-level system should be used for easier risk stratification, which we actually adopted in this investigation.

In our series we analyze the risk of presenting histopathological factors of poor prognosis in relation to the degree of budding, taking the Bd3 category as a reference, with the hypothesis that this constitutes in itself an adverse prognosis factor. This is justified in the face of that there is not a "no budding" category that permits to contrast the different degrees of Bd.

Both for the vascular invasion as for the presence of lymph nodes metastasis the OR includes the value 1 (Table 3), so it could not be determined the existence of a decreased or increased risk depending on the degree of Bd. This absence of statistical significance could be explained by the reduced sample size and therefore the low power of the study. However, according to what was observed (Table 2), a certain tendency to the existence of vascular invasion and lymph node involvement is noted in the presence of Bd3, as well as a tendency to observe absence of these elements when a low Bd grade is present, especially Bd1.

With regard to the parietal invasion, something similar is observed. The low number of cases studied determines the absence of observations in certain categories, for what the OR cannot be established with validity. Nevertheless, a higher proportion of Bd2 and Bd3 occurred with a higher degree of invasion (T3 and T4).

The high-degree budding (Bd2 and/or Bd3) also seemed be associated with an increased risk of local and/or distant recurrence compared to low-grade budding (Bd1).

The main limitation of this study, in addition to being retrospective, is the small sample size (patients from a low-volume colon cancer center) and the exclusion of cases with worse prognosis, whose scope was already indicated at the time of determining the existence and magnitude of the risk association.

While the study period is long, the follow-up time in some patients may be relatively short for correctly assess the development of recurrence.

CONCLUSIONS

Our findings demonstrate that TB is a reproducible indicator and suggest that it is also a predictor of parietal,

nodal and vascular invasion in patients with rectal cancer in accordance with the existing bibliography, although it could not be rigorously demonstrated statistically.

Pathologists should routinely adopt evaluating TB in the histological examination of rectal cancer specimens, which would give more predictive power to the final prognostic evaluation. This could help selecting those patients with a more aggressive disease, thus justifying meticulous postoperative follow-up, and possibly adjuvant therapy.

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COMMENT

Dr. Chinelli together with his collaborators from the Maciel Hospital in Montevideo present a series of 27 patients operated on for rectal cancer, with a mean follow-up of 47 (17/77) months. Patients with neoadjuvant were excluded and most tumors were located above the peritoneal reflection.

Although the n of the sample is small and this generates that statistically significant results cannot be obtained, the work it is very interesting.

The importance of tumor budding in relation with the prognosis of colorectal cancer patients is currently debatable.

However, this type of work serves to highlight the importance of the multidisciplinary approach to rectal cancer that should involve surgeons, gastroenterologists, imaging specialists, pathologists, among other specialties.

In this way, the quality of medical care is improved and this has an impact on the survival of patients.

Mariano Laporte
Hospital Aleman de Buenos Aires. C.A.B.A., Argentina