Techniques in Coloproctology (2019) 23:333–342
https://doi.org/10.1007/s10151-019-01959-2

ORIGINAL ARTICLE

The prognostic value of tumor budding in patients who had surgery for rectal cancer with and without neoadjuvant therapy

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Received: 11 October 2017 / Accepted: 2 March 2019 / Published online: 21 March 2019

Abstract

Background The aim of this study was to investigate the prognostic value of tumor budding (TB) in rectal cancer patients. TB in the specimens of patients who received neoadjuvant chemoradiotherapy was specifically analyzed.

Methods This study was conducted on rectal cancer patients treated at Dokuz Eylul University Hospital, Turkey, between January 2000 and June 2010. Prospectively recorded clinicopathological data and the oncological outcomes of patients who received neoadjuvant chemoradiotherapy (CRT) ($n=117$) and also patients who did not receive it ($n=113$) were analyzed. TB was defined as an isolated single cancer cell or a cluster of cells composed of less than 5 cells of a "budding focus". Budding intensity was scored as follows: none (0), mild (1–5 buds), moderate (6–10 buds), and severe (> 10 buds). Two tumor budding intesity groups were created, TB-1 (none, few) and TB-2 (moderate, severe) for statistical analysis.

Results The median follow-up time was 40.12 ± 27.5 months. The 5-year overall and disease-free survival (DFS) rates were 66% and 62%, respectively. Multivariate analysis of overall survival in all patients showed that TB intensity (HR 2.64; 95% CI 1.46–4.77) and radial margin status (HR 2.16; 95% CI 1.18–3.96) were independent predictors of decreased overall survival. In patients who received CRT, TB (HR 4.87; 95% CI 2.10–11.28) and distant metastasis (HR 4.31; 95% CI 1.81–10.22) were predictive of survival while in patients who did not receive CRT, TB (HR 4.28; 95% CI 1.60–11.49), distant metastasis (HR 2.33; 95% CI 1.19–4.60), radial margin status (HR 2.53; 95% CI 1.09–5.91), and venous invasion (HR 4.48; 95% CI 2.14–9.39) were significantly independent predictors of survival. In multivariate analysis of all patients decreased DFS was correlated with lymph node involvement (HR 2.78; 95% CI 1.60–4.87), venous invasion (HR 1.76; 95% CI 1.00–3.09), and with radial margin status (HR 2.31; 95% CI 1.27–4.22). In multivariate analysis in the CRT group, decreased DFS was significantly associated with lymph node involvement (HR 4.39; 95% CI 1.70–11.33) and radial margin status (HR 2.56; 95% CI 1.12–5.90) while only lymph node involvement (HR 2.33; 95% CI 1.16–4.66) was a significant predictor of decreased DFS in patients who did not receive CRT.

Conclusions TB has prognostic value as important as lymph node involvement and radial margin status and it may be a helpful prognostic indicator even after CRT. TB should be included in the TNM classification and may be used in planning adjuvant therapy.

Keywords Rectal cancer · Tumor budding · Rectal cancer prognosis · Overall survival · Disease-free survival

Introduction

Pathologic staging systems such as Dukes', Astler-Coller's, and the UICC/TNM classifications [1–3], which consider only parameters regarding depth of tumor penetration and lymph node involvement, are most widely used to predict long-term survival after a potentially curative resection in colorectal cancer (CRC). However, patients with the same stage of disease do not always have similar oncologic outcomes even if complete radical surgery was performed. This prognostic difference is thought to be due mainly to variations in the biological aggressiveness of primary tumors of the same stage [4].

CRC is not a homogeneous disease; rather it contains different molecular and pathological entities expressing a wide range of clinical behavior. Therefore, traditional pathological staging systems are insufficient to estimate the biological

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behaviour of rectal cancer. Concerning the prognosis of rectal cancer, it is thought that the qualitative characteristic of the true biological activity of the tumor, e.g. tumor budding (TB), can have important value *t*. TB, defined as undifferentiated cancer cells in the form of small aggregates existing on the invasive side of the lesion, is a characteristic microscopic feature of tumor dedifferentiation, which is the first sign of tumor invasion [5, 6]. In recent years, the meaning of TB in CRC has begun to be researched in terms of tumor biology, invasion, and metastasis. The prognostic importance of TB has been investigated in series of patients with CRC in various stages [7]. TB in CRC patients is related to both their biological status and clinical condition. Recent studies have demonstrated that the intensity of TB at the invasive site of CRC has a correlation with local recurrence, lymph node metastasis, and 5-year-survival. However, most of the studies have inherent limitations because of the small number of patients, short follow-up time, undeclared full oncological outcomes, and the lack of standard pathological reporting of TB [8–12].

For rectal cancer the clinical and prognostic importance of TB has not been investigated in resected specimens from both patients who had received neoadjuvant chemoradiotherapy (CRT) and those who had not. It has been a well-known assumption that unexpected and unpredictable local recurrence and distant metastasis in patients with prognostically a "grey zone" stage are due to the undetermined biological aggressiveness of the primary tumor in case of no additional treatment. Hence, the pathological evaluation of TB may be very important in determining which patients are going to receive neoadjuvant CRT.

The aim of this study was to investigate the following:

- the prognostic importance of TB in patients who received and neoadjuvant CRT and those who did not,
- the association between TB and oncological outcomes,
- whether a relationship between TB and the other well-investigated pathological prognostic factors could be demonstrated or not, in rectal cancer patients.

Materıals and methods

This study was approved by Dokuz Eylul University, Faculty of Medicine Research Ethics Committee (approval no: 381-GOA).

Patients

The clinicopathological data and oncological outcomes of 437 patients who had surgery for rectal cancer at Dokuz Eylul University Hospital between January 2000 and June 2010 were evaluated. Exclusion criteria were: tumors other than adenocarcinomas, synchronous or metachronous cancer, cancer complicating familial adenomatous polyposis or inflammatory bowel disease and ypT0N0 tumors. The preoperative work-up included general clinical examination, digital rectal examination, a complete blood test, carcinoembryonic antigen (CEA), colonoscopy and directed tumor biopsy, computed tomography (CT) scan of the chest and abdomen, and pelvic phase-array magnetic resonance imaging (MRI). Tumor location was determined by rigid proctosigmoidoscopy. The treatment regimen was planned according to the patient's age, tumor location, clinical stage, and World Health Organization performance status in a multidisciplinary team approach. All patients with locally advanced rectal cancer (cT_3–T_4N0 or $cT_{any}N+$) received neoadjuvant chemoradiotherapy (1.8 Gy/day, 5 days/week to a total of 25 fractions over a period of 5 weeks for a total of 4500 + 5-fluorouracil (FU) 225 mg/m^2/day infusion for 5 days/week during a period of 5 weeks). All included patients had radical surgery strictly according to the principles of total mesorectal excision (TME) [13]. In the CRT group patients had surgery 8 weeks after the completion of CRT. All patients were followed up with physical examination and serial assay of the serum concentrations of CEA every 3 months for 2 years and every 6 months thereafter. Colonoscopy, abdominal ultrasound/CT scan were additionally performed every 6 months for 2 years and yearly thereafter. Disease progression was defined as local recurrence and/or development of distant metastasis.

Histopathology

Histopathology slides of radically resected specimens were prospectively evaluated by two experienced pathologists who were blinded to the clinical data and patient outcomes. Formalin-fixed and paraffin-embedded tissue was sectioned and stained with hematoxylen and eosin for microscopic examination. TB along the invasive margin was examined in addition to routine pathological findings. The presence of budding was determined according to the criteria proposed by Hase at al. [6], whereby budding is defined as an isolated single cancer cell or a cluster composed of ≤ 5 undifferentiated cancer cells appearing to bud from a large cancer gland on the invasive side. Tumor slides were initially scanned at 20× magnification for areas with the highest density of tumor buds. The selected area was then examinated under 400× magnification and number of the tumor buds was determined with the same light microscope for each case. The resulting number of tumor buds was considered to be the degree of TB and the term "budding intensity" was used for this number. Budding intensity was scored as follows: none (0), mild (1–5 buds), moderate (6–10 buds), and severe (> 10 buds) (Fig. 1). Two groups were created, TB-1 (none, mild) and TB-2 (moderate, severe), for statistical analysis

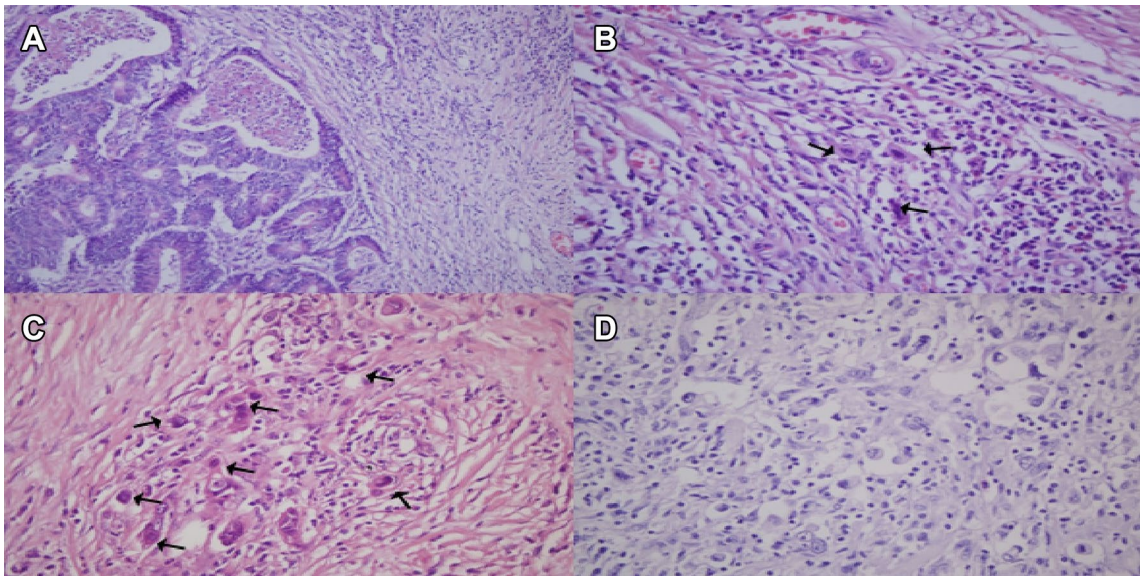

Fig. 1 Tumor budding indicated with arrows, showing small clusters of poorly differentiated or undifferentiated cells, ahead of the invasive front of rectal cancer; **a** no budding (H&E ×200); **b** mild (1–5 buds) (H&E ×400); **c** moderate (6–10 buds) (H&E ×400); **d** severe (> 10 buds) (H&E ×400)

[14]. The pathologic stage was evaluated according to the 7th UICC/TNM staging system.

Statistical analysis

An independent variables *t* test, the Mann–Whitney *U* test, and the Chi square test were used during data evaluation for single variable analysis. A model was composed using the significant relation detected in the univariate analysis and then the multivariate logistic regression analysis was performed. The Kaplan–Meier method was used for the survival analysis and the log rank test was applied for the comparison of the groups. The significantly different variables were evaluated by the Cox regression model. The results were evaluated as 95% confidence interval and significance was set at $p < 0.05$.

Results

A total of 230 patients out of 432 patients were included in this study; 117 patients with cT_3–T_4N0 or $cT_{any}N(+)$ locally advanced rectal cancer who received neoadjuvant CRT, and 113 patients with cT_2–T_3N0 rectal cancer who did not receive CRT preoperatively. There were 96 (41.7%) women, and 134 (58.3%) men and the mean age was 62.1 ± 12 years. Excluding 15 patients without follow-up data, median follow-up time was 40.1 ± 27.5 months. Local recurrence had occurred in 13 patients during the follow-up period. For all patients the 5-year overall survival (OS) and disease-free survival (DFS) rates were 66% and 62%, respectively.

The association between the intensity of TB and clinicopathological variables in all patients is shown in Table 1. In multivariate analysis, the presence of lymphatic invasion (HR 3.99; 95% CI 1.84–8.69), venous invasion (HR 2.48; 95% CI 1.04–5.92), local recurrence (HR 3.87; 95% CI 1.05–14.26), and the number of metastatic lymph nodes (HR 1.16; 95% CI 1.02–1.32) were shown to be independent prognostic factors for TB.

Multivariate Cox regression analysis revealed that radial margin status (HR 2.16; 95% CI 1.18–3.96) and intensity of TB (HR 2.64; 95% CI 1.46–4.77) had an independent effect on overall survival (Table 2).

The association between clinicopathological variables and overall survival in patients who received neoadjuvant CRT and those who did not is shown in Table 3. In multivariate analysis, TB (HR 4.28; 95% CI 1.60–11.49), radial margin status (HR 2.53; 95% CI 1.09–5.90), distant metastasis (HR 2.33; 95% CI 1.18–4.60), and venous invasion (HR 4.48; 95% CI 2.14–9.39) were strong prognostic factors of overall survival for the patients who underwent CRT while TB (HR 4.87; 95% CI 2.10–11.28) and distant metastasis (HR 4.30; 95% CI 1.81–10.22) were independent predictors in patients not undergoing CRT.

In the Cox proportional hazards model for all patients, worse disease free survival was correlated with involved lymph nodes (HR 2.78; 95% CI 1.60–4.87), venous invasion (HR 1.76; 95% CI 1.00–3.09), and with radial margin status (HR 2.31; 95% CI 1.27–4.22) as shown in Table 4.

Table 1 The association between clinicopathological variables and tumor budding

Variable	TB-1 (none or mild)		TB-2 (moderate or severe)		Univariate analysis[a,b] *p*	Multivariate analysis[c] (HR, 95% CI)	*p*
	N	% (mean ± SD)	*N*	% (mean ± SD)			
Sex					0.232[a]		
Female	71	74	25	26			
Male	108	80.6	26	19.4			
Neoadjuvant chemoradiotherapy					0.005[a]		
Absent	79	69.9	34				
Present	100	85.5	17				
Local recurrence					0.034[a]	3.87 (1.05–14.26)	0.042
Absent	170	79.1	45	20.09			
Present	7	53.8	6	46.2			
T stage					0.041[a]		
T1	14	100	0	0			
T2	45	84.9	8	15.1			
T3	90	75.6	29	24.4			
T4	30	68.2	14	31.8			
N stage					< 0.001[a]		
N0	119	86.2	19	13.8			
N1	42	76.4	13	23.6			
N2	18	48.6	19	51.4			
Distant metastasis					0.009[a]		
Absent	134	82.2	29	17.8			
Present	43	66.2	22	33.8			
Grade					0.794[a]		
Low	100	78.7	27	21.3			
High	65	80.2	16	19.8			
Radial margin					0.187[a]		
Negative	152	79.6	39	20.4			
Positive	17	68	8	32			
Lymphatic invasion					< 0.001[a]	3.99 (1.84–8.69)	< 0.001
Absent	123	89.1	15	10.9			
Present	47	58	34	42			
Perineural invasion					0.062[a]		
Absent	140	80.5	34	19.5			
Present	34	68	16	32			
Venous invasion					< 0.001[a]	2.48 (1.04–5.92)	0.041
Absent	151	84.4	28	15.6			
Present	20	47.6	22	52.4			
Age, years	179	62 ± 11	51	62 ± 13	0.905[b]		
Tumor location (cm)[d]	177	9.5 ± 4.5	51	9.5 ± 4.6	0.971[b]		
No. of lymph nodes examined	179	14.4 ± 8.5	51	15.9 ± 9.6	0.287[b]		
No. of metastatic lymph nodes	179	0.9 ± 2	51	5.3 ± 9	0.002[b]	1.16 (1.02–1.32)	0.024
Maximum diameter of tumor (cm)	164	3.3 ± 1.6	48	3.4 ± 1.5	0.583[b]		
Radial margin status (mm)	127	7 ± 6.4	32	5 ± 5.9	0.114[b]		

[a]Chi square test

[b]*t* test

[c]Logistic regression

[d]The average distance between the distal pole of the tumor to the anal verge

Table 2 The association between clinicopathological variables and overall survival

Variable	Alive		Dead		Univariate analysis[a, b] *p*	Multivariate analysis[c] (HR, 95% CI)	*p*
	n	% (mean ± SD)	*n*	% (mean ± SD)			
Sex					0.117[a]		
Female	68	75.6	22	24.4			
Male	82	65.6	43	34.4			
Neoadjuvant chemoradiotherapy					0.003[a]		
Absent	59	59.6	40	40.4			
Present	91	78.4	25	21.6			
Local recurrence					0.056[a]		
Absent	144	71.3	58	28.7			
Present	6	46.2	7	53.8			
T stage					0.002[a]		
T1	10	83.3	2	16.7			
T2	43	82.7	9	17.3			
T3	78	70.3	33	29.7			
T4	19	47.5	21	52.5			
N stage					< 0.001[a]		
N0	103	79.8	26	20.2			
N1	34	63	20	37			
N2	13	40.6	19	59.4			
Intensity of tumor budding					< 0.001[a]		
None	82	82.8	17	17.2			
Mild	50	72.5	19	27.5		2.38 (0.81–6.96)	0.111
Moderate	11	36.7	19	63.3		18.66 (4.72–73.74)	< 0.001
Severe	7	41.2	10	58.8		8.97 (1.37–58.56)	0.022
Intensity of tumor budding							
No budding	82	82.8	17	17.2			
Budding	68	58.6	48	41.4	< 0.001[a]	2.64 (1.46–4.77)	0.002
Grade					0.648[a]		
Low	87	70.7	36	29.3			
High	48	67.6	23	32.4			
Radial margin					0.001[a]		
Negative	133	73.1	49	26.9			
Positive	9	39.1	14	60.9			
Lymphatic invasion					0.005[a]		
Absent	101	76.5	31	23.5			
Present	42	57.5	31	42.5			
Perineural invasion					0.006[a]		
Absent	120	74.1	42	25.9			
Present	25	53.2	22	46.8			
Venous invasion					< 0.001[a]		
Absent	128	76.6	39	23.4			
Present	16	41	23	59			
Age, years	150	(61 ± 10.6)	65	63 ± 14	0.167[b]		
Tumor location (cm)[d]	148	(9.1 ± 4.6)	65	(10.1 ± 4.1)	0.162[b]		
No. of lymph nodes examined	150	(14.5 ± 8.9)	65	(15.5 ± 8.9)	0.446[b]		
No. of metastatic lymph nodes	150	(0.9 ± 2.3)	65	(4.1 ± 8.1)	0.003[b]		
Maximum diameter of tumor (cm)	139	(3.2 ± 1.6)	62	(3.5 ± 1.5)	0.216[b]		
Radial margin status (mm)	107	(8.2 ± 6.7)	45	(3.3 ± 3.4)	< 0.001[b]	2.16 (1.18–3.96)	< 0.001

[a]Chi square test

[b]*t* test

[c]Logistic regression

[d]The average distance between the distal pole of the tumor to the anal verge

Table 3 The association between clinicopathological variables and overall survival in the CRT (+ve) and CRT (−ve) groups

	Neoadjuvant chemoradiotherapy (+)				Univariate analysis[a,b] *p*	Multivariate analysis[c] (HR, 95% CI) *p*	Neoadjuvant chemoradiotherapy (−)				Univariate analysis[a,b] *p*	Multivariate analysis[c] (HR, 95% CI) *p*
	Alive		Dead				Alive		Dead			
	N	% (mean ± SD)	*n*	% (mean ± SD)			*n*	% (mean ± SD)	*n*	% (mean ± SD)		
Sex					0.418[a]						0.187[a]	
Female	41	82	9	18			27	67.5	13	32.5		
Male	50	75.8	16	24.2			32	54.2	27	45.8		
Local recurrence					0.305[a]						0.184[a]	
Absent	88	79.3	23	20.7			56	61.5	35	38.5		
Present	3	60	2	40			3	37.5	5	62.5		
T stage					0.016[a]						0.237[a]	
T1	3	100	0	0			7	77.8	2	22.2		
T2	30	93.8	2	6.3			13	65	7	35		
T3	51	75	17	25			27	62.8	16	37.2		
T4	7	53.8	6	46.2			12	44.4	15	55.6		
N stage					0.011[a]						0.007[a]	
N0	63	86.3	10	13.7			40	71.4	16	28.6		
N1	20	71.4	8	28.6			14	53.8	12	46.2		
N2	8	53.3	7	46.7			5	29.4	12	70.6		
Intensity of tumor budding					0.001[a]						0.008[a]	
None	54	85.7	9	14.3			28	77.8	8	22.2		
Mild	30	83.3	6	16.7			20	60.6	13	39.4		3.13 (0.66–14.80) 0.150
Moderate	5	41.7	7	58.3			6	33.3	12	66.7		25.36 (2.89–221.91) 0.003
Severe	2	40	3	60.0			5	41.7	7	58.3		21.07 (1.62–273.41) 0.020
Intensity of tumor budding												
No budding	28	77.8	8	22.2			54	85.7	9	14.3		
Budding	31	49.2	32	50.8	0.005[a]		37	69.8	16	30.2	0.038[a]	1.64 (0.66–4.05) 0.065
Grade					0.884[a]						0.697[a]	
Low	53	76.8	16	23.2			34	63	20	37		
High	25	78.1	7	21.9			23	59	16	41		
Radial margin					0.013[a]						0.026[a]	
Negative	83	81.4	19	18.6			50	62.5	30	37.5		
Positive	6	50	6	50			3	27.3	8	72.7		

Table 3 (continued)

	Neoadjuvant chemoradiotherapy (+)				Univariate analysis[a,b] *p*	Multivariate analysis[c] (HR, 95% CI) *p*	Neoadjuvant chemoradiotherapy (−)				Univariate analysis[a,b] *p*	Multivariate analysis[c] (HR, 95% CI) *p*
	Alive		Dead				Alive		Dead			
	N	% (mean ± SD)	*n*	% (mean ± SD)			*n*	% (mean ± SD)	*n*	% (mean ± SD)		
Lymphatic invasion					0.003[a]						0.557[a]	
Absent	69	85.2	12	14.8			32	62.7	19	37.3		
Present	17	58.6	12	41.4			25	56.8	19	43.2		
Perineural invasion					0.010[a]						0.180[a]	
Absent	73	83	15	17			47	63.5	27	36.5		
Present	14	58.3	10	41.7			11	47.8	12	52.2		
Venous invasion					0.009[a]						< 0.001[a]	
Absent	74	83.1	15	16.9			54	69.2	24	30.8		
Present	12	57.1	9	42.9			4	22.2	14	77.8		2.14 (0.89–5.16) 0.032
Age, years	91	(60.1 ± 9.5)	25	(58.9 ± 0)	0.762[b]		59	(62.8 ± 11.9)	40	(67.1 ± 13.7)	0.104[b]	
Tumor location (cm)[d]	89	(7.1 ± 3.9)	25	(9.4 ± 4.3)	0.015[b]		59	(12.2 ± 3.9)	40	(10.5 ± 4.1)	0.045[b]	
No. of lymph nodes examined	90	(11.8 ± 6.7)	25	(13.5 ± 8)	0.431[b]		59	(18.6 ± 10.2)	40	(16.8 ± 9.2)	0.368[b]	
No. of metastatic lymph nodes	91	(0.8 ± 1.9)	25	(3.6 ± 7)	0.004[b]		59	(1 ± 2.9)	40	(4.5 ± 8.9)	0.023[b]	
Maximum diameter of tumor (cm)	84	(2.6 ± 1.3)	23	(3 ± 1)	0.041[b]		55	(4.1 ± 1.7)	39	(3.8 ± 1.6)	0.355[b]	
Radial margin status (mm)	65	(8 ± 5)	16	(2.7 ± 3.1)	< 0.001[b]	0.71 (0.57–0.87) 0.001	42	(8.5 ± 8.9)	29	(3.6 ± 3.6)	0.002[b]	0.49 (0.18–1.33) 0.060
Tumor budding grade	91	(1.6 ± 2.5)	25	(4.5 ± 4.8)	0.003[b]		59	(2.8 ± 3.7)	40	(5.4 ± 4.7)	0.005[b]	
Tumor regression grade	91	(1.9 ± 0.5)	25	(2.3 ± 0.5)	0.016[e]							
Quality of mesorectum	90	(0.4 ± 0.8)	25	(1.1 ± 1.4)	0.019[e]		46	(0.3 ± 0.8)	24	(0.5 ± 1.0)	0.818[d]	

CRT chemoradiotherapy

[a]Chi square test

[b]*t* test

[c]Logistic regression

[d]The average distance between the distal pole of the tumor to the anal verge

[e]Mann–Whitney *U* Test

Table 4 Determination of prognostic factors for disease-free survival using Cox regression analysis

Prognostic factors	*B*	SE	*p*	HR	95% CI for HR	
					Lower	Upper
Tumor budding	0.164	0.325	0.615	1.17	0.62	2.22
Age	0.006	0.012	0.593	1.00	0.98	1.03
Gender	0.066	0.290	0.820	1.06	0.60	1.88
Depth of invasion	0.665	0.419	0.113	1.94	0.85	4.42
Lymph node involvement	0.916	0.311	0.003	2.49	1.35	4.60
Tumor grade	−0.093	0.300	0.758	0.91	0.50	1.64
Radial margin status	0.762	0.348	0.029	2.14	1.08	4.24
Perineural invasion	0.428	0.300	0.154	1.53	0.85	2.76
Venous invasion	0.394	0.321	0.221	1.48	0.79	2.78
Chemo-radiotherapy	0.380	0.301	0.207	1.46	0.81	2.63

In the subgroup of patients who had CRT decreased disease free survival was associated with lymph node involvement (HR 4.39; 95% CI 1.70–11.33) and radial margin status (HR 2.56; 95% CI 1.12–5.90) while only lymph node involvement (HR 2.33; 95% CI 1.16–4.66) was significant predictor of DFS in patients who had not received CRT (Table 5).

A separate analysis of pT_2–T_3NO rectal tumors was performed. In multivariate analysis, the presence of venous invasion (HR 6.20; 95% CI 1.99–19.36) was shown to be an independent predictor for TB in pT_2–T_3N0 rectal cancer. Multivariate Cox regression analysis showed that venous invasion (HR 26.22; 95% CI 2.63–261.76) was the only independent risk factor for overall survival. In multivariate analysis, no factor was independently predictive of survival in the CRT-treated group, while venous invasion (HR 15.60; 95% CI 1.34–182.09) was a strong prognostic factor in patients with pT_2–T_3N0 rectal cancer who did not receive CRT. In the Cox proportional hazards model, TB (HR 4.40; 95% CI 1.10–17.74) was significantly associated with decreased DFS in CRT-treated patients with T_2–T_3N0 tumors.

Discussion

To date, the prognostic and clinical importance of tumour budding have not been investigated in the irradiated macroscopic specimens of rectal cancer from patients who received neoadjuvant chemotherapy. The literature indicates that TB in CRC cases was investigated before 2000 when neoadjuvant CRT was uncommon [7, 11, 15–17]. Our study is important, because it is, to the best of our knowledge, the first study to evaluate the potential prognostic relationship of TB in a relatively large series of CRT-treated patients with locally advanced rectal cancer. Our results showed that as the intensity of TB increased, the OS rates sharply decreased. When patients who received CRT were grouped with patients who did not receive CRT, there was a significant relationship between TB and worse OS but such a robust association was lacking between TB and DFS. In a study by Kinoshita et al. [18], TB was reduced after preoperative CRT. In our study we observed that in patients who had received neoadjuvant CRT TB also affected OS. TB was not identified as an independent prognostic factor for DFS in both patients who underwent CRT and patients who did not. However, TB was identified as a a prognostic factor for DFS in the subset of patients with T_2–T_3N0 tumors who underwent CRT.

It is remarkable that TB had prognostic value in early stage rectal cancer given the fact that adjuvant chemotherapy

Table 5 Multivariate analysis of the prognostic factors of DFS in the CRT (+ve) and CRT (−ve) groups

Prognostic factors	Neoadjuvant chemoradiotherapy (+)				Neoadjuvant chemoradiotherapy (−)			
	p	HR	95% CI		*p*	HR	95% CI	
			Lower	Upper			Lower	Upper
pN	0.002	4.39	1.70	11.33	0.017	2.33	1.16	4.66
Radial margin status	0.026	2.56	1.12	5.90				

DFS disease-free survival, *CRT* chemoradiotherapy

and radiotherapy is not administered in early stage cancer TB may be used as an indication for such treatment. TB in CRC patients with T_3N0 was also reported to be an independent prognostic factor in a study by Wang et al. [19]. TB must be evaluated and reported in pathology reports and should be considered in the multidisciplinary tumor board discussions to assist in decision making about additional treatment.

The oncologic literature indicates that as the depth of tumor invasion increases the OS rate of rectal cancer patients decreases [20]. Our study also confirmed that the OS rates were higher in patients with early stage rectal cancer.

Findings in our study are consistent with those in studies supporting the notion that the presence of lymphatic invasion and venous invasion reduces OS in patients with CRC [21]. Lymphatic invasion and venous invasion are the established prognostic factors defined as Category I in the classification made by College of American Pathologists [22].

TB was more frequent in patients who developed a local recurrence. In a study by Hase et al [6], patients with moderate and severe TB had significantly higher local recurrence rates and significantly lower 5-year and 10-year survival rates than patients with no TB or mild TB. Also according to this study, 5-year survival of Dukes B cases with moderate or severe tumor budding was reported to be lower than that of Dukes C cases with no TB or mild TB. In our study, the presence of distant metastasis was found to negatively affect OS whether patients had received neoadjuvant CRT or not. Distant metastasis in patients with CRC was proved to be an indicator of poor prognosis for survival by several studies [23].

The presence of lymph node involvement reduces OS significantly in patients with CRC and the higher the numbers of involved lymph nodes the worse the effect on survival rates. Lymph node involvement is defined as the second most powerful "postoperative outcome indicator", the first one being the occurence of distant metastasis [3]. As well as the number of involved lymph nodes, the number of total lymph nodes in the surgical specimen affects the prognosis of both stage II (lymph node negative) and stage III (lymph node positive) disease directly [24]. In our study, lymph node involvement was found to be a factor that reduced OS and DFS rates through all groups in univariate analysis. In multivariate analysis, lymph node involvement was found to be an independent prognostic factor for DFS whether patients received neoadjuvant CRT or not.

The intactness of the mesorectum and the radial margin status are among the most important prognostic factors for local and distant metastasis and for survival [25, 26]. In a study by Birbeck et al. [27], the effect of radial margin involvement in rectal cancer on the survival rate has been investigated. A higher rate of local recurrence was reported in cases of radial margin involvement. It has been demonstrated that radial margin status could be used as the prognosticator of survival after rectal cancer surgery. In our study, radial margin involvement reduced OS significantly in all patients whether they had received neoadjuvant CRT or not. Moreover, when the radial margin was involved, DFS rates decreased in patients treated with CRT.

Although not used for staging, pre-treatment CEA levels, radial margin status, the presence/absence of perineural invasion, the microsatellite instability status and tumor regression grade (for CRT-treated patients) are the recommended prognostic factors according to the TNM guidelines [3]. Despite the fact that many studies in the literature report the prognostic value of TB, it is still not used sufficiently in clinical practice.

The drawbacks of this study are mainly the non-measured interobserver agreement and the relatively small number of patients in the series, particularly in the subgroup analysis.

Conclusions

TB has a prognostic value at least as strong as lymph node involvement. We recommend that TB be included in the routine pathological examination of rectal carcinoma. Because of its independent prognostic value the intensity of TB should be used to determine whether adjuvant treatment is indicated after chemotradiation and may be used as a biological criterion for patients who are unresponsive to treatment and for the use of more intensive systemic chemotherapy protocols. More studies are needed to confirm the prognostic significance of TB in rectal cancer patients, especially in the subset treated with CRT. If our results are confirmed TB should be included among the negative prognostic factors listed in the TNM classification.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by instutional ethical comittee.

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