Original paper

Tumour budding – an additional prognostic factor in colorectal cancer survival

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Tumour budding (TB) in cancer is a phenomenon of tumour cells forming clusters, and it is associated with an epithelial-mesenchymal transition into the extracellular matrix of the tumour. It has been shown that the presence of TB in colorectal cancer (CRC) is associated with worse overall survival, higher possibility for vessel invasion, lymph node involvement, and distant metastases appearance.
In this retrospective study TB presence in operated patients for CRC is analysed. In the data from 81 patients, 26 presented with TB.
Analysis revealed high statistical significance of the effect of TB presence on the number of metastatic lymph nodes, and the lymphovascular and perineural invasion. A statistically meaningful correlation was found between the presence of TB and CRC survival ($p = 0.016$). Patients with right-sided colon cancer presented with worse overall survival ($p = 0.011$). The patients who presented lymph node metastases and TB presence had worse overall survival ($p = 0.026$ and $p = 0.021$, respectively). Tumour budding, tumour location, and age over 64 years are found to be the independent prognostic factors in CRC patients. Tumour budding is an important prognostic factor in CRC patients that will contribute to treatment. Pathological examination must consider TB in detail.

Key words: colorectal cancer, prognosis, survival, tumour budding.

Introduction

Tumour budding (TB) is defined with the presence of a single tumour cell and/or up to 5 cells organized in clusters (Figs. 1–3), a phenomenon associated with an epithelial-mesenchymal transition into the extracellular matrix of the tumour [1]. According to the location of the tumour buds, they are classified as peritumoural and intratumoural [2]. As a result, higher possibility for vessel invasion and lymph node and distant metastases occurrence has been shown [3]. Therefore, the relationship between TB with the progression of the primary tumour, local recurrence, and distant metastases appearance was proved [4].

Several publications imposed the need and importance for the inclusion of TB in the staging systems due to its prognostic value [5–9]. Its role in the prediction of prognosis in colorectal cancer (CRC) with a standardized scoring system was also proposed by the International Tumour Budding Consensus Conference (ITBCC) in 2016. Additionally, the presence of tumour buds plays a role in indicating an oncologic resection in pT1 CRC tumours, and at the same time, in the recommendation for adjuvant therapy in stage II of CRC [2]. It has been demonstrated that there is strong prognostic impact of the poorly differentiated clusters of TB in patients with colorectal metastases, and at the same time, they are a strong and indepen-

dent predictor of the overall survival and the disease-free survival after colorectal metastasis surgery in stage IV patients [7].

Material and methods

The medical data of patients who underwent surgery due to CRC between 2015 and 2020 were examined. The data of 81 patients was analysed for TB presence in the pathology report. Inclusion criteria were defined for CRC patients (solitary and synchronous) with proven presence of TB in the pathology report in every stage of the disease. Exclusion criteria were applied in patients with mortality within one month of the surgery and in patients with present other organ primary tumours. Data of demographic and tumour characteristics (localization of tumour, tumour size, histopathologic features of the tumour) were collected. Statistical analysis was conducted on the overall survival and the clinicopathological features of patients, age, gender, localization of tumour, perineural invasion, lymphovascular invasion, total number of harvested lymph nodes, metastatic lymph nodes, and the presence of TB. Age and tumour size cut-off values were defined by using the receiver operating characteristic curve.

SPSS v. 18.0.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Clinical and pathological parameters were analysed with the χ^2 test, Fisher exact test, Mann-Whitney *U* test, Kaplan-Meier, and multivariable Cox regression methods. Statistical significance was set at a p-value of ≤ 0.05.

Results

In the group of 81 patients operated due to colorectal cancer, 32 were female and the remaining 49 were male. In the female group TB was identified in 8, while in males TB was reported by the pathologist in 18 patients. Colorectal cancer localization presented as follows: 22 patients with right colon cancer, 5 with transverse colon cancer, 20 with left colon cancer, 4 patients with sigmoid presentation of tumour, and 28 patients with rectal cancer. In 2 patients a synchronous tumour was treated. Tumour budding was identified in 26 patients in total. Statistically, there was no influence of TB on the patients' age, tumour size and location, T stage, and the total number of harvested lymph nodes. However, the analysis showed high statistical significance of TB presence on the number of metastatic lymph nodes, and the lymphovascular and perineural invasion (Table I).

All patients included in this study were followed with a mean follow-up period of 23.07 months. In terms of the influence of TB presence on the overall survival, different parameters were analysed.

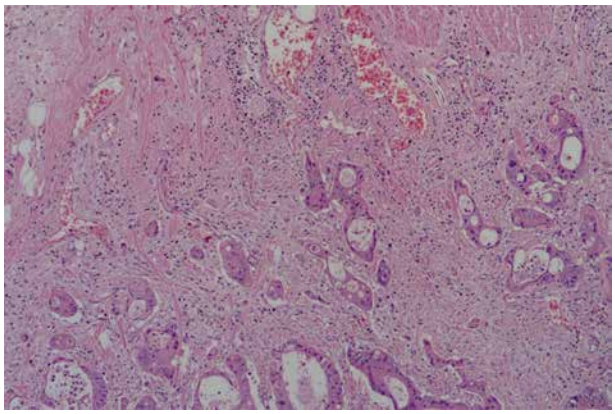

Fig. 1. Isolated cells and small groups making tumour budding (X10 HE)

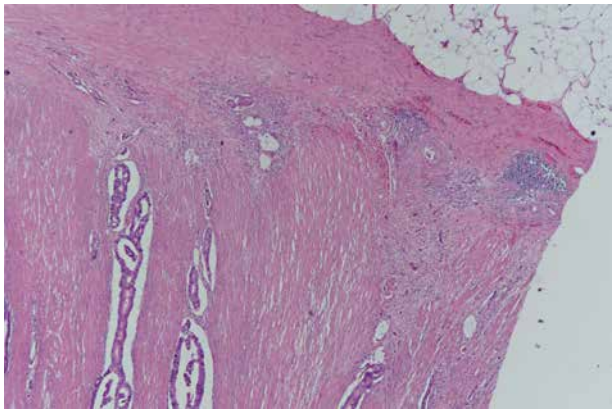

Fig. 2. High-grade tumour budding around tumour (X4 HE)

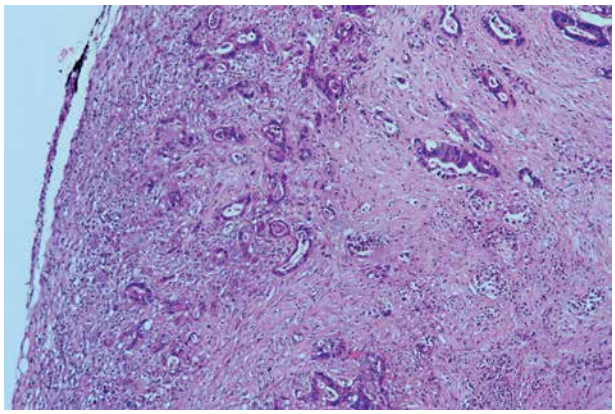

Fig. 3. High-grade tumour budding around tumour invasion border (X10 HE)

The tumour size, T stage, lymphovascular and perineural invasion, and lymph node metastases did not present with statistical significance in both groups. On the other hand, age with a cut-off value of 64 years presented with significance in terms of worse overall survival ($p = 0.004$).

The anatomic location of the tumour also differed, with the left colon tumours having the better overall survival ($p = 0.011$). Patients with lymph node metastases had significantly worse outcomes in the case

Table I. Demographic and tumour characteristics correlation with tumour budding presence

Parameters	No tumour budding	Tumour budding present	*p*-value
Age (mean)	64.05	64.2	0.95
Gender			
Female	24	8	0.334
Male	31	18	
Tumour location			
Right colon	12	10	
Left colon	14	6	
Sigmoid colon	2	2	0.499
Rectum	21	7	
Transverse colon	4	1	
Synchronous tumour	2	0	
Tumour size (cm)	5.783	5.808	0.876
T stage			
T1	2	1	
T2	9	6	0.837
T3	37	15	
T4	6	4	
Tumour in situ	1	0	
Harvested lymph nodes (mean)	24.35	29.00	0.373
Metastatic lymph nodes (mean)	1.64	6.62	**0.000**
Lymphovascular invasion			
(–)	32	5	**0.002**
(+)	23	21	
Perineural invasion			
(–)	38	6	**0.000**
(+)	17	20	

Table II. Overall survival according to demographic and tumor characteristics

Parameters	Values, location, pathological features		*p*-value
Age (cut-off value 64)	> 64	< 64	**0.042**
Tumour (cut-off value 5)	> 5 cm	< 5 cm	**0.64**
Left vs. right colon cancer	Descending, sigmoid, rectum	Ascending, transverse	**0.011**
T stage			0.42
Lymph node metastases	Present	Absent	**0.026**
Lymphovascular invasion	Present	Absent	**0.002**
Perineural invasion	Present	Absent	**0.018**
Tumour budding	Present	Absent	**0.021**

of TB presentation ($p = 0.021$). The division of tumour location (left vs. right colon cancer) also presented a statistically significant difference in survival ($p = 0.011$) (Table II). The overall survival was significantly shorter in patients with TB ($p = 0.016$) (Fig. 3).

Multivariate Cox regression analysis for independent prognostic factors revealed that TB presence, tumour location, and age over 64 years are independent prognostic factors for survival in CRC, ($p = 0.016$, $p = 0.029$, and $p = 0.004$, respectively) (Table III).

Discussion

Although being the most important independent prognostic factor in CRC, the postoperative TNM staging of the tumour in different patients with the same tumour stage shows differences in terms of the prognosis and survival [10]. Therefore, one of the proposed additional prognostic markers as an addition to the TNM system is TB [1]. Besides TB, other prognostic biomarkers were proposed: Kristen rat sarcoma viral oncogene homologue (KRAS), microsatellite instability (MSI), and homebox protein CDX2. It has been shown that all of them are independent factors in the CRC prognosis [11, 12].

The relationship between TB and gender, age, tumour size, and T stage has been shown to be without statistical significance to date [13–17]. This study also presented the absence of an influence of TB on the patient demographics, tumour diameter and location, and T stage.

Regarding the lymphovascular and perineural invasion, in most of the reports the positivity of the invasion is significantly correlated with TB presence [15, 16, 18–20]. In this study the lymphovascular and perineural invasion was significantly correlated with the TB presence in the CRC.

The relationship of TB with positive lymph node number was proven to be significant in previous studies. In most of them, the association between TB presence and lymph node involvement was proven [6, 9, 16, 18]. In his meta-analysis, Cappellesso *et al.* showed this strong association in 41 studies with an OR value of 6.44 (95 % CI: 5.26–7.87; $p < 0.0001$) [21]. High significance of this association was also proved in this study.

The lymph node involvement from CRC generally is reported to be a bad prognostic factor [22]. In addition, the prognosis is even worse with concomitant TB. It has been shown that TB presence is associated with cancer-related death within 5 years (OR 4.51, 95% CI: 2.55–7.99, $p < 0.00001$) [3]. In his review, Petrelli presented 4 studies including only pT3N0M0 cases where TB was associated with a worse 5-year risk of death (OR = 5.55, 95% CI: 3.09– 9.94, $p < 0.00001$) [23]. Ohtsuki *et al.* showed a significantly lower disease-free survival rate in patients with or without present TB (40.9% and 75.1%, respectively) [19]. In our study the presence of TB in the colorectal carcinoma significantly influenced the overall survival.

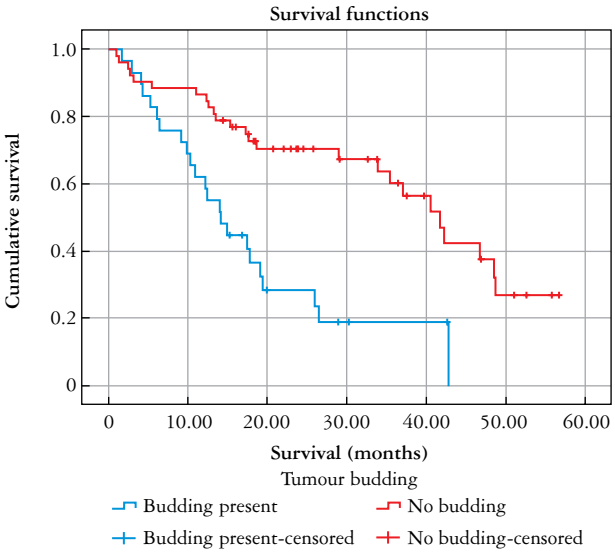

Fig. 4. Kaplan-Meier curve for overall survival according to the presence of tumour budding

Furthermore, the proposed criteria for TB subgrouping were analysed in terms of the differences in budding and its influence on the prognosis prediction [24]. According to ITBCC guidelines, there are 3 different budding grades defined and termed with BD1,

Table III. Multivariate Cox regression analysis for independent prognostic factors

Variables in the equation	B	SE	Wald	df	Sig.	Exp (B)	95 % CI for exp (B)	
							Lower	Upper
Age 64 years (cut-off)	0.977	0.369	7.026	1	0.004	2.656	1.290	5.471
T stages			2.991	4	0.559			
T stage: T1: 1, T2: 2, T3: 3, T4: 4 (1)	1.451	0.864	2.818	1	0.093	4.267	0.784	23.221
T stage: T1: 1, T2: 2, T3: 3, T4: 4 (2)	1.129	0.808	1.954	1	0.162	3.094	0.635	15.075
T stage: T1: 1, T2: 2, T3: 3, T4: 4 (3)	0.901	0.901	1.001	1	0.317	2.462	0.421	14.386
T stage: T1: 1, T2: 2, T3: 3, T4: 4 (4)	–10.102	413.280	0.001	1	0.980	0.000	0.000	.
Lymphovascular invasion	0.863	0.552	2.445	1	0.118	2.369	0.804	6.986
Perineural invasion	0.556	0.340	2.670	1	0.102	1.744	0.895	3.396
Lymph node involvement	–0.230	0.574	0.161	1	0.688	0.794	0.258	2.444
Tumour location	–0.717	0.328	4.768	1	0.029	0.488	0.257	0.929
Tumour budding (+, –)	0.872	0.368	5.614	1	0.016	2.391	1.163	4.916

B – coefficient B value, df. – degree of freedom, exp – hazard ratio, SE – standard error, sig. – significance

BD2, and BD3 depending on the number of buds in a hotspot of 0.785 mm^2 [2]. Recently, Zlobec *et al*. proposed the necessity for a fourth BD category in colorectal cancers (BD0 or "zero-budding" category) [25].

It has been shown that differences in TB categorization influence on the colorectal cancer-specific deaths and the overall mortality [26]. Van Wyk *et al*. proved that TB stratifies cancer survival in patients with primary operable CRC. They showed significant association of high vs. low TB and cancer-specific survival ($p < 0.001$) [6]. Ozer *et al*. also proved a significant difference in survival rates in patients without TB, or with low and moderate TB scores ($p < 0.001$, $p < 0.001$, and $p = 0.021$, respectively) [16]. By using a cut-off of 10 buds for low- and high-grade budding score in 150 stage II CRC patients, Koelzer *et al*. found statistical difference in the disease-free survival ($p = 0.0095$) [15]. Recently, Basile *et al*. recommended mandatory use of the TB scoring system according to ITBCC in every pathology report in stage III CC patients whereas TB is an emerging prognostic biomarker in colon cancer [27]. In this study TB subgroups were not defined in the methodology.

The International Tumour Budding Consensus Conference recommendations were incorporated into the College of American Pathologists (CAP) cancer protocol [28], and its use was advised along with the 8th edition of the American Joint Committee on Cancer [29]. The same pathologic methodology is up to be a standard in our clinic as well.

This study proved the high statistical significance of TB presence on the number of metastatic lymph nodes and lymphovascular and perineural invasion. It showed differences in overall survival according to the age, tumour location, lymph node metastases presence, lymphovascular and perineural invasion, and TB presence. It also revealed the independent prognostic factors for CRC survival as follows: age over 64 years, tumor location and TB.

Conclusions

In recent years more individualized approaches for treatment planning (microsatellite stability indexes, RAS mutations and others) have been proven to affect the prognosis. Tumour budding is also an additional parameter with no extra cost. It represents a serious prognostic factor besides the established other ones. With its addition in the pathology reports, the decision-making for the treatment modality of patients with CRC and malignant colorectal polyps will be reliable and more certain.

Acknowledgment

We would like to sincerely express our condolences and dedicate this manuscript to the memory of our esteemed co-author, Dr. Sedat Kamalı, who sadly passed away during the editorial revision process. His invaluable contributions to this research will be greatly missed, and his legacy will continue to inspire us in our scientific endeavors.

The authors declare no conflict of interest.

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