



# Prognostic significance of tumor budding in muscle invasive urothelial carcinomas of the bladder

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## ABSTRACT

**Objective:** The aim of this study was to evaluate the prognostic significance of tumor budding in muscle invasive urothelial carcinoma of bladder (MIBC).

**Material and methods:** A total of 60 patients who underwent radical cystectomy and cystoprostatectomy for MIBC were included in the study. The correlations between tumor budding, and tumor necrosis, lympho-vascular invasion (LVI), perineural invasion (PNI) and histopathological data with distant metastasis were evaluated. The correlation between progression free (PFS) and overall survival (OS) rates and the presence, and grade of tumor budding was investigated.

**Results:** A statistically significant correlation was not seen between tumor budding, necrosis, LVI, and PNI. There was a strong correlation between distant organ metastasis, and presence of tumor necrosis. There was no statistically significant correlation between PFS, OS and tumor budding. A statistically significant relationship was observed between OS and tumor stage, lymph node metastasis, and distant organ metastasis.

**Conclusion:** In our study, statistically significant effect of tumor budding on survival rates in MIBCs was not observed. Also, no significant correlation was observed between tumor budding and tumor necrosis, LVI, and PNI.

**Keywords:** Bladder; tumor budding; urothelial carcinoma.

## Introduction

Urothelial cancer is the second most frequent neoplasia of the urogenital system, and accounts for the death of nearly 150.000 patients annually.<sup>[1,2]</sup> Nearly half of muscle-invasive urothelial carcinomas (MIBCs) which have been reported to have occult distant metastases at the time of diagnosis are responsible from most of these deaths.<sup>[2]</sup> In the literature several studies have investigated the factors effective on post-cystectomy recurrence, and survival rates. Pathologic stage of the tumor and lymph node metastasis (LNM) have been reported to be the most important prognostic factors.<sup>[3-5]</sup> However, detection of diverse

clinical outcomes in the same disease stage suggests the presence of different factors affecting prognosis.<sup>[6]</sup>

The histopathological evaluation of growth patterns of the cells at the tumor invasion front in urothelial carcinoma of bladder (UCB) was first reported by Jimenez et al.<sup>[7]</sup> in 2000 and they classified invasive UCB into three groups as: nodular, trabecular, and infiltrative.<sup>[7-11]</sup> Tumor budding is a pathological condition at the tumor invasion front in which individual tumor cells and/or small clusters of up to 5 tumor cells invade the stroma. Budding resembles to the infiltrative pattern described in the earliest classification of UCB, and the terminology of

budding was firstly defined for colorectal adenocarcinomas.<sup>[12]</sup> The prognostic value of tumor budding in various tumors has been reported in the literature.<sup>[13-15]</sup> Although some studies have indicated prognostic significance of this histopathological parameter in pT1 urothelial carcinomas of bladder, only limited

number of studies have been performed about its prognostic significance in MIBCs.<sup>[11,7-10]</sup> Herein, the correlation between tumor budding, and lymphovascular invasion (LVI), perineural invasion (PNI), necrosis, and the impact of tumor budding on progression-free (PFS) and overall survival (OS) has been investigated in 60 patients with MIBC.

**Table 1. Demographic and histopathological features of the cases**

	n (%)
<b>Gender</b>	
Female	8 (13.3)
Male	52 (86.7)
<b>Overall Survival</b>	
Alive	12 (20)
Dead	48 (80)
<b>Stage</b>	
Stage 2	14 (23.3)
Stage 3	25 (41.7)
Stage 4	21 (35)
<b>LNM</b>	
Negative	39 (65)
Positive	21 (35)
<b>Distant metastasis</b>	
Negative	31 (52)
Positive	29 (48)
<b>Tumor budding</b>	
Negative	8 (13.3)
Positive	52 (86.7)
<b>Grade of tumor budding</b>	
Negative	8 (13.3)
Mild	18 (30)
Moderate	13 (21.7)
Marked	21 (35)
<b>Necrosis</b>	
Negative	28 (46.7)
Positive	32 (53.3)
<b>LVI</b>	
Negative	8 (13.3)
Positive	52 (86.7)
<b>PNI</b>	
Negative	21 (35)
Positive	39 (65)

LNM: lymph node metastasis; LVI: lymphovascular invasion; PNI: perineural invasion

## Material and methods

A total of 60 patients who had a diagnosis of MIBC (pT2) after histopathological examination of transurethral resection specimens, and then underwent radical cystectomy (RC) (n=9), and cystoprostatectomy (n=51) were included in the study. Bilateral pelvic lymph node dissection was performed excluding three cases. Information about age, and gender of the patients, and LNM were retrieved from histopathology reports, and those about tumor stage, distant organ metastases, and survival were harvested from archives of the urology clinic. H&E stained slides were revised by two different pathologists.

Lymphatic invasion was defined as the presence of tumor within an endothelial-lined lymphatic space without muscle in its wall, vascular invasion as tumor in a blood vessel which has muscle in its wall and PNI as tumor within the perineural sheath. H&E stained sections of the patients with UC were taken from adjacent tumor areas including deepest invasive margin. Isolated single tumor cells and clusters composed of fewer than five cells were defined as budding foci as previously described. The presence and extent of budding was evaluated using light microscopy at high magnification (X400). The degree of tumor budding was classified into three grades according to the extent of invasion of surgical margin as follows: mild, <1/3; moderate, 1/3–2/3; marked, >2/3 as stated by Kanazawa.<sup>[16]</sup>

## Statistical analysis

The correlation between tumor budding, and tumor necrosis, LVI, PNI and also between histopathological data with distant organ

**Table 2. The relationship between tumor budding, LVI, PNI, and tumor necrosis**

	LVI negative	LVI positive	p
<b>Tumor budding</b>			
Negative	1 (12.5)	7 (87.5)	
Positive	7 (13.5)	45 (86.5)	1.000
	PNI (-)	PNI (+)	p
<b>Tumor budding</b>			
Negative	5 (62.5)	3 (69.2)	
Positive	16 (30.8)	36 (69.2)	0.114

Chi-square test. LVI: lymphovascular invasion; PNI: perineural invasion

metastasis were evaluated using chi-square test. The correlation of PFS and OS with the presence, and grade of tumor budding was investigated using Kaplan-Meier method, and log rank analysis.  $P < 0.05$  was considered as the level of statistical significance. Statistical analysis was performed using Statistical Package of Social

Sciences version 24 (IBM Corp.; Armonk, NY, USA). The study has been approved by the ethics committee. All included patients have declared their informed consent in writing.

## Results

A total of 60 (female,  $n=8$ , 13.3%, and male,  $n=52$ ; 86.7%) cases with a median age of 65.6 (min: 42, max: 82) consisted the study population. Twelve (20%) patients survived, and 48 (80%) of them died with a median survival time of 23.4 (min: 3 months, max: 86 months) months.

Tumor stages of the cases, LNM, distant organ metastases, and histopathological data derived from HE stained sections (tumor necrosis, LVI, PNI, presence, and grade of tumor budding) are summarized in Table 1.

The relationship between tumor budding, LVI, PNI, and tumor necrosis is summarized in Table 2. A statistically significant correlation was not determined between tumor budding, necrosis, LVI, and PNI ( $p=1.000$ ,  $p=0.114$ , and  $p=0.712$ , respectively).

The correlation between histopathological data and distant organ metastasis is summarized in Table 3. A statistically significant correlation was detected between distant organ metastasis, and tumor necrosis ( $p=0.001$ ). A statistically significant correlation was not observed between distant organ metastasis and presence, and grade of tumor budding, LVI, and PNI ( $p=0.465$ ,  $p=0.419$ ,  $p=1.000$ , and  $p=0.935$ , respectively) (Table 3).

Relationship between histopathologic data and PFS is summarized in Table 4. A statistically significant correlation was de-

**Table 3. The correlation between histopathological data and distant organ metastasis**

	LVI negative	LVI positive	p
<b>Necrosis</b>			
Negative	21 (67.7)	7 (24.1)	
Positive	10 (32.3)	22 (75.9)	0.001
<b>Tumor budding</b>			
Negative	3 (9.7%)	5 (17.2)	
Positive	28 (90.3%)	24 (82.8%)	0.465
<b>Grade of tumor budding</b>			
Negative	3 (9.7%)	5 (17.2%)	
Mild	12 (38.7%)	6 (20.7%)	
Modarete	7 (22.6%)	6 (20.7%)	
Marked	9 (29%)	12 (41.4%)	0.419
<b>LVI</b>			
Negative	4 (12.9%)	4 (13.8%)	
Positive	27 (87.1%)	25 (86.2%)	1.000
<b>PNI</b>			
Negative	11 (35.5%)	10 (34.5%)	
Positive	20 (64.5%)	19 (65.5%)	0.935

Chi-square test. DM: distant metastasis; LVI: lymphovascular invasion; PNI: perineural invasion

**Table 4. Relationship between histopathologic data of the cases with progression free survival**

	Progresyon Negative	Progresyon Positive	Hazard Ratio	95% CI	p
<b>Necrosis</b>					
Negative	21 (75%)	7 (25%)	2.091	4.902-13.098	<0.001
Positive	10 (31.3%)	22 (68.7%)			
<b>LVI</b>					
Negative	4 (50%)	4 (50%)	3.286	10.56-23.44	0.476
Positive	27 (51.9%)	25 (48.1%)			
<b>PNI</b>					
Negative	11 (52.4%)	10 (47.6%)	3.561	10.02-23.979	0.872
Positive	20 (51.3%)	19 (48.7%)			
<b>Tumor budding</b>					
Negative	3 (37.5%)	5 (62.5%)	6.041	5.16-28.84	0.791
Positive	28 (53.8%)	24 (46.2%)			

Kaplan Meier method, and log rank analysis. LVI: lymphovascular invasion; PNI: perineural invasion

**Table 5. Relationship between histopathologic data of the cases with OS**

	Alive	Dead	p
<b>Stage</b>			
Stage 2	3 (21.4%)	11 (78.6%)	
Stage 3	8 (32%)	17 (68%)	
Stage 4	1 (4.8%)	20 (95.5%)	0.047
<b>LNM</b>			
Negative	11 (28.2%)	28 (71.8%)	
Positive	1 (4.8%)	20 (95.2%)	0.019
<b>DM</b>			
Negative	12 (38.7%)	19 (61.3%)	
Positive	0	29 (100%)	0.005
<b>Necrosis</b>			
Negative	8 (28.6%)	20 (71.4%)	
Positive	4 (12.5%)	28 (87.5%)	0.099
<b>Tumor budding</b>			
Negative	2 (25%)	6 (75%)	
Positive	10 (19.2%)	42 (80.8%)	0.331
<b>Grade of tumor budding</b>			
Negative	2 (16.7%)	6 (12.5%)	
Mild	5 (41.7%)	13 (27.1%)	
Moderate	3 (25%)	10 (20.8%)	
Marked	2 (16.7%)	19 (39.6%)	0.457
<b>LVI</b>			
Negative	2 (25%)	6 (75%)	
Positive	10 (19.2%)	42 (80.8%)	0.729
<b>PNI</b>			
Negative	7 (33.3%)	14 (66.7%)	
Positive	5 (12.8%)	34 (87.2%)	0.230

Kaplan-Meier method, and log rank analysis. LVI: lymphovascular invasion; PNI: perineural invasion

tected between tumor necrosis, and PFS ( $p < 0.001$ ). There was no statistically significant correlation between PFS, and tumor budding, LVI, and PNI ( $p = 0.791$ ,  $p = 0.476$ , and  $p = 0.872$ , respectively).

The correlation between histopathologic data with OS is summarized in Table 5. A statistically significant correlation between OS, and tumor necrosis, presence and grade of tumor budding, LVI and PNI was not detected ( $p = 0.099$ ,  $p = 0.331$ ,  $p = 0.457$ ,  $p = 0.729$ , and  $p = 0.230$ , respectively). A statistically significant

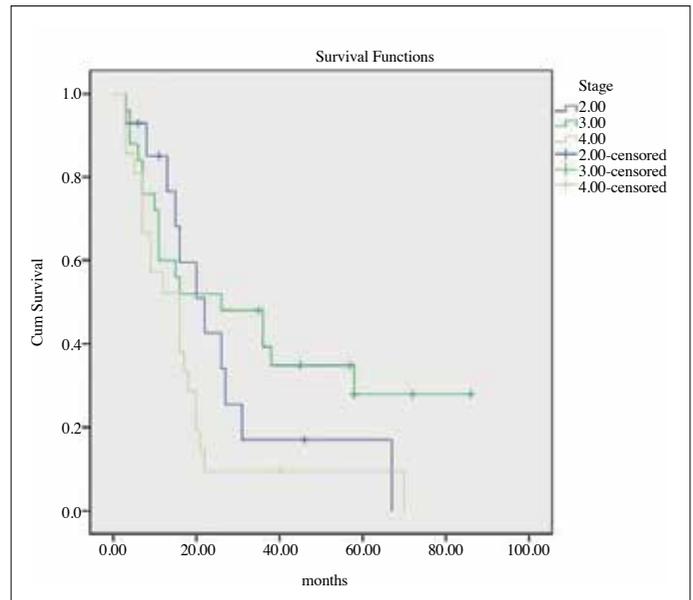


Figure 1. Kaplan-Meier OS in patients according to tumor stage

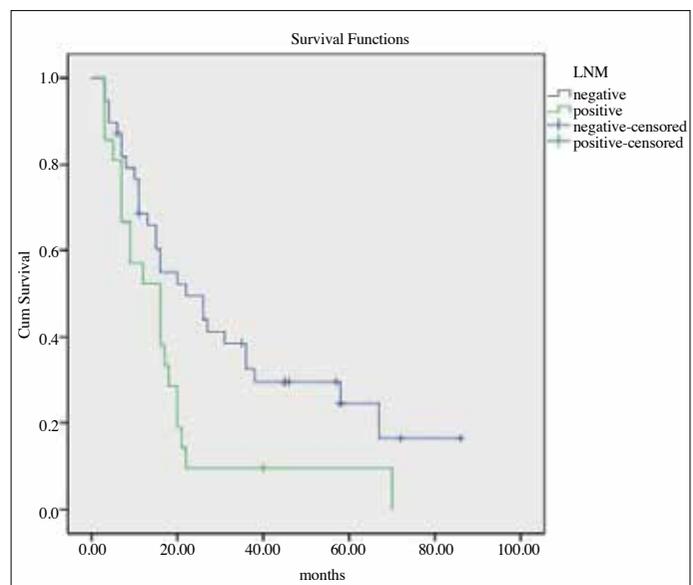


Figure 2. Kaplan-Meier OS in patients according to lymph node metastasis (LNM)

correlation between OS and tumor stage, LNM, and distant organ metastasis was determined ( $p = 0.005$ ,  $p = 0.047$ , and  $p = 0.019$ , respectively) (Table 4) (Figure 1-3).

## Discussion

Radical cystectomy with bilateral lymph node dissection is the gold standard treatment of MIBC.<sup>[6]</sup> In the literature, many studies have investigated the factors effecting post-RC recurrence, and survival rates.<sup>[3-9,11]</sup>

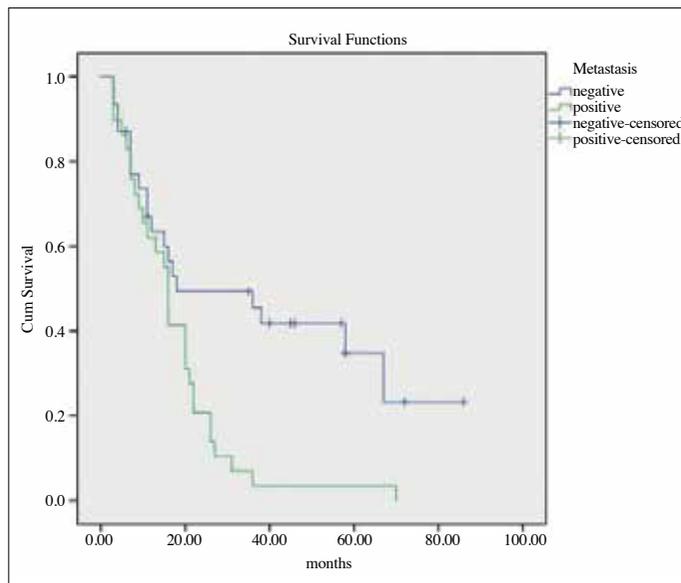


Figure 3. Kaplan-Meier OS in patients with distant metastasis

In recent years, many studies have been performed about the prognostic significance of tumor budding in various tumor types.<sup>[13]</sup> Tumor buddings were firstly described in colorectal adenocarcinomas, and defined as small clusters of tumors consisting of single or up to 5 tumor cells at the invasive front of the tumor.<sup>[12]</sup> Morphologically tumor buddings are more atypical in appearance than the main tumor cells. They are thought to be migratory cancer stem cell (CSC) population which underwent epithelial mesenchymal transition. In several studies, the association of CSC with aggressive characteristics such as increased invasion, and resistance to treatment has been reported.<sup>[17]</sup>

Miyake et al.<sup>[11]</sup> demonstrated that COL4A1 and COL13A1 production by cancer cells plays a pivotal role in tumor invasion through the induction of tumor budding. They mentioned that blocking of these collagens may be an attractive therapeutic approach for the treatment of human urothelial cancer of the bladder.

Tumor budding has been found to be associated with LNM, local and distant metastases, LVI, and poor prognosis in colorectal cancers (CRCs) at all pathologic stages.<sup>[18]</sup> Based on the studies performed, World Health Organization (WHO), and International Union Against Cancer (UICC) have defined tumor budding as a prognostic factor for CRC.<sup>[19]</sup> Still many other studies have reported about the prognostic significance of tumor budding.<sup>[13-15]</sup> However limited number of studies have investigated the impact of tumor budding on the prognosis of urothelial tumors of the bladder. Fukumoto et al.<sup>[20]</sup> reported that tumor budding is an independent predictor of stage progression in non-muscle invasive T1 UCs. Whereas in our study a statistically significant correlation was not detected between tumor budding, and distant

organ metastases, PFS, and OS in MIBC. Besides, we did not determine any correlation between tumor budding, LVI, PNI, and tumor necrosis.

Some studies have reported tumor necrosis as a prognostic factor for bladder UC.<sup>[6]</sup> In parallel with literature, also in our study, a statistically significant correlation was detected between tumor necrosis, distant metastasis, and PFS.

In the literature, prognostic significance of LVI, and PNI in patients with bladder carcinomas treated with cystectomy is controversial.<sup>[21]</sup> In MIBCs, incidence of LVI ranges between 35-55%.<sup>[6]</sup> In their multivariate analyses, Muppa et al.<sup>[21]</sup> demonstrated the presence of a correlation between LVI, and cancer-specific survival in 1504 UC patients treated with RC. In the literature some studies reported LVI as a poor prognostic factor, while others did not.<sup>[6,21]</sup> In the present study a statistically significant correlation was not determined between LVI, and OS, or PFS.

Some other authors have reported PNI as a prognostic factor in univariate analysis in UCs, and stated that PNI lost its statistical significance in multivariate analyses<sup>[6,21]</sup> But in some studies lack of any statistically significant correlation between PNI, and OS has been reported. Also in our study a statistically significant correlation was not seen between PNI and OS or PFS in cases with MIBC.

Tumor stage, and LNM have been reported as the most important prognostic factors in various studies.<sup>[3,5]</sup> Also in our study a statistically significant correlation was determined between tumor stage, LNM, and distant organ metastasis, and OS in parallel with the literature.

In conclusion, the prognostic significance of tumor budding has been accepted for both CRC and other different types of tumors but in our study, any significant effect of tumor budding on survival rates in MIBC was not observed. Also, no significant correlation was determined between tumor budding and tumor necrosis, LVI, and PNI. While recent literature suggests tumor budding as an indicator of predicting stage progression in T1 bladder cancer, more studies are needed to confirm this correlation in T2 bladder cancer. However, based on histopathological data, a strong correlation between tumor necrosis, distant organ metastasis, and PFS was observed in this population. Also in our study in parallel with the literature a significant correlation was detected among tumor stage, LNM, distant organ metastasis, and survival.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the Ethics Committee of University of Health Sciences, Tepecik Training and Research Hospital (09.05.2018, 2018/4-7).

**Informed Consent:** Written informed consent was obtained from all patients who participated in this study.

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