UROONCOLOGY
Original Article

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

Ülkü Küçük[1], Sümeyye Ekmekçi[1], Ebru Çakır[2], Zübeyde Ekin[3], Batuhan Ergani[4], Gökhan Rahmi Ekin[5]

Cite this article as: Küçük Ü, Ekmekçi S, Çakır E, Ekin Z, Ergani B, Ekin GR. Prognostic significance of tumor budding in muscle invasive urothelial carcinomas of the bladder. Turk J Urol 2019; 45(4): 273-8.

ORCID ID of the author:
Ü.K. 0000-0003-2916-0123;
S.E. 0000-0003-1607-500X;
E.Ç. 0000-0001-7959-3491;
Z.E. 0000-0002-9429-9394;
B.E. 0000-0002-4667-855X;
G.R.E. 0000-0001-7341-1212.

[1]Department of Pathology, University of Health Sciences, Tepecik Training and Research Hospital, İzmir, Turkey

[2]Department of Pathology, University of Katip Çelebi, Atatürk Training and Research Hospital, İzmir, Turkey

[3]Department of Pathology, Ege University School of Medicine, İzmir, Turkey

[4]Department of Urology, University of Health Sciences, Tepecik Training and Research Hospital, İzmir, Turkey

[5]Department of Urology, Urla State Hospital, İzmir, Turkey

This manuscript has presented as poster presentation in 27. European Congress of Pathology, 5-9 September 2015, Belgrade, Serbia

Submitted:
24.04.2018

Accepted:
05.06.2018

Available Online Date:
31.08.2018

Corresponding Author:
Ülkü Küçük
E-mail:
kucukulku@hotmail.com

Available online at
www.turkishjournalofurology.com

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, lymphovascular 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.[1,2] 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.[2] 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.[3-5] However, detection of diverse clinical outcomes in the same disease stage suggests the presence of different factors effecting prognosis.[6]

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.[7] in 2000 and they classified invasive UCB into three groups as: nodular, trabecular, and infiltrative.[7-11] 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.[12] The prognostic value of tumor budding in various tumors has been reported in the literature.[13-15] Although some studies have indicated prognostic significance of this

Turk J Urol 2019; 45(4): 273–8
DOI:10.5152/tud.2018.58269

histopathological parameter in pT1 urothelial carcinomas of bladder, only limited number of studies have been performed about its prognostic significance in MIBCs.[1,7-10] 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 (%)
Gender	
Female	8 (13.3)
Male	52 (86.7)
Overall Survival	
Alive	12 (20)
Dead	48 (80)
Stage	
Stage 2	14 (23.3)
Stage 3	25 (41.7)
Stage 4	21 (35)
LNM	
Negative	39 (65)
Positive	21 (35)
Distant metastasis	
Negative	31 (52)
Positive	29 (48)
Tumor budding	
Negative	8 (13.3)
Positive	52 (86.7)
Grade of tumor budding	
Negative	8 (13.3)
Mild	18 (30)
Moderate	13 (21.7)
Marked	21 (35)
Necrosis	
Negative	28 (46.7)
Positive	32 (53.3)
LVI	
Negative	8 (13.3)
Positive	52 (86.7)
PNI	
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 transuretral 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.[16]

Statistical analysis

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

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

	LVI negative	LVI positive	p
Tumor budding			
Negative	1 (12.5)	7 (87.5)	
Positive	7 (13.5)	45 (86.5)	1.000
	PNI (-)	**PNI (+)**	**p**
Tumor budding			
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

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.

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

	LVI negative	LVI positive	p
Necrosis			
Negative	21 (67.7)	7 (24.1)	
Positive	10 (32.3)	22 (75.9)	0.001
Tumor budding			
Negative	3 (9.7%)	5 (17.2)	
Positive	28 (90.3%)	24 (82.8%)	0.465
Grade of tumor budding			
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
LVI			
Negative	4 (12.9%)	4 (13.8%)	
Positive	27 (87.1%)	25 (86.2%)	1.000
PNI			
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

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 detected 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 tu-

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

	Progresyon Negative	Progresyon Positive	Hazard Ratio	95%CI	p
Necrosis					
Negative	21 (75%)	7 (25%)	2.091	4.902-13.098	<0.001
Positive	10 (31.3%)	22 (68.7%)			
LVI					
Negative	4 (50%)	4 (50%)	3.286	10.56-23.44	0.476
Positive	27 (51.9%)	25 (48.1%)			
PNI					
Negative	11 (52.4%)	10 (47.6%)	3.561	10.02-23.979	0.872
Positive	20 (51.3%)	19 (48.7%)			
Tumor budding					
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

Turk J Urol 2019; 45(4): 273-8
DOI:10.5152/tud.2018.58269

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

	Alive	Dead	p
Stage			
Stage 2	3 (21.4%)	11 (78.6%)	
Stage 3	8 (32%)	17 (68%)	
Stage 4	1 (4.8%)	20 (95.5%)	0.047
LNM			
Negative	11 (28.2%)	28 (71.8%)	
Positive	1 (4.8%)	20 (95.2%)	0.019
DM			
Negative	12 (38.7%)	19 (61.3%)	
Positive	0	29 (100%)	0.005
Necrosis			
Negative	8 (28.6%)	20 (71.4%)	
Positive	4 (12.5%)	28 (87.5%)	0.099
Tumor budding			
Negative	2 (25%)	6 (75%)	
Positive	10 (19.2%)	42 (80.8%)	0.331
Grade of tumor budding			
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
LVI			
Negative	2 (25%)	6 (75%)	
Positive	10 (19.2%)	42 (80.8%)	0.729
PNI			
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

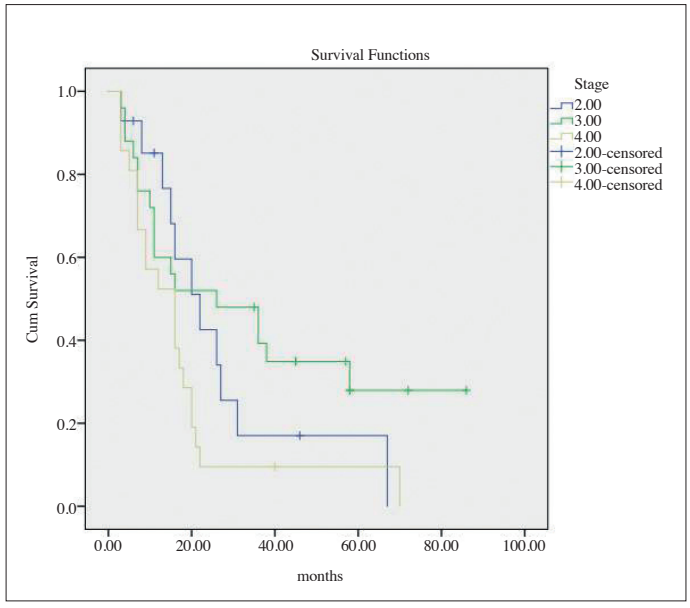

Figure 1. Kaplan-Meier OS in patients according to tumor stage

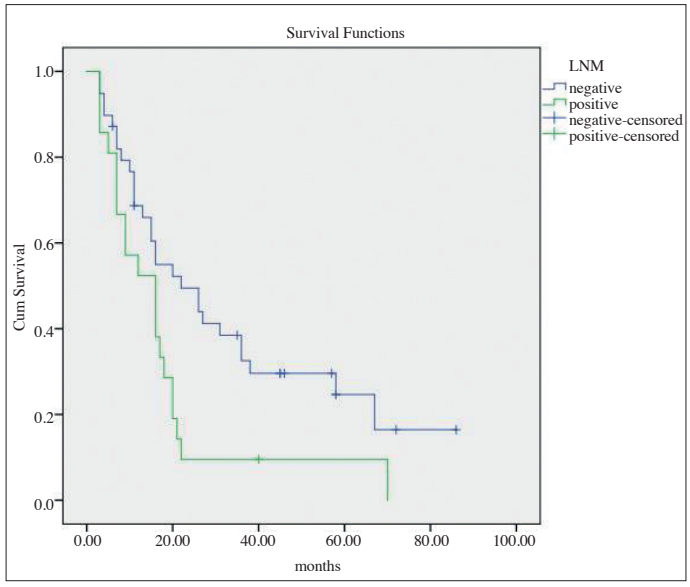

Figure 2. Kaplan-Meier OS in patients according to lymph node metastasis (LNM)

mor 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 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.[6] In the literature, many studies have investigated the factors effecting post-RC recurrence, and survival rates.[3-9,11]

In recent years, many studies have been performed about the prognostic significance of tumor budding in various tumor types.[13] 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.[12] Morphologically tumor buddings are more atypical in appearance than the main tumor cells. They are thought to be migratory

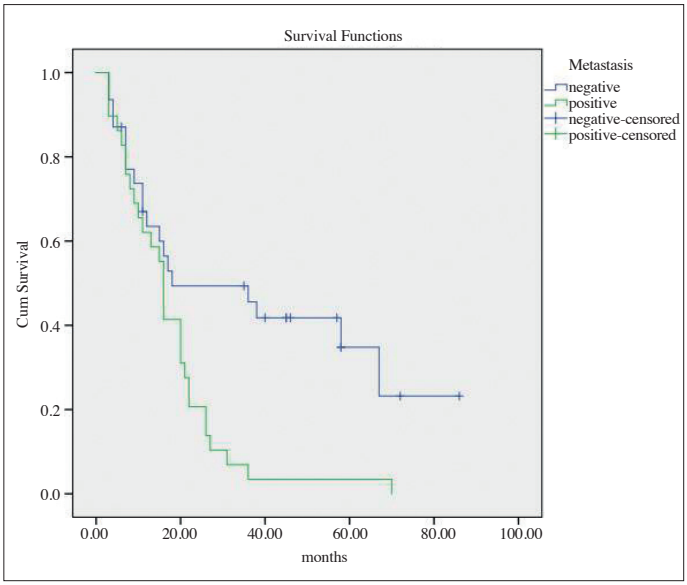

Figure 3. Kaplan-Meier OS in patients with distant metastasis

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.[17]

Miyake et al.[1] 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.[18] 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.[19] Still many other studies have reported about the prognostic significance of tumor budding.[13-15] However limited number of studies have investigated the impact of tumor budding on the prognosis of urothelial tumors of the bladder. Fukumoto et al.[20] 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.[6] 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.[21] In MIBCs, incidence of LVI ranges between 35-55%.[6] In their multivariate analyses, Muppa et al.[21] 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.[6,21] 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[6,21] 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.[3,5] 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.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – Ü.K., E.Ç., S.E.; Design – Ü.K.; Supervision – Ü.K., E.Ç., S.E.; Resources – G.R.E., B.E.; Materials – Z.E., B.E.; Data Collection and/or Processing – Z.E., S.E.; Analysis and/or Interpretation – Ü.K., E.Ç., S.E.; Literature Search – Ü.K., Z.E.; Writing Manuscript – Ü.K., S.E., E.Ç.; Critical Review – Ü.K., E.Ç., S.E.

Turk J Urol 2019; 45(4): 273–8
DOI:10.5152/tud.2018.58269

Conflict of Interest: Authors have no conflicts of interest to declare.

Financial Disclosure: The authors have declared that they didn't receive any financial support for this study.

References

1. Miyake M, Hirao S, Mibu H, Tanaka M, Takashima K, Shimada K, et al. Clinical significance of subepithelial growth patterns in non-muscle invasive bladder cancer. BMC Urol 2011;11:17. [CrossRef]
2. Yun SJ, Kim WJ. Role of the epithelial-mesenchymal transition in bladder cancer: from prognosis to therapeutic target. Korean J Urol 2013;54:645-50. [CrossRef]
3. Bruins HM, Arends TJH, Pelkman M, Hulsbergen-van de Kaa H, van der Heijden AG, Witjes JA. Radical cystectomy in a Dutch University Hospital: Long term outcomes and prognostic factors in a homogenous surgery-only series. Clin Genitourinaty Cancer 2013;12:190-5. [CrossRef]
4. Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, et al. Radical cystectomy in the treatment of invasive bladder cancer: Long-term results in 1.054 patients. J Clin Oncol 2001;19:666-75. [CrossRef]
5. Xylinas E, Rink M, Robinson BD, Lotan Y, Babjuk M, Brisuda A, et al. Impact of histological variants on oncological outcomes of patients with urothelial carcinoma of the bladder treated with radical cystectomy. Eur J Cancer 2013;49:1889-97. [CrossRef]
6. Kucuk U, Pala EE, Cakır E, Sezer O, Bayol U, Divrik RT, et al. Clinical, demographic and histopathological prognostic factors for urothalial carcinoma of the bladder. Cent European J Urol 2015;68:30-6.
7. Jimenez RE, Gheiler E, Oskanian P, Tiguert R, Sakr W, Wood DP Jr, et al. Grading the invasive component of urothelial carcinoma of the bladder and its relationship with progression-free survival. Am J Surg Pathol 2000;4:980-7. [CrossRef]
8. Krüger S, Noack F, Böhle A, Feller AC. Histologic tumor growth pattern is significantly associated with disease-related survival in muscle-invasive transitional cell carcinoma of the urinary bladder. Oncol Rep 2004;12:609-13. [CrossRef]
9. Bircan S, Candir O, Kapucuoglu N. The effect of tumor invasion patterns on pathologic stage of bladder urothelial carcinomas. Pathol Oncol Res 2005;11:87-91. [CrossRef]
10. Denzinger S, Burger M, Fritsche HM, Bertz S, Hofstädter F, Wieland WF, et al. Prognostic value of histopathological tumour growth patterns at the invasion front of T1G3 urothelial carcinoma of the bladder. Scand J Urol Nephrol 2009;43:282-7. [CrossRef]
11. Gofrit ON, Shapiro A, Pode D, Katz R, Yutkin V, Zorn KC, et al. Subepithelial growth patterns in urothelial carcinoma-frequency and prognostic significance. Urol Oncol 2010;30:49-54. [CrossRef]
12. Hase K, Shatney C, Johnson D, Trollope M, Vierra M. Prognostic value of tumour 'budding' in patients with colorectal cancer. Dis. Colon Rectum 1993;36:627-35. [CrossRef]
13. Angadi PV, Patil PV, Hallikeri K, Mallapur MD, Hallikerimath S, Kale AD. Tumor budding is in independent prognostic factor for prediction of lymph node metastasis in oral squamous cell carcinoma. Int J Surg Pathol 2015;23:102-10. [CrossRef]
14. Karamitopoulou E, Zlobec I, Born D, Kondi-Pafiti A, Lykoudis P, Mellou A, et al. Tumour budding is a strong and independent prognostic factor in pancreatic cancer. Eur J Cancer 2013;49:1032-9. [CrossRef]
15. Salhia B, Trippel M, Pfaltz K, Cihoric N, Grogg A, Lädrach C, et al. High tumor budding stratifies breast cancer with metastatic properties. Breast Cancer Res Treat 2015;150:363-71. [CrossRef]
16. Kanazawa H, Mitomi H, Nishiyama Y, Kishimoto I, Fukui N, Nakamura T, et al. Tumour budding at invasive margins and outcome in colorectal cancer. Colorectal Dis 2008;10:41-7.
17. Dawson H, Lugli A. Molecular and pathogenetic aspects of tumor budding in colorectal cancer. Front Med (Lausanne) 2015;2:1-11. [CrossRef]
18. Lugli A, Karamitopoulou E, Zlobec I. Tumour budding: a promising parameter in colorectal cancer. Br J Cancer 2012;106:1713-7. [CrossRef]
19. Horcic M, Koelzer VH, Karamitopoulou E, Terracciano L, Puppa G, Zlobec I, et al. Tumor budding score based on 10 high-power fields is a promising basis for a standardized prognostic scoring system in stage II colorectal cancer. Hum Pathol 2013;44:697-705. [CrossRef]
20. Fukumoto K, Kikuchi E, Mikami S, Ogihara K, Matsumoto K, Miyajima A, et al.Tumor budding, a novel prognostic indicator for predicting stage progression in T1 bladder cancers. Cancer Sci 2016;107:1338-44. [CrossRef]
21. Muppa P, Gupta S, Frank I, Boorjian SA, Karnes RJ, Thompson RH, et al. Prognostic significance of lymphatic, vascular and perineural invasion for bladder cancer patients treated by radical cystectomy. Pathology 2017;49:259-66. [CrossRef]