Tumor budding in cervical carcinoma: associations with some clinical and pathological factors

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SUMMARY

Background: *Tumor budding is recognized as an important independent prognostic factor in colorectal carcinoma. The aim of this study was to evaluate the grade of tumor budding and association with other clinical and pathological features in patients with cervical carcinoma.* **Material and methods:** *We evaluated pathohistological data from 91 cervical carcinoma patients (mean age: 53.8 years) who underwent radical hysterectomy and pelvic lymphatic dissection at the Oncology Institute of Vojvodina between January 2010 and December 2018. Tumor budding was evaluated in invasive front of the tumor. Based on the number of bud counts/10 high power field, three groups were formed: with no budding, with less than 15 buds, and with more than 15 buds.* **Results:** *Eighty (87.91%) of evaluated cervical carcinomas were squamous-cell type, while 12.09% were adenocarcinomas. All carcinomas were graded (HG1-HG3). Average diameter of the tumors was 25 mm (81.6% < 4 cm and 18.4% > 4 cm). Metastases in lymph nodes were present in 30 (32.9%) cases. Based on the number of bud counts/10 high power field there were 35.1% with no budding, 32.9% with less than 15 buds and 37.3% with more than 15 buds. There was a significant association between tumor budding grade and histological grade (p=0.04), as well as with tumor budding grade and the diameter of the tumor (p=0.04).* **Conclusion:** *As a quantitative measure of cancer cell dissociation, tumor budding is associated with poor prognosis in cervical carcinoma and should be considered as a prognostic factor.*

KEY WORDS: *cervix, cancer, tumor budding, cell dissociation*

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INTRODUCTION

Cervical cancer is a main cause of cancer-related deaths in women in low-income countries (1). Predicted European cervical cancer prevalence in 2018 was 27.9 by age standardised rate (ASR) per 100 000 inhabitants, and with highest recorded ASR in Latvia (32.2), Estonia (32.0) and Romania (31.2) (2).

Squamous, adeno- and adenosquamous types of carcinoma are major histologic types of cervical carcinoma, squamous carcinoma being the most common among all other types (3,4).

It is well known that both squamous and adenocarcinoma of the cervix are associated with human papillomavirus infection, that plays vital role in pathogeneses of pre-neoplastic and neoplastic lesions of the cervix. (5).

Cervical squamous cell carcinoma is often unpredictable disease, starting from slowly progressing to aggressive form (6). The histologic grade of cervical carcinoma is, primarily based on keratinization, cytological features, mitotic activity, as well as pattern of invasion (7-10).

However, these studies have not affirmed the prognostic significance of any proposed grading systems. Nowadays, grading systems taking into account pattern on the invasive front of the tumor have been proposed (11). The latest one, 3-stage scoring system of infiltrative growth of cervical carcinoma was proposed with: closed, finger- and spray-like pattern of invasion (Figure 1) (11). Closed pattern of invasion is consisted of solid, cohesive areas of atypical cells. A finger-like pattern of invasion is characterized by trabecular systems with spherical edges at the same time as the spray-like pattern of invasion is defined by the small cell groups or single cells infiltrating cervical stroma (12-14). In case of adenocarcinoma, invasive front is divided into 3 distinct categories: group-A is defined by glandular shape without presence of single invasive cells, group-B is consisted of trabecular structures accompanied with early invasion of surrounding stroma and group-C is described by presence of solid structure or diffuse infiltration (4).

Tumor budding, first described in 1950s, was defined as a remote single cancer cell or microscopic small cell cluster in the front of the invasive margin of the tumor (15,16). Those isolated cancer cells on the invasive front of the tumor were related to more aggressive form of disease. Histologically, tumor budding is explained as a sign of cancer cell motility (17). These motile single cancer cells infiltrate surrounding tissues and in this process they are able to infiltrate vascular spaces, this being the critical point in initiating haematogenous spread of disease and development of metastases (17).

Based on results of our study tumor budding should be considered as a strong prognostic factor for poor prognosis in cervical carcinoma and viewed on as a primary step of metastatic process.

MATERIAL AND METHODS

This retrospective study included data from 91 cervical cancer patients who were treated at the Oncology Institute of Vojvodina from January 2010 to December 2018. After preoperative biopsy, radical hysterectomy with wide lymph node resection was performed in all patients. Postoperatively, histological parameters such as: tumor grade, tumor diameter, morphologic type of growth, presence of lymphovascular and perineural invasion, lymphocytic infiltrate, tumor necrosis, regional lymphonodal status and tumor budding grade were evaluated.

Full blocked hematoxylin-eosin (H&E) stained slides of every cervical cancer case were evaluated. All cervical carcinomas were graded (G1–G3) in accordance to the WHO classification for cervical cancer. In each tumor case, the characteristics of invasive margin were evaluated within a zone of one low-power field width (5.0 mm diameter, ×40 magnification) along the invasive margin of carcinoma by placing the deepest point of invasion at the center of field. Invasive pattern was classified as closed (or pushing margin), finger-like, and spray-like pattern, based on previous descriptions in case of squamous cell carcinoma and pattern A, B and C in case of adenocarcinoma (4, 11-14). Dissociation of small

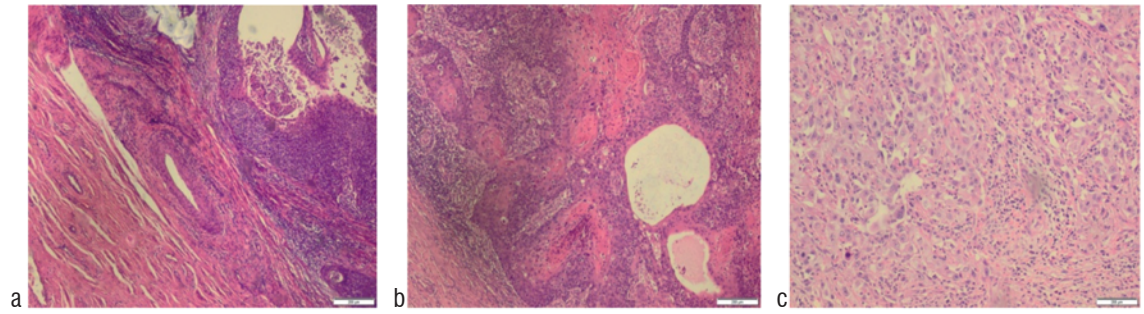

Figure 1. Three level system of infiltrating growth of squamous carcinoma: a) "closed" pattern of invasion, b) "finger like" pattern of invasion and c) "spray like" pattern of invasion

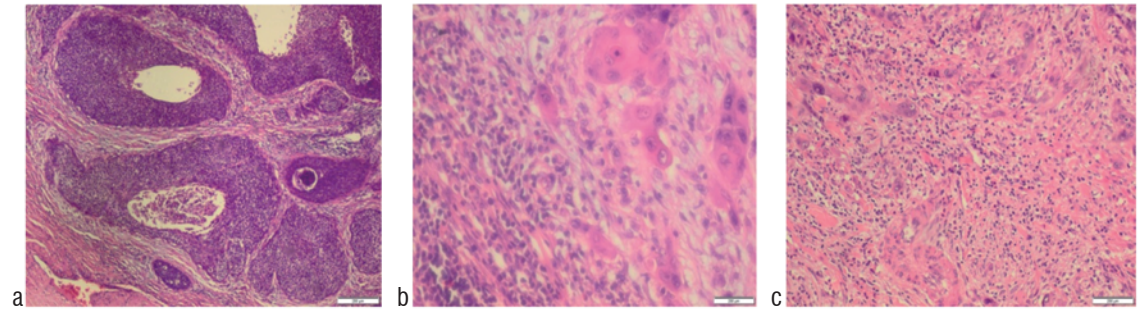

Figure 2. Tumor budding score: a) tumor without budding activity, b) tumor with low budding activity (<15 buds per 10 HPF) and c) tumor with high budding frequency (more than 15 buds per 10 HPF)

cluster of tumor cells (<5 cells) that ''bud'' in peritumoral stroma was defined as tumor budding (15,16). Tumor budding activity was evaluated in whole tumor area and scored within the tumor area showing the highest buding activity. We used a novel grading system based on grading activity: tumors without budding activity were scored 1, tumors with low budding activity (<15 buds per 10 high power field, HPF) were scored 2 and tumors with high budding frequency (15 buds per 10 HPF) were scored 3 (Figure 2)(15-17).

STATISTICAL ANALYSIS

The associations between the clinico-pathological characteristics and tumor budding were analyzed using contingency tables. Statistical significance was evaluated using χ^2 test. P values <0.05 were considered statistically significant.

Variable	No. of patients (%)	Tumor Budding Count			P value
	N=91	1 (no budding) n= 30	2 (<15 budds) n=27	3 (>15 budds) n=34	
SquamousCA	80				
closed	34 (42.5%)	17(56.66%)	11 (40.75%)	6 (20.00%)	
finger like	32 (40%)	5 (16.66%)	10 (37.03%)	17 (63.33%)	**0.1**
spray like	14 (17.5%)	1 (3.33%)	2 (7.40%)	11 (16.67%)	
AdenoCA	11				
pattern A	5 (45.45%)	3 (10.00%)	2 (7.40%)	0	
pattern B	5 (45.45%)	4 (13.35%)	1 (3.71%)	0	**0.08**
pattern C	1 (9.10%)	0	1 (3.71%)	0	

Table 1. Correlation between growth pattern and tumor budding activity

RESULTS

The median age of patients was 53.8 years. Eighty (87.91%) of all evaluated cervical carcinomas were squamous, and 11 (12.09%) were adeno-histological subtype of cervical carcinoma. Majority of patients (87.91%) had squamous cervical carcinoma. Equal amounts of these patients had closed (42.5%) and finger-like (40%) pattern of invasion. According to histological grade 14 cases (15.38%) were G1, 57 cases (62.63%) were G2 and 20 cases (21.99%) were G3. Average diameter of the tumor was 25 mm (79.13% < 4 cm and 20.87% > 4 cm). Clinicopathological characteristics of observed cervical carcinomas were showed in Tables 1 and 2. Association between tumor budding grade and histological grade of the tumor (p value=0.04) was presented in Figure 3. These results showed that 55.88% of patients with tumor budding grade 3 had moderately differentiated carcinoma and 44.11% of them had a poorly differentiated one. Also, the results showed significant association between the diameter of the tumor and tumor budd count (p=0.04). Our results showed that none of the well differentiated cervical carcinomas had high tumor budding grade.

As it has been sad, in majority of patients squamous cervical carcinoma was diagnosed, with equal amount of closed and finger-like pattern of invasion. There was not statisticaly significant association between pattern of invasion and tumor budding grade in squamous carcinoma, although 57.15% of patients with spray-like pattern had more than 15 tumor budds (grade 3). The majority (more than two thirds) of adenocarcinoma patients had a pattern A of invasion. There was not significant association between tumor budding grade and type of growth of invasive front of the adenocarcinoma determined.

The presence of lymphovascular invasion (LVI) and perineural invasion (PNI) was evaluated and there was significant association between those parameters and tumor bud count (p =0.02). Tumor necrosis was present in 79.12% and lymphocitic infiltration in 60.43% cases. These parameters were not significantly associated with tumor budding grade (p=0.07).

Thirty (32.96%) of cervical cancer patients had a metastases in lymph nodes (15.3% with grade 2 tumor budding and 13.18% with grade 3). Lymph node metastases were not present in case of cervical carcinomas with low bud count. Tumor budding grade was not significantly associated with a presence of metastases in regional lymph nodes (Table 2).

DISCUSSION

There is a medical significance of tumor budding and cellular nest size as a measure of tumor cellular dissociation (15,16).

Tumor budding has been explained as a signal of cellular motility in most cancers and as a crucial step in the metastatic process (18). Process of epithelial to mesenchymal transition (EMT) and the reverse process of mesenchymal to epithelial transition (MET) are critical (19). The transformation of epithelial cells into mesenchymal is vital because in this process epithelial cells gain some new functions, along with motility. The essential moment in metastatic process is reverse transition, from mesenchymal to epithelial cells. This is critical due to the fact that by regaining epithelial features, those cells can accommodate to new environment and coordinate with surrounding cells (18,19).

Previous research have investigated tumor budding as a likely prognostic element in colorectal and oesophageal carcinoma (20). Preceding research have shown that presence of tumor budding related with lymphovascular invasion can be a massive predictor of nodal metastases (21,22). This study has shown no significant association between tumor budding grade and presence of locoregional development of disease. In our study patients with carcinomas that were classified as high tumor budding grade did not have higher locoregional spread of disease. Some previous studies have managed to correlate high tumor budding grade with probability of recurrence, even after radical treatment (23). Prognostic value of tumor budding have also been determined in cases of pulmonary and oral carcinomas (6). Tumor budding have been associated with locoregional lymph node metastases and local recurrence (21-24).

Jesinghaus and colleagues have studied cases of squamous carcinoma of the cervix and proposed a unique grading system that uses tumor budding grade and cell nest size. Diameter of single cluster of atypical cells has been proven to be a prognostic factor of massive importance and, in cooperation with tumor budding, have had a big impact in overall survival. Combination of these factors can be more harmful than occurrence of these factors alone. Therefore, high budding grade have been highly associated with presence of lymphovascular invasion. Consequently, it can be used as a predictor of local metastases (6). We determined a correlation among tumor budding grade and the dimensions of the tumor (Table 1). Other studies have showed that high tumor budding grade (more than 15 buds/ 10 HPF) is associated with higher histologic grade of the tumor (25,26). In our study, significant correlation between tumor histologic grade and tumor budding grade was proven.

There is clear evidence that pattern of invasion is a strong prognostic factor in cervical carcinoma. Invasion pattern with highest amount of cell dissociation (in case of squamous carcinoma – spray like pattern of invasion) is related with worse prognosis (11). In adenocarcinomas, aggressive pattern C of invasion is characterized, requiring more aggressive treatment modalities (4). Our study showed no significant association between tumor budding grade and the pattern of invasion. Furthermore, we estimated significant association (Table 1) between tumor budding grade and presence of lymphovascular and perineural invasion.

We based our study on tumor budding as indicator of single cell dissociation in the front of the tumor. Some researchers have reported that intratumoral as well as peritumoral budding can be a strong indicator for worse diagnosis (27-29). In association with other established, independent indicators of worse prognosis like histological grade, tumor diameter, lymphovascular and perineural invasion tumor budding have also had implications on overall survival (30-32). One previous study has shown that there has been statistically significant 3 years survival rate

Variable	No. of patients (%)	Tumor Budding Count			P value
	N=91	1 (no budding) n= 30	2 (<15 budds) n=27	3 (>15 budds) n=34	
Histologic grade					
G1	14 (15.38%)	7 (50%)	7 (50%)	0 (0%)	**0.04**
G2	57 (62.63%)	20 (35.08%)	18 (31.57%)	19 (33.35%)	
G3	20 (21.99%)	3 (15.00%)	2 (10.00%)	15 (65.00%)	
Lymphovascular invasion					
YES	33 (36.26%)	10 (30.30%)	8 (24.24%)	15 (45.46%)	**0.02**
NO	58 (63.74%)	20 (34.48%)	19 (32.76%)	19 (32.76%)	
Perineural invasion					
YES	20 (21.97%)	7 (35.00%)	5 (25.00%)	8 (40.00%)	**0.02**
NO	71 (78.03%)	23 (32.39%)	22 (30.98%)	26 (36.63%)	
Tumor necrosis					
YES	19 (20.87%)	6 (31.57%)	3 (15.78%)	10 (52.65%)	**0.07**
NO	72 (79.13%)	24 (33.33%)	24 (33.33%)	24 (33.33%)	
Inflammatory infiltrate					
YES	36 (39.56%)	17 (47.22%)	6 (16.66%)	13 (36.12%)	**0.07**
NO	55 (60.44%)	13 (23.63%)	21(38.18%)	21 (38.18%)	
Lymph node metastases					
YES	30 (32.96%)	4 (13.33%)	14 (46.66%)	12 (40.01%)	**0.07**
NO	61 (67.04%)	26 (42.62%)	13 (21.31%)	22 (36.07%)	

Table 2. Correlation between established prognostic factors and tumor budding acitivity

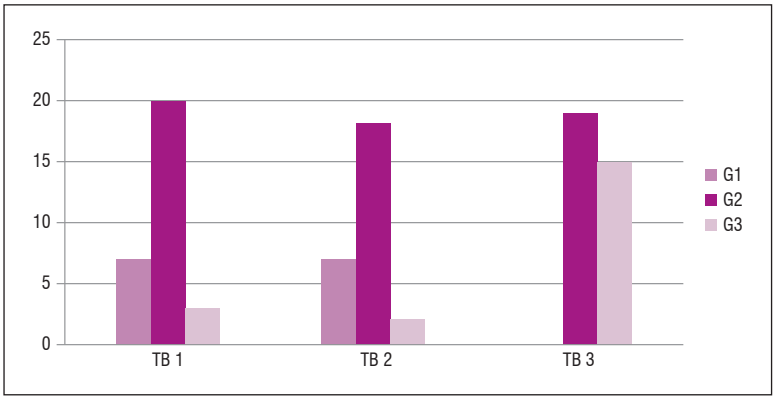

Figure 3. Correlation between tumor budding grade and histological grade of the tumor

TB1 - tumors without budding activity
TB2 - tumors with low budding activity (<15 buds per 10 HPF) and
TB3 - tumors with high budding frequency (≥15 buds per 10 HPF)

between patients with low and high grade tumor budding i.e. patients with high grade budding have had a substantially shorter survival compared to patients with low budding grade (23). This result may be attributed to substantial correlation between tumor budding and different well known factors of poor prognosis of disease (33).

CONCLUSION

There is strong evidence to suggest that cervical cancer tumor budding is parameter that can help to stratify patients into more meaningful risk groups than TNM staging alone, and, even more importantly, has the potential to guide decision making.

As a quantitative measure of cancer cell dissociation, tumor budding is highly associated with already established prognostic factors for poor prognosis in cervical cancer and therefore should be considered as one of them.

Declaration of Interests

Authors declare no conflicts of interest.

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