RESEARCH ARTICLE

Prognostic Value of Tumor Budding in Early-Stage Cervical Adenocarcinomas

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Abstract

Background: Tumor budding has recently been reported as an independent adverse prognostic factor for colorectal adenocarcinomas and other types of carcinoma in the digestive tract. This study aimed to evaluate the prognostic value of tumor budding in patients with early-stage cervical adenocarcinomas and any associations with other clinical and pathological features. **Methods:** Histological slides of patients with early-stage (IB-IIA) usual-type endocervical adenocarcinoma who underwent radical hysterectomy and pelvic lymph node dissection, without preoperative chemotherapy, between January 2006 and December 2012 were reviewed. Tumor budding was evaluated in routinely-stained sections and defined as detached single cells or clusters of fewer than 5 cells in a tumor invasive front and was stratified based on the number of bud counts in 10-high-power fields as low (<15 buds) and high (≥15 buds). Correlations between tumor bud count and other clinical and pathological variables including follow-up outcomes were assessed. **Results:** Of 129 patients, a high tumor bud count was observed in 15 (11.6%), positively associated with histologic grade 3 ($p<0.001$), invasive pattern C (Silva System) ($p=0.004$), lymph node metastasis ($p=0.008$), stage IB2-IIA ($p=0.016$), and tumor size >2 cm ($p=0.036$). Kaplan-Meyer analysis showed a significant decrease in both disease-free survival and cancer-specific survival for patients with a high tumor bud count ($p=0.027$ and 0.031, respectively). On multivariate analysis, histologic grade 3 was the only independent predictor for decreased disease-free survival ($p=0.004$) and cancer-specific survival ($p=0.003$). **Conclusions:** A high tumor budding count based on assessment of routinely-stained sections was found to be associated with decreased disease-free and cancer-specific survival in patients with early-stage cervical adenocarcinomas. However, it was not found to be an independent prognostic predictor in this study.

Keywords: Uterine cervix- adenocarcinoma- tumor budding- prognosis

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Introduction

Cervical cancer is the third most common cancer in women worldwide, and is the second leading cause of cancer death in women of developing countries (Torre et al., 2015). In Thailand, cervical cancer is the second most common cancer with an average age-standardized rate of 14.4 per 100,000 women (Wilailak and Lertchaipattanakul, 2016).

Squamous cell carcinoma is the predominant histological type of cervical cancer, accounting for approximately 80% of cases (Wilailak and Lertchaipattanakul, 2016). Although adenocarcinoma is a less common histologic type, its incidence has been increasing up to 20-25% during the last two decades in the Western women population (Roma et al., 2015). In general, clinicopathological prognostic factors in patients with cervical carcinoma include patient's age, the International Federation of Gynecology and Obstetrics (FIGO) tumor stage, tumor size, histologic type and grade, lymphovascular space invasion (LVSI), depth of cervical wall invasion, parametrial involvement, lymph node metastasis, and treatment modality (Biewenga et al., 2011; Intaraphet et al., 2013). The prognosis of patients with adenocarcinoma may be worse than those with squamous cell carcinoma even in early-stage patients (Intaraphet et al., 2013). In addition to tumor stage, histologic grade, and lymph node metastasis (Baalbergen et al., 2004), the prognostic variables that determine an appropriate management and disease outcome in patients with early-stage cervical adenocarcinoma remain to be determined.

Tumor budding has recently been a well-established independent adverse prognostic factor in colorectal adenocarcinoma (Karamitopoulou et al., 2013).

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High-grade (or high number) of tumor budding is associated with lymph node metastasis, local recurrence, distant metastasis, and worsened disease-free and overall survival (Zlobec and Lugli, 2010). The prognostic value of tumor budding has been reported in patients with all stages of colorectal adenocarcinoma, and tumor budding may be used for risk stratification of patients, for justification of adjuvant chemotherapy, and probably for prediction of response to targeted therapy (Mitrovic et al., 2012). Tumor budding has also been found to be an independent prognostic variable in other types of carcinoma in the digestive system, including the esophagus (Teramoto et al., 2013; Landau et al., 2014), stomach (Gulluoglu et al., 2015), ampulla (Ohike et al., 2010), and pancreas (Karamitopoulou, 2013; O'Connor et al., 2015).

To our knowledge, the evaluation of tumor budding and its clinical significance in cervical adenocarcinoma has not been reported. This study was aimed to evaluate the prognostic value of tumor budding in patients with early-stage cervical adenocarcinoma and the association of tumor budding with other clinical and pathological features.

Material and Methods

This study was approved by the Institutional Research Ethics Board. The surgical pathology records of the Department of Pathology, Faculty of Medicine, Chiang Mai University, were searched for the cases of cervical adenocarcinoma who underwent radical hysterectomy and pelvic lymph node dissection between January 2006 and December 2012. The cases diagnosed between 2006 and 2011 had been included as a part of our previous study of cervical adenocarcinoma without the assessment of tumor budding (Pongsuvareeyakul et al., 2015). Data of the medical records of the identified cases were retrieved. The inclusion criteria were: surgery performed in Chiang Mai University Hospital; histology of endocervical adenocarcinoma of usual type; and FIGO stage IB to IIA. The exclusion criteria were: adenosquamous carcinoma or non-usual types of cervical adenocarcinoma (Wibur et al., 2014); patients who received preoperative chemotherapy or radiation therapy; patients who died within 30 days postoperatively; or incomplete histological material available for review.

In each case, pathological information was abstracted from the pathology reports including tumor size, histologic grade, fraction of cervical stromal invasion, residual stromal thickness, LVSI, parametrial involvement, vaginal margin status, and lymph node metastasis. The pathologic examinations in all cases were performed by a team of gynecological pathologists (S.S., S.K., J.S, and K.S.). Tumor size was determined by the pathological measurement of maximal dimension of invasive adenocarcinoma. If invasive adenocarcinoma was present in both conization and hysterectomy specimens, the larger dimension in any specimen represented the tumor size. Histologic grade was stratified using a three-tiered grading system (Baalbergen et al., 2004). LVSI was further classified as extensive when there were more than 10 foci of involvement. Parametrial involvement was defined by the presence of direct invasion of carcinoma into parametrial tissue, metastasis in parametrial lymph node, or the presence of tumor embolus in a parametrial vessel.

The histologic slides were reviewed by one pathologist (N.S.) for the invasive pattern of and tumor budding, blinded to the clinical outcome and previously reported pathological features. In each case, all slides containing adenocarcinoma were first assessed using low-power magnification. The invasive pattern was classified based on the recently proposed risk stratification system for usual-type endocervical adenocarcinoma (Silva System) into 3 types: A, B, and C (Diaz De Vivar et al., 2013; Roma et al., 2015). In brief, the invasive pattern A is defined by well-demarcated glands with the absence of single invasive cells or destructive stromal invasion, LVSI, and solid growth. The invasive pattern B is characterized by early destructive stromal invasion arising from well-demarcated glands, with or without LVSI. The invasive pattern C, which is associated with the worst prognosis, is characterized by diffuse destructive invasion or the presence of confluent epithelial growth (>5 mm) or solid architecture. One or two slides representing the invasive pattern were selected for further evaluation of interobserver agreement.

Tumor budding was defined as detached single cells or clusters of less than 5 cells at the invasive tumor front (Karamitopoulou et al., 2013) (Figure 1). In each case, the most representative slide with the highest number of budding foci was selected, and tumor buds were counted under high magnification (40x objective or 400x) in 10 high-power fields (HPF) which showed the highest density of buds (Karamitopoulou et al., 2013). Due to the lack of previous information regarding tumor budding in cervical cancer, the cut-off number for stratification of tumor budding into high and low bud count was determined based on the performance in predicting the clinical outcomes, using the Youden's index and receiver operating characteristic analysis with a consideration for high specificity (>90%). The cut-off value for a high bud count was determined as ≥15 buds in 10 HPF.

For the assessment of interobserver agreement, another pathologist (S.K.) evaluated the selected slides and scored the invasive pattern (pattern C versus pattern A or B) and the tumor bud count (high versus low). In the cases with disagreement of the results, a third pathologist (K.S.) evaluated the slides. The final results of invasive pattern and tumor budding used in the study were based on the agreement of at least 2 pathologists.

Clinical information including patient age, treatment modalities and clinical follow-up outcomes or cause of death was retrieved from medical records or data obtained from the cancer registry. Regarding adjuvant treatment, the patients were justified for adjuvant chemotherapy and/or radiation therapy due to the presence of any high-risk pathological features (positive lymph nodes, surgical margins, or parametrial involvement) or a combination of at least 2 intermediate-risk pathological features (deep stromal invasion, extensive LVSI, or tumor size >4 cm).

The data were analyzed using STATA version 11 (StataCorp LP, College Station, TX, USA). The interobserver agreement between pathologists (N.S.

and S.K.) was assessed using the Kappa statistic. The associations of tumor bud count and other clinical and pathological variables were assessed using the Fisher's exact test. The Kaplan-Meier method and log-rank test were used for analysis and comparison of survival curves. Cox regression models were used to perform univariate and multivariate analyses of disease-free survival and cancer-specific survival. The variables with a p value of less than 0.25 in the univariate analysis were further evaluated in the multivariate analysis. A p value of less than 0.05 was considered statistically significant. Disease-free survival was defined as the time period between the date of surgery until disease recurrence (locoregional or distant), the last follow-up, or censoring. Cancer-specific survival was defined as the time period between the date of surgery until death from cancer, the last follow-up, or censoring.

Results

A total of 129 patients were included into the study. The mean age was 45.4±9.4 years. The FIGO stage was IB1 in 89 patients (69.0%), IB2 in 28 patients (21.7%), and IIA in 12 patients (9.3%). Lymph node metastasis was detected in 24 patients (18.6%). The follow-up duration ranged from 2 to 120 months (median 60 months). Twenty-three patients (17.8%) were known to have tumor recurrence, and 18 (14.0%) had cancer-related death.

The invasive pattern A was observed in 18 patients (16.0%), pattern B in 39 patients (29.8%), and invasive pattern C in 71 patients (54.2%). Lymph node metastasis was present in none of patients with invasive pattern A (0%), 3 of 36 (8.3%) of patients with pattern B, and 21 of 75 (27.6%) patients with pattern C. The difference in the rates of lymph node metastasis was significant between pattern C and pattern B (p=0.015), and between pattern C and pattern A (p=0.010), but not between pattern B and pattern A (p=0.543). The agreement of invasive pattern scoring between both pathologists was observed in 102 cases (79.1%). The Kappa statistic was 0.56, consistent with moderate agreement.

The number of tumor bud count ranged from 0 to 35 in 10 HPF (median 2). A high tumor bud count was observed in 15 patients (11.6%), and a low bud count in 114 patients (88.4%). The agreement of tumor bud count (high versus low) was observed in 110 cases (85.3%), with Kappa statistic of 0.30 which indicated fair agreement.

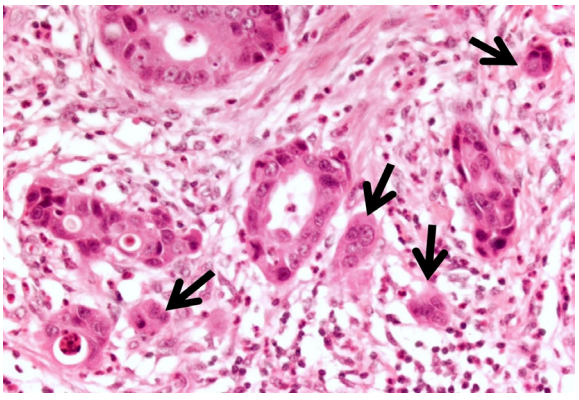

Figure 1. Each of Tumor Buds (Arrows) is Composed of Less Than 5 Carcinoma Cells (400x Magnification)

The association of tumor budding with other clinical and pathological features is shown in Table 1. A high tumor bud count was significantly associated with histologic grade 3 (p<0.001), invasive pattern C, lymph node metastasis, stage IB2-IIA, and tumor size >2 cm. In comparison with the patients who had a low tumor bud count, those with a high bud count had higher

Table 1. Association of Tumor Budding and Clinical and Pathological Variables in 129 Patients

Variable	No. of patients (%), n=129	Tumor budding count		p value
		Low number (%), n = 114	High number (%), n = 15	
Age				
≤45 years	71 (55.0)	61 (53.5)	10 (66.7)	0.414
>45 years	58 (45.0)	53 (46.5)	5 (33.3)	
Stage				
IB1	89 (69.0)	83 (72.8)	6 (40.0)	0.016
IB2-IIA	40 (31.0)	31 (27.2)	9 (60.0)	
Tumor size				
≤2.0 cm	42 (32.6)	41 (36.0)	1 (6.7)	0.036
>2.0 cm	87 (67.4)	73 (64.0)	14 (93.3)	
Histologic grade				
Grade 1-2	113 (87.6)	105 (92.1)	8 (53.3)	<0.001
Grade 3	16 (12.4)	9 (7.9)	7 (46.7)	
Pattern of invasion				
Patterns A and B	54 (41.9)	53 (46.5)	1 (6.7)	0.004
Pattern C	75 (58.1)	61 (53.5)	14 (93.3)	
Depth of invasion				
Inner or middle third	54 (41.9)	48 (42.1)	6 (40.0)	>0.99
Outer third	75 (58.1)	66 (57.9)	9 (60.0)	
Residual stroma <3mm				
No	78 (60.5)	72 (63.2)	6 (40.0)	0.098
Yes	51 (39.5)	42 (36.8)	9 (60.0)	
Lymphovascular space invasion				
No	56 (43.4)	53 (46.5)	3 (20.0)	0.058
Yes	73 (56.6)	61 (53.5)	12 (80.0)	
Parametrial involvement				
No	107 (82.9)	95 (83.3)	12 (80.0)	0.720
Yes	22 (17.1)	19 (16.7)	3 (20.0)	
Vaginal margin involvement				
No	124 (96.1)	110 (96.5)	14 (93.3)	0.467
Yes	5 (3.9)	4 (3.5)	1 (6.7)	
Lymph node metastasis				
No	105 (81.4)	97 (85.1)	8 (53.3)	0.008
Yes	24 (18.6)	17 (14.9)	7 (46.7)	

Table 2. Univariate and Multivariate Analysis of Clinical and Pathological Variables on Disease-Free Survival

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p value	HR (95% CI)	p value
Age				
≤45 years	Reference	0.448	-	-
>45 years	1.4 (0.6-3.1)			
Stage				
IB1	Reference	0.009	Reference	0.738
IB2-IIA	3.0 (1.3-6.8)		1.3 (0.3-4.8)	
Tumor bud count				
Low number	Reference	0.035	Reference	0.937
High number	2.7 (1.1-6.9)		1.1 (0.3-3.7)	
Tumor size				
≤2.0 cm	Reference	0.025	Reference	0.139
>2.0 cm	5.3 (1.2-22.4)		3.8 (0.6-23.2)	
Histologic grade				
Grade 1-2	Reference	0.001	Reference	0.004
Grade 3	2.1 (1.3-3.2)		2.5 (1.3-4.5)	
Pattern of invasion				
Patterns A and B	Reference	0.140	Reference	0.056
Pattern C	2.0 (0.8-5.1)		0.3 (0.1-1.0)	
Depth of invasion				
Inner or middle third	Reference	0.019	Reference	0.181
Outer third	3.6 (1.2-10.7)		2.8 (0.6-12.4)	
Residual stroma <3mm				
No	Reference	0.084	Reference	0.499
Yes	2.1 (0.9-4.7)		0.7 (0.2-2.2)	
Lymphovascular space invasion				
No	Reference	0.006	Reference	0.123
Yes	5.5 (1.6-18.4)		2.9 (0.7-11.9)	
Parametrial involvement				
No	Reference	0.013	Reference	0.722
Yes	3.0 (1.3-7.1)		1.3 (0.4-4.4)	
Vaginal margin involvement				
No	Reference	0.971	-	-
Yes	1.0 (0.1-7.2)			
Lymph node metastasis				
No	Reference	0.001	Reference	0.088
Yes	4.1 (1.8-9.3		2.8 (0.9-9.4)	
Adjuvant therapy				
No	Reference	0.002	Reference	0.936
Yes	4.7 (1.7-12.7)		1.1 (0.3-4.1)	

HR, hazard ratio ; CI, confidence interval

Table 3. Univariate and Multivariate Analysis of Clinical and Pathological Variables on Cancer-Specific survival

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p value	HR (95% CI)	p value
Age				
≤45 years	Reference	0.98	-	-
>45 years	1.0 (0.4-2.5)			
Stage				
IB1	Reference	0.077	Reference	0.981
IB2-IIA	2.3 (0.9-5.8)		1.0 (0.2-4.6)	
Tumor bud count				
Low number	Reference	0.040	Reference	0.910
High number	3.0 (1.1-8.3)		1.1 (0.3-4.1)	
Tumor size				
≤2.0 cm	Reference	0.071	Reference	0.132
>2.0 cm	3.9 (0.9-16.9)		4.1 (0.7-26.1)	
Histologic grade				
Grade 1-2	Reference	0.001	Reference	0.003
Grade 3	2.2 (1.4-3.6)		2.7 (1.4-5.2)	
Pattern of invasion				
Patterns A and B	Reference	0.225	Reference	0.108
Pattern C	1.9 (0.7-5.3)		0.3 (0.1-1.3)	
Depth of invasion				
Inner or middle third	Reference	0.104	Reference	0.462
Outer third	2.5 (0.8-7.6)		1.8 (0.4-9.2)	
Residual stroma <3mm				
No	Reference	0.156	Reference	0.769
Yes	2.0 (0.8-5.0)		0.8 (0.2-3.4)	
Lymphovascular space invasion				
No	Reference	0.013	Reference	0.090
Yes	6.4 (1.5-27.8)		4.2 (0.9-21.9)	
Parametrial involvement				
No	Reference	0.042	Reference	0.824
Yes	2.8 (1.0-7.4)		1.2 (0.3-5.5)	
Vaginal margin involvement				
No	Reference	0.784	-	-
Yes	1.3 (1.2-10.0)			
Lymph node metastasis				
No	Reference	0.003	Reference	0.158
Yes	4.1 (1.6-10.4)		2.8 (0.8-11.7)	
Adjuvant therapy				
No	Reference	0.027	Reference	0.651
Yes	3.2 (1.1-9.0)		0.7 (0.2-3.2)	

HR, hazard ratio ; CI, confidence interval

proportions of LVSI and residual stroma <3 mm, but the differences were not statistically significant. The presence of extensive LVSI was not significantly associated with a high tumor bud count (40.0% in high count vs 26.3% in low count, p=0.357). There was a marginal difference in the proportion of patients who received adjuvant therapy between the groups with high and low tumor bud counts (66.7% vs 41.2%, p=0.095).

The patients with a high tumor bud count had significantly higher rates of recurrence and cancer-specific death than those with a low bud count (recurrence: 40.0% vs 14.9%, p=0.033; death: 33.3% vs 11.4%, p=0.037). The Kaplan-Meier analysis showed a significant association of high tumor bud count with decreased disease-free

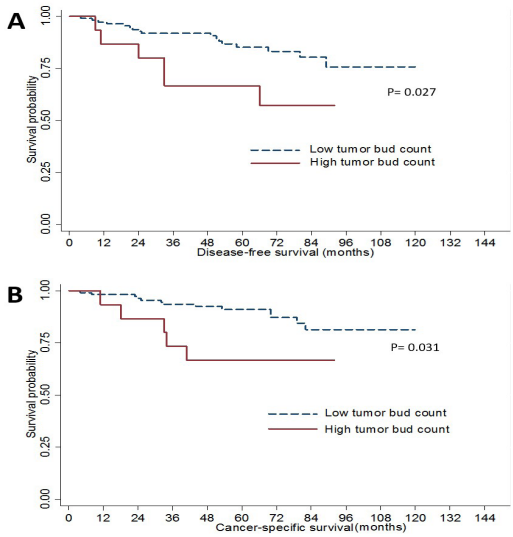

Figure 2. Kaplan-Meier Plots for Survival Stratified by Tumor Bud Count. A) disease-free survival, B). cancer-specific survival

survival (p=0.027) and decreased cancer-specific survival (p=0.031) (Figure 2).

On univariate for disease-free survival, most of previously known prognostic variables showed significant association with survival; including lymph node metastasis, histologic grade, adjuvant therapy, LVSI, stage, parametrial involvement, deep stromal invasion, and tumor size (Table 2). Tumor budding was also significantly associated with disease-free survival (p=0.035) on univariate analysis. On multivariate analysis, histologic grade 3 was the only independent predictor for decreased disease-free survival (p=0.004), whereas lymph node metastasis showed only a marginal significance.

On univariate analysis for cancer-specific survival, histologic grade, lymph node metastasis, LVSI, adjuvant therapy, tumor budding, and parametrial involvement showed a significant association with survival (Table 3). On multivariate analysis, histologic grade 3 was the only independent predictor of decreased cancer-specific survival (p=0.003), whereas LVSI and showed a marginal association with survival. Invasive pattern C did not adversely affect cancer-specific survival.

Discussion

The prognostic significance of the pathologic characteristics of invasive front have been shown in previous studies in many types of carcinomas, including cervical cancer (Horn et al., 2008; Khunamornpong et al., 2013). Tumor budding at the invasive front has recently been established as an independent prognostic predictor in patients with colorectal cancer (Mitrovic et al., 2012). Tumor budding may reflect the process of epithelial-mesenchymal transition, which allows neoplastic epithelial cells to acquire a mesenchymal phenotype with increased migratory capacity and invasiveness, increased resistance to apoptosis, and increased production of extracellular matrix molecules (Zlobec and Lugli, 2010).

Although the prognostic value of tumor budding has been extensively studied in colorectal cancer, the methodologies and scoring systems for "high-grade" tumor budding are not always uniform across the studies (Mitrovic et al., 2012; Horcic et al., 2013). The counting method using 10 HPF area in the present study was based on the recent proposal for the assessment of tumor budding in colorectal cancer (Karamitopoulou et al., 2013). High-grade tumor budding as classified using an average bud count ≥10 per HPF was found to have an independent prognostic value (Horcic et al., 2013; Karamitopoulou et al., 2013). However, cervical adenocarcinoma in the present study had a much lower number of tumor budding than colorectal cancer as none of the 129 patients had an average bud count reaching 10 per HPF.

In this study, high tumor bud count in early-stage cervical adenocarcinoma, as defined by ≥15 buds in 10 HPF of routinely-stained histologic sections, was associated with the well-recognized adverse prognostic features, particularly histologic grade 3 and lymph node metastasis, as well as the recently described invasive pattern C (Baalbergen et al., 2004; Roma et al., 2015). Tumor budding was significantly associated with worsened disease-free and cancer-specific survivals, although it was not found to be an independent prognostic predictor on multivariate analysis. The assessment for interobserver variability showed only fair agreement on tumor bud count based on the examination of routinely-stained slides. The finding indicates that immunohistochemical stain for cytokeratin may be necessary for a more reliable evaluation of tumor budding in cervical adenocarcinoma (Mitrovic et al., 2012).

The recently proposed pattern-based classification system for cervical adenocarcinoma of usual type has shown a better performance in predicting lymph node metastasis than the measurement of invasive extent (Diaz De Vivar et al., 2013). The risk for lymph node metastasis was different between the 3 invasive patterns: pattern A (risk 0%), pattern B (risk 4.4%), and pattern C (risk 23.8%) (Diaz De Vivar et al., 2013). In this study, the case proportion of each invasive pattern and the rate of lymph node metastasis in each pattern were in keeping with the previously reported findings. However, the invasive pattern C was not found to be an adverse prognostic predictor on multivariate analysis. The invasive pattern C even showed some protective effect with hazard ratios of 0.3 for both disease-free survival and cancer-specific survival. Although the explanation for this finding is unclear, it may be possible that the adverse prognostic impact of invasive pattern C is driven by other prognostic variables that it is associated with. The moderate agreement between both pathologists in the diagnosis of invasive pattern in this study is in keeping with the recently reported finding, which suggests that further refinement of the diagnostic criteria may be needed (Rutgers et al., 2016).

This study had several limitations. We did not evaluate the predictive performance of the other methods for tumor bud counting and scoring systems which have been used in previous studies on colorectal and other types of cancers (Ohike et al., 2010; Horcic et al., 2013;

Teramoto et al., 2013; Landau et al., 2014). Further studies may be necessary to clarify whether the other scoring methods could improve the prognostic significance of tumor budding in patients with cervical adenocarcinoma. Cytokeratin immunohistochemistry was not used in the assessment of tumor budding in this study. This immunostain may help to highlight the presence of cancer cells in the stroma and to reduce a misinterpretation of reactive non-epithelial cells as cancer cells, leading to a more accurate detection of tumor buds. A previous study has shown that interobserver agreement on the grading of tumor budding was improved from fair agreement using routinely-stained sections to substantial or almost perfect agreement using cytokeratin immunostaining (O'Connor et al., 2015). It should also be noted that in the present study, several well-recognized prognostic factors in cervical cancer patients were not found to have significant prognostic impact. It is possible that the fine selection of patients for adjuvant therapy based on the levels of risk variables may be related to the loss of prognostic significance of the risk factors in this study.

In conclusion, a high count of tumor budding based on the assessment of routinely-stained sections was associated with decreased disease-free and cancer-specific survivals in the patients with early-stage cervical adenocarcinoma. However, it was not found to be an independent prognostic predictor in this study.

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