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Tumor budding in tumor tissue among operatively treated patients with lung adenocarcinoma

Pupljenje tumora u tumorskom tkivu kod bolesnika operisanih zbog adenokarcinoma pluća

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Abstract

Background/Aim. The histological phenomenon of tumor budding is being recognized as an important determinant of disease progression and poor prognosis in various types of carcinoma. We aimed to evaluate the clinicopathological significance of tumor budding in adenocarcinoma of the lung. **Methods.** The study included 114 patients operatively treated for lung adenocarcinoma in a one-year period. Microscopic analysis of routine histological slides was performed to establish the presence and density of tumor buds. These results were compared in relation to gender, age, tumor size, nodal status, and pathological stage. **Results.** The budding-positive group included 34 (53.1%) men and 27 (54%) women. There were 30 (46.9%) men and 23 (46%) women in the budding-negative group. No statistical significant difference in age was found between males (64.3 ± 6.59 years) and females (63.1 ± 6.53 years) in the budding-positive group, nor in the budding-negative group (males 63.3 ± 6.02 years; females 63.2 ± 6.72 years). Statistically significant result in tumor size was found in females with the presence of tumor budding ($p < 0.05$). The budding-positive group of patients in nodal stage N1 and stage III of the disease pointed to the statistical significance ($p < 0.05$). **Conclusion.** With the statistical significance confirmed between the higher nodal status, higher pathological stage, and tumor budding found in this study, this histological phenomenon is still relatively new for the diagnostics domain of pathology. However, it increasingly receives attention as an adverse prognostic factor. These results may help tumor budding incorporate into the existing staging systems in addition to other factors known to be predictors of worse outcome.

Key words:
lung neoplasms; adenocarcinoma; neoplasm invasiveness; neoplasm staging; postoperative period; neoplasm recurrence, local.

Apstrakt

Uvod/Cilj. Fenomen tumorskog pupljenja sve više biva prepoznat kao značajna determinanta progresije i loše prognoze različitih tipova karcinoma. Cilj studije je bila klinički-patološka evaluacija ovog fenomena u adenokarcinomu pluća. **Metode.** Studija je obuhvatila 114 bolesnika operisanih od adenokarcinoma pluća u periodu od jedne godine. Prisustvo i gustina tumorskih pupoljaka analizirani su mikroskopski. Dobijeni rezultati su upoređivani u odnosu na pol i starost bolesnika, veličinu primarnog tumora, nodalni status i stadijum bolesti. **Rezultati.** U grupi bolesnika sa potvrđenim prisustvom tumorskog pupljenja bilo je 34 (55,7%) muškaraca i 27 (44,3%) žena, a u grupi bez prisustva tumorskog pupljenja 30 (56,6%) muškaraca i 23 (43,4%) žena. Nije uočena statistički značajna razlika u starosnoj dobi između muškog (64,3 ± 6,59 godina) i ženskog pola (63,1 ± 6,53 godina) kod bolesnika sa prisustvom tumorskih pupoljaka u tumorskom tkivu, kao ni kod muškog (63,3 ± 6,02 godina) i ženskog pola (63,2 ± 6,72 godina) kod bolesnika bez tog prisustva. Primarni tumor bio je značajno veći ($p < 0.05$) kod bolesnica sa fenomenom tumorskog pupljenja. U grupi bolesnika u čijem je tumorskom tkivu dokazan fenomen pupljenja dominirao je nodalni stadijum N1 ($p < 0,05$) i stadijum III bolesti ($p < 0,05$). **Zaključak.** Sa potvrđenom statističkom značajnošću između višeg nodalnog statusa, stadijuma bolesti i tumorskog pupljenja, pokazano je da ovaj fenomen, iako relativno nov u dijagnostičkom domenu patologije, privlači dodatnu pažnju kao značajan prognostički faktor. Dobijeni rezultati bi mogli pomoći u integrisanju ovog fenomena u postojeće skoring sisteme kao dodatka ostalim prediktorima koji ukazuju na lošiju prognozu bolesti.

Ključne reči:
pluća, neoplazme; adenokarcinom; neoplazme, invazivnost; neoplazme, određivanje stadijuma; postoperativni period; neoplazme, lokalni recidiv.

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Introduction

Lung cancer is confirmed to be the leading cause of cancer-related deaths worldwide, with a generally unfavorable outcome, even with a successful surgery. In recent years, the number of lung adenocarcinoma cases has been increasing. According to many studies, lung adenocarcinoma often comes with aggressive biological behavior, noting recurrence or distant metastasis soon after curative resection [1–5]. A better understanding of changes in malignant neoplasm biology that result in a more aggressive neoplastic behavior may help identify patients with a high risk of recurrent diseases and influence treatment algorithms.

In an arsenal of parameters essential in the outcome of patients, the target area in the present study was the phenomenon of tumor budding, investigated in terms of having an important role in risk stratification in lung adenocarcinoma.

The term tumor budding has been applied to the detachment and migration of single tumor cells or small clusters of cells from the neoplastic epithelium on the invasive front of tumor [6]. With an unknown molecular background, tumor budding is associated with a high incidence of local invasion and distant metastasis. Previously known as tumor dedifferentiation, this phenomenon has been likened to an epithelial-mesenchymal transition, thereby increasing cell migration and invasion [7–10]. This phenomenon is speculated to be a morphological expression of an invasive growth process that includes detachment between tumor cells, migration, and active invasion of surrounding stroma. From a morphological point of view, these groups of tumor cells tend to appear more atypical than cells in the main tumor body and may be visualized with difficulties on hematoxylin and eosin (H&E) routine slides. In addition, these cells may be obscured by a peritumoral inflammatory reaction and hardly distinguished from the reactive stromal cells [11, 12]. There are no well-established criteria used to determine how many cells should be in a cluster, therefore, it can be called a tumor bud. To date, the majority of studies used the 5-cell cutoff value. This criterion is often regarded to the presence and intensity of tumor budding in colorectal adenocarcinoma, while the number of studies referred to the budding in lung adenocarcinoma is rather small [13].

Tumor budding cannot be identified as tumor dedifferentiation [14]. The pattern composed of solid areas with numerous detached cells is often found in poorly differentiated tumors. Furthermore, high-grade tumors do not have or may have an insignificant number of tumor buds [15].

The differences in morphological features between cells in tumor buds and cells in the main tumor body are major. Budding cells have a tendency of progressively losing epithelial-cell features and resembling mesenchymal cells, therefore, they get long and spindle. In addition, these cells may degrade the extracellular matrix. Markers of motility, chemotaxis, and angiogenesis may be present on the cell surface. By performing immunohistochemistry, a positive reaction on mesenchymal cell markers may be confirmed. All the features mentioned are not found among cells in the central tumor area [9, 10, 15].

A limited number of studies, mostly in the colorectal oncology domain, recognize the presence of tumor budding as an important determinant of disease progression and poor prognosis [16–18]. Nevertheless, many disagreements are present among authors. The majority of authors consider budding as an indicator that drives aggressiveness and affects the disease-free and overall survival. Other authors, on the contrary, reported the presence of tumor budding in cases with already anticipated unfavorable prognosis due to the lymphatic and vascular invasion, as well as the infiltration of serosa.

As budding is often thought to have an independent prognostic value in patients with primary operable lung adenocarcinoma, the purpose of this study was to evaluate the clinicopathological significance of budding in adenocarcinoma of the lung.

Methods

From January 1 to December 31, 2018, a total of 114 patients with primary lung adenocarcinoma were treated by surgical resection in the regional hospital for pulmonary diseases. The cases of 64 male patients and 50 female patients who had undergone complete resection of lung adenocarcinoma were reviewed in this retrospective study. The study protocol was approved by the Research Ethics Committee of our hospital (January 26, 2018, No. 73-I/23). These cases were selected sequentially. The patients diagnosed with lung adenocarcinoma, with histological slides available for histological evaluation, and with complete follow-up data were included in this study. The exclusion criteria used in this study referred to patients who received neoadjuvant chemotherapy before the surgery and patients whose follow-up data was incomplete. The patients' characteristics that were assessed included gender, age, tumor size, stage of the disease, and histological subtypes of lung adenocarcinoma. The clinicopathological data were obtained from routine medical reports.

The histological diagnosis of primary lung adenocarcinoma was based on the 2015 World Health Organization Classification of Lung Tumors [19]. Tumor size was measured as the maximal diameter on the cut sections of the lung. The tumor subtypes, as well as the pathological stage, were determined according to the newest 2015 World Health Organization Classification of Lung Tumors and the 2014 IASLC/ATS/ERS Lung Adenocarcinoma Classification [20].

The lung tissue surgical specimens for the histological analysis were fixed by 10% neutral formalin, then routinely paraffin-embedded. The tumors were cut at approximately 5 mm intervals, sliced to 4 μm thick sections, and stained with (H&E. Full-section H&E slides were used to evaluate the presence and intensity of tumor budding, characterized by isolated tumor cells or small clusters that migrate a short distance into the neoplastic stroma at the advancing edge of neoplasms. Tumor budding was evaluated semiquantitatively, using a 20x objective lens by two pulmonary pathologists (AL and MP). In the first step, all of the slides were evaluated to determine the most representative tumor area. In a his

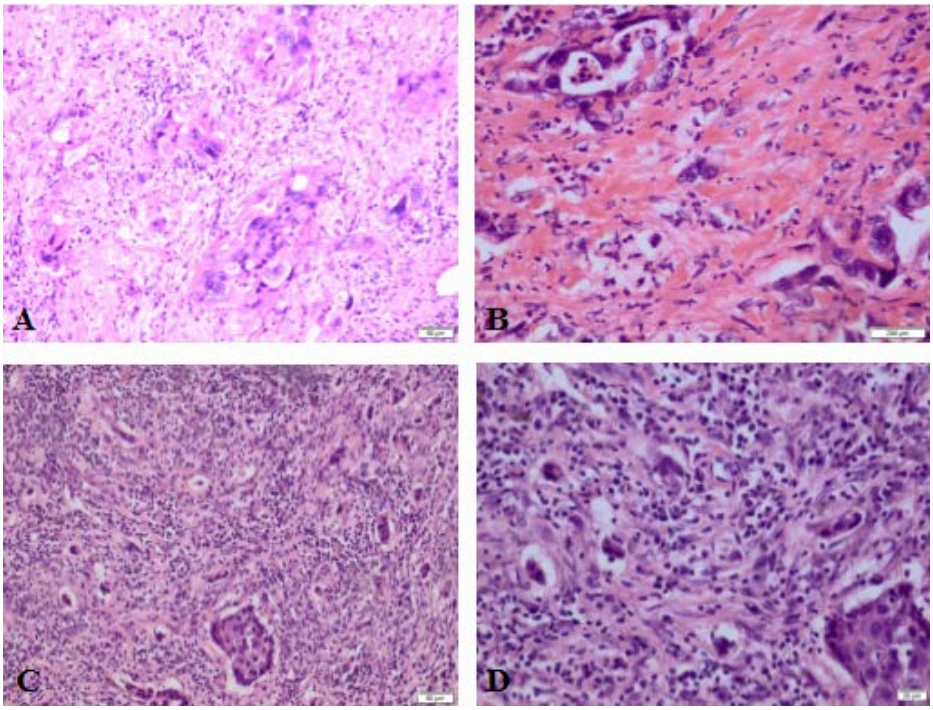

Fig. 1 – Tumor budding in lung adenocarcinoma: A, B) hematoxylin and eosin (H&E), ×20; C, D) H&E, ×40.

tological section, the maximal intensity of tumor budding was selected on the slide, and the number of tumor buds in that field was counted using a 20× objective lens. According to the presence of tumor buds per field, 2 major groups of patients were formed: 1) a budding-positive group of patients (Figure 1), and 2) a budding-negative group of patients.

In order to investigate the relationship between tumor budding and clinicopathological characteristics of the patients, we compared these results in relation to demographic parameters (gender, age), as well as with histological subtypes, tumor size, nodal status, and pathological stage of the tumor.

The data were processed in the IBM SPSS (*Statistical Package for Social Sciences*) program, version 23. Data analysis methods used descriptive and inferential statistics. Numerical variables were presented by the arithmetic mean and standard deviation, and the categorized variables through the frequencies and percentages. To determine the existence of a difference in variables between the study groups, Student's *t*-test and χ^2-test were used. Cumulative survival rates were calculated by the Kaplan-Meier method. The log-rank test was used to evaluate differences between the survival curves. All the differences were considered significant when the *p*-value was less than 0.05. The results were shown as tables and figures.

Results

Clinicopathological characteristics and histologic examination

Summarized characteristics of all 114 cases were presented through percentages. Within both groups, male patients were dominant regarding the tumor budding (Table 1). Sixty-one cases were classified as the acinar subtype, 42 as the solid subtype, 5 as the papillary subtype, 4 as the mucinous subtype, and 2 as the lepidic subtype. Tumor budding was found in 61 cases, and it was most frequently detected in the acinar subtype of lung adenocarcinoma (Table 1).

Table 2 shows the distribution of 114 cases by age and by tumor size within budding-positive and budding-negative groups. The arithmetic mean and standard deviation were calculated for these numerical variables, but, as the results

Table 1

Clinicopathological characteristics of patients with tumor budding

Characteristics of patients	All cases, n	Budding (+), n (%)	Budding (-), n (%)
Number	114	61 (53.5)	53 (46.5)
Gender			
male	64	34 (53.1)	30 (46.9)
female	50	27 (54)	23 (46)
Histological subtypes			
acinar	61	35 (57.3)	26 (42.7)
solid	42	22 (52.3)	20 (47.7)
papillary	5	3 (60)	2 (40)
mucinous	4	1 (25)	3 (75)
lepidic	2	0 (0)	2 (100)

Table 2

Distribution of patients by age and tumor size in study groups

Parameters	All cases (n)	Budding (+) mean ± SD	Budding (-) mean ± SD	Student's *t*- test	*p*-value
Age (years)					
male	64	64.3 ± 6.59	63.3 ± 6.02	0.627	0.533
female	50	63.1 ± 6.53	63.2 ± 6.72	-0.053	0.958
Tumor size (cm)					
male	64	4.12 ± 1.93	4.78 ± 2.50	-1.227	0.224
female	50	4.79 ± 2.77	3.33 ± 1.32	2.304	0.026

SD – standard deviation.

Table 3

Association between nodal and pathological stages and the presence of tumor budding

Parameters	All cases (n)	Budding (+) n (%)	Budding (-) n (%)	χ^2-test	*p*-value
Nodal stage					
N0	78	35 (44.9)	43 (55.1)	7.407	0.08
N1	20	17 (85)	3 (15)	9.669	0.02
N2	16	9 (56.2)	7 (43.8)	0.056	1
Pathological stage					
I	50	23	27	2.019	0.109
II	31	15	16	0.449	0.323
III	33	23	10	4.893	0.022

showed, the average age and tumor size were not significantly associated with the presence of tumor budding.

Table 3 shows the results of the χ^2-test used to determine the existence of a difference in nodular stage and pathological stage between the study groups. These two parameters and the presence of tumor budding were analyzed for associations, and significant associations were found between N1 status and stage III and the presence of tumor budding.

Survival analysis

From the Kaplan-Meier plots, it can be concluded that the cumulative survival proportions vary between the examined parameters. The cumulative survival proportion appeared to be much higher in the population without tumor budding compared to the population with tumor budding. It was shown that patients without tumor budding had better chances of survival (Figure 2A). Secondly, the cumulative survival proportion appeared to be equal in all nodular stages (Figure 2B). Moreover, the cumulative survival proportion appeared to be much higher in stage II compared to stage I and stage III, which did not appear to differ considerably. It was shown also that patients with the second stage of the disease had better chances of survival (Figure 2C). A log-rank test was run to determine if there were differences in the survival distribution for these three parameters. Survival distributions were not significantly different (for tumor budding: $\chi^2(2) = 1.556$, $p = 0.212$; for nodal stage: $\chi^2(2) = 1.236$, $p = 0.539$; for pathological stage: $\chi^2(2) = 5.939$, $p = 0.051$).

Discussion

The histological phenomenon of tumor budding was first described in the Japanese medical literature in 1949 [21] but revised after more than 2 decades among patients with colorectal adenocarcinoma. It is still not a part of the routine medical access and does not have a definite role in evaluating the prognosis of patients with different types of carcinoma because no consensus for the finest and most precise definition of tumor budding and the unique methodology for

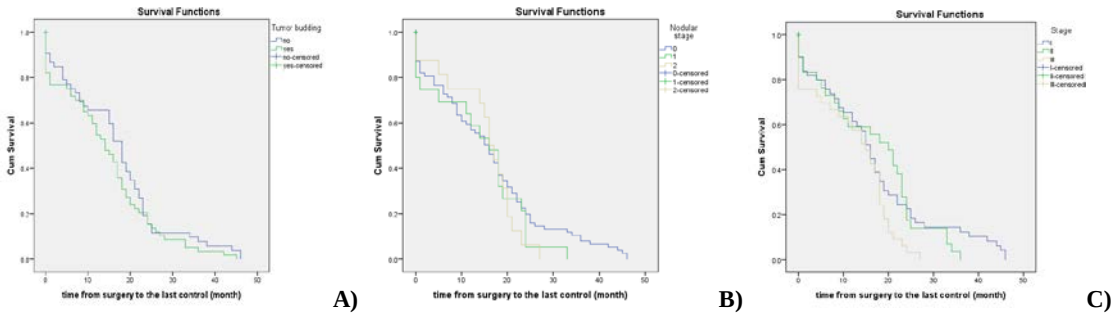

Fig. 2 – A) Cumulative overall survival curves stratified by the presence or absence of tumor budding; B) Cumulative overall survival curves stratified by the nodal stages N0, N1, and N2; C) Cumulative overall survival curves stratified by the pathological stages I, II, and III.

scoring has been formed [22]. The desire to conduct this retrospective study was based on the findings of multivariate analysis studies that show a stronger relationship between tumor budding and poor overall prognosis, unlike the singly used tumor-node-metastasis (TNM) classification [18]. In spite of these results, budding has still not been fully accepted as a factor that correlates directly with the biological behavior of the tumor.

Various ways can be used to define a histological structure as a tumor bud and to exclude bud-looking structures that are not true buds. Ueno et al. [16] defined buds as isolated malignant cells or ≤ 4 clustered malignant cells in the stroma at the invasive front of the tumor. Some authors slightly changed this definition and increased the cutoff value to foci of ≤ 5 clustered malignant cells [23, 24], thus they set the value many other authors tend to favor [25–27]. Along with the 2002 original publication of Ueno et al. [16] that was widely used in literature, there are 4 most cited methods for tumor budding assessment: Hase et al. (1993) [28], Nakamura et al. (2005) [29], and conventional method and rapid method by Wang et al. (2009) [30].

A total of 114 patients in this study were divided into two groups based on the budding-positive or budding-negative findings. The result of a dominant male distribution between the study groups may be related to the conventional fact of men being more frequently diagnosed with lung carcinoma than women. However, the study from 2015 indicated a relationship between females and low-grade budding in lung adenocarcinoma [31]. In the present study, no association between gender and the presence of tumor budding has been confirmed. The median age of the patients in our study was 63, with the range from 46 to 78 years, which is consistent with the observations of the 2016 study, where the median age was 66 (66 ± 9.9) years [32]. However, the consistency between age and tumor budding was not found.

Our attention was also dedicated to histological subtype analysis, and it was proved that the acinar subtype was dominant in male patients in both study groups, while the acinar and solid subtype were equally found in female patients, which makes these results corresponding to the reports of Kadota et al. [31] study. Tumor budding was not found in the lepidic subtype of lung adenocarcinoma [33]. These results suggest that the biological mechanism by which tumor budding is induced may vary with histological subtype.

The mean tumor size in the budding-positive group of patients was 4 cm (4.4 ± 2.34 cm), as well as in the budding-negative group (4.2 ± 2.18 cm), thus the statistical significance was not confirmed. Yamaguchi et al. [33] reported the findings of tumor budding in cases with adenocarcinoma bigger than 3 cm.

One of the most significant parameters to which tumor budding is connected is nodal status. The current result revealed that N1 status was significantly associated with the presence of tumor budding. In contrast, the absence of lymphatic invasion resulted in other studies conducted on N0 status, as opposed to our study [31]. Moreover, we analyzed the presence of tumor budding and pathological stage for associations, and significant associations were found between stage III and tumor budding. Comparing our results to other studies' results is difficult due to different stage analyses in other studies (mostly stage I) [33]. The reason why tumor budding is significantly associated with parameters that lead to a poor prognosis is not clarified. One of the satisfactory explanations is that budding cell phenotype represents a component of distant tumor invasion [22]. Taken together may explain the more aggressive behavior of the tumors that show this feature.

The overall number of studies demonstrating the presence and intensity of tumor budding in primary lung adenocarcinoma is rather small, especially because the use of corresponding immunohistochemistry methods is often required. The biggest obstacle for considering tumor budding as an integrated category in pathology reports is not having enough well-defined criteria for its evaluation. In addition, this has been pointed out in various types of carcinoma. In this manner, the budding aspect as a prognostic factor has been attracting interest [34–39]. Furthermore, it is believed that budding represents a histological basis for tumor cells to detach and invade locally and systemically [22]. According to the data reported previously, budding has been strongly linked to adverse clinicopathological features, poor overall prognosis, and disease-free survival.

Conclusion

With statistical significance confirmed between a higher nodal status, higher pathological stage, and tumor budding found in our study, this histological phenomenon is still relatively new for the diagnostics domain of pathology. However, it is receiving increasing attention as an adverse prognostic factor. It is imperative to add more clinicopathological features used to assess the risk of overall prognosis and to facilitate optimal clinical management through planning the treatment prior to surgery. These results may help tumor budding incorporate into the existing staging systems as it is associated with other factors known to portend worse outcomes, such as infiltrating tumor border, scirrhous stromal type, lymphatic, vascular, perineural and pleural invasion, nodal and distant metastases.

It is widely noted that additional studies will be needed to further define the methodology and uniform reporting of tumor budding through the most reproducible scoring method. The significance of tumor budding will need to be further evaluated in a multidisciplinary setting until further data become available.

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