Original Article

The Relationship between Tumor Budding and Clinicopathological Parameters in Patients with Gastric Adenocarcinoma

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Abstract

Background: Gastric cancer is the third most common cause of cancer-related death worldwide. Tumor budding is an easy to detect histopathological feature associated with a poor prognosis in patients with several types of cancer. The present study aimed to determine the relationship between tumor budding and clinicopathological parameters in gastric adenocarcinoma patients. **Materials and Methods:** This study retrospectively analyzed the H and E-stained slides of 146 patients that were diagnosed with gastric adenocarcinoma between 2013 and 2017. Tumor budding, large cell invasion, mitosis, fibrosis, and peritumoral lymphocytic response were recorded in all cases. The relationship between tumor budding and clinicopathological prognostic parameters was statistically analyzed. **Results:** Increased tumor budding density (≥10 tumor buds) was observed in 62 (42.5%) of the patients. There was a significant relationship between increased tumor budding density and histological grade ($P < 0.001$), lymphovascular invasion ($P = 0.016$), perineural invasion ($P < 0.001$), lymph node involvement ($P = 0.015$), and tumor invasion depth (pT stage) ($P < 0.001$). There was also a significant relationship between a high fibrosis rate, and lymphovascular invasion ($P < 0.001$), lymph node involvement ($P = 0.030$), and pT stage ($P = 0.002$); however, there wasn't a significant association between prognostic parameters, and large cell invasion, the mitotic count, and peritumoral lymphocytic response. **Conclusions:** The present findings suggest that increased tumor budding density in gastric adenocarcinoma patients may be used to predict poor prognosis.

Keywords: *Gastric adenocarcinoma, prognosis, tumor budding*

Introduction

Gastric adenocarcinoma is a malignant epithelial tumor of the gastric mucosa that exhibits glandular differentiation.[1] It is the fifth most common cancer worldwide and ranks third among cancer-related deaths.[2] Tumor budding has been investigated in case series, especially in patients with colorectal cancer, as well as lung adenocarcinoma, laryngeal carcinoma, esophageal carcinoma, ampullary carcinoma, and head and neck squamous cell carcinoma. Increased tumor budding is associated with a high tumour, node, metastasis (TNM) stage and tumor grade, lymphovascular invasion, lymph node involvement, and distant metastasis.[3-5] It is suggested that the biological significance of tumor budding might be related to epithelial-mesenchymal transformation, thereby increasing the migration and invasion characteristics of cancer cells.[6,7]

The present study aimed to determine the relationship between tumor budding and some morphological parameters, including large cell invasion, mitosis, fibrosis and peritumoral lymphocytic response, and clinicopathological prognostic parameters in gastric adenocarcinoma patients.

Materials and Methods

H and E-stained slides of 146 patients that were diagnosed with gastric adenocarcinoma between 2013 and 2017 were retrospectively analyzed via light microscopy. Patients with gastric tumors of nonepithelial origin, metastasis to the stomach, invasive tumors originating from other organs, and tumors that received preoperative treatment were excluded. Single isolated cancer cells or clusters of <5 cells at the invasive border of the tumor were evaluated. Tumor slides were scanned at ×100 magnification, and the area with maximum tumor budding density was selected for enumeration at (0.785 mm^2)

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×200 magnification. Tumor budding was categorized as low density (0–4 buds), moderate density (5–9 buds), and high density (≥10 buds) [Figures 1-3]. Tumor budding density and its relationship with clinicopathological parameters, namely age, gender, differentiation, lymphovascular and perineural invasion, Lauren classification, tumor invasion depth (pT), lymph node involvement stage (pN), metastasis, and overall survival were statistically analyzed.

Large cell invasion was defined as the presence of tumor cells with a nuclear diameter ≥4-fold the size of the lymphocyte at ×400 magnification. The presence and absence of mitosis were evaluated based on the mean mitotic count in 10 high-power fields (HPFs) as >15 mitotic figures and ≤15 mitotic figures, respectively. Fibrosis was considered mild (<30%), moderate (30%–60%), and severe (>60%) at ×100 magnification. Lastly, peritumoral lymphocytic response was evaluated as none, mild-moderate, and marked. The study protocol was approved by the Health Sciences University, Ankara Dışkapı Yıldırım Beyazıt Training and Research Hospital Ethics Committee (no. 40/07, dated July 24, 2017).

Statistical analysis

Data were analyzed using SPSS Statistics for Windows v. 17.0 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics are expressed as mean ± standard deviation or median (range) for discrete numerical variables, whereas categorical or ordinal variables are shown as number of observations and percentage. The significance of the difference in mean values between groups was examined via Student's *t*-test when there were 2 independent groups and one-way analysis of variance (ANOVA) in cases of >2 independent groups. The significance of the difference in ordinal variables between groups was assessed using the Mann–Whitney *U* test when there were 2 independent groups and the Kruskal–Wallis test in the case of >2 independent groups. In cases where one-way ANOVA or the Kruskal–Wallis test statistics were significant, the condition(s) that caused the difference were determined using the *post hoc* Tukey's Honestly Significantly Difference (HSD) test or Conover's multiple comparison test. Categorical variables were evaluated using Pearson's Chi-square test and Fisher's exact probability or likelihood ratio test. The level of statistical significance was set at $P < 0.05$.

Results

Among the patients, 102 (69.9%) were male and 44 (30.1%) were female. Mean age of the patients was 65.0 ± 12.0 years (range: 27–87 years). The clinical and pathological characteristics of the patients are given in Table 1. High tumor budding density (≥10 tumor buds) was noted in 62 (42.5%) of the patients. Mean age of the patients with high tumor budding density was significantly lower than in those with without ($P < 0.001$). Among the patients with high tumor budding density, the diffuse type was more common, based on Lauren classification ($P < 0.001$) [Table 2].

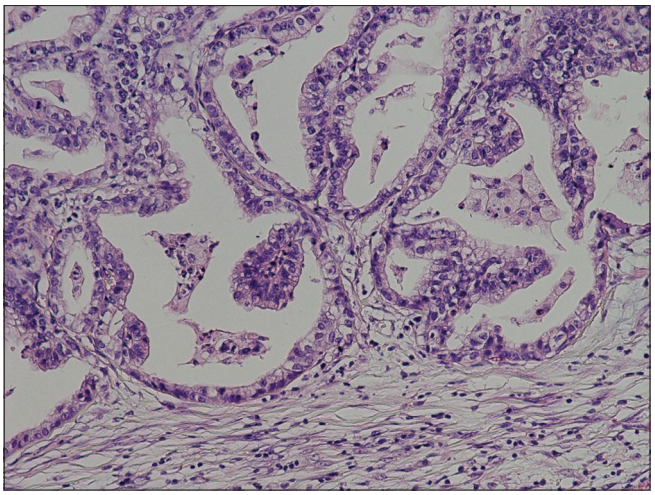

Figure 1: Gastric adenocarcinoma (pT3 N2), no tumor budding (H and E, ×200)

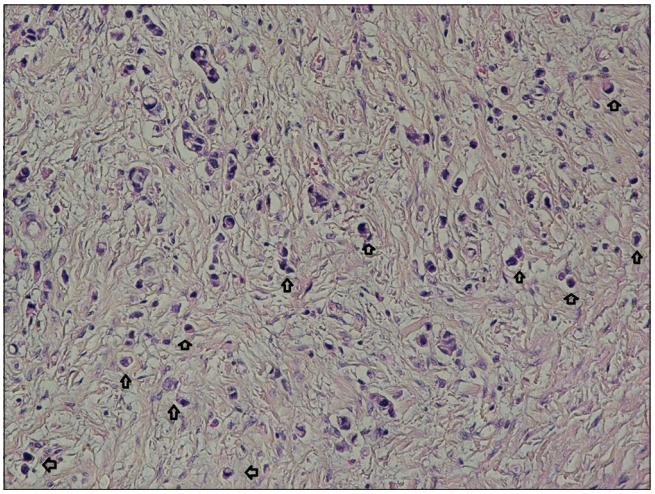

Figure 2: Gastric adenocarcinoma (pT3 N3), moderate tumor budding density indicated by arrows (H and E, ×200) The arrows show the budding cells

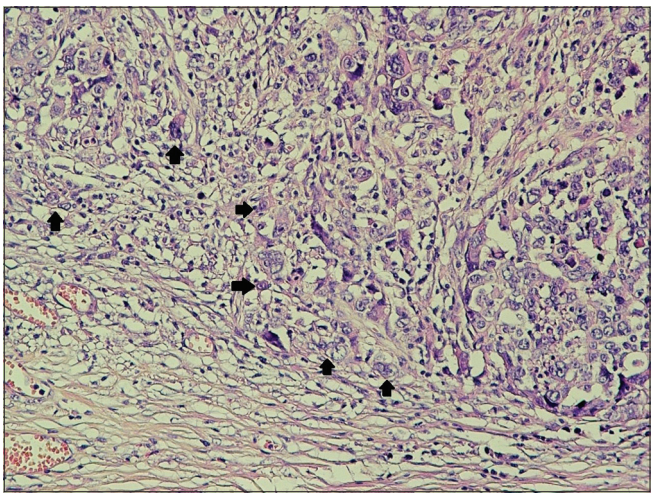

Figure 3: Gastric adenocarcinoma (pT4 N1), high tumor budding density indicated by arrows (H and E, ×200). The arrows show the budding cells

Table 1: Patient clinical and pathological findings (*n*=146)

Variable	*n* (%)
Age (years), mean±SD (range)	65.0±12.0 (27-87)
Gender	
Male	102 (69.9)
Female	44 (30.1)
Lauren classification	
Intestinal	113 (77.4)
Diffuse	33 (22.6)
Histological type	
Tubular	85 (58.2)
Papillary	12 (8.2)
Mucinous	16 (11.0)
Poorly cohesive	33 (22.6)
Localization	
Cardial	23 (15.8)
Corpus	18 (12.3)
Lesser curvature	45 (30.8)
Greater curvature	11 (7.5)
Antrum	48 (32.9)
Antrum-corpus	1 (0.7)
Differentiation	
Well	3 (2.1)
Moderate	66 (45.2)
Poor	77 (52.7)
Perineural invasion	90 (61.6)
Lymphovascular invasion	97 (66.4)
Lymph node involvement	
N0	41 (28.1)
N1	20 (13.7)
N2	24 (16.4)
N3	61 (41.8)
Material	
Total gastrectomy	103 (70.5)
Subtotal gastrectomy	43 (29.5)
Tumor size (cm)	5 (1-16)
T stage	
T1	19 (13.0)
T2	21 (14.4)
T3	43 (29.5)
T4	63 (43.2)
Metastasis	5 (3.4)
Pathological stage	
Stage I	28 (19.2)
Stage II	28 (19.2)
Stage III	85 (58.2)
Stage IV	5 (3.4)
Survival	
Alive	86 (58.9)
Deceased	60 (41.1)

SD: Standard deviation

The frequency of perineural invasion ($P < 0.001$) and lymphovascular invasion ($P = 0.016$) increased as tumor budding density increased [Figures 4 and 5]. In addition, there was a significant increase in depth of invasion (pT) ($P < 0.001$) and lymph node involvement stage (pN) ($P = 0.015$) as the number of tumor buds increased [Figures 6 and 7]. A significant correlation was observed between high tumor budding density and loss of tumor differentiation ($P < 0.001$); however, there weren't any significant correlations between tumor budding, and gender, metastasis, or survival [Table 3].

Large cell invasion was observed in 67 (45.9%) of the patients. In total, 98 (67.1%) of the patients had ≤ 15 mitotic figures/10 HPFs, whereas the remaining 48 (32.9%) patients had >15 mitotic figures/10 HPFs. Fibrosis was mild in 48 (32.9%) patients, moderate in 39 (26.7%), and severe in 59 (40.4%). There wasn't a peritumoral lymphocytic response in 31 (21.2%) patients, whereas this response was mild to moderate in 77 (52.7%) and marked in 38 (26.0%). There was a significant correlation between a high fibrosis rate, and lymphovascular invasion ($P < 0.001$), lymph node involvement ($P = 0.030$), and T stage ($P = 0.002$); however, there wasn't a significant correlation between prognostic parameters, and large cell invasion, the mitosis count, or peritumoral lymphocytic response.

Discussion

The most important prognostic parameter in cases of gastric cancer is tumor stage;[8] however, variation in the clinical course of gastric cancer patients with the same stage indicates that additional prognostic parameters are required for adequate evaluation. Tumor budding is an accepted prognostic parameter in colorectal cancer patients and its prognostic significance has also been shown in patients with lung adenocarcinoma, laryngeal carcinoma, esophageal carcinoma, ampullary carcinoma, and head and neck squamous cell carcinomas.[3-5] Increased tumor budding is associated with a high TNM stage and tumor grade, lymphovascular invasion, lymph node involvement, and distant metastasis, and is associated with a poor prognosis.[3-5] Nevertheless, the literature includes only a few studies on the relationship between tumor budding and gastric cancer.[9-12]

Studies on patients with colorectal cancer and other malignancies have used various grading (double or triple) systems and cut-off values for tumor budding. Some studies have enumerated tumor buds based on 1 HPF and 10 HPFs.[13] The International Tumor Budding Consensus Conference defines tumor budding as a single tumor cell or a cluster of <5 tumor cells, and recommends evaluating tumor budding based on a 3-tier system (0–4 buds: low density; 5–9 buds: moderate density; ≥1 buds: high density) using the "hot spot" technique, in which buds are enumerated where tumor budding density is highest (0.785 mm^2) at ×200 magnification,[14] as used in the present study. These recommendations for colorectal cancers can be applied to other gastrointestinal system cancers in the future and standardization of tumor budding evaluation can be achieved for other cancers.

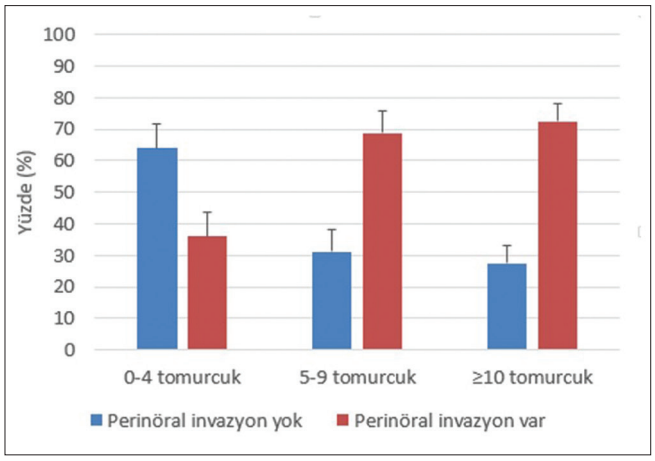

Figure 4: The relationship between perineural invasion and tumor budding

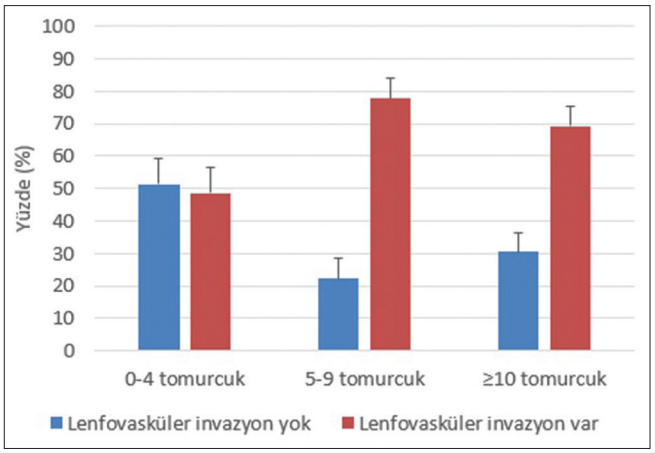

Figure 5: The relationship between lymphovascular invasion and tumor budding

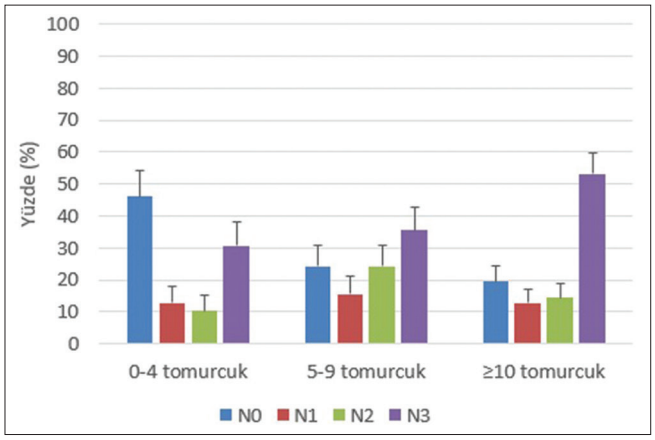

Figure 6: The relationship between lymph node involvement stage and tumor budding

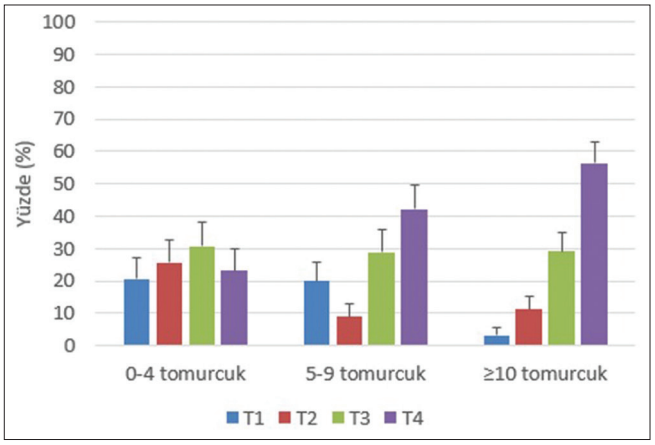

Figure 7: The relationship between tumor invasion depth and tumor budding

Table 2: The relationship between tumor budding and clinical findings

	0-4 buds (*n*=39)	5-9 buds (*n*=45)	≥10 buds (*n*=62)	*P*
Age (years)	69.8±9.0[a]	66.8±11.4[b]	60.6±12.7[a,b]	<0.001[†]
Gender, *n* (%)				
Male	27 (69.2)	31 (68.9)	44 (71.0)	0.969[‡]
Female	12 (30.8)	14 (31.1)	18 (29.0)	
Lauren classification, *n* (%)				
Intestinal	37 (94.9)[a]	40 (88.9)[b]	36 (58.1)[a,b]	<0.001[‡]
Diffuse	2 (5.1)[a]	5 (11.1)[b]	26 (41.9)[a,b]	

[†]One-way ANOVA, [‡]Pearson's Chi-squared test, Likelihood ratio test: [a]Significant difference between 0 and 4 buds and ≥10 buds (P<0.05), [b]Significant difference between 5-9 buds and ≥10 buds (P<0.05). ANOVA: Analysis of variance

In the present study H and E-stained slides were used to evaluate tumor budding, as previously used to investigate the relationship between gastric cancer and tumor n budding;[9,12] however, some studies on colorectal cancer used immunohistochemical analysis to investigate tumor budding.[13] It has been reported that immunohistochemical evaluation is the superior method due to its high inter-observer consistency and reproducibility, whereas others reported no difference.[13] In addition, the cost effectiveness of H and E staining for analysis makes it more appropriate for investigating tumor budding. It was reported that immunohistochemistry may be useful in cases in which peritumoral inflammatory infiltration makes evaluation difficult, and for differentiation of tumors from reactive stromal cells and in the presence of confusing findings, such as glandular fragmentation.[14]

As tumor budding density increased in the present study the incidence of lymphovascular invasion and perineural invasion, tumor invasion depth (pT), and lymph node involvement stage (pN) also increased significantly (P = 0.016, P < 0.001, P < 0.001, and P = 0.015, respectively). In addition, there was a significant correlation between high tumor budding density and loss of tumor differentiation (P < 0.001). These findings are consistent with earlier findings.[9-12] If the relationship between tumor budding and clinicopathological prognostic parameters in gastric cancer patients can be confirmed by larger scale, multi-center case series, tumor budding could

Table 3: The relationship between tumor budding and pathological findings

	0-4 buds (n=39), *n* (%)	5-9 buds (*n*=45), *n* (%)	≥10 buds (*n*=62), *n* (%)	*P*
Differentiation				
Well	1 (2.6)	1 (2.2)	1 (1.6)	<0.001†
Moderate	28 (71.8)[a]	25 (55.6)[b]	13 (21.0)	
Poor	10 (25.6)	19 (42.2)	48 (77.4)[a,b]	
Perineural invasion	14 (35.9)[a,c]	31 (68.9)[c]	45 (72.6)[a]	<0.001‡
Lymphovascular invasion	19 (48.7)[a,c]	35 (77.8)[c]	43 (69.4)[a]	0.016‡
Lymph node involvement				
N0	18 (46.2)	11 (24.4)	12 (19.4)	0.015†
N1	5 (12.8)[a]	7 (15.6)	8 (12.9)	
N2	4 (10.3)	11 (24.4)	9 (14.5)	
N3	12 (30.8)	16 (35.6)	33 (53.2)[a]	
T stage				
T1	8 (20.5)	9 (20.0)	2 (3.2)	<0.001†
T2	10 (25.6)	4 (8.9)	7 (11.3)	
T3	12 (30.8)[a]	13 (28.9)[b]	18 (29.0)	
T4	9 (23.1)	19 (42.2)	35 (56.5)[a,b]	
Metastasis	2 (5.1)	2 (4.4)	1 (1.6)	0.552**
Survival				
Alive	28 (71.8)	28 (62.2)	30 (48.4)	0.057‡
Deceased	11 (28.2)	17 (37.8)	32 (51.6)	

†One-way ANOVA, ‡Pearson's Chi-squared test, **Likelihood ratio test: [a]Significant difference between 0 and 4 buds and ≥10 buds (P<0.05), [b]Significant difference between 5 and 9 buds and ≥10 buds (P<0.05). [c]Significant difference between 0 and 4 buds and between 5 and 9 buds (P<0.01). ANOVA: Analysis of variance

then be used as a parameter that aids the follow-up of such patients.

Che *et al*.[11] reported in 2017 that there is a significant relationship between tumor budding and metastasis in patients with gastric cancer. The same year Olsen *et al*.[12] reported that there is a relationship between tumor budding, and high pT, pN stage, and grade, and recurrence in intestinal gastric cancer patients, but not between tumor budding and metastasis. In the present study there wasn't a significant relationship between metastasis and tumor budding, but this could be misleading, as only 5 of the 146 patients had metastasis, some patients were lost to follow-up, and epicrisis reports for patients other than those that underwent surgery due to metastasis were not available. Additional research is needed to obtain more precise data on the relationship between tumor budding and metastasis.

Che *et al*.[11] reported that the overall survival in gastric cancer patients with high-density tumor budding is shorter than in those with low-density tumor budding. In the present study there wasn't a significant relationship between overall survival and tumor budding; overall survival was calculated based on the death notification system and each patient was evaluated as alive or deceased. Although the patients' dates of death were available, the causes of death and recurrence dates were not obtained; thus, only the total survival rate was calculated without disease-specific and recurrence-free survival calculations. Furthermore, the lack of postsurgery follow-up data is another a limiting factor; as such, the relationship between tumor budding, and overall survival, disease-specific survival, and recurrence-free survival in gastric cancer patients needs to be investigated in large-scale case series supported by clinical data.

In the present study diffuse-type tumors (based on Lauren classification) had high tumor budding density. Diffuse-type cancers are noncohesive and present with dense tumor budding. It was thought that the significantly lower mean age in the patients with high tumor budding density might be consistent with the fact that tumors in this age group are often hereditary and of the diffuse type.

Pronounced desmoplasia in gastric cancer patients is indicative of aggressive behavior and peritoneal spread.[1] Zhou *et al*.[15] reported in 2017 that cases with thick and wide collagen bands are poorly differentiated, and had deeper mural invasion, increased lymph node metastasis, and increased recurrence, as compared to those with thin collagen bands. The present study also investigated the relationship between fibrosis and clinicopathological parameters. Lymphovascular invasion, lymph node involvement stage (pN), and tumor invasion depth (pT) were significantly higher in the present study's patients with marked fibrosis, as compared to those with mild fibrosis (P < 0.001, P = 0.030, and P = 0.002, respectively); however, there wasn't a significant correlation between fibrosis, and perineural invasion, metastasis, or overall survival.

Che *et al*.[11] observed a significant relationship between large cell invasion, and TNM classification, pathological stage, and overall survival in patients with gastric cancer. In the present study there wasn't a significant association between large cell invasion, and perineural invasion, lymphovascular invasion, tumor invasion depth (pT), lymph node involvement stage (pN), metastasis, or overall survival; therefore, it may be warranted for future studies to investigate the relationship between large cell invasion and prognosis in gastric cancer patients.

Lee *et al*.[16] showed that the density of lymphocytes (especially regulator T cells) that infiltrate the tumor in gastric cancer patients is associated with lymph node metastasis and overall survival. Additionally, the increase in lymphocytes that infiltrate the tumor were associated with longer survival and a lower rate of lymph node metastasis. In contrast, Setälä *et al*.[17] did not observe a relationship between lymphoplasmacytic infiltration density, and tumor stage or other histological parameters. Similarly, in the present study there wasn't a significant

correlation between peritumoral lymphocytic response, and perineural invasion, lymphovascular invasion, tumor invasion depth (pT), lymph node involvement stage (pN), metastasis, or overall survival.

The prognostic significance of the number of mitotic figures has been reported in many studies on neoplasia (breast-bladder cancer); however, only a limited number of studies investigated the relationship between the rate of mitosis and prognostic parameters in gastric cancer patients.[17,18] Tabuchi *et al*.[18] reported that gastric cancer patients with high mitotic activity died earlier than those with low mitotic activity. Setälä *et al*.[17] observed that the higher rate of mitosis in patients with intestinal cancers (based on Lauren classification) was associated with lymphatic invasion, but that it was not related to other histological features. In the present study there wasn't a significant correlation between mitosis, and perineural invasion, lymphovascular invasion, tumor invasion depth (pT), lymph node involvement stage (pN), metastasis, or overall survival. In light of these findings, it is considered that the mitotic count does not have strong prognostic significance in gastric cancer patients.

Conclusions

The present findings show that increased tumor budding density in gastric adenocarcinoma patients is associated with a high TNM stage and tumor grade, lymphovascular invasion, and lymph node involvement. Increased tumor budding density can be a useful microscopic indicator of poor prognosis in cases of gastric adenocarcinoma.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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