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Tumor budding in invasive breast carcinoma: correlation with clinicopathological parameters, hormone receptor status, and survival: an observational study

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SUMMARY

OBJECTIVE: Tumor budding is currently thought to be associated with worse prognosis. This study aims to examine tumor budding in invasive ductal-type breast carcinoma and its relationship with other clinicopathological parameters and overall survival.

METHODS: All the H&E slides of 198 patients were re-evaluated for the histological grade, angiolymphatic invasion, perineural invasion, lymph node status, extranodal extension, multicentricity, pT, presence of the tumor budding, tumor budding score (i.e., low, intermediate, or high). Overall survival was considered the period after surgery until death. SPSS was used for statistical analysis.

RESULTS: Tumor budding was identified in 98 (49.5%) patients. Tumor budding score was low in 41 (41.8%) of 98 cases, intermediate in 25 (25.5%), and high in 32 (32.7%). We determined a strong correlation between tumor budding and poor prognostic variables such as tumor size, pT stage, angiolymphatic invasion, perineural invasion, number of metastatic axillary lymph nodes, overall survival, and extranodal tumor extension in metastatic lymph nodes. This strong correlation was also present for the tumor budding score.

CONCLUSION: Tumor budding may be a prognostic indicator for breast cancer.

KEYWORDS: Breast cancer. Breast tumor. Carcinoma, invasive ductal, breast. Survival.

INTRODUCTION

Breast cancers are the most common cause of mortality in women worldwide[1]. They are heterogeneous and have variable morphological and biological features and thus clinical behavior and therapeutic outcome. The histopathological assessment aims to provide an accurate diagnosis of the disease and prediction of tumor behavior to facilitate clinical and oncologic decision-making. Invasive ductal carcinoma constitutes the majority and is the cause of a great clinical burden[2]. In spite of the availability of treatment protocols, relapse and metastasis are known to occur. Therefore, additional and more efficient prognostic markers are required to predict prognosis and survival and also for individual treatment approaches[3-5].

Tumor budding (TB), which has previously been reported to predict survival in several solid organ tumors, is currently thought to be associated with worse prognosis[6,7].

TB is defined as the formation of single malignant cells or cell clusters of fewer than five malignant cells at the invasive tumor front and is associated with tumor invasion and distant metastasis[4]. The 2019 World Health Organization classification of colorectal cancer introduces TB as a second major grading criterion[8]. Studies reported that TB is also a novel prognostic indicator independent of tumor stage and grade in esophageal, gastric, ürinary bladder, and pancreatic tumors[9,10]. In invasive ductal carcinoma, a high number of tumor buds are associated with angiolymphatic invasion (LVI), lymph node metastasis, and shorter survival[11]. Extranodal extension (ENE) is defined as tumor cells penetrating through the capsule of a lymph node into the perinodal tissue. The importance of ENE in axillary lymph nodes in breast carcinoma was first reported in a series between 1936 and 1941[12]. From that day on, lots of studies conducted about this phenomenon and ENE has been found in association with worse prognosis in breast carcinoma. While we were doing this study, there was no study in the literature investigating the relationship between TB and ENE.

Therefore, this study aims to examine TB in invasive ductal-type breast carcinoma, and its relationship with other clinicopathological parameters, especially hormone receptor status, LVI, perineural invasion (PNI), metastatic lymph node status, ENE, and overall survival.

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METHODS

Study design and case selection

From June 2014 to January 2022, patients who had undergone breast carcinoma surgery at the Bolu Abant Izzet Baysal Training and Research Hospital were retrospectively scanned from the electronic database in the present observational study. Among them, some cases were excluded for any of the following criteria: (1) those whose diagnosis was not invasive ductal carcinoma, (2) those whose H&E-stained slides were not reached or available for review, (3) those who received neoadjuvant chemotherapy or radiotherapy, (4) those who died due to post-operative complications in the first month after surgery, and (5) those whose clinical data not to be reached. A total of 198 patients were included in the study.

Clinicopathological information, which included age, tumor size (TS), stage, and nodal status, was retrieved from pathological reports. Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 proliferation index analysis were retrieved from immunohistochemistry reports and slides were re-evaluated.

All cases were divided into molecular subtypes (i.e., Luminal A, Luminal B, HER2, and Triple-negative) according to the ER, PR, HER2, and Ki-67 immunohistochemical staining patterns. Overall survival was considered the period after surgery until the death of the patient. Death records were completed on August 2022.

All the H&E slides were re-evaluated for the histological grade (Modified Bloom-Richardson), LVI, PNI, lymph node status, ENE, multicentricity, pT, presence of the TB, and tumor budding score (TBS). TB was considered single tumor cells or cell clusters of up to four cells in the peripheral advancing tumor front, as indicated by ITBCC 2016. First, the cases were grouped as "tumor budding absent (TBA)" or "tumor budding present (TBP)." Then, TBS was evaluated with a three-tier score; low (0–4 buds), intermediate (5–9 buds), and high (10 or more buds) as also defined in ITBCC. TB was assessed by selecting a "hotspot area" chosen after a review of all available slides. The total number of buds was reported in an area measuring 0.950 mm^2, which corresponds to 20′ field in the Olympus CX43 microscope (Figure 1). We grouped lymph nodes into three groups as follows: negative, positive without ENE, and positive with ENE. ENE length was not measured in ENE-positive lymph nodes.

Ethical approval

The study was approved by the Clinical Researches Ethics Committee of the Bolu Abant Izzet Baysal University (Decision number: 164/2022).

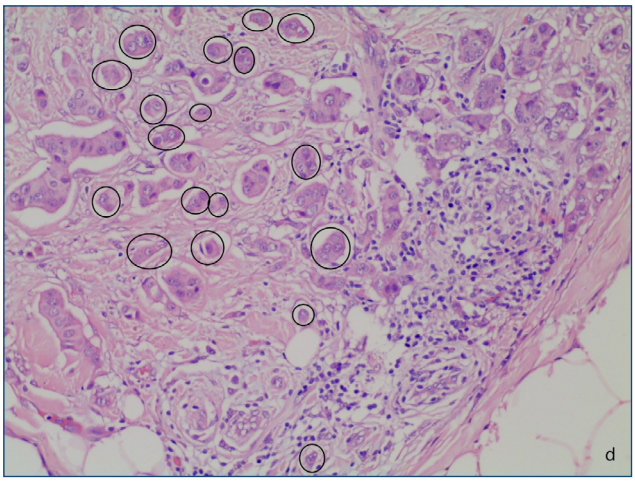

Figure 1. Tumor budding at the invasive front of invasive ductal carcinoma (H&E 200×). High tumor budding.

Statistical analysis

SPSS 15.0 for Windows was used for statistical analysis. Kolmogorov-Smirnov test was applied to the study variables for normality analysis. The variables with normal distribution were compared with the independent-samples t-test between two groups and with the one-way ANOVA test for three or more groups. These variables were expressed as mean±SD. Variables without normal distribution were compared by the Mann-Whitney U test in two groups and by the Kruskall-Wallis test in three or more groups. These variables were expressed as median (min–max). The comparison of categorical variables was conducted with the chi-square test. These variables were expressed as numbers and percentages. Pearson's correlation analysis test was used to observe the correlation between study variables. The sensitivity and specificity of study variables in determining TB were analyzed using receiver operative characteristics curve analysis. Kaplan-Meier analysis was used in the survey analysis of study variables. Statistical significance was accepted as $p<0.05$.

RESULTS

In this study, 198 samples of invasive ductal carcinoma were assessed. TB was identified in 98 (49.5%) patients and not identified in 100 (50.5%). TBS was low in 41 (41.8%) of 98 cases, intermediate in 25 (25.5%), and high in 32 (32.7%).

The average TS of the TBA and TBP groups was 19.5 mm (2–70 mm) and 25 mm (8–170 mm), respectively, and it was statistically significant ($p<0.001$). The median value of TS is 20 (8–105) mm in patients with low TBS, 25 (12–52) mm in patients with moderate TBS, and 40 (12–170) mm in patients with high TBS. TS significantly increased as the TBS increased ($p=0.001$).

A total of 51 patients were evaluated as pT1, 43 patients as pT2, 5 patients as pT3, and 1 patient as pT4 in the TBA group. A total of 24 patients were evaluated as pT1, 56 patients as pT2, 13 patients as pT3, and 5 patients as pT4 in the TBP group. It was statistically significant that patients with TB were in the advanced pT stage (p=0.002). TB was detected in 62 (72.9%) of 85 cases with LVI and 29 (76.3%) of 38 cases with PNI. Both were significant (p<0.001 and p<0.001) (Table 1).

In addition, the relationship of TBS with LVI and PNI was statistically significant (p=0.004 and p=0.01, respectively). The number of metastatic lymph nodes was between 0 and 51, and the average metastatic lymph node number was 4. The median value of the metastatic axillary lymph node in the TBA group was 0 (0–51) and in the TBP group was 2 (0–36), and this was statistically significant (p<0.001). As well as the TBS increased, metastatic lymph node count increased statistically (p<0.001).

ENE was detected in 35 patients (35.7%) in the TBP group and was detected in 14 patients (14%) in the TBA group. There was a significant correlation between TB and ENE (p<0.001). The rates of presence of ENE in the TBS groups were as follows: 6 cases (17%) in the low, 8 cases (23%) in the intermediate, and 21 cases (60%) in the high group. As the TBS increased, the presence of ENE increased statistically (p<0.001).

The mean follow-up period of the patients was 39.6 months, and the follow-up interval ranged from 3 to 97 months. The relationship between TB and cumulative survival was significant; 22 (22.4%) of the patients with TB died, and 76 (77.6%) were still alive (p=0.002). The median survival time was 43 (5–97) months in the TBA group and 27 (3–97) months in the TBP group (p=0.001) (Figure 2). In the overall survival analysis, mean survival times were significantly lower in the TBP group and TBS was also high (p<0.001 and p=0.004). The association of TB with age, molecular subtypes, multicentricity, and histological grade was not significant.

DISCUSSION

Invasive ductal carcinomas are heterogeneous and have variable morphological and biological features and thus clinical

Table 1. Tumor budding and clinicopathological parameters.

		Tumor budding present	Tumor budding absent	p-Value
Patients, n (%)		98 (49.5)	100 (50.5)	
Age (years)		55.9 (±13.2)	56.5 (±11.7)	0.20
Tumor size (mm, min–max)		25 (8–170)	19.5 (2–70)	<0.001
Molecular subtype (n)	Luminal A	33	49	0.08
	Luminal B	46	33	
	HER2	15	11	
	Triple Negative	4	7	
Histological grade, n (%)	Grade 1	22 (9.1)	33 (16.7)	0.053
	Grade 2	50 (25.3)	45 (22.7)	
	Grade 3	30 (15.2)	22 (11.1)	
Multicentricity, n (%)		13 (13.3)	10 (10)	0.47
pT stage (n)	pT1	24	51	0.002
	pT2	56	43	
	pT3	13	5	
	pT4	5	1	
Angiolymphatic invasion, n (%)		62 (72.9)	23 (27.1)	<0.001
Perineural invasion, n (%)		29 (76.3)	9 (23.7)	<0.001
Metastatic lymph node, n (min–max)		2 (0–36)	0 (0–51)	<0.001
Extranodal extension, n (%)		35 (35.7%)	14 (14%)	<0.001
Cumulative survival, n (%)	Alive	76 (77.6)	93 (93)	0.002
	Dead	22 (22.4)	7 (7)	
Survival time (month)		27 (3–97)	43 (5–97)	0.001

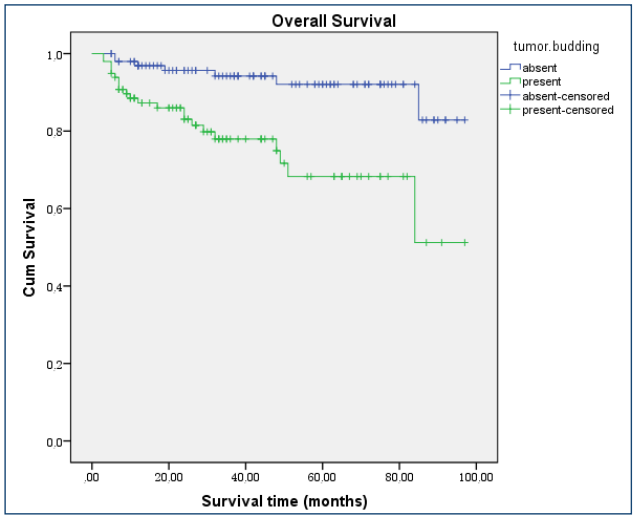

Figure 2. Kaplan-Meier overall survival curve for tumor budding.

behavior and therapeutic outcome. Therefore, additional and more efficient prognostic markers are required to predict prognosis and survival and also for individual treatment approaches[3-5]. TB is a histological process, which was described in colorectal carcinoma first by Imai in 1954[7]. In the ensuing years, it has been studied in many solid organ malignancies as a prognostic marker[4,13,14]. Therefore TB is advocated as a more sensitive prognostic factor, a predictor of aggressiveness and a worse outcome[4,6,15].

At the ITBCC, the method of evaluating, scoring, and reporting TB was standardized and detailed in colorectal carcinomas[7]. For other organ malignancies, this is still a subject of debate. So far, different studies have utilized different methods for the assessment of TB[16]. Most of them assessed TB in the low-high bud group with different cutoff values[4,17]. In spite of various evaluation methods, all these studies showed that high TBS was associated with poor prognosis and decreased survival[6,8,17]. In our study, TB was assessed by selecting a "hotspot area" measuring 0.950 mm^2 and scoring TB counts with a three-tier scoring system, as defined in ITBCC. Some studies used immunohistochemistry for evaluating buds, and some of them did not[4,11,18,19]. We did not perform immunohistochemistry as recommended at ITBCC.

In this study, TB was statistically associated with TS, pT stage, LVI, PNI, number of metastatic axillary lymph nodes, ENE, and overall survival. However, TB was not associated with age, molecular subtype, histological grade, and multicentricity. Previous studies investigated the association of hormone receptor status with TB. Some of them have found significant relationship with ER positivity[17,19], but Okcu et al. have not[4]. Similar to our study, Masilamani et al. evaluated the association between molecular subtype groups and have not found a significant association[20].

Therefore, many previous studies have found similar associations between TB and LVI, pT, and axillary lymph node metastasis[4,6,8,15,17-20]. In line with them, we found a significant association. Also in our TBS groups, as TBS increased, LVI and metastatic axillary lymph nodes increased.

Only a few studies focused on the association of PNI with TBS. While Okcu et al. found no relationship[4], we found a strong association between them. As TBS increases, PNI will also increase, which is a new contribution to the literature.

While we performed our study, there was no study that assessed the association between ENE and TB. In our study, there was a significant correlation between TB and ENE ($p<0.001$). In addition, as the TBS increased, the presence of ENE increased statistically ($p<0.001$).

Various studies investigated the association of TB with survival. Survival is evaluated as overall or cancer-specific. They have found a strong relationship, especially high TB groups have had lower survival times[4,6,8,17]. Li et al. have found that TB was an independent prognostic factor of cancer-specific survival[6]. In line with the literature, we found a significant relationship between TB and overall survival. Survival time was reduced in patients with TB, and as the TBS increased, survival time decreased.

Other methods are also useful in breast carcinoma survival. Preoperative magnetic resonance image has beneficial effects on survival rates of breast cancer patients[21]. Moreover, extracapsular extension in sentinel lymph node biopsy is also considered a predictor of survival[22]. Similarly, we found that TB was associated with survival in breast cancer cases.

Retrospective design and single-center nature of the study are limitations of this study. Yet, the strength is being the first study in the literature that reported the association between TB and breast cancer.

CONCLUSION

This study provides an extensive assessment of TB, and there is a strong correlation between TB and poor prognostic variables such as TS, pT stage, LVI, PNI, lymph node metastasis, overall survival, and ENE, which has not been the subject of the previous studies. This strong correlation was also present for the TBS. Based on all these results of this study, we can say that TB is a prognostic indicator, and the assesment of TB utilizing routine pathological slides is relatively easy and it does not bring additional cost. However, it needs standardization for evaluation and scoring.

ETHICS STATEMENT

This study has been approved by the directorate of the institution under decision number 164/2022.

AUTHORS' CONTRIBUTIONS

SPO: Conceptualization, Data curation, Formal Analysis, Writing – original draft, Writing – review & editing.

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