Original paper

Tumour budding in invasive breast carcinoma of no special type – relationship with clinicopathological parameters

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Each breast cancer is a heterogeneous tumour with different clinicopathological feature, and thus they all have different prognoses. Tumour budding (TB), considered as the first step in tumour metastasis, is the most critical factor for poor prognosis and is associated with the epithelial-mesenchymal transition (EMT). Tumour budding and its clinicopathological features in invasive breast carcinoma of no special type (NST).

Patients who underwent surgery for invasive breast carcinoma (NST) between January 2018 and 2022 were retrospectively reviewed from the database, haematoxylin and eosin-stained slides were retrieved and reevaluated.

The study included 200 patients. The mean number of TB was 12.8 ±9.6. The number of TB was significantly lower in patients who underwent neoadjuvant chemotherapy treatment ($p = 0.002$). There was a weak positive correlation between TB count and tumour size ($r = 0.177$). Triple-negative patients had significantly lower TB counts ($p = 0.001$). No significant difference was observed between histological grade, nuclear grade, presence of ductal carcinoma in situ, stromal tumour-infiltrating lymphocytes, perineural invasion, lymph node metastasis, and number of TB ($p > 0.05$).

The number of TB was higher in oestrogen receptor positive tumours ($p = 0.015$). There were more TB in patients with angiolymphatic invasion, which supports the pathophysiological relationship between tumour budding, metastasis, and EMT. Clarification of the mechanism of TB with more studies is promising in terms of treatment options.

Key words: invasive breast carcinoma of no special type (NST), tumour budding, clinicopathological parameters.

Introduction

Breast cancer (BC) is the most common malignant tumour among women worldwide and is the second leading cause of cancer death [1, 2].

Breast cancers have 4 main intrinsic molecular subtypes: luminal A, luminal B, HER2-positive, and triple-negative. These BC all have different proliferation and metastasis abilities with metabolic, genotypes, and phenotypes [3].

Tumour budding (TB) refers to the detachment of tumour cells, individually or in small clusters, from neoplastic glands at the invasive front. Although its importance has been studied mainly in the field of colorectal cancer, there are studies suggesting it

is associated with an aggressive course in other solid cancers [4].

Tumour budding is defined as clusters of less than 5 cells, which are separated and isolated from the tumour mass. Tumour budding has been described as a feature of cancer cell motility and thus as first step in the metastatic process [5].

In this study, we evaluated the relationship between TB and tumour size, histological grade, axillary lymph node metastasis, angiolymphatic invasion, lymphocytic infiltration, and immunohistochemical staining (oestrogen receptor – ER, progesterone receptor – PR, HER2) patterns.

Material and methods

Study population

We studied 200 patients diagnosed with invasive breast carcinoma of no special type resected between 2018 and 2022 at the Department of Pathology, Prof. Dr. Cemil Taşcıoğlu City Hospital. Clinical information was gathered using the institute's database records.

Pathological examination

Haematoxylin and eosin stain slides of cases were retrieved from the pathology archive and evaluated. The diagnosis was based on morphology in haematoxylin and eosin-stained sections. We followed the recommendations of the International Tumour Budding Consensus Conference held in 2016. Tumour budding was counted by selecting a "hot spot" chosen after viewing all available slides, and counts were made on haematoxylin and eosin-stained sections over 10 high-magnification fields (Fig. 1, 2).

The cases were divided into 3 groups according to the number of tumour buds: low budding (0–4), intermediate budding (5–9), and high budding (> 10).

Statistical analysis

SPSS (Statistical Package for the Social Sciences) v. 25.0 software was used for statistical analysis of the data. Categorical measurements were summarised as numbers and percentages, and continuous measurements as mean and standard deviation (median and minimum-maximum where appropriate). The Shapiro-Wilk test was used to determine whether the parameters in the study showed normal distribution. The Mann-Whitney *U* test was used in double group analyses, and the Kruskal-Wallis test was used in analyses of more than 2 groups. The Spearman correlation test was used to determine the relationship between continuous measurements. Statistical significance was considered as 0.05 in all tests.

Compliance with ethical standards

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Appropriate research ethics and review board permissions were obtained from Prof. Dr. Cemil Taşcıoğlu City Hospital under protocol #283 in October 2022. For this type of study, formal consent is not required.

Result

The study included 200 patients. The mean number of TB was 12.8 ±9.6, and the mean tumour size was 2.27 ±1.0 cm.

The histologic grade distribution of invasive ductal carcinomas in the study was Grade I in 10 (5.1%), Grade II in 117 (59.1%), and Grade III in 71 (35.8%) patients. Nuclear grade distribution was Grade II in 52 (26.0%) and Grade III in 148 (74.0%) patients.

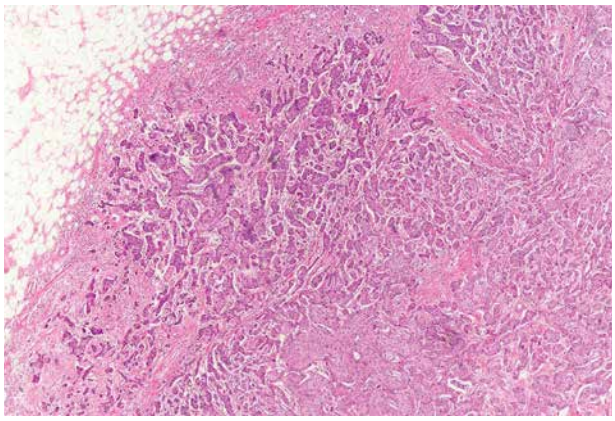

Fig. 1. Tumour buds containing fewer than 5 cells near the invasive front (haematoxylin and eosin stain 4O×)

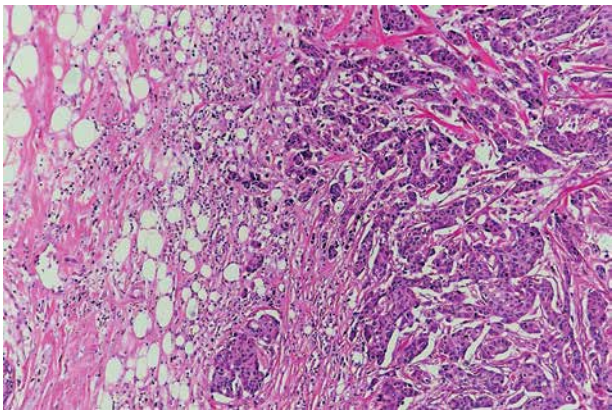

Fig. 2. Tumour buds containing fewer than 5 cells near the invasive front (haematoxylin and eosin stain 100×)

The accompanying ductal carcinoma *in situ* grades were Grade II in 52 (26.0%) patients and Grade III in 119 (59.5%) patients; 29 (14.5%) patients did not have accompanying ductal carcinoma *in situ*. Lobular carcinoma *in situ* was found in 10 (5.0%) patients (Table I).

Hormone profiles showed that 177 (88.5%) patients were ER positive; ER staining intensities were mild in 3 (1.7%), moderate in 90 (47.5%), and strong in 84 (50.8%) patients. Progesterone receptor positivity was detected in 141 (70.5%) patients; PR staining intensities were mild in 15 (10.6%), moderate in 106 (75.2%), and strong in 20 (14.2%) patients (Table I).

Human epidermal growth factor receptor 2/HER2 (c-erbB2) positivity was seen in 13 (6.5%) patients (Table I).

The presence of lymph node metastasis was detected in 86 (43.0%) patients.

Macrometastasis was detected in 62 (31.0%) patients, and the mean macrometastatic lymph node size (cm) was 1.93 ±1.0 cm. The mean macrometastatic focus size (cm) was 1.44 ±1.1 cm. Extra-nodal extension was detected in 33 (16.5%) patients. Micrometastasis was detected in 16 (8.0%) patients, and the mean micrometastatic lymph node size (cm) was 1.32 ±0.8 cm. The mean micrometastatic focus size (mm) was 0.84 ±0.5 mm (Table I).

Stromal tumour infiltrating lymphocytes were detected in 101 (50.5%) patients; the mean lymphocyte infiltration response (%) was 10.7 ±20.7 (Table I).

The presence of perineural invasion was observed in 61 (30.5%) patients. Oestrogen receptor-negative, PR-negative, and c-erbB2-negative staining was seen in 20 (10.0%) patients, and triple-negative patients had significantly lower TB counts ($p = 0.001$). Oestrogen receptor and PR negativity and cc-erbB2-positivity were present in 3 (1.5%) patients with no significant association with TB. Oestrogen receptor-positive, PR-positive, and c-erbB2-negative status was found in 134 (67%) patients. No significant association with TB was found. The number of TB was higher in ER-positive tumours ($p = 0.015$).

Twenty-seven (13.5%) patients received neoadjuvant chemotherapy. The tumour treatment response was Grade 1 in 4 (14.8%), Grade 2 in 5 (18.5%), Grade 3 in 14 (51.9%), and Grade 4 in 4 (14.8%) according to Miller-Payne grade, respectively (Table I). The number of TB was significantly lower in patients who received neoadjuvant chemotherapy treatment (NACT) ($p = 0.002$).

Peritumoural angiolymphatic invasion was detected in 57 (28.5%) patients (Table I). Patients with peritumoural angiolymphatic invasion had significantly higher TB counts ($p = 0.004$).

Perineural invasion was observed in 61 (30.5%) patients (Table I).

Table I. Clinicopathological parameters

Parameters	*n*	Percentage
Histologic grade		
Grade I	10	5.1
Grade II	117	59.1
Grade III	71	35.8
Nuclear grade		
Grade II	52	26.0
Grade III	148	74.0
Ductal carcinoma in situ grade		
None	29	14.5
Grade II	52	26.0
Grade III	119	59.5
ER		
Negative	23	11.5
Positive	177	88.5
ER intensity of staining		
Weak	3	1.7
Moderate	90	47.5
Strong	84	50.8
PR		
Negative	59	29.5
Positive	141	70.5
PR intensity of staining		
Weak	15	10.6
Moderate	106	75.2
Strong	20	14.2
CERB2		
Negative	187	93.5
Positive	13	6.5
ER, PR, CERB2 negative	20	10.0
ER, PR negative, CERBB2 positive	3	1.5
ER, PR positive, CERBB2 negative	134	67
Metastatic lymph node	86	43.0
Macrometastatic lymph node	62	31.0
Extranodal extension	33	16.5
Micrometastatic lymph node	16	8.0
Isolated tumour cells	10	5.0
Stromal tumour-infiltrating lymphocytes	101	50.5
NACT treatment effect	27	13.5
NACT treatment effect		
Miller -Payne Grade 1	4	14.8
Miller-Payne Grade 2	5	18.5
Miller-Payne Grade 3	14	51.9
Miller-Payne Grade 4	4	14.8
Lobular carcinoma in situ	10	5.0
Peritumoural lymphovascular invasion	57	28.5
Perineural invasion	61	30.5

ER – oestrogen receptor, NACT – neoadjuvant chemotherapy treatment, PR – progesterone receptor

There was a weak positive correlation between TB count and tumour size ($r = 0.177$) and a weak negative correlation with ER staining rate (%) ($r = –0.150$) ($p = 0.012$; $p = 0.048$, respectively).

No significant difference was observed between histological grade, nuclear grade, presence of ductal carcinoma in situ, stromal tumour-infiltrating lymphocytes, perineural invasion, lymph node metastasis, and number of TB ($p > 0.05$).

There was a weak positive correlation between the number of TB and tumour size ($r = 0.177$) and a weak negative correlation with ER staining intensity (%) ($r = –0.150$) ($p = 0.012$; $p = 0.048$, respectively).

When the cases were divided into 3 groups as low, medium, and high according to tumour bud numbers, a statistically significant correlation was observed between tumour bud and ER expression ($p = 0.021$), peritumoural angiolymphatic invasion ($p = 0.001$), and perineural invasion ($p = 0.006$) (Table II).

Discussion

Tumour budding has been pathophysiologically defined as an indicator of cancer cell motility and the first step of metastasis [5]. In addition, TB has recently been increasingly recognised as an aggressive behaviour and adverse prognostic factor in many cancers [6–9].

It has been emphasised that the degree of TB is an important independent prognostic factor in BC, such as tumour size, lymph node status, and lymphovascular invasion [10]. In the study by Salhia *et al*., a relationship was found between high TB and regional lymph node metastasis [11]. Also, Kumarguru *et al*. showed a significant relationship between high TB and lymphovascular invasion, lymph node metastasis, primary tumour staging, regional lymph node staging, and necrosis [12]. However, no significant association was found between TB and lymph node metastasis in our study. The difference between our study and the studies of Salhia *et al*. and Kumarguru *et al*. may be due to the rate of metastatic lymph nodes. In our patient group it was 43%, while the rates of metastatic lymph nodes in their studies were 54% and 46%, respectively [11, 12].

In our study, the number of TB was higher in patients with angiolymphatic invasion ($p = 0.004$). This may be explained by the fact that some BC metastasise to systemic sites before entering the lymph nodes, and tumours may cause direct invasion of blood vessels [13]. At metastatic sites, the reverse process occurs when cells regain their epithelial properties and reconnect with neighbouring cells with the help of cues in their new microenvironment [14]. In fact, cancer-associated epithelial-mesenchymal transition (EMT)/mesenchymal to epithelial transition (MET) is believed to confer stem cell properties to cells, thus promoting the interaction between epithelial and mesenchymal states during their metastatic journey; this interaction may help them to change along the spectrum [15]. Cells less than 5 cells, tumour buds, preserve their cell connections, and there may be a partial EMT state in which the bud cells remain connected and move together through the circulation to the metastatic site. However, buds may sometimes represent the first step of detachment. Individual cells may then further detach from other bud cells and move separately. Both cases have been observed experimentally [16, 17]. In addition, studies have shown that oestrogen is involved in EMT in BC cell lines with stem cell properties, and oestrogen also plays a role in breaking the tight junction and increasing cell motility. Sun *et al*. and Jiménez-Salazar *et al*. demonstrated that oestrogen is involved in EMT in BC cell lines with stem cell properties, and it was shown that oestrogen is involved in disruption of tight-junction and increased cell motility [18, 19]. The number of TB was higher in ER-positive patients, and this may explain the statistically significant difference between TB and LVI ($p = 0.015$). In our study, patients with triple-negative BC had lower TB counts ($p = 0.001$). Also, Rathod *et al*. showed that the majority of cases with ER positivity were associated with high tumour budding, and it was statistically significant ($p = 0.04$) [20].

Neoadjuvant chemotherapy has become the standard treatment for locally advanced BC. The main advantage of preoperative chemotherapy is to shrink the tumour and thus facilitate respectability and negative surgical margins. Response to the chemotherapy also gives information about the prognosis of the tumour, which helps doctors to adjust the patient's treatment according to this chemotherapy response [21, 22]. In their study, Mozarowski *et al*. showed TB was frequent in BC patients receiving neoadjuvant therapy, but in our study, we found that TB counts of patients who received NACT were low ($p = 0.002$) [23].

Conclusions

Our results showed that tumour budding is an important prognostic factor. A standardised evaluation method should be determined, and more studies are needed with larger cohort sizes. Clarification of the mechanism of TB at the molecular level will also be useful in terms of development of alternative treatment options.

The authors declare no conflict of interest.

Table II. Tumour budding groups (low, intermediate, high) and associated clinicopathological parameters

Parameters	Low budding		Intermediate budding		High budding		
	Count	Column *N* (%)	Count	Column *N* (%)	Count	Column *N* (%)	*p*-value*
Histological grade							
1	2	7.1	2	3.1	2	2.0	0.454
2	16	57.1	35	54.7	66	65.3	
3	10	35.7	27	42.2	33	32.7	
Nuclear grade							
2	5	16.7	14	23.3	32	31.4	0.219
3	25	83.3	46	76.7	70	68.6	
Tumour size							
1	15	50.0	35	54.7	49	46.2	0.794
2	15	50.0	28	43.8	55	51.9	
3	0	0.0	1	1.6	2	1.9	
ER							
0	6	20.0	11	17.2	6	5.7%	0.021
1	24	80.0	53	82.8	100	94.3	
ER intensity							
1	1	4.2	1	1.9	1	1.0	0.179
2	8	33.3	24	45.3	58	58.0	
3	15	62.5	28	52.8	41	41.0	
PR							
0	8	26.7	22	34.4	29	27.4	0.582
1	22	73.3	42	65.6	77	72.6	
PR intensity							
1	3	13.6	3	7.0	9	11.5	0.318
2	16	72.7	37	86.0	54	69.2	
3	3	13.6	3	7.0	15	19.2	
CERBb2							
0	28	93.3	59	92.2	100	94.3	0.858
1	2	6.7	5	7.8	6	5.7	
Ki-67 proliferation index							
1	12	40.0	18	29.0	36	34.3	0.562
2	18	60.0	44	71.0	69	65.7	
Lymph node metastasis							
0	20	66.7	35	54.7	59	55.7	0.506
1	10	33.3	29	45.3	47	44.3	
Extra nodal extension							
0	24	80.0	56	87.5	87	82.1	0.558
1	6	20.0	8	12.5	19	17.9	
Micro metastasis							
0	29	96.7	57	89.1	98	92.5	0.434
1	1	3.3	7	10.9	8	7.5	

Table II. Cont.

Parameters	Low budding		Intermediate budding		High budding		
	Count	Column *N* (%)	Count	Column *N* (%)	Count	Column *N* (%)	*p*-value*
Isolated tumour cells							
0	29	96.7	58	90.6	103	97.2	0.149
1	1	3.3	6	9.4	3	2.8	
Lobular neoplasia/lobular carcinoma in situ							
0	22	91.7	44	95.7	77	92.8	0.759
1	2	8.3	2	4.3	6	7.2	
Peritumoural angiolymphatic invasion							
0	29	96.7	47	73.4	66	62.9	0.001
1	1	3.3	17	26.6	39	37.1	
Perineural invasion							
0	22	73.3	53	82.8	62	59.6	0.006
1	8	26.7	11	17.2	42	40.4	

* *p-value below 0.05 is statistically significant.*

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