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The Effect of Intratumoral Budding and Other Histological Features in Predicting Treatment Response in Breast Cancer Patients Receiving Neoadjuvant Therapy

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ABSTRACT

Introduction: Neoadjuvant chemotherapy (NAC) is used with increasing frequency in breast cancers. Various clinicopathological parameters predict treatment response (TR). Tumor budding (TB), which is a prognostic parameter in many cancers, can be considered the first stage of the metastatic process. In our study, the relationship between TR and clinicopathological parameters and TB in core biopsy samples of patients before NAC was investigated.

Methods: Seventy-four patients were included in our study. The association between the patients' TR and clinicopathological parameters such as estrogen (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), Ki67, molecular subtype, axilla metastasis was examined. All core biopsy specimens of the cases were evaluated, subsequently the area with the highest intratumoral budding (ITB) was determined, and finally ITB was counted in one area in a 20x objective. Cut-off was determined according to receiver operating characteristic analysis and cases were grouped as <4: low budding; ≥4: high ITB.

Results: High nuclear grade, ER and PR negativity, HER2 positivity, molecular groups, and absence of angiolymphatic invasion or axillary lymph node metastasis were statistically correlated with a complete response to treatment ($p<0.05$). ITB was not associated with TR ($p>0.05$). ITB correlated significantly with ER, PR positivity and luminal group molecular subtype ($p<0.05$).

Conclusion: ER, PR negativity, HER2 positivity, high Ki67, and high nuclear grade, invasive ductal carcinoma histological subtype were associated with complete TR, whereas ITB was not associated with TR. Further studies are required to elucidate the prognostic significance of ITB in core biopsy specimens.

Keywords: Intratumoral budding, neoadjuvant chemotherapy, treatment response, breast cancer, core biopsy

Introduction

Tumor budding (TB) can be considered as the initial stage of the metastatic process. In the metastatic process, cells lose their epithelial properties and gain mesenchymal properties, later invade and metastasize, ultimately in the tissue they metastasize, they gain epithelial properties again through the cancer stem cell properties in the tissue. It has been supported by many studies that TB, which can be easily evaluated in hematoxylin-eosin (H&E) sections, is a prognostic parameter, especially in colon cancers (1-3). In rectal tumors, tumors for which neoadjuvant chemotherapy (NAC) is commonly used, pre-NAC TB has been proven to be a predictive factor for poor response to NAC (4). Similarly, TB in esophageal squamous cell carcinoma has been reported to be associated with poor prognosis after neoadjuvant chemo-radiotherapy (5).

Although many studies have shown that TB in pre-NAC materials is a predictive parameter for treatment response in many cancers; there are a limited number of studies investigating the relationship of pre-NAC TB with treatment response in breast cancers. (4,5). Parameters associated with treatment response in pre-NAC core biopsy materials provide invaluable information to predict patients' NAC response and guide subsequent treatment selection in BCs.

Methods

Patient Selection and Clinicopathologic Evaluation

The study was approved by the Recep Tayyip Erdoğan University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 2021/210, date: 27.12.2021). Written informed consent was obtained from each patient.

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By using the hospital database, patients who received NAC after core biopsy between January 2010 and December 2021 were identified. Among these patients, 74 patients who were operated on in our hospital after NAC were included in our study. The exclusion criteria were as follows: cases for which H&E sections of core biopsy and resection materials could not be found in the pathology archive, and cases whose clinical information and follow-up data could not be reached.

The age, gender, development of metastasis and recurrence, and survival information of the cases were obtained from the hospital database, and tumor size and axillary lymph node status were acquired from the pathology reports. Tumor grade, presence of angiolymphatic invasion, and perineural invasion were determined by re-evaluating H&E sections.

Our cases were divided into molecular subtypes as luminal A (LA), luminal B (LB), human epidermal growth factor receptor 2 (HER2), and triple negative (TN). Over 1% staining for estrogen (ER) and progesterone receptor (PR) in immunohistochemical staining and more than 10% complete and membranous staining for HER2 in tumor cells were considered positive. The cut-off for the Ki67 proliferation index was taken as 14% and below this value was accepted as low and above it as high (6). In our study, there were cases diagnosed with invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) according to the World Health Organization breast cancer classification (7).

Cases were graded according to the Nottingham histological-grade scoring system (7). The response to treatment in the resection materials of the cases was classified into three categories, according to the American Joint Committee on Cancer. Accordingly, the cases were classified as having no residual tumor, partial response, and no response to treatment in resection material (8). Since the number of cases was low, cases with no response and cases with partial pathological responses to treatment were included in the same category. The relationship between the patient's response to treatment and clinicopathological parameters such as ER, PR, HER2, Ki67, molecular subtype, and axilla metastasis was investigated.

Assessment of Tumor Budding

Although TB has been defined differently in diverse studies, it is generally defined as up to 5 tumor cell groups separated from the main tumor mass (9,10). If TB is evaluated at the invasive edge of the main tumor, it can be named peritumoral budding. If it is evaluated within the main mass of the tumor, it can be named intratumoral budding (ITB). In the literature, TB was studied solely in IDC cases in studies based on tumor morphology. In our study, ITB was evaluated only in cases of IDC, since only morphology was evaluated without additional immunohistochemical studies. Due to its easy application in routine pathology practice, ITB counting was performed as recommended by the International Tumor Budding Consensus Conference 2016. All core biopsy specimens of the cases were evaluated by two pathologists (Ç.Ö., S.D.Ö.), the area with the highest ITB in these samples was determined in a 10x objective, and ITB was counted in one area in a 20x objective (Olympus, BX-51, ocular 22 mm, field size 0.950 mm^2) (11). The cases with different scores were re-evaluated under the double-headed microscope, and a third pathologist opinion (O.O.) was obtained where no consensus could be reached. Examples of cases with low and high budding are seen in Figure 1a, b. The ITB cut-off value was determined by performing a receiver operating characteristic (ROC) analysis based on the response to treatment. Cases were divided into low and high ITB groups according to the determined cut-off (Figure 1c).

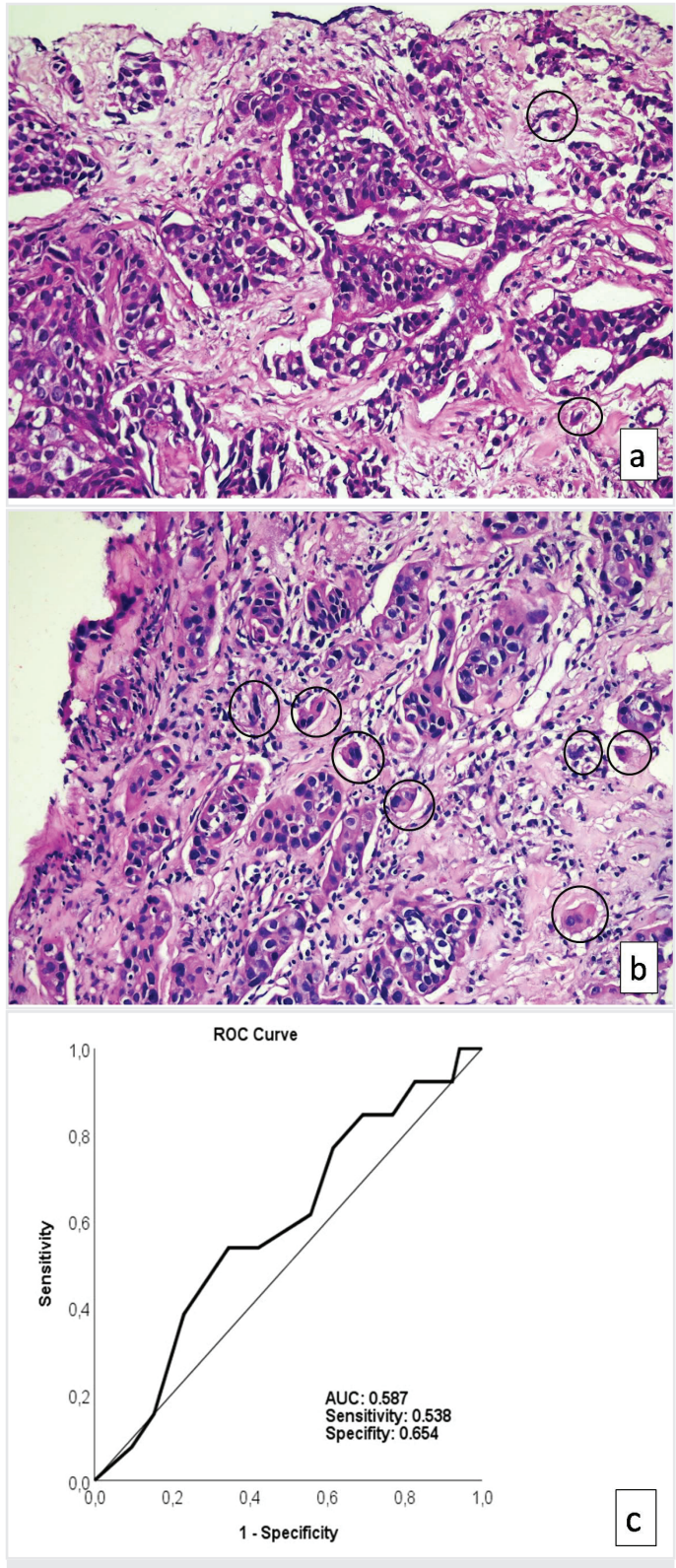

Figure 1. Low (a), high (b) intratumoral budding samples of patients (H&E, x200) (tumor budding shown in circle) and ROC analysis (c) with tumor budding values and treatment response status
H&E: Hematoxylin-eosin, ROC: Receiver operating characteristic, AUC: Area under the curve

Statistical Analysis

Statistical analyses were performed using the SPSS program (IBM, SPSS Inc., Version 23.0, Chicago, USA). The optimum cut-off value was established by ROC analysis for the number of tumor buds of the patients, considering treatment response status. Based on the defined cut-off value, the patients were split into two categories as low and high TB groups. Descriptive statistics of categorical variables were reported as frequency and percentage (n, %). The relationship between categorical variables was evaluated with the chi-square test (Pearson chi-square and Fisher's exact test) considering the size of the patient groups in the categories.

Kaplan-Meier method and Log-rank test were carried out to analyze the correlation between tumor buds and survival in patients with breast cancer. A p-value of <0.05 was considered for statistical significance.

Results

General Characteristics

All 74 patients were female and their ages ranged from 33 to 84, with a mean age of 57. Among the core biopsy samples examined, 67 patients (90.5%) were diagnosed with IDC, whereas 7 patients (9.5%) were diagnosed with ILC. While 20 cases (27.0%) were LA, 35 cases (47.3%) were LB. 8 cases (10.8%) were HER2+, 11 cases (14.9%) were TN. When the response to treatment was evaluated, no response was observed in 8 cases (10.8%), a partial response was observed in 37 cases (50.0%), and complete response was observed in 29 cases (39.2%).

Treatment Response and Clinicopathological Parameters

When the relationship between the response and treatment and clinicopathological parameters was examined, the ratio of complete response to treatment was statistically low in ILCs ($p<0.05$). Additionally, high nuclear grade, ER, PR negativity, HER2 positivity, molecular groups, absence of angiolymphatic invasion, and axillary lymph node metastasis were statistically associated with a complete response to treatment ($p<0.05$). ITB and treatment responses were not correlated ($p>0.05$). The relationship between the response to treatment and clinicopathological parameters is shown in Table 1.

ITB and Prognostic Associations with Outcome

In the ROC analysis performed with ITB and treatment response, the low and high ITB cut-off were found to be 3.5/0.950 mm^2 (area under the curve: 0.587; 0.538 and 0.654 for sensitivity and specificity) (Figure 1c). Accordingly, 42 (≥4; 62.7%) of the cases contain high ITB, 25 (<4; 37.3%) low ITB.

Thirty-eight (56.7%) patients had partial or no response to treatment; 29 (43.3%) had a complete response to treatment. There was partial or no response in 26 patients (61.9%) with high ITB, whereas 16 patients (38.1%) had a complete response. No statistically significant correlation was found between ITB and response to treatment ($p=0.267$) (Table 1).

When the relationship between ITB and clinicopathological parameters was examined, it was found that high ITB was associated with ER, PR positivity, and the luminal group ($p<0.05$). The relationship between ITB and clinicopathological parameters is shown in Table 2.

Even when performing Kaplan-Meier curve for DFS and OS; trend was seen toward a worse DFS and OS for patients with low ITB compared to patients with high ITB ($p=0.118$, $p=0.309$); however, there was no statistically significant difference.

Discussion

NAC is used with increasing frequency in locally advanced breast cancers (10). While the survival of patients with a complete pathological response to NAC is excellent, the recurrence and death rates of patients without a complete response are higher (13). For this reason, it is critical to examine all pathological features in the core biopsy materials of patients before NAC to determine the parameters that predict treatment response.

The most common histological subtype of all breast cancers is IDC, and NAC regimens are well established in IDCs. However, in other histological subtypes, which are less frequent, the information on the relationship between NAC and treatment response is unclear yet (14-16). Nagao et al. (14) evaluated 562 patients in their study and found that IDCs responded better to NAC than other special types. Similarly, most patients in our study were IDC, s and IDCs had a better response to NAC than ILC. These results may lead to the development of new treatment options according to the histological subtype in the selection of NAC.

Nottingham histological scoring system is used for grading breast carcinomas, and the interobserver agreement is low in this system (17). Therefore, most of the cases in our study were in grade 2. However, when the relationship between treatment response and grade was investigated, the rate of complete response to treatment in grade 1 cases was low. There was a complete response to treatment in approximately 77% of grade 3 cases. In a study of 353 patients, Jarzab et al. (17) found that high nuclear grade, mitosis, Ki67, ER, PR positivity, and TN status were associated with a complete response to treatment. The nuclear grade was an independent prognostic factor in their study (17). These results contribute to the predictive value of nuclear grade in the NAC response.

Ki67, which is a marker that indicates cell proliferation, reflects the G1, S, G2, and M phases of cells and is not expressed in resting-phase G0 cells. NAC's target cells. Therefore, it is an expected result that patients with a high Ki67 will benefit more from NAC (18). Many studies with contradictory results have investigated the relationship between the Ki67 level and NAC response. While some studies have argued that high Ki67 is not associated with a complete response; many other studies found the Ki67 as an independent prognostic factor for NAC response (18-20). As expected, the rate of complete response to treatment was high in patients with high Ki67 in our study.

The predictivity of many parameters in predicting the NAC response is still controversial. However, the predictive value of molecular subtypes has been proven. Accordingly, HER2 and TN subtypes respond better to NAC than ER+ luminal types. In our study, the ratio of a complete response to NAC was significantly lower in ER, PR positivity, and HER2 negativity. Based on these results, the distribution according to molecular subtypes was evaluated, the complete response ratio was significantly lower in the ER+ luminal group, whereas the complete

Table 1. Correlation between treatment response and clinicopathological parameters

		Treatment response				
		No response/partial response		Complete response		p-value
		Count	Column, (n %)	Count	Column, (n %)	
Histological types	IDC	38	84.4	29	100.0	0.038
	ILC	7	15.6	0	0.0	-
Intratumoral budding	Low budding	12	48.0	13	52.0	0.267
	High budding	26	61.9	16	38.1	-
Nuclear grade	Grade 1	15	33.3	2	6.9	0.005
	Grade 2	27	60.0	19	65.5	-
	Grade 3	3	6.7	8	27.6	-
ER expression	ER negative	8	17.8	13	44.8	0.012
	ER positive	37	82.2	16	55.2	-
PR expression	PR negative	10	22.2	16	55.2	0.004
	PR positive	35	77.8	13	44.8	-
HER2 expression	HER2 negative	30	66.7	15	51.7	0.023
	HER2 positive	10	22.2	14	48.3	-
	HER2 unknown	5	11.1	0	0.0	-
Ki67	Low	13	30.2	1	4.3	0.024
	High	30	69.8	22	95.7	-
Molecular subtype groups	LA + LB	38	84.4	17	58.6	0.013
	HER2 + TN	7	15.6	12	41.4	-
Anjiolymphatic invasion	Negative	24	53.3	24	82.8	0.01
	Positive	21	46.7	5	17.2	-
Perineural Invasion	Negative	36	80.0	28	96.6	0.078
	Positive	9	20.0	1	3.4	-
Lymph node metastasis	Negative	17	37.8	20	69.0	0.017
	Positive	28	62.2	9	31.0	-
Metastasis	Negative	27	65.9	28	96.6	0.002
	Positive	14	34.1	1	3.4	-
Death status	Alive with/no disease	35	85.4	28	96.6	0.226
	Death of disease	6	14.6	1	3.4	-

IDC: Invasive ductal carcinoma, ILC: Invasive lobular carcinoma, ER: Estrogen, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2, LA: Luminal A, LB: Luminal B, TN: Triple negative

response ratio was significantly higher in the HER2 and TN groups in line with the literature (21,22).

TB in breast cancers, especially in TN breast cancers, was associated with a worse prognosis compared to the ER+ subtype (23). Similarly, although studies in which high TB presence was associated with poor prognosis in breast cancers are available in the literature, limited studies have investigated the relationship between treatment response and prognosis in pre-NAC TB. Mozarowski et al. (1), in their study comprising 75 patients with different molecular subtypes, argued that ITB did not predict the effectiveness of NAC. Although the rate of complete response to treatment decreased in the presence of high ITB in our study, this was not statistically significant. However, in survival analyses, it was noted that patients with a high ITB in both OS and DFS tended to have better survival. When the relationship between ITB and clinicopathological parameters was evaluated, ER, PR positivity, and accordingly, luminal group status was associated with high ITB. Similar to our results, Gujam et al. (9) found that ER, PR positivity, and HER2 negativity were associated with high TB in their study consisting of 471 patients. Additionally, in a study by Salhia et al. (23) ER, PR positivity and HER2 negativity were associated with high budding (24).

While a significant relationship was found in resection materials between TB and prognostic parameters, the reason why no relationship was found in our study may be the difficulty of distinguishing ITB in core biopsy materials. Because TB defines cells that lose their epithelial properties and gain mesenchymal properties, these cells are evaluated in the intratumoral area since the invasive margin cannot be selected in the core biopsy materials. In the intratumoral area, it is almost impossible to distinguish cells that acquire mesenchymal features from small tumor nests. It has been reported in the literature that antigens such as ZEB1, ZEB2, SNAIL, TWIST can be used to indicate epithelial-

Table 2. Intratumoral budding and clinicopathological parameters

		Intratumoral budding				
		Low budding (<4)		High budding (≥4)		p-value
		Count	Column (n, %)	Count	Column (n, %)	
Nuclear grade	Grade 1	3	12.0	11	26.2	0.09
	Grade 2	15	60.0	27	64.3	-
	Grade 3	7	28.0	4	9.5	-
ER expression	ER negative	14	56.0	7	16.7	0.001
	ER positive	11	44.0	35	83.3	-
PR expression	PR negative	15	60.0	9	21.4	0.001
	PR positive	10	40.0	33	78.6	-
HER2 expression	HER2 negative	18	72.0	20	47.6	0.071
	HER2 positive	7	28.0	17	40.5	-
	HER2 unknown	0	0.0	5	11.9	-
Ki67	Low	2	8.7	9	25.0	0.174
	High	21	91.3	27	75.0	-
Molecular subtype groups	LA + LB	11	44.0	37	88.1	<0.001
	HER2 + TN	14	56.0	5	11.9	-
Anjiolymphatic invasion	Negative	16	64.0	27	64.3	0.981
	Positive	9	36.0	15	35.7	-
Perineural invasion	Negative	20	80.0	38	90.5	0.277
	Positive	5	20.0	4	9.5	-
Lymph node metastasis	Negative	17	68.0	18	42.9	0.046
	Positive	8	32.0	24	57.1	-
Metastasis	Negative	18	72.0	34	85.0	0.202
	Positive	7	28.0	6	15.0	-
Death status	Alive with/no disease	21	84.0	37	92.5	0.415
	Death of disease	4	16.0	3	7.5	-

ER: Estrogen, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2, LA: Luminal A, LB: luminal B, TN: Triple negative

mesenchymal transition (25). However, since immunohistochemistry is not cost-effective, our study was based only on the light microscopic examination to obtain an opinion.

Study Limitations

Our study included various limitations. The cases were not homogeneously distributed and we may not have been able to obtain significant results because of the small number of different molecular subtypes, particularly HER2 and TN breast cancer cases.

Conclusion

Core biopsy materials are the only material reflecting the morphological features of pre-NAC cases, therefore all features in these samples are precious. ER, PR negativity, HER2 positivity, high Ki67, and high nuclear grade, IDC histological subtype was associated with a complete response to treatment, whereas ITB was not associated with treatment. More reliable results may be obtained if additional immunohistochemical studies are conducted to differentiate ITB from small tumor cell nests.

Ethics Committee Approval: The study was approved by the Recep Tayyip Erdoğan University Faculty of Medicine Non-Interventional Clinical Research Ethics Committee (approval number: 2021/210, date: 27.12.2021).

Informed Consent: Written informed consent was obtained from each patient.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions: Concept - Ç.Ö., O.O., A.İ.Y., R.B.; Design - Ç.Ö., S.D.Ö., O.O., B.Ş., R.B.; Data Collection or Processing - Ç.Ö., S.D.Ö., O.O., B.Ş., R.B.; Analysis or Interpretation - Ç.Ö., S.D.Ö., B.Ş., A.İ.Y., R.B.; Literature Search - Ç.Ö., A.İ.Y.; Writing - Ç.Ö.

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