Original article

doi:10.5633/amm.2026.0108

Tumor budding as a prognostic indicator in invasive breast carcinoma

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Tumor budding (TB) represents a histopathological manifestation of epithelial–mesenchymal transition (EMT) and is increasingly recognized as an indicator of tumor invasiveness and metastatic potential. Although extensively validated in colorectal carcinoma, its prognostic significance in breast cancer remains insufficiently explored. To evaluate the prognostic value of TB in invasive breast carcinoma (IBC-NST) by correlating budding grades with clinicopathological parameters including tumor size, nodal status, hormone receptor and HER2 expression, and visceral metastases. The study included 60 patients with IBC-NST who had not received neoadjuvant therapy. Histopathological and immunohistochemical analyses were performed on formalin-fixed, paraffin-embedded tissue samples. TB was assessed on hematoxylin and eosin (H&E)-stained sections using the International Tumor Budding Consensus Conference (ITBCC 2016) three-tier grading system (low, intermediate, high). Statistical analyses included the Chi-square, Mann–Whitney U, and Kruskal–Wallis tests, with $p<0.05$ considered significant. High TB grades were significantly associated with lymph node positivity and visceral metastases ($p<0.05$), while no correlation was observed with tumor size, ER, PR, or HER2 receptor expression. These findings indicate that TB reflects invasive and metastatic behavior rather than tumor bulk or receptor status. TB represents a promising histopathological marker of aggressiveness in invasive breast carcinoma. Its assessment provides valuable prognostic information beyond conventional parameters. Further large-scale, multicenter studies are warranted to validate its integration into standardized prognostic models for breast cancer.

Key words: tumor budding, breast cancer, prognostic marker

Originalni rad

doi:10.5633/amm.2026.0108

Tumorsko pupljenje kao prognostički pokazatelj kod invazivnog karcinoma dojke

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Tumorsko pupljenje (TB) predstavlja histopatološku manifestaciju epitelno-mezenhimalne tranzicije (EMT) i sve se više prepoznaje kao pokazatelj invazivnosti i metastatskog potencijala tumora. Iako je njegova prognostička uloga detaljno potvrđena kod kolorektalnog karcinoma, značaj TB kod karcinoma dojke još uvek nije dovoljno ispitan. Cilj ovog istraživanja bio je da se proceni prognostička vrednost TB kod invazivnog karcinoma dojke (IBC-NST) kroz analizu povezanosti stepena pupljenja sa kliničko-patološkim parametrima, uključujući veličinu tumora, status limfnih čvorova, hormonske receptore, HER2 ekspresiju i prisustvo visceralnih metastaza. Studija je obuhvatila 60 pacijentkinja sa IBC-NST koje nisu bile podvrgnute neoadjuvantnoj terapiji. Histopatološke i imunohistohemijske analize urađene su na uzorcima tkiva fiksiranim u formalinu i uparafiniranim blokovima. TB je procenjivan na hematoksilin-eozin (HE) obojenim preparatima prema kriterijumima Međunarodne konferencije o tumor budding-u (ITBCC 2016) koristeći trostepeni sistem (nizak, srednji, visok). Statistička analiza obuhvatila je Hi-kvadrat, Mann–Whitney U i Kruskal–Wallis test, sa nivoom značajnosti $p<0,05$. Visok stepen TB bio je značajno povezan sa pozitivnim limfnim čvorovima i prisustvom visceralnih metastaza ($p<0,05$), dok nije utvrđena korelacija sa veličinom tumora, ER, PR ili HER2 statusom. Ovi nalazi ukazuju da TB odražava invazivno i metastatsko ponašanje tumora, a ne njegovu veličinu ili receptorni profil. TB predstavlja obećavajući histopatološki marker agresivnosti kod invazivnog karcinoma dojke i može pružiti dodatne prognostičke informacije u odnosu na standardne parametre.

Ključne reči: tumorsko pupljenje, karcinom dojke, prognostički marker

Introduction

Breast cancer remains the most commonly diagnosed malignancy among women and represents the leading cause of cancer-related mortality worldwide (1,2). Despite advances in early detection and systemic therapies that have improved survival, many patients still experience disease recurrence, metastatic spread, and therapeutic resistance (3). As a result, breast cancer continues to impose the highest burden in terms of Disability-Adjusted Life Years (DALYs) among female malignancies(1).

The heterogeneity of breast cancer is reflected in its molecular and histological subtypes, each associated with distinct biological behaviors and clinical outcomes. According to the World Health Organization (WHO), the predominant histological subtype is invasive breast carcinoma of no special type (IBC-NST) (4). Prognostic assessment is traditionally based on tumor size, grade, lymph node involvement, lymphovascular invasion, hormone receptor and human epidermal growth factor receptor 2 (HER2) status, proliferation index (Ki-67), and patient age (5). However, these factors are not always sufficient to fully capture tumor aggressiveness and patient prognosis.

Tumor budding (TB), initially described by Imai in gastric carcinoma (6), has since been validated as a robust prognostic marker in colorectal, gastric, and lung cancers (7-9). It is defined as the presence of single cells or small clusters (≤5) of tumor cells detaching from the main tumor mass at the invasive front (10). The International Tumor Budding Consensus Conference (ITBCC) 2016 established standardized scoring for colorectal carcinoma, demonstrating strong correlations with lymph node metastasis, lymphovascular invasion (LVI), and poor survival (11,12). Biologically, TB is linked to epithelial–mesenchymal transition (EMT), enabling tumor cells to acquire migratory and invasive properties (13,14).

In breast cancer, TB has only recently attracted attention as a potential prognostic factor. Emerging evidence suggests that higher TB counts are associated with adverse features such as larger tumor size, lymph node involvement, and aggressive molecular subtypes (15–17). Nevertheless, its clinical significance remains underexplored, underscoring the need for further investigation.

Aim

This study aimed to assess the prognostic value of TB in invasive breast carcinoma by examining its relationship with established clinicopathological parameters, including tumor size, nodal involvement, hormone receptor and HER2 expression, and visceral metastases.

Material and methods

The study included 60 patients with invasive breast carcinoma who had not undergone neoadjuvant therapy. Tumor excision was performed at the Clinic for Surgery, University Clinical Center Niš. The tissue specimens were fixed in 10% buffered neutral formalin for 24–36 hours and subsequently processed using standard histological techniques at the Center for Pathology, University Clinical Center Niš. The definitive histopathological diagnosis was established on hematoxylin and eosin (H&E)-stained tissue sections and included the assessment of tumor size, lymph node involvement, and the presence or absence of visceral metastases.

Following diagnosis, immunohistochemical analysis was performed for estrogen receptor (ER; clone EP1, ready-to-use; DAKO, Glostrup, Denmark), progesterone receptor (PR; clone PgR636, ready-to-use; DAKO, Glostrup, Denmark) and HER2 (HercepTest™, DAKO, Glostrup, Denmark).

TB was assessed on H&E-stained sections according to the recommendations of the ITBCC 2016 for assessing TB in colorectal cancer. This scoring system employs a three-tier grading scale based on the number of tumor buds present in a defined area at the invasive front of the tumor. Evaluation is typically conducted within a field of 0.785 mm^2. The scoring categories are as follows: 0–4 buds indicate low budding (Bd1), 5–9 buds represent intermediate budding (Bd2), and 10 or more buds are classified as high budding (Bd3) (16-17).

All patients were categorized into three groups according to TB grade: low, intermediate, and high. Statistical analyses were performed to assess correlations between TB grade and clinicopathological parameters, including tumor size, lymph node metastasis, visceral metastasis, estrogen receptor (ER) status, progesterone receptor (PR) status, and HER2 status.

Follow-up of patients and correlation with the obtained results will be carried out at the Clinic for Oncology, University Clinical Center Niš, using available medical records.

Statistical analysis

The Chi-square test was used to determine the presence of statistically significant differences in nodal status and the presence of visceral metastases. Differences in ER, PR, and HER2 expression between the examined groups were assessed using the Mann–Whitney U test. A p-value of less than 0.05 was considered statistically significant.

Results

Using the Chi-square test, a statistically significant difference in nodal status was observed among all examined groups. A significant difference in the presence of visceral metastases was found between the first and second (low and intermediate) groups, as well as between the second and third (intermediate and high) groups. (Table 1.)

Table 1. The classification of samples according to tumor size and tumor budding grade.

Budding	Size of tumor					Total
	Ia	Ib	Ic	II	III	
Low	0	5	4	8	0	**17**
Intermediate	0	2	6	11	0	**19**
High	0	2	12	6	4	**24**

Differences in ER, PR, and HER2 expression were analyzed using the Mann–Whitney U test and the Kruskal–Wallis test, since the normality test was negative. No statistically significant differences in receptor expression were observed among the studied groups. (Figure 1., Figure 2., Figure 3.)

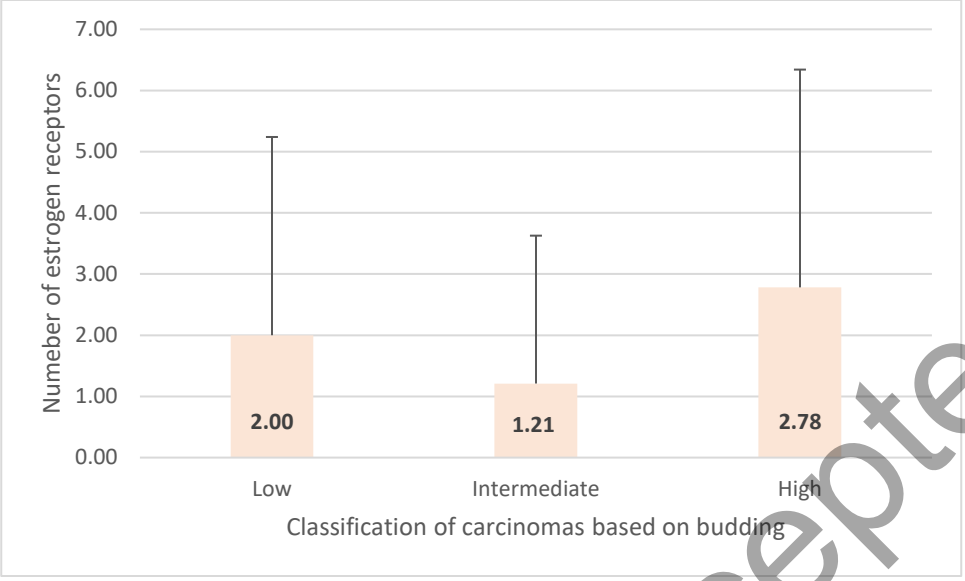

Figure 1. The mean number of estrogen receptors across the examined groups.

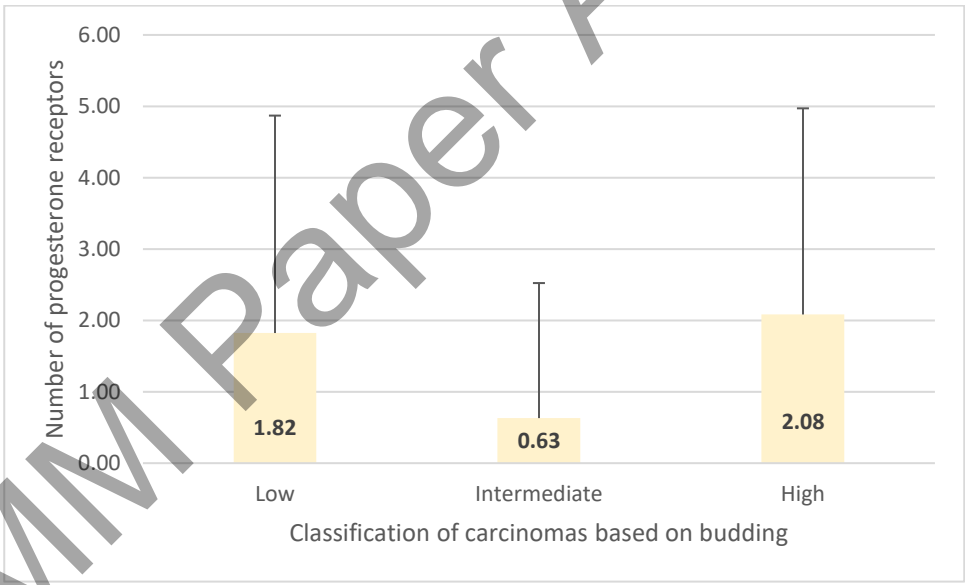

Figure 2. The mean number of progesterone receptors across the examined groups.

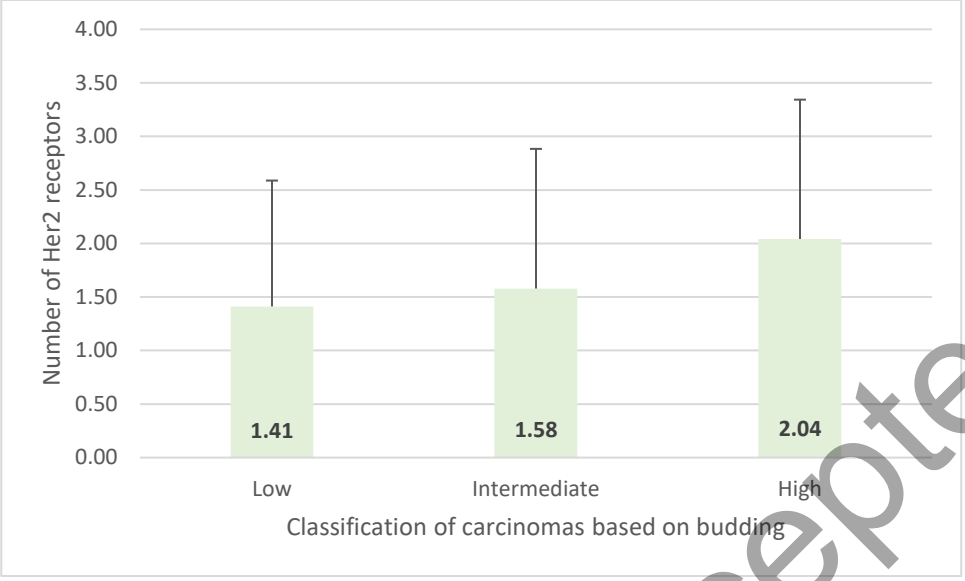

Figure 3. The mean number of HER2 receptors across the examined groups.

The tumor tissue on Fig.4 shows a diffuse growth pattern without glandular or tubule-like structures, consistent with poor differentiation, with solid nests and irregular clusters of pleomorphic cells separated by a fibrotic stroma. Tumor cells exhibit marked nuclear atypia, hyperchromasia, coarse chromatin, prominent nucleoli, and frequent mitotic figures, while focal necrosis is also present. At the invasive front, extensive (high) TB is observed, with single cells and small clusters detaching from the main tumor mass, accompanied by a moderate lymphocytic infiltrate. In addition, lymphovascular invasion is evident, further supporting the aggressive biological behavior. Overall, the morphological features are consistent with a poorly differentiated invasive ductal carcinoma of the breast, classified as high-grade (G3).

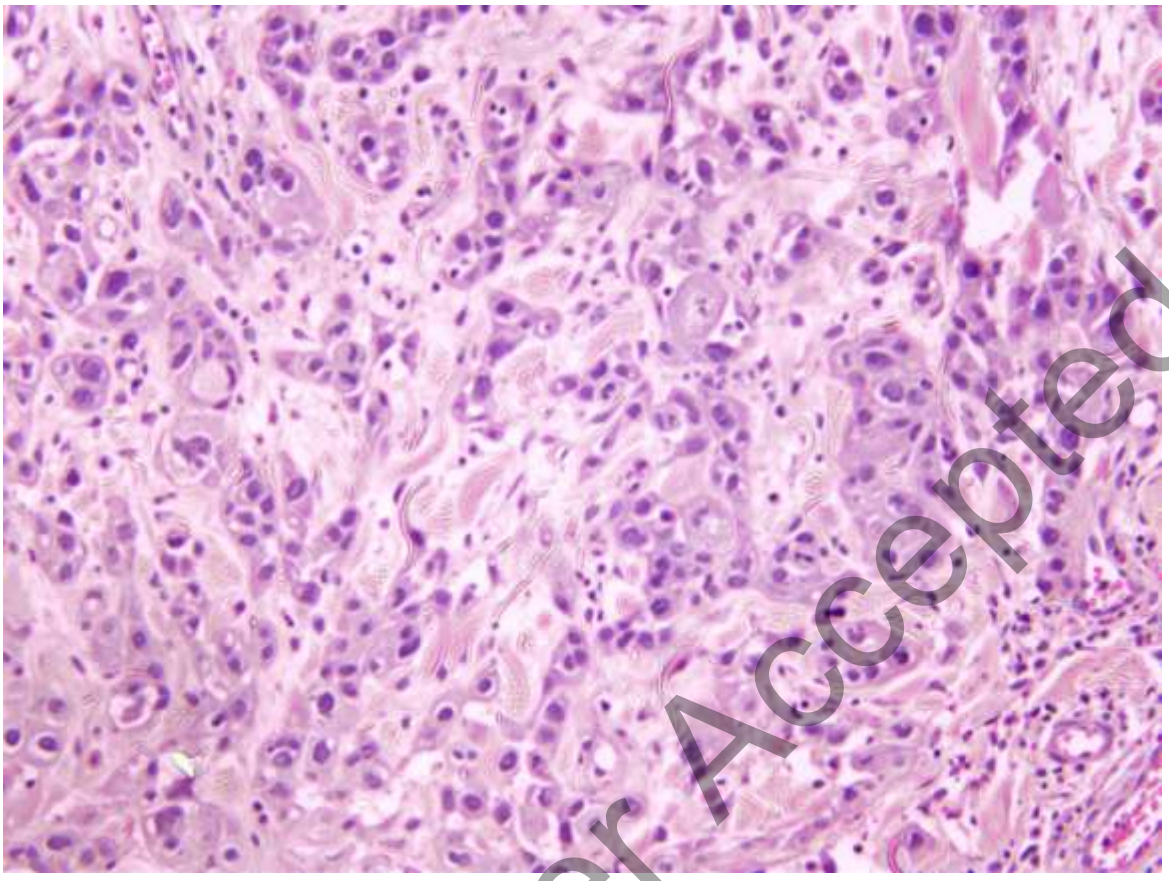

Figure 4. Histological section of poorly differentiated invasive ductal carcinoma of the breast (H&E, ×200). The invasive front demonstrates high TB, with numerous single cells and small clusters detaching from the main tumor mass, accompanied by lymphocytic infiltrates and lymphovascular invasion, consistent with an aggressive phenotype.

The histological section Fig 5. reveals a poorly differentiated invasive ductal carcinoma of the breast composed of pleomorphic tumor cells arranged in irregular solid nests and sheets, separated by fibrotic stroma. The cells exhibit enlarged, hyperchromatic nuclei with conspicuous nucleoli and frequent atypical mitotic figures. At the invasive front, intermediate tumor budding is evident, with 5–9 small clusters and single cells detaching from the main tumor mass and infiltrating the surrounding stroma. A moderate stromal lymphocytic infiltrate is present, and focal lymphovascular

invasion can also be observed. These findings are consistent with an aggressive biological behavior, supporting classification as a high-grade tumor (G3).

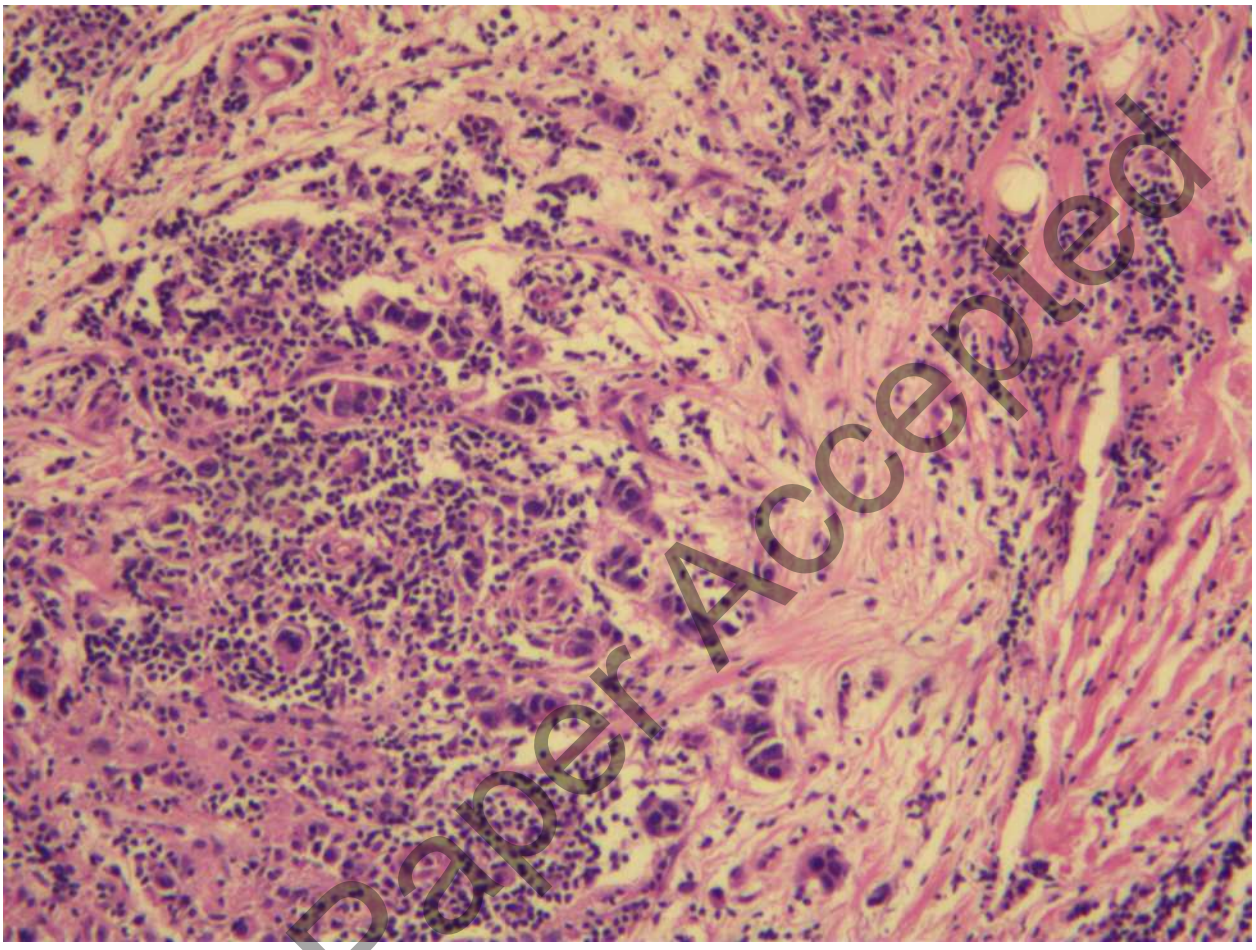

Figure 5. Poorly differentiated invasive ductal carcinoma of the breast (H&E, ×100). The invasive margin demonstrates intermediate TB with small clusters and single tumor cells detaching from the main mass, accompanied by strong stromal lymphocytic infiltration and focal lymphovascular invasion.

This histological section on Fig 6. shows an invasive ductal carcinoma of the breast with mucinous differentiation composed of solid and irregular nests of malignant cells within a fibrotic stroma. Tumor cells display marked nuclear atypia, hyperchromasia, and frequent mitotic figures. At the

invasive front, tumor budding is scarce**,** with only a few single cells detaching from the main tumor mass, consistent with low TB. The stroma demonstrates moderate fibrosis and a mild lymphocytic infiltrate, while no clear lymphovascular invasion is observed. Overall, the findings confirm a high-grade malignancy but with a low level of TB activity in the evaluated area.

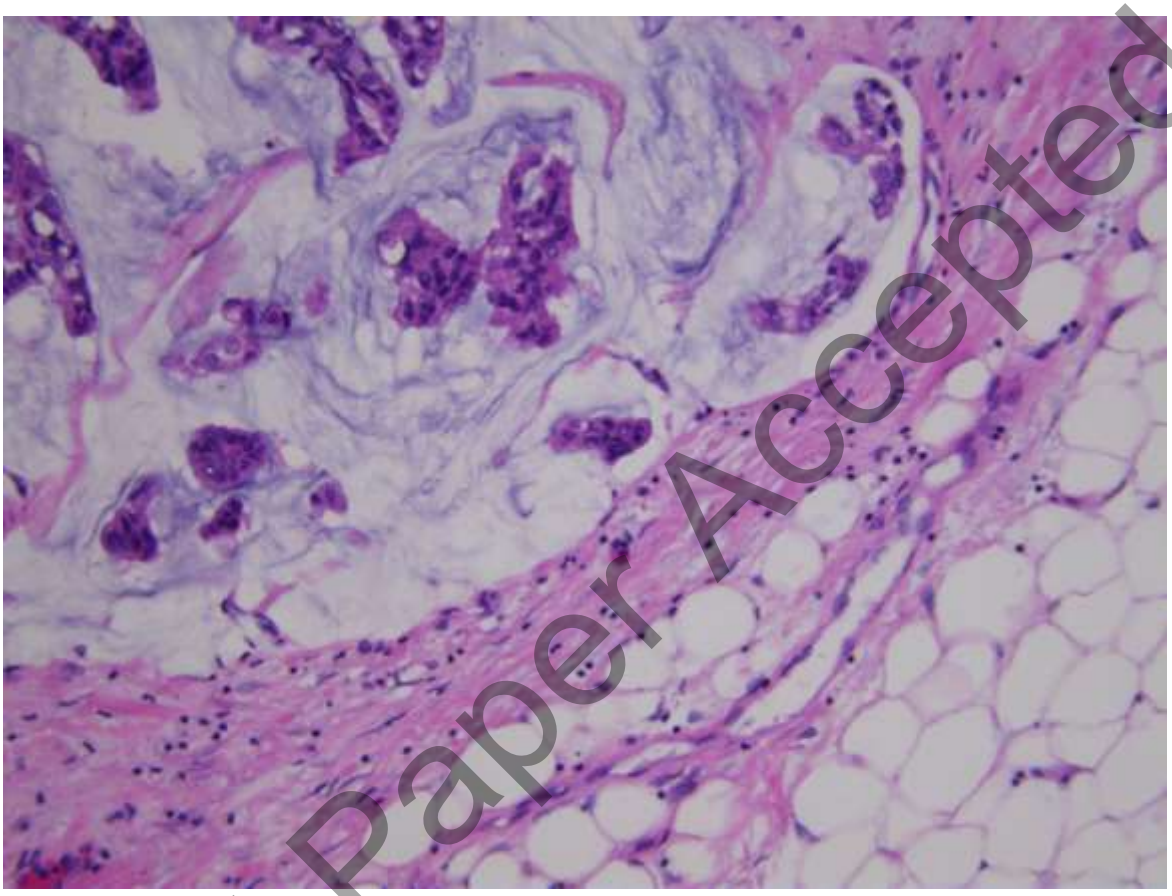

Figure 6. Invasive ductal carcinoma of the breast with mucinous differentiation (H&E, ×100) represents low TB, showing only a few isolated tumor cells and small clusters at the invasive margin, accompanied by mild stromal lymphocytic infiltration.

Discussion

This cohort study of IBC-NST used the ITBCC three-tier system to classify tumors into low, intermediate and high TB categories which showed that high TB correlated with nodal positivity and visceral metastases but not with tumor size or ER/PR status or HER2 expression (18–20). The

results confirm that TB indicates the invasive and metastatic potential of cancer cells rather than tumor size or receptor status which is consistent with multiple recent studies and meta-analyses (21–25).

Tumor Budding and Lymph Node Metastasis

Research findings demonstrate that tumors with higher budding scores tend to develop more LVI and spread to axillary lymph nodes (21–23). The research by Usta et al. on 278 patients established that high TB acts as an independent factor that predicts both lymph node involvement and distant cancer spread (23). The study by Ranaee et al. (24) showed that high-grade budding is linked to worse 5-year survival rates for both overall survival and disease-free survival. The 2023 meta-analysis by Buch et al. analyzed 13 studies with 1,763 participants to show that high-grade budding leads to 2.25 times more nodal metastasis and three times more LVI (25). The research supports our findings by showing that budding serves as a strong histological indicator for cancer spread.

Correlation with Other Clinicopathologic Features

The analysis in our study showed TB did not show any significant relationship with tumor dimensions or with ER, PR or HER2 status which indicates that budding provides different information than traditional prognostic markers. Research findings show that high budding rates occur more frequently in triple-negative and HER2-enriched tumors according to two studies. Still, another study found no significant relationship after controlling for grade and LVI (28). The absence of tumor size correlation in this study supports the theory that TB indicates invasive potential rather than tumor growth because small tumors may already have high budding activity and early metastatic potential while large tumors can remain cohesive with low budding activity (21,25).

Biological Mechanisms and Tumor Microenvironment

Tumor budding functions as a key histological indicator of EMT, as it is associated with loss of E-cadherin, activation of EMT transcription factors (ZEB1, SNAIL, TWIST), and detachment of tumor cells (26,27). These changes enable malignant cells to infiltrate surrounding tissues, penetrate lymphovascular structures, and disseminate to distant sites. At the invasive front, TB is supported

by cancer-associated fibroblasts (CAFs), extracellular matrix remodeling, and increased lymphovascular density, which together explain its correlation with lymph node and visceral metastases (26,27). Pan-cytokeratin immunohistochemistry has been shown to improve the detection of tumor buds in areas with inflammation or desmoplasia, while digital image analysis and artificial intelligence (AI)-based systems further enhance precision and reproducibility of tumor bud assessment (28). Biologically, TB represents the initial step of metastasis and is closely linked to epithelial–mesenchymal plasticity, a dynamic and reversible process observed not only in cancer progression but also in embryogenesis and wound healing (29–31).

Clinical Implications

Our research results produce immediate medical applications. The presence of high TB in early-stage (cT1–T2) breast cancer helps physicians determine which patients need sentinel node biopsy or axillary staging procedures (18,23,25). The connection between TB and visceral metastases indicates its potential to help doctors determine distant recurrence risk which would affect treatment intensity and monitoring plans for patients with minimal primary cancer (23,24). The connection between TB and EMT biology creates potential applications for identifying patients who would benefit from EMT pathway or stromal interaction or TGF-β signaling targeted therapies (26,27,29).

Future Directions

Future research should focus on standardizing scoring systems while developing prognostic models that integrate TB with LVI, histological grade, tumor-infiltrating lymphocytes (TILs), and molecular subtype (30–32). The independent prognostic value of TB needs verification through multivariable modeling which includes logistic regression for nodal status and Cox proportional hazards for survival outcomes. The implementation of pan-cytokeratin-assisted counts, digital pathology, and AI-based quantification methods will enhance reproducibility and enable the use of this method in standard pathology reports worldwide.

Conclusion

TB is an emerging histopathological feature with prognostic relevance in breast carcinoma. In this study, higher budding grades were associated with adverse clinicopathological parameters, including larger tumor size, lymph node and visceral metastases. These findings support TB as a marker of tumor aggressiveness and indicate the need for its further validation. Large-scale, multicenter studies are required to confirm these results and to assess the integration of TB into standardized prognostic models for breast cancer.

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