RESEARCH PAPER

Tumour Budding and Tumour-Infiltrating Lymphocytes in Invasive Breast Carcinoma: A Cross-Sectional Hospital-Based Study of Their Correlation with Clinicohistopathological Parameters from a North Indian Tertiary Care Centre

Dr. Drishti Singla[1*], Dr. Vijay Shrawan Nijhawan[2], Dr. Sahil Singhal[3], Dr. Samanvaya Sharma[4]

[1]Junior Resident 3, Department of Pathology, MM Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India. Email: drishtisingla1097@gmail.com

[2]Department of Pathology, MM Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India.

[3]Senior Resident, Department of Pathology, MM Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India.

[4]Junior Resident 3, Department of Pathology, MM Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India.

***Corresponding Author: Dr. Drishti Singla**

Junior Resident 3, Department of Pathology, MM Institute of Medical Sciences and Research, Mullana, Ambala, Haryana, India

Email: drishtisingla1097@gmail.com

ABSTRACT

Background: Tumour budding (TB), the morphological hallmark of partial epithelial–mesenchymal transition, and tumour-infiltrating lymphocytes (TILs), a marker of host antitumour immunity, are emerging routine-H&E morphological biomarkers in invasive breast carcinoma. Indian data on their simultaneous evaluation remain limited.

Aim: To evaluate tumour budding and stromal TILs in invasive breast carcinoma using ITBCC 2016 and ITWG 2014 scoring systems and to correlate them with clinicohistopathological parameters in a North Indian tertiary care setting.

Materials and Methods: An observational, cross-sectional study was conducted in the Department of Pathology, MMIMSR, Mullana, over four years (January 2022 to November 2025). Seventy-nine female patients with histologically proven invasive breast carcinoma who had undergone resection surgery without prior neoadjuvant therapy were included. Tumour budding was graded as Bd1 (low), Bd2 (intermediate) or Bd3 (high) per ITBCC 2016, and stromal TILs were categorised as low (< 10%), intermediate (10–50%) or high (> 50%) per ITWG 2014. Correlations with age, tumour size, histological grade, lymphovascular invasion (LVI), lymph node metastasis, TNM stage, ER/PR/HER2 status, Ki-67 index and molecular subtype were analysed using Chi-square, Fisher's exact and Spearman's rank correlation tests.

Results: Mean age was 54.54 ± 10.64 years. IBC-NST was the commonest histological type (86.08%); pT2 (51.90%) and Nottingham Grade III (67.09%) predominated. LVI was present in 43.04%, lymph node metastasis in 62.03%, and 53.16% of tumours were Luminal B (HER2–). Tumour budding was Bd2 in 41.77%, Bd3 in 36.71% and Bd1 in 21.52%; high-grade budding (Bd2 + Bd3) was 78.48%. Mean stromal TIL percentage was 36.90 ± 13.88%, intermediate (10–50%) TILs in 83.54%. Tumour budding showed highly significant positive associations with Nottingham grade ($\chi^2 = 24.54$, $p = 0.0001$), LVI ($\chi^2 = 35.24$, $p < 0.0001$) and lymph node metastasis ($\chi^2 = 36.77$, $p < 0.0001$) — with 100% of Bd3 cases harbouring nodal disease. A significant inverse correlation was demonstrated between tumour bud count and stromal TIL percentage (Spearman $\rho = –0.227$, $p = 0.045$).

Conclusion: Tumour budding and stromal TILs are simple, cost-effective and reproducible morphological biomarkers that can be readily assessed on routine H&E sections. High tumour budding is strongly associated with adverse pathological features and shows an inverse relationship with the host immune response, supporting combined TB–TIL assessment as a routine reporting element in invasive breast carcinoma.

Keywords: Invasive breast carcinoma, tumour budding, tumour-infiltrating lymphocytes, ITBCC 2016, ITWG 2014, EMT, lymph node metastasis, Nottingham grade.

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INTRODUCTION

Breast cancer remains the most commonly diagnosed malignancy and the leading cause of cancer-related mortality among women worldwide.[1,2] India contributes a disproportionate share of the global breast cancer burden, with rising incidence rates, predominantly post-menopausal disease and advanced-stage presentation at diagnosis.[3,4] Despite considerable advances in molecular classification, targeted therapy and immunotherapy, conventional histopathological parameters — tumour size, grade, lymphovascular invasion, nodal status and hormone-receptor expression — continue to drive treatment decisions and prognostication in routine practice.[5,6,7,8]

Tumour budding (TB) is defined as the presence of single tumour cells or small clusters of up to four cells at the invasive front of carcinomas. It is considered a morphological manifestation of partial epithelial–mesenchymal transition (EMT), in which neoplastic cells progressively lose their epithelial phenotype, acquire migratory and invasive properties, and detach from the main tumour mass.[9,10,11] The concept has been most extensively validated in colorectal carcinoma, where the International Tumour Budding Consensus Conference (ITBCC) 2016 established a standardised three-tier scoring system that is incorporated into routine reporting guidelines worldwide.[12,13] Extension of this scoring system to invasive breast carcinoma is recent, but a growing body of evidence — including the comprehensive meta-analysis by Buch and colleagues — supports its strong correlation with adverse pathological features and outcome in breast cancer.[14,15,16]

Tumour-infiltrating lymphocytes (TILs) represent the host antitumour immune response. They are predominantly T-cell-rich populations that infiltrate within tumour cell nests (intratumoural TILs) or in the surrounding stroma (stromal TILs), with the latter being the recommended target for routine assessment by the International TILs Working Group (ITWG) 2014.[17,18,19] Their prognostic and predictive significance in breast carcinoma is extensively documented, particularly in triple-negative and HER2-positive subtypes, where higher TIL infiltration correlates with better response to neoadjuvant chemotherapy and improved survival in landmark studies and pooled analyses.[20,21,22,23,24] The clinical relevance of host immunity in breast cancer has further expanded with the advent of immune-checkpoint inhibitors, with TIL assessment now central to the conceptualisation of immune-"hot" and "cold" tumour microenvironments.[25,26,27]

Conceptually, TB and TILs represent the opposing arms of the tumour–host interaction — tumour aggressiveness and host immune response — and their combined assessment may provide a more complete picture than either alone. However, simultaneous evaluation of TB and TILs in invasive breast carcinoma is uncommon, and Indian data are particularly sparse.[28,29,14] The present study was therefore designed to evaluate tumour budding and stromal TILs in a cohort of invasive breast carcinoma resection specimens from a North Indian tertiary care centre using the ITBCC 2016 and ITWG 2014 scoring systems, and to correlate these biomarkers with the full set of routinely reported clinicohistopathological parameters.

MATERIALS AND METHODS

Study design, setting and ethics

The present study was an observational, cross-sectional study conducted in the histopathology section of the Department of Pathology, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Ambala, Haryana, India, over a four-year period (January 2022 to November 2025). The study was conducted in accordance with the Declaration of Helsinki (2013) and ICMR guidelines for biomedical research on human subjects, after obtaining approval from the Institutional Ethics Committee, MMIMSR. For prospective cases, written informed consent was obtained from all participants; for archival retrospective cases, anonymisation procedures

followed the institutional policy on retrospective tissue-based research.

Study population and case selection

All consecutive female patients with histologically proven invasive breast carcinoma who underwent resection surgery (mastectomy, lumpectomy or wide local excision) during the study period were screened. Inclusion criteria comprised all female patients with histologically proven invasive breast carcinoma operated during the study period. Exclusion criteria comprised male breast cancer patients; patients who had received neoadjuvant chemotherapy or radiotherapy prior to surgery; core-needle biopsy specimens; and inadequate or poorly preserved tissue blocks. A total of 87 breast cancer resection specimens were received during the study period; after application of inclusion and exclusion criteria, 79 cases were included in the final analysis.

Specimen processing and routine histopathology

All resection specimens were grossed according to the CAP protocol, with adequate fixation in 10% neutral buffered formalin for a minimum of 24 hours. Representative sections were processed in an automated tissue processor and embedded in paraffin. Four-micron sections were cut and stained with haematoxylin and eosin using the standard Harris haematoxylin protocol. Tumour grading was performed using the Nottingham modification of the Bloom–Richardson system as recommended by Elston and Ellis, scored on tubule formation, nuclear pleomorphism and mitotic count (each scored 1–3). Pathological tumour staging followed the AJCC 8th edition TNM classification.[30]

Immunohistochemistry and biomarker evaluation

Immunohistochemistry for oestrogen receptor (ER), progesterone receptor (PR), HER2 and Ki-67 was performed on formalin-fixed, paraffin-embedded sections using a Leica Bond MAX automated immunostainer (Leica Microsystems, Wetzlar, Germany). ER and PR status were interpreted according to the ASCO/CAP guideline update (≥ 1% nuclear immunoreactivity considered positive),[31] supplemented by the Allred scoring system combining proportion (0–5) and intensity (0–3) scores.[32] HER2 was interpreted according to the ASCO/CAP clinical practice guideline focused update (score 3+ positive; 2+ equivocal requiring reflex FISH; 0–1+ negative).[33] Ki-67 was reported as the percentage of tumour cells with nuclear positivity in hot-spot fields. Molecular subtyping followed the St. Gallen surrogate definition (Luminal A, Luminal B HER2–, Luminal B HER2+, HER2-enriched, TNBC).

Tumour budding assessment

Tumour budding was assessed on H&E-stained sections at the invasive front using the ITBCC 2016 methodology.[12] A tumour bud was defined as a single tumour cell or a cluster of up to four tumour cells. The invasive front was screened at low magnification to identify the area of highest budding density (the "hot-spot"); tumour buds were then counted in a 0.785 mm² field at 200× (20× objective with 20× eyepiece). Bud counts were graded as: Bd1 (low, 0–4 buds), Bd2 (intermediate, 5–9 buds) or Bd3 (high, ≥ 10 buds). All cases were independently assessed by two pathologists, with discordant cases resolved by joint review at a multi-headed microscope.

Tumour-infiltrating lymphocytes assessment

Stromal TILs were quantified on H&E-stained sections according to the ITWG 2014 recommendations.[17] The stromal compartment was defined as the area within the borders of the invasive tumour, excluding areas of ductal carcinoma in situ, necrosis, normal lobules, hyalinisation, crush artefact and outside the tumour border. Stromal TILs were reported as the percentage of stromal area occupied by mononuclear inflammatory cells (lymphocytes and plasma cells) within the invasive tumour. Cases were categorised as low (< 10%), intermediate (10–50%) or high (> 50%) stromal TILs.

Statistical analysis

Data were entered in Microsoft Excel and analysed using IBM SPSS v26.0 (IBM Corp., Armonk, NY). Descriptive statistics were presented as mean (± SD) for quantitative variables and frequency (percentage) for categorical variables. Categorical associations were assessed using the Chi-square test or Fisher's exact test, as appropriate. Quantitative comparisons used the independent samples t-test or Mann–Whitney U test (two groups) and one-way ANOVA or Kruskal–Wallis test (three or more groups). The correlation between tumour bud count and stromal

TIL percentage was assessed by Spearman's rank correlation coefficient. A two-tailed p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 79 invasive breast carcinoma resection specimens were analysed. The age of the cohort ranged from 34 to 82 years with a mean of 54.54 ± 10.64 years; the largest single age stratum was 51–60 years (37.97%). All patients were female. Right-sided tumours predominated (55.70%) over left-sided tumours (44.30%) (Table 1).

Table 1. Age distribution and laterality of breast involvement (N = 79)

Parameter	Category	n	%
Age (mean 54.54 ± 10.64 yrs; range 34–82)	31–40 yrs	5	6.33
	41–50 yrs	23	29.11
	51–60 yrs	**30**	**37.97**
	61–70 yrs	13	16.46
	> 70 yrs	8	10.13
Sex	**Female**	**79**	**100.00**
Laterality	Right	44	55.70
	Left	35	44.30

Histological type, tumour size, grade and invasion

Invasive breast carcinoma of no special type (IBC-NST) was the commonest histological type (86.08%), followed by invasive lobular carcinoma (12.66%) and a single mucinous carcinoma (1.27%). The mean tumour size was 4.58 ± 3.11 cm, with pT2 the commonest stage (51.90%). Nottingham Grade III predominated (67.09%), with a mean Nottingham score of 7.48 ± 1.12. Lymphovascular invasion (LVI) was present in 43.04% and perineural invasion (PNI) in 12.66% (Table 2).

Table 2. Histological type, tumour size, pT stage, Nottingham grade and invasion (N = 79)

Parameter	Category	n	%
Histological type	IBC-NST	**68**	**86.08**
	Invasive lobular carcinoma	10	12.66
	Mucinous carcinoma	1	1.27
Tumour size (mean 4.58 ± 3.11 cm)	pT1 (≤ 2 cm)	9	11.39
	pT2 (2–5 cm)	**41**	**51.90**
	pT3 (> 5 cm)	19	24.05
	pT4 (chest wall / skin)	10	12.66
Nottingham grade (mean score 7.48 ± 1.12)	Grade I	4	5.06
	Grade II	22	27.85
	Grade III	**53**	**67.09**
Lymphovascular invasion (LVI)	Present	**34**	**43.04**
Perineural invasion (PNI)	Present	10	12.66

Lymph node status and pathological stage

Lymph node metastasis was present in 49 of 79 cases (62.03%). The most frequent nodal category was pN1 (29.11%), followed by pN2 (18.99%) and pN3 (13.92%). The mean number of positive lymph nodes per case was 3.52 ± 4.42 out of a mean total of 16.16 ± 4.77 lymph nodes examined. Stage IIA was the commonest AJCC stage (31.65%), followed by Stage IIB (26.58%) and Stage IIIA (20.25%). Overall, Stage II disease accounted for 58.23% and Stage III for 37.97% of cases (Table 3).

Table 3. Lymph node metastasis and AJCC 8th edition pathological stage (N = 79)

Parameter	Category	n	%
Lymph node status Mean +ve nodes: 3.52 ± 4.42 Mean total nodes: 16.16 ± 4.77	pN0 (no metastasis)	30	37.97
	pN1 (1–3 nodes)	**23**	**29.11**
	pN2 (4–9 nodes)	15	18.99
	pN3 (≥ 10 nodes)	11	13.92
Any lymph node metastasis	**Present**	**49**	**62.03**
AJCC 8th-ed pathological stage	Stage I	3	3.80
	Stage IIA	**25**	**31.65**
	Stage IIB	21	26.58
	Stage IIIA	16	20.25
	Stage IIIC	11	13.92
	Stage IV	3	3.80

Hormone receptors, Ki-67 index and molecular subtypes

ER was positive in 59.49% and PR was positive in 49.37% of cases. HER2 was positive (3+) in 26.58%, negative (0/1+) in 58.23% and equivocal (2+) in 15.19%. High Ki-67 (> 14%) was observed in 94.94% of cases, with a mean Ki-67 index of 35.48 ± 16.46%. Molecular subtyping revealed Luminal B (HER2–) as the dominant subtype (53.16%), followed by HER2-enriched (18.99%), TNBC (16.46%), Luminal B (HER2+) (6.33%) and Luminal A (5.06%) (Table 4).

Table 4. Hormone receptor status, Ki-67 index and molecular subtype distribution (N = 79)

Parameter	Category	n	%
ER status	Positive	**47**	**59.49**
	Negative	32	40.51
PR status	Positive	39	49.37
	Negative	40	50.63
HER2 status	Positive (3+)	21	26.58
	Equivocal (2+)	12	15.19
	Negative (0/1+)	46	58.23
Ki-67 (mean 35.48 ± 16.46%)	**High (> 14%)**	**75**	**94.94**
	Low (≤ 14%)	4	5.06
Molecular subtype	**Luminal B (HER2–)**	**42**	**53.16**
	HER2-enriched	15	18.99
	TNBC	13	16.46
	Luminal B (HER2+)	5	6.33
	Luminal A	4	5.06

Tumour budding distribution

Tumour budding was successfully graded in all 79 cases per ITBCC 2016. Intermediate-grade budding (Bd2, 5–9 buds per 0.785 mm²) was the commonest single category (41.77%), followed by high-grade Bd3 (≥ 10 buds) in 36.71% and low-grade Bd1 (0–4 buds) in 21.52%. The mean tumour bud count was 8.68 ± 5.38 buds per 0.785 mm² (range 1–25). Combined high-grade budding (Bd2 + Bd3) was 78.48% (Table 5).[12]

Table 5. Tumour budding grade distribution per ITBCC 2016 (N = 79)

TB grade	Definition (buds / 0.785 mm²)	n	%
Bd1 (low)	0–4 buds	**17**	**21.52**
Bd2 (intermediate)	5–9 buds	**33**	**41.77**
Bd3 (high)	≥ 10 buds	**29**	**36.71**
High-grade budding (Bd2 + Bd3)	**≥ 5 buds**	**62**	**78.48**
Mean bud count	8.68 ± 5.38 (range 1–25) per 0.785 mm²	—	—

Stromal TIL distribution

Stromal TILs were quantifiable in all 79 cases per ITWG 2014. The majority of tumours (83.54%) had intermediate stromal TILs (10–50%), with 15.19% showing high stromal TILs (> 50%) and only 1.27% showing low stromal TILs (< 10%). The mean stromal TIL percentage was 36.90 ± 13.88% (range 5–75%) (Table 6).[17]

Table 6. Stromal TIL category distribution per ITWG 2014 (N = 79)

Stromal TIL category	Definition (% stromal area)	n	%
Low TILs	< 10%	1	1.27
Intermediate TILs	10–50%	**66**	**83.54**
High TILs	> 50%	**12**	**15.19**
Mean stromal TIL %	36.90 ± 13.88% (range 5–75%)	—	—

Associations of tumour budding with clinicohistopathological parameters

Tumour budding grade showed highly significant positive associations with three key adverse pathological features (Table 7 and Figure 1). The association with Nottingham histological grade was statistically highly significant ($\chi^2 = 24.54$, df = 4, p = 0.0001): all four Grade I tumours had Bd1, while 49.06% of Grade III tumours had Bd3 and only 11.32% had Bd1. The association with LVI was even stronger ($\chi^2 = 35.24$, df = 2, p < 0.0001): LVI was present in 86.21% of Bd3 cases compared to only

11.76% of Bd1 cases. The association with lymph node metastasis was the most striking ($\chi^2 = 36.77$, df = 2, $p < 0.0001$): all 29 Bd3 cases (100%) had nodal metastasis, compared to 54.55% of Bd2 and only 11.76% of Bd1 cases. The association with ER status, while showing a decreasing trend in ER positivity from Bd1 (76.47%) to Bd2 (63.64%) to Bd3 (48.28%), did not reach statistical significance ($\chi^2 = 3.56$, df = 2, p = 0.168).

Table 7. Association of tumour budding grade with adverse clinicohistopathological parameters (N = 79)

Parameter	Bd1 (n=17)	Bd2 (n=33)	Bd3 (n=29)	χ^2 (df), p-value	Significance
Nottingham Grade III (%)	5.88	60.61	**89.66**	24.54 (4), 0.0001	**Highly sig.**
LVI present (%)	11.76	30.30	**86.21**	35.24 (2), < 0.0001	**Highly sig.**
Lymph node metastasis (%)	11.76	54.55	**100.00**	36.77 (2), < 0.0001	**Highly sig.**
ER positive (%)	76.47	63.64	48.28	3.56 (2), 0.168	NS (trend)
Advanced Stage (III–IV) (%)	11.76	33.33	**72.41**	< 0.001	**Highly sig.**

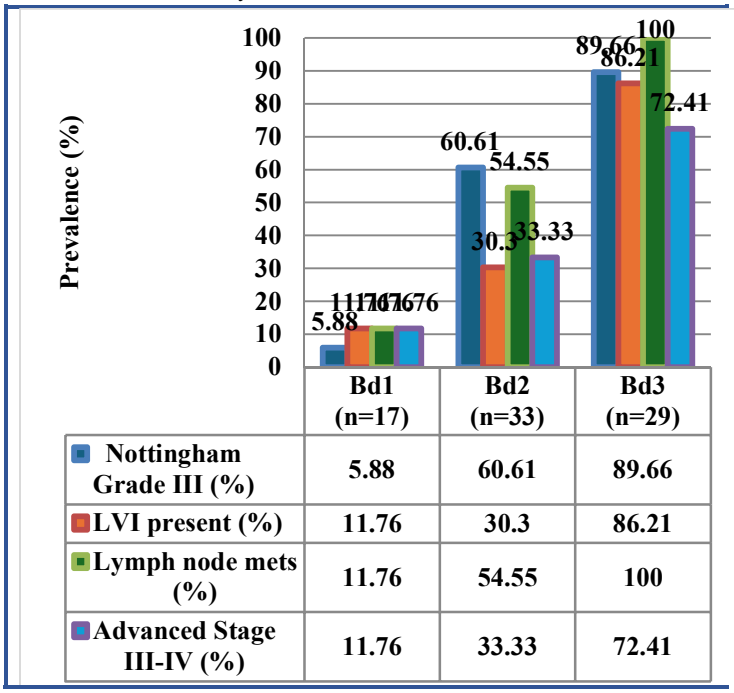

Figure 1. Prevalence (%) of adverse pathological features (Nottingham Grade III, LVI, lymph node metastasis, advanced stage) across the three tumour budding grades. All four parameters show a striking stepwise increase with rising tumour budding grade, with lymph node metastasis reaching 100% in the Bd3 stratum.

Inverse correlation between tumour budding and stromal TILs

A clinically relevant inverse relationship was demonstrated between tumour budding and stromal TILs (Table 8 and Figure 2). Cases with Bd1 showed the highest mean stromal TIL percentage (44.18 ± 16.87%), Bd2 showed intermediate stromal TILs (36.36 ± 12.78%), and Bd3 showed the lowest (32.55 ± 12.21%). Spearman's rank correlation between continuous tumour bud count and continuous stromal TIL percentage confirmed a statistically significant inverse correlation ($\rho = -0.227$, $p = 0.045$). This is consistent with the conceptual framework in which an EMT-active tumour phenotype (high TB) coexists with a relatively suppressed local immune response (low TILs), suggestive of an immune-evasive "cold" microenvironment.

Table 8. Inverse association of tumour budding grade with mean stromal TIL percentage (N = 79)

TB grade	n	Mean stromal TIL %	SD	Statistical test
Bd1 (low)	17	**44.18**	± 16.87	Kruskal–Wallis

Bd2 (intermediate)	33	**36.36**	± 12.78	**p < 0.05**
Bd3 (high)	29	**32.55**	± 12.21	Spearman ρ = –0.227
Overall	**79**	**36.90**	± 13.88	**p = 0.045**

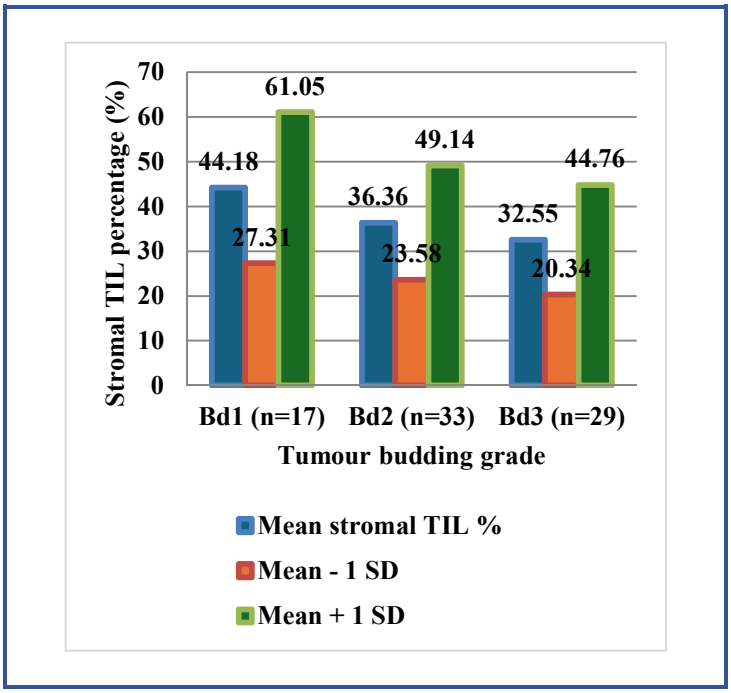

Figure 2. Mean stromal TIL percentage across the three tumour budding grades (Bd1 → Bd2 → Bd3). The monotonic decline from 44.18% (Bd1) to 32.55% (Bd3) confirms the inverse relationship between tumour aggressiveness (TB) and host immune response (stromal TILs); Spearman ρ = –0.227, p = 0.045.

DISCUSSION

Demographic and clinicopathological profile

The mean age of 54.54 ± 10.64 years and predominance of the 51–60 year age stratum (37.97%) in the present cohort are concordant with previously published Indian invasive breast carcinoma series. Shah and colleagues from a North Indian tertiary care centre reported a comparable mean age,[34] Rathod and colleagues reported a similar age distribution,[35] Agarwal and colleagues from Delhi also reported a 51–60 year peak,[36]Kumarguru and colleagues from South India reported broadly comparable demographics,[37] and Bandyopadhyay and Krishna reported similar age findings in their combined TB–TIL study.[28] The dominance of IBC-NST (86.08%) is consistent with the global preponderance of this histological type, and the predominance of Grade III tumours (67.09%) is comparable to the 52–70% Grade III rates reported by Shah and colleagues and Rathod and colleagues.[34,35]

Tumour budding distribution and prognostic relevance

Tumour budding has emerged in the last decade as a histopathological hallmark of aggressive tumour biology in invasive breast carcinoma, having been first described and validated in colorectal cancer.[9,12,14] The 78.48% prevalence of high-grade tumour budding (Bd2 + Bd3) in the present cohort is consistent with rates reported in published Indian breast cancer series, which range broadly from 35% to 80% depending on case mix.[38,39,40] Rathod and colleagues reported high-grade budding in approximately 60% of their Indian cohort,[35]Devashwar and colleagues reported similar proportions,[41] and Vasudevan and colleagues from South India reported a comparable mean tumour bud count.[42] International comparators by Liang and colleagues, Salhia and colleagues, and Gujam and colleagues report broadly comparable distributions across European, North American and East Asian cohorts.[43,44,45]

Association of tumour budding with histological grade

The present study demonstrated a highly significant association between tumour budding grade and Nottingham histological grade (χ^2 = 24.54, p = 0.0001). All four Grade I tumours had Bd1 while nearly half of Grade III tumours showed Bd3. This finding is in agreement with multiple published Indian and international studies. Kumarguru and colleagues, Singh and colleagues, Shah and colleagues, Devashwar and colleagues, and Francis and Sharma all reported a highly significant positive association between tumour budding and Nottingham grade.[37,46,34,41,47] Buch and colleagues, in their meta-analysis, confirmed a statistically significant positive correlation between high tumour budding and Nottingham Grade III in pooled analysis.[15] The biological basis of this association is consistent with the EMT framework: poorly differentiated tumours have a higher fraction of cells with diminished cell–

cell adhesion and acquired migratory capacity, manifesting as higher tumour bud counts at the invasive front.[10,11]

Association of tumour budding with lymphovascular invasion and lymph node metastasis

The present study demonstrated highly significant positive associations between tumour budding and both LVI ($\chi^2 = 35.24$, $p < 0.0001$) and lymph node metastasis ($\chi^2 = 36.77$, $p < 0.0001$). Notably, all 29 Bd3 cases (100%) had nodal metastasis compared to only 11.76% of Bd1 cases. This finding closely parallels published international data. Liang and colleagues reported a significant association between high TB and LVI in their Chinese cohort ($p = 0.001$),[43]Gujam and colleagues demonstrated a strong association of high tumour budding with poor outcomes including nodal disease,[45] Gabal and colleagues from Egypt reported a significant association between tumour budding and LVI and nodal status,[48]Salhia and colleagues showed that high tumour budding stratifies breast cancer with metastatic properties,[44]Sriwidyani and colleagues correlated tumour budding with E-cadherin loss and metastasis risk,[49] and Buch and colleagues confirmed in their meta-analysis a strong pooled odds ratio for nodal metastasis in high-TB tumours.[15] The 100% nodal metastasis rate in our Bd3 stratum is amongst the most striking reported figures in the literature and likely reflects the advanced-stage case mix of this hospital-based North Indian cohort.

Association of tumour budding with hormone receptor status

The present study demonstrated a clear decreasing trend in ER positivity from Bd1 (76.47%) through Bd2 (63.64%) to Bd3 (48.28%), although the Chi-square test did not reach statistical significance ($p = 0.168$). This finding is concordant with the published literature. Rathod and colleagues, Francis and Sharma, Agarwal and colleagues, and the meta-analysis by Buch and colleagues all reported a similar trend of declining ER positivity with rising tumour budding grade.[35,47,36,15] Kundu and colleagues observed a similar trend in their North Indian cohort,[39] Masilamani and Kanmani noted lower hormone receptor positivity in high-TB tumours,[50] and Ozer reported significant decreasing ER positivity with increasing tumour budding in a Turkish observational study.[51] The lack of statistical significance in our cohort likely reflects the modest sample size, although the 28 percentage-point drop from Bd1 to Bd3 is biologically meaningful.

Distribution and prognostic relevance of stromal TILs

Stromal TILs are now widely accepted as a prognostic and predictive biomarker in invasive breast carcinoma, with their assessment standardised by the ITWG 2014 recommendations.[17,18] The 36.90% mean stromal TIL percentage and 83.54% intermediate-TIL prevalence in our cohort are consistent with the bell-shaped distribution typical of unselected invasive breast carcinoma series. Denkert and colleagues, in their pooled analysis of 3771 patients, demonstrated that higher stromal TILs were strongly predictive of pathological complete response across breast cancer subtypes.[21] Yu and colleagues, in their meta-analysis of 12,968 cases, showed that high TILs are associated with improved disease-free and overall survival, particularly in triple-negative and HER2-positive subtypes.[24] Loi and colleagues from the BIG 02–98 trial demonstrated significant prognostic value of TILs in node-positive disease,[22]Pruneri and colleagues confirmed the clinical validity of TIL analysis in TNBC,[23] and Angelico and colleagues showed predictive significance of TILs for hormone receptor expression and proliferative activity in ER-positive disease.[52] Agarwal and colleagues from India reported similar prognostic relevance of TILs in advanced-stage Indian breast cancer.[53]

Inverse relationship between tumour budding and TILs

The present study demonstrated a statistically significant inverse correlation between tumour bud count and stromal TIL percentage (Spearman $\rho = -0.227$, $p = 0.045$). Bd1 cases had the highest mean stromal TIL percentage (44.18%) while Bd3 cases had the lowest (32.55%). Bandyopadhyay and Krishna were among the first to simultaneously evaluate TB and TILs in invasive breast carcinoma and reported a similar inverse relationship.[28]Gujam and colleagues had earlier observed that high tumour budding co-occurred with reduced stromal lymphocyte infiltrate.[45] Xiang and colleagues

confirmed that breast cancer classification incorporating both TB and stem cell-related signatures provides superior prognostic stratification.[29] Conceptually, this inverse relationship is consistent with the partial-EMT model in which tumour cells undergoing EMT acquire immunosuppressive properties — increased PD-L1 expression, secretion of immunosuppressive cytokines, and reduced antigen presentation — creating an immune-evasive "cold" microenvironment.[10,11,26]

Strengths, limitations and clinical implications

The present study has several strengths. First, it is one of the few North Indian studies to simultaneously assess both tumour budding and stromal TILs in invasive breast carcinoma using internationally recommended scoring systems (ITBCC 2016 and ITWG 2014). Second, all assessments were independently scored by two pathologists with consensus resolution of discordant cases. Third, the comprehensive clinicopathological dataset permitted multivariate analysis across all routinely reported parameters. Several limitations should be acknowledged. First, the modest sample size (n = 79) limits subgroup analyses, particularly within molecular subtypes. Second, the cross-sectional design precludes the assessment of disease-free and overall survival outcomes. Third, the absence of breast-specific consensus guidelines for tumour budding necessitated the use of the ITBCC 2016 scoring system, acknowledged as a current necessity pending breast-specific guidelines.[50,42,14] Fourth, interobserver variability in tumour budding assessment, while minimised by dual scoring, remains a recognised challenge, with Öztürk and colleagues demonstrating that small variations in bud-count cut-offs can alter prognostic stratification.[54] Fifth, the relatively short follow-up does not allow correlation with survival, an outcome assessed by Ranaee and colleagues and Voutsadakis in their long-term studies.[55,16] Despite these limitations, the strong associations of tumour budding with adverse pathological features — particularly the 100% nodal metastasis rate in Bd3 cases — and the demonstrated inverse correlation with stromal TILs strongly support the routine reporting of tumour budding and stromal TILs as cost-effective, easily assessable H&E-based prognostic biomarkers. A consolidated comparison of our findings with the published literature is presented in Table 9.

CONCLUSION

The present observational cross-sectional study of 79 invasive breast carcinoma cases from a North Indian tertiary care centre demonstrates that tumour budding and stromal tumour-infiltrating lymphocytes are valuable, cost-effective and reproducible morphological biomarkers that can be assessed on routine H&E sections without additional immunohistochemistry or molecular testing. High-grade tumour budding (Bd2 + Bd3) was observed in 78.48% of cases and showed highly significant positive associations with Nottingham histological grade ($p = 0.0001$), lymphovascular invasion ($p < 0.0001$) and lymph node metastasis ($p < 0.0001$), with all Bd3 cases harbouring nodal disease. A statistically significant inverse correlation was demonstrated between tumour bud count and stromal TIL percentage (Spearman $\rho = –0.227$, $p = 0.045$), supporting the conceptual framework in which an aggressive EMT-active tumour phenotype coexists with a suppressed local immune response. The combined morphological assessment of tumour aggressiveness (TB) and host immune response (TILs) provides a holistic view of the tumour–host interaction that may help refine risk stratification and inform treatment decisions in routine pathology practice. Routine reporting of tumour budding using ITBCC 2016 and stromal TILs using ITWG 2014 is recommended for invasive breast carcinoma resection specimens, while breast-specific consensus guidelines for tumour budding remain awaited.

DECLARATIONS

Ethics approval and consent to participate: Approval was obtained from the Institutional Ethics Committee, Maharishi Markandeshwar Institute of Medical Sciences and Research, Mullana, Ambala. Written informed consent was obtained from prospective participants; archival cases were anonymised per institutional policy.

Conflict of interest: The authors declare no conflict of interest.

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