Tumor budding in invasive breast cancer - An indispensable budding touchstone

B. N. Kumarguru, Anikode S. Ramaswamy, Shahanuma Shaik, Aruna Karri, Venugopal Sandeep Srinivas, B. M. Prashant

Department of Pathology, PES Institute of Medical Sciences and Research, Kuppam, Chittoor, Consultant Pathologist, Ashwini Diagnostics, Pogathota, Nellore, Consultant Pathologist, Lotus Hospitals, Sriampuram, Vishakapatnam, Consultant Pathologist, Medall Diagnostics, Medall Health Care Pvt Ltd, Nellore, Andhra Pradesh, Department of Pathology, Karwar Institute of Medical Sciences, Karwar, Karnataka, India

Address for correspondence: Dr. Anikode S. Ramaswamy, Department of Pathology, PESIMSR, Kuppam, Chittoor, Andhra Pradesh, India. E-mail: as.ramaswamy@gmail.com

ABSTRACT

Background: Tumor budding denotes a phenomenon in which the tumor cells, singly or in small aggregates, become detached from the neoplastic glands at the invasive front of adenocarcinoma. Tumors with budding cells have a significantly more aggressive clinical course. Significance of tumor budding has mainly been examined in the field of colorectal cancer. Aims: To document the number tumor buds at the invasive front of invasive breast cancer. To correlate the number of tumor buds with other histopathological parameters, and available clinical details. Setting and Study Design: Analytical study at a rural tertiary care referral institute. Materials and Methods: It was a retrospective study of invasive breast cancer cases from January 2012 to April 2015. Tumor buds were counted in H and E stained sections in 10 High Power Fields (HPFs). Association of tumor budding with histological parameters and available clinical details were analyzed statistically. Statistical Analysis Used: Frequencies, Chi-Square Test and Crosstabs were used for calculation. Results: 50 cases of invasive breast carcinoma were analyzed. Invasive ductal carcinoma constituted predominant histological type (92%). Low tumor budding (tumor buds ≤20/10HPFs) constituted 20 cases. High tumor budding (tumor buds >20/10HPFs) constituted 30 cases. Association of high tumor budding with lympho-vascular invasion, lymph node metastasis, primary tumor staging, regional lymph node staging, necrosis and Monckeberg medial sclerosis was statistically significant. Conclusion: Tumor budding may be incorporated as a new parameter in reporting protocols. Tumor budding serves as an indispensable touchstone in evaluating cases of invasive breast cancer.

KEY WORDS: Adenocarcinoma, lymph node, metastasis, necrosis

INTRODUCTION

Worldwide, breast cancer is the most common cancer in women.1,2 It has ranked number one cancer among Indian women with an age adjusted rate of 25.8 per 100,000 women and mortality of 12.7 per 100,000 women.3 The term, tumor budding denotes a phenomenon in which the tumour cells, singly or in small aggregates, become detached from the neoplastic glands at the invasive front of adenocarcinoma. The tumour budding has been strongly advocated as an important topic of research and a promising prognostic factor.4 The current study was undertaken to find out the significance of tumor budding in invasive breast cancer.
ductal carcinoma were reviewed. The cases were reviewed from December 2016 to March 2017 for a total period of four months. The lesions were classified according to WHO classification of tumors of breast 4th edition (2012).[^4]

There were 50 cases that were analysed. All invasive ductal carcinoma confirmed by histopathological examination were included in the study. Those cases in which only small (core) biopsies were performed were excluded from the study.

**Evaluation of tumor buds was done as follows**

1. The invasive front of invasive breast carcinoma was identified in scanner power (X4 objective)
2. Tumor buds were searched in low power (X10 objective)
3. Details of tumor buds were examined under high power (X40 objective) corresponding to an area of 0.159 mm². [Figure 1a-d]
4. Under high power (X40 objective), the possibility of mimickers of tumor buds [inflammatory cells (macrophages), multinucleated giant cells, fibroblasts, endothelial cells, smooth muscle cells and artifacts (floaters)] were excluded. [Figure 2a and b]
5. The nuclear and cytoplasmic characteristics of tumor cells were compared with those of invasive tumor cells (tumor proper). Those cells which exhibited similar morphology (except for the polarity) were considered, because polarity of tumor bud cells may vary
6. The presence of tumor bud (1-5 cells) was confirmed in high power (X40 objective)
7. Number of tumor buds were counted in 10 high power fields (HPF) and documented
8. The number of tumor buds was correlated with histopathological parameters [tumor size, tumor grade, lymph node status, lympho-vascular invasion and others features] and available clinical details
9. Association between the tumor budding and histological parameters and available clinical details were analyzed by statistical methods.

**Statistical analysis of data**

The socio-demographic variables were represented using frequencies and percentages. The cut-off value for classifying tumor budding into high tumor budding and low tumor budding was decided by using receiver operating characteristic (ROC) curve. The data analysis of association between the tumor budding and histological parameters and available clinical details were done by Chi-square test. All statistical calculations were done through statistical software STATA version 14.1.

**RESULTS**

In the present study, 50 cases of invasive breast carcinoma were analyzed. The lesions were seen in females in the range of 24–77 years. Clustering of cases was seen in sixth decade (mean = 53.14 years). Invasive breast carcinomas more commonly involved left breast [27 cases (54%)] than right breast [23 cases (46%)]. The most common site of involvement was central area [25 cases (50%)], followed by upper outer quadrant [9 cases (18%), lower outer quadrant [3 cases (6%)], upper inner quadrant [3 cases (6%)], upper quadrants [3 cases (6%)], outer quadrants [3 cases (6%)], lower quadrants [2 cases (4%)], lower inner quadrant [1 case (2%)], and inner quadrants [1 case (2%)].

Invasive carcinoma of no special type (Ductal carcinoma NST) was the most common histological type constituting 46 cases (92%), followed by invasive lobular carcinoma [2 cases (4%)], invasive cribriform carcinoma [1 case (2%)] and invasive mucinous...
carcinoma [1 case (2%)]. Additional lesions were seen in seven cases (14%). Ductal carcinoma in situ (DCIS) was the most common additional lesion constituting 4 cases (8%) followed by Paget disease [1 case (2%)], fibroadenoma [1 case (2%)], and benign phyllodes tumor [1 case (2%)].

Tumor budding was evaluated in all the 50 cases. Tumor buds ranged in number from 5–32 tumor buds/10 HPF with a mean of 20.06. Tumor budding was classified into high tumor budding (Tumor buds >20/10 HPF), and low tumor budding (Tumor buds ≤20/10 HPF). The cut-off value for classifying the number of tumor buds into high tumor budding and low tumor budding was decided based on ROC curve with a sensitivity of 92.59% and specificity of 69.57%. The presence of lymph node metastasis was considered positive and the absence of lymph node metastasis was considered negative. The area under ROC curve was 0.8543.

High tumor budding was seen in 30 cases (60%), and low tumor budding was seen in 20 cases (40%). High tumor budding was seen more in Invasive ductal carcinoma and invasive lobular carcinoma. Low tumor budding was seen in invasive cribriform carcinoma and invasive mucinous carcinoma. Table 1

The association of high tumor budding with lymph node metastasis (LN METS), lympho-vascular invasion (LVI), necrosis (NEC), and combination of parameters [LN METS, and LVI, LVI, and perineural invasion (PNI), LN METS, and PNI, LN METS, LVI, and PNI] was highly significant. [Figure 3a and b] Association of high tumor budding with Monckeberg medial sclerosis (MMS) was statistically significant. However, the association of high tumor budding with parameters like age of the patients, perineural invasion, tubule formation score, nuclear pleomorphism score, mitotic rate score, total Nottingham score and overall histological grade was statistically not significant [Table 2]. The association of high tumor budding with primary tumor staging was also statistically significant. Lympho-vascular invasion show highest odds ratio. The association of high tumor budding with regional lymph node staging was highly significant [Table 3].

Table 1: Tumor budding in various histological types of invasive breast carcinoma

| Lesions                               | Cases | High tumor budding (Tumor buds >20/10 HPF) | Low tumor budding (Tumor buds ≤20/10 HPF) |
|---------------------------------------|-------|--------------------------------------------|------------------------------------------|
| Invasive carcinoma of no special type [invasive ductal carcinoma nst] | 46 [92%] | 28 [56%] | 18 [36%] |
| Invasive lobular carcinoma            | 2 [4%] | 2 [4%] | 0 [0%] |
| Invasive cribriform carcinoma         | 1 [2%] | 0 [0%] | 1 [2%] |
| Invasive mucinous carcinoma           | 1 [2%] | 0 [0%] | 1 [2%] |
| Total                                 | 50 [100%] | 30 [60%] | 20 [40%] |

Table 2: Association of tumor budding and histopathological parameters

| Parameters                         | High tumor budding (Tumor buds >20/10 HPF) | Low tumor budding (Tumor buds ≤20/10 HPF) | P    | Odds ratio |
|------------------------------------|--------------------------------------------|------------------------------------------|------|------------|
| Lymph node metastasis [LN METS]    | Present 23 [46%] | Absent 7 [23.33%] | Present 4 [20%] | Absent 16 [80%] | P<0.001 13.14 |
| Lympho-vascular invasion [LVI]     | Present 26 [86.67%] | Absent 4 [13.33%] | Present 3 [15%] | Absent 17 [85%] | P<0.001 36.83 |
| Necrosis [NEC]                     | Present 28 [93.33%] | Absent 2 [6.67%] | Present 12 [60%] | Absent 8 [40%] | P=0.004 9.33 |
| Monckeberg medial sclerosis [MMS]  | Present 15 [50%] | Absent 15 [50%] | Present 3 [15%] | Absent 17 [85%] | P=0.012 5.67 |
| LN METS + LVI                      | Present 30 [100%] | Absent 0 [0%] | Present 7 [35%] | Absent 13 [65%] | P<0.001 |
| LVI + PNI                          | Present 29 [96.67%] | Absent 1 [3.33%] | Present 7 [35%] | Absent 13 [65%] | P<0.001 |
| LN METS + PNI                      | Present 23 [46%] | Absent 7 [23.33%] | Present 8 [40%] | Absent 12 [60%] | P=0.009 |
| LN METS + LVI + PNI                | Present 30 [100%] | Absent 0 [0%] | Present 10 [50%] | Absent 10 [50%] | P=0.001 |
| AGE [CUT OFF - 53 YEARS]           | Present 15 [50%] | Absent 15 [50%] | Present 9 [45%] | Absent 11 [55%] | P=0.729 |
| Perineural invasion [PNI]          | Present 8 [26%] | Absent 22 [73.33%] | Present 4 [20%] | Absent 16 [80%] | P=0.589 |
| Tubule formation score [1, 2, 3]   | P=0.141 |
| Nuclear pleomorphism score [1, 2, 3] | P=0.949 |
| Mitotic rate score [1, 2, 3]       | P=0.878 |
| Total nottingham score [4-8]       | P=0.850 |
| Overall histological grade         | P=0.884 |
DISCUSSION

Tumor budding is increasingly being recognized as a strong adverse prognostic factor. Prognostic significance of tumor budding has mainly been examined in the field of colorectal cancer and several solid organs. Breast cancer represents a group of highly heterogeneous lesions consisting of many morphologically distinct types. The current study was undertaken to evaluate the role of tumor budding in invasive breast cancer.

The total number of cases was highest in study conducted by Gujam FJA et al. In contrast to other studies, the present study had less number of cases. In contrast to the present study, Sriwidyani NP et al. documented clustering of cases in fifth decade. Other studies had not specified clearly. Most of the studies considered only invasive breast cancer-NOS. In contrast, the present study included various histological types of invasive breast cancer. Table 4 This is because, Man YG had suggested that tumor cell budding from a focally disrupted tumor capsule is likely to represent a common pathway shared by all breast cancer subtypes for their invasion. The author had utilized double immunostained sections to demonstrate the findings.

Srihia et al., Sriwidyani NP et al., and the present study used X40 (high power) objective to count the tumor buds. In contrast, Liang F et al., and Gujam FJA et al. used X20 objective. By using X20 objective, it would be difficult to differentiate tumor buds cells from their mimickers [fibroblasts or inflammatory cells] on H and E stained sections. So, Liang F et al. used IHC stained slides to overcome this practical problem. Hence, it may be suggested that it would be better if the tumor buds are examined under X40 objective to exclude the mimickers, confirm the presence of tumor buds and count on H and E sections.

Salhia et al. and the present study counted tumor buds in ten field. In contrast, Liang F et al., Gujam FJA et al., and Sriwidyani NP et al. counted tumor buds in five fields. It may be suggested that it would be better if tumor buds are counted in ten fields for better accuracy. Gujam FJA et al. and the present study had similar cut off value for tumor budding. Other studies have used different cut off values. Tumor budding varied in range for different studies. This is because: different studies used different methods for counting tumor buds.

Gujam FJA et al. and the present study counted tumor buds on H and E stained sections. In contrast, Salhia et al., and Sriwidyani NP et al., and counted tumor buds on IHC stained sections. Liang F et al. utilized both. Table 4 It may be suggested that, tumor buds may be counted routinely in H and E stained sections and IHC may be utilized only in selected cases where it would be difficult to distinguish between tumor buds and its mimickers;

### Table 3: Association of tumor budding with primary tumor staging and lymph node staging

| Staging | Primary tumor staging |
|---------|-----------------------|
| pT1     | 6 (12%)               |
| pT2     | 18 (36%)              |
| pT3     | 19 (38%)              |
| pT4     | 7 (14%)               |
| Total   | 50 (100%)             |

| Staging | Regional lymph node staging |
|---------|-----------------------------|
| pN0     | 19 (41.3%)                  |
| pN1     | 13 (28.26%)                 |
| pN2     | 5 (10.87%)                 |
| pN3     | 9 (19.57%)                 |
| Total   | 46 (100%)                   |

### Table 4: Comparison of socio-demographic variables and tumor budding parameters

| Parameters                      | Present study [India] | Sriwidyani NP et al.[9] [Indonesia, 2016] | Gujam FJA et al. [8] [UK, 2015] | Salhia B et al. [7] [USA, 2015] | Liang F et al. [5] [China, 2013] |
|--------------------------------|-----------------------|------------------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Total number of cases          | 50                    | 70                                       | 474                           | 148                           | 160                           |
| Age                            | Sixth decade [53.14 yr] | Fifth decade [48.6 yr]                   | > 50 yr (70%)                 | 61 yr [median]                | > 35 [92.5%]                  |
| Lesion                         | Invasive breast carcinoma [NOS] | Invasive breast carcinoma [NOS] | Invasive breast carcinoma [NOS] | Invasive breast carcinoma [NOS] | Invasive ductal carcinoma [NOS] |
| Power of objective             | X 40 [HPF]            | X 40 [HPF]                               | x 20                          | X 40 [HPF]                    | X 20                          |
| Number of fields               | 10                    | 5                                        | 10                            | 5                             | 5                             |
| Tumor budding cut off value    | > 20                  | > 10                                      | > 20                           | > 4                            | > 7                            |
| Tumor budding range            | 5-32 per 10 HPF       | 2-40 per 5 HPF                           | H&E                           | IHC [cytokeratin]              | H and E, IHC [pan-cytokeratin] |
| Staining                       | H&E                   | IHC [pan-cytokeratin]                    | H&E                           | H and E, IHC [pan-cytokeratin] | H and E, IHC [pan-cytokeratin] |
and the tumor bud count is borderline (near the cut off range) as high tumor budding indicate significant association with various histopathological parameters. It is intended to use to only count the tumor buds, IHC may be better avoided in cases clearly showing high tumor budding on initial H and E stained sections. Hence, IHC should be used judiciously.

It is quite obvious, that different studies have used different methods for evaluating the tumor buds. Hence, the cut off value, the number of fields counted, the power of the objective used for counting, the stain used for assessing tumor buds and range of tumor buds were different and consequently show variation in observations. This calls for standardization of criteria for evaluating the tumor buds to bring uniformity in assessment.

The association of high tumor budding with lymph node metastasis and lympho-vascular invasion was significant in all the studies. In the present study, the association of high tumor budding with necrosis was highly significant. In contrast, it was not significant in the study conducted by Gujam FJA et al.[8] Association of high tumor budding with primary tumor staging was significant in studies conducted by Liang F et al.[5] Sriwidyani NP et al.[9] and the present study. Association of high tumor budding with regional lymph node staging was significant in studies conducted by Salhia et al.[6] and the present study. Association of high tumor budding with age group distribution was not significant in studies conducted by Liang F et al.,[5] Gujam FJA et al.[8] and the present study. Association of high tumor budding with overall histologic grade was not significant in studies conducted by Liang F et al.,[5] Gujam FJA et al.[8] and the present study. In contrast, it was significant in the study conducted by Sriwidyani NP et al.[9] [Table 5].

In the present study, the association of high tumor budding with Monckeberg medial sclerosis was statistically significant. This was an unexpected and unique observation which had not received much attention in other studies. It may be hypothesized that Monckeberg medial sclerosis could be related to the aging process or it may occur as a pathological phenomenon operating as a parallel mechanism accelerated under the influence of tumor microenvironment. However, the hypothesis remains enigmatic and needs to be elucidated by molecular studies.

Biologically, tumour budding is closely related to the epithelial-mesenchymal transition. Epithelial-mesenchymal transition (EMT) refers to trans-differentiation of epithelial cells into mesenchymal cells. EMT and Mesenchymal-epithelial transition (MET) regulate embryonic stem cell differentiation, induced pleuripotency and cancer stem cell behaviour. During EMT, Epithelial cells lose their junctions and apical-basal polarity, reorganize their cytoskeleton, undergo a change in

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**Table 5: Comparison of association of clinico-pathological parameters with tumor budding**

| PARAMETERS            | Present study [India] | Sriwidyani NP et al.[9] [Indonesia, 2016] | Gujam FJA et al.[8] [UK, 2015] | Salhia B et al.[8] [USA, 2015] | Liang F et al.[7] [China, 2013] |
|-----------------------|-----------------------|------------------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Lymph node metastasis [LN METS] | P<0.001 [highly significant] | P<0.003 [highly significant] | P<0.009 [highly significant] | 0.05 [just significant] | P<0.001 [highly significant] |
| Lympho-vascular invasion [LVI] | P<0.001 [highly significant] | P<0.001 [highly significant] | P<0.001 [highly significant] | P=0.015 [significant] | P<0.001 [highly significant] |
| Necrosis [NEC] | P=0.004 [highly significant] | P<0.001 [highly significant] | P<0.001 [highly significant] | P=0.107 [not significant] | P=0.012 [significant] |
| Monckeberg medial sclerosis [MMS] | P=0.012 [significant] | P=0.010 [not significant] | P=0.009 [highly significant] | P=0.004 [significant] | P=0.012 [significant] |
| LN METS + LVI | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.009 [highly significant] | P=0.001 [highly significant] |
| LVI + PNI | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.009 [highly significant] | P=0.001 [highly significant] |
| LN METS + LVI + PNI | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.009 [highly significant] | P=0.001 [highly significant] |
| Primary tumor staging | P=0.024 [significant] | P<0.001 [highly significant] | P=0.001 [highly significant] | P=0.003 [significant] | P=0.014 [significant] |
| Regional lymph node staging | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.001 [highly significant] | P=0.003 [significant] | P=0.014 [significant] |
| Age | P=0.729 [not significant] | P=0.08 [not significant] | P=0.017 [not significant] | P=0.012 [not significant] | P=0.163 [not significant] |
| Perineural invasion [PNI] | P=0.589 [not significant] | P=0.141 [not significant] | P=0.949 [not significant] | P=0.878 [not significant] | P=0.850 [not significant] |
| Tubule formation score [1, 2, 3] | P=0.141 [not significant] | P=0.949 [not significant] | P=0.878 [not significant] | P=0.850 [not significant] | P=0.884 [not significant] |
| Nuclear pleomorphism score [1, 2, 3] | P=0.589 [not significant] | P=0.141 [not significant] | P=0.949 [not significant] | P=0.878 [not significant] | P=0.850 [not significant] |
| Mitotic rate score [1, 2, 3] | P=0.850 [not significant] | P=0.878 [not significant] | P=0.878 [not significant] | P=0.850 [not significant] | P=0.884 [not significant] |
| Total nottingham score [4-8] | P=0.850 [not significant] | P=0.878 [not significant] | P=0.878 [not significant] | P=0.850 [not significant] | P=0.884 [not significant] |
| Overall histologic grade [1, 2, 3] | P=0.850 [not significant] | P=0.878 [not significant] | P=0.878 [not significant] | P=0.850 [not significant] | P=0.884 [not significant] |
signalling programmes that define cell shape and reprogramme gene expression. This increases the motility of individual cells and enables the development of invasive phenotype. The switch in cell differentiation and behaviour is mediated by key transcription factors such as Snail homolog 1 (SNAIL), Zinc-finger E-box binding (ZEB), and basic helix-loop-helix transcription factors, and the function of which are finely regulated at the transcriptional, translational and post translational levels. Transforming growth factor β (TGF β) family signalling plays a predominant role in EMT.\(^{[11]}\) Tumor budding is one of the important mechanisms of cancer invasion and metastasis.\(^{[9]}\)

**LIMITATIONS OF THE STUDY**

The number of cases was relatively less in comparison with other studies. Perineural invasion was an important observation noted in invasive breast cancer cases. But, it was statistically not significant when analysed independently. But it was found to be significant when perineural invasion was statistically analyzed in combination with other parameters [lymph node metastasis and lympho-vascular invasion]. It may be suggested that additional sections may be evaluated and diligent search has to be made to detect perineural invasion, if a case shows high tumor budding.

**CONCLUSION**

High tumor budding is significantly associated with lympho-vascular invasion, lymph node metastasis, primary tumor staging, regional lymph node staging, Monckeberg medial sclerosis and necrosis. Criteria for evaluating the tumor buds need to be standardized. Tumor budding may be incorporated as a new parameter in the reporting protocols for reporting breast cancer cases, if it is consistently found to be an useful tool by future studies. Tumor budding may serve as an indispensable touchstone in evaluating invasive breast cancer cases.

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