Section: Pathology

Original Research Article

HISTOMORPHOLOGICAL STUDY OF POORLY DIFFERENTIATED CLUSTERS AND ITS CORRELATION WITH TUMOUR BUDDING IN INVASIVE BREAST CANCER

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Abstract

Background: Breast cancer is a malignant disease with a heterogenous prognosis. Standard histological parameters continue to be the most useful prognostic indicators. The tumor invasive front is a key interface of tumor-host interactions, and its features are hypothesized to regulate tumor growth. Tumor budding (TB) and poorly differentiated clusters (PDC) are examples of tumor invasion. The description and predictive usefulness of the TB, PDC grading of breast cancer have not been investigated. The current study attempted to correlate PDC with tumor budding as well as PDC with other histopathological prognostic characteristics such as age, size, grade, lymph node status, lymphovascular invasion, perineural invasion, tumor necrosis, and tumor staging. **Materials and Methods:** It is a retrospective study of 70 female patients with IDC-NOS who underwent curative resections between June 2019 and May 2022. Clinicopathological data was gathered from recruitment records and by analyzing archival H & E slides. The histological characterisation of invasive ductal carcinoma was performed in accordance with WHO tumor histological typing criteria and reported in accordance with College of American pathologists guidelines. The cut-off for tumour buds and PDC average was determined using ROC curve analysis. The chi-square test was used to perform a univariate study between tumor buds and clinicopathological characteristics. **Result:** Of the 70 patients, 41 (58.57%) had low tumor budding and 29 (41.43%) have high tumor budding. Out of 70 cases, 44 (62.86%) have a low PDC count and 26 (37.14%) have a high PDC count. The correlation between tumor budding and PDC is statistically significant (p=0.007). On investigation of clinicopathological characteristics of breast cancer with Tumour budding and poorly differentiated clusters, both demonstrate statistical significance with lymph node involvement (p=0.007, p=0.0001 respectively) and tumor staging (p=0.024, p=0.025 respectively). **Conclusion:** PDCs and tumor budding are both reproducible and important prognostic factor in Invasive ductal carcinoma not otherwise specified (IDC-NOS). Good interobserver agreement is seen in PDC than tumor budding. PDC are easy to access with the help of light microscopy with such a high predictivity. More research is needed, such as establishing a consistent evaluation approach and verifying the predictive significance of PDC in bigger sample sizes.

INTRODUCTION

Breast cancer is a malignant disease with a heterogeneous prognosis. Evaluating potential prognostic indicators such as tumor histological grading, cell proliferation index, estrogen receptor status, and lymph node status is gaining popularity. Additional prognosticators are required to improve individualized treatment and, in particular, to overcome over and undertreatment of patients.[1] The adoption of additional hallmarks as a supplement to conventional assessment could help the management of patients with breast cancer.[2] The tumor invasive front is a key interface of tumor-host interactions, and its features are hypothesized to govern tumor growth. Tumor budding and poorly differentiated clusters (PDC) are manifestations of tumor invasion.[3] Tumor budding is a pathological morphologic candidate index that has been applied to evaluate the prognosis of colorectal cancer, breast

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cancer, and other cancers.[4] By International tumour Budding Consensus Conference (ITBCC) criteria, it is formally defined as a single tumor cell or a cluster of fewer than five tumour cells dissociated from the main tumour at the invasive front, whereas clusters of five or more tumour cells without gland formation are defined as poorly differentiated clusters.[3] High intensity tumor budding reflects malignant progression and is a promising prognostic factor for low survival rate. Tumor budding is considered to be related to the biological processes of cancer invasion and metastasis and was also postulated as the histological representation of epithelial mesenchymal transition. However, the use of tumor budding as a prognostic factor has limitations: budding can be observed only in the actively invasive frontal region; Identifying tumor budding is difficult for single cancer cells or fairly small cell clusters in routine sections. Poorly differentiated clusters (PDCs), a novel histopathologic indicator, provide additional tumor bioinformation in addition to tumor budding. These are cancer clusters composed of five or more cancer cells and lacking gland like structures. The number of PDCs is highly relevant to survival and the incidence of nodal involvement in invasive colorectal cancer. A grading system based on PDCs successfully stratifies colorectal cancer cases by survival outcome and is believed to be useful in determining therapeutic strategies.[5] Compared with tumor budding, counting larger clusters (≥5 cancer cells) in the whole tumor tissue stained with H & E is a sufficiently easy process. So far, the description and prognostic value of the TB and PDC grading of breast cancer has not been explored.

We evaluated the Tumor budding and PDC grading in 70 patients with invasive ductal carcinoma, that was not otherwise specified (IDC-NOS), which is the most common histological type of invasive breast cancer, and determined the relationship between PDC grading and other known prognostic parameters.

Hence our aim is to correlate PDC with tumour budding and to correlate PDC and tumor budding with other histopathological prognostic parameters like age, size, grade, lymph node status, lymphovascular invasion, perineural invasion, tumor necrosis, tumor staging.

MATERIALS AND METHODS

It is retrospective study conducted in the Department of Pathology, Sri Venkateswara Medical College, Tirupati. The study included 70 female patients with IDC-NOS who underwent curative resections from June 2019 to May 2022, Clinicopathological data were obtained from recruiting records and by reviewing the archival H & E. Inclusion criteria consists of all invasive ductal carcinoma not otherwise specified (IDC-NOS) confirmed by histopathological report on mastectomy specimens. Core biopsies and other types of breast cancers along with faded slides or improper complete record of cases were excluded from the study. The histological characterization of invasive ductal carcinoma was done according to WHO histological typing of tumors and reported according to College of American pathologists [CAP] guidelines.

Definition and assessment of tumor budding

Tumor budding was determined as a single cancer cell or as cancer clusters with fewer than five cancer cells at the invasive front. To determine the degree of tumor budding, like the counting method of PDCs, the clusters were counted under the × 40 objective lens in a field where budding was most intensively distributed. Tumors with fewer than 2.5 and greater than 2.5 were classified as low and high tumour buds respectively

Definition and assessment of poorly differentiated Clusters

Cancer clusters composed of five or more cancer cells and lacking gland-like structures were defined as PDCs. Using Magnus microscope, the entire tumor, including its advancing edge, was first scanned at a lowerpower magnification, to identify the five densest PDC areas. Subsequently, the clusters were counted under themicroscopic field of a × 40 objective lens (field size 0.95 mm2), and the highest count of five areas per case was used as the number of PDCs. Tumors with fewer than four and greater than four as low pdc and high pdc respectively.

Statistical analysis was performed, ROC curve analysis was done for deriving cut-off for tumour buds and PDC grading. A univariate analysis was done between the tumour buds, PDC and clinicopathological parameters using chi-square test. All statistical analysis was two-sided and significance was defined as p-valve <0.05.

RESULTS

The patient's age ranged from (29 - 77) years The patients were divided into 2 categories: 1) Age group < 40years:2) Age group >40 years. Out of 70 cases, in 37 cases the lesion was located in the left breast and in 33 cases it was located in the right breast are observed. The tumors were predominantly located in the central quadrant [26 cases (37.14%)], the next in frequency being the outer upper quadrant [21cases (30%)]. Tumour size range from 1cm to 15 cm, tumour size was divided into 3 categories;

1) Tumor size < 2cm; 2) Tumor size 2.1 to 5 cm; 3) tumour size >5cm.

Out of 70 cases, majority are of >5cm size that constituting 32cases (45.71%).Nottingham's scoring system was used, with this consistent Nottinghams grade was given for all the cases and grouped under G1,G2 and G3. Majority of cases are of grade 2 in the present study which constitute 46 cases

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(65.71%). Lymphnode status was analysed by dividing into two categories

1) Node negative for metastasis (N0)
2) Node positive for metastasis (Np)

In the present study, cases of nodal negative are 34 in number which constitute 48.57% and nodal positive are 36 in number that constitute 51.43%. Lymphovascular invasion is analysed in all cases according to cap guidelines, in present study equal number of cases showing presence and absence of lymphovascular invasion of which each constitute 50% perineural invasion (PNI) is analyzed in all cases according to cap guidelines Out of 70 cases, perineural invasion was seen in 17 cases which constitute 24.29%. All the cases are analysed for tumour necrosis Out of 70 cases, 45 cases showed necrosis, which constitute 64.29%. Number of tumour buds are counted for 10 fields in high power and documented. Average of 10 fields is calculated and a cutoff of 2.5 was derived using ROC curve analysis. Out of 70 cases, 41 cases (58.57) are showing tumour buds < 2.5 and 29 cases (41.43%) showed tumour buds greater than 2.5. Relationship of histopathological parameters with tumour budding groups and its significance was calculated using chi square test.

Table 1: Distribution of Tumour Budding in Conjugation to ROC Derived Cut Off

Tumour budding Groups	Frequency	Percentage
Low tumour budding (< 2.5)	41	58.57
High tumour budding (> 2.5)	29	41.43
Total	70	100

Table 2: Relationship between Clinicopathological characteristics and tumor budding in Patients with Invasive Ductal Breast Cancer (NOS)

Parameters	Patients N (%)	Tumour Budding <25 N=41(58.57%)	Tumour Budding >25 N=29(41.43%)	P-Value
Age (<40/>40 years)	9(12.86%)/61(87.14%)	5/36	4/25	0.84
Size (<2 /2-5/ >5cm)	7(10%)/31(44.29%)/ 32(45.71%)	5/16/20	2/15/12	0.522
GRADE (I/II/III)	10(14.29%)/46(65.71%)/ 14(20%)	7/25/9	3/21/5	0.587
Involved lymph nodes(negative/positive)	34(48.57)/ 36(51.43%)	25/16	9/20	0.007 Significant
Tumour Necrosis (Absent/Present)	25(35.71%)/45(64.29%)	13/28	12/17	0.405
Lymphovascular invasion (absent/present)	35(50%)/35(50%)	16/25	19/10	0.029 Significant
Perineural invasion (absent/present)	53(75.71%)/17(24.29%)	34/7	19/10	0.094

Relationship with clinicopathological characteristics similar to tumour budding were analysed by univariate analysis for poorly differentiated clusters too. By analysis of mean, PDC are divided into two categories; Cases with PDC < 4 are grouped as low PDC, and with > 4 are grouped as high PDC.

Table 3: Distribution of poorly differentiated clusters

Poorly differentiated clusters	Frequency	Percentage
Low PDC (<4)	44	62.86%
High PDC (> 4)	26	37.14%
TOTAL	70	100%

Table 4: Correlation of tumor budding with PDC

Tumour budding	Patients n (%)	Low PDC	High PDC	P value
Low TB	44 (58.57%)	30	14	0.007
High TB	26(41.43%)	11	15	

Table 5: Relationship between clinicopathological characteristics and poorly differentiated clusters (PDC) in patients with invasive ductal breast cancer (NOS).

Parameters	Patients, n (%)	Poorly differentiated clusters (PDC) <4 n=44 (62.86%)	Poorly differentiated clusters (PDC) >4 n=26(37.14%)	P value
Age (<40/>40 years)	9 (12.86%)/ 61(87.14%)	7/37	2/24	0.321
Size (<2 /2-5/ >5cm)	7(10%)/ 31(44.29%)/ 32(45.71%)	7/18/19	0/13/13	0.100
Grade (I/II/III)	10(14.29%)/ 46(65.71%)/ 14(20%)	7/25/12	3/21/2	0.092
Involved lymphnodes (negative/positive)	34(48.57%)/ 36(51.43%)	32/12	2/24	0.0001
Tumour Necrosis (Absent/Present)	25(35.71%)/45(64.29%)	15/29	10/16	0.712
Lymphovascular Invasion (Absent/Present)	35(50%)/35(50%)	24/20	11/15	0.322
Perineural Invasion (Absent/Present)	53(75.71%)/17(24.29%)	36/8	17/9	0.121

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Tumour Budding Lowtb/Hightb	44(58.57%)/26 (41.43%)	30/11	14/15	0.007
TS(primary tumour staging) T1/T2/T3/T4	8(11.43%)/28 (40%)/23 (32.86%)/11(15.71%)	8/16/16/4	0/12/7/7	0.025

Table 6: Comparison of association of clinic-pathological parameters with tumor budding

Study	Lymphnode metastasis	Lymphovascular invasion	Primary tumour staging	Poorly differentiated clusters
Liang F et al China, 2013	P=0.05 significant	P<0.001 significant	significant	-
Salhia B et al.USA, 2015	-	P<0.015 significant	-	-
Gujam FJA et al.UK, 2015	P=0.009 significant	P<0.001 significant	-	-
Sriwidyani NP et al. Indonesia, 2016	P=0.003 significant	P<0.001 significant	significant	-
Kumarguru et al. India, 2022	P=0.001	P<0.001 significant	significant	-
Present study	P=0.007 significant	P=0.029 significant	P=0.025 significant	P=0.007, significant

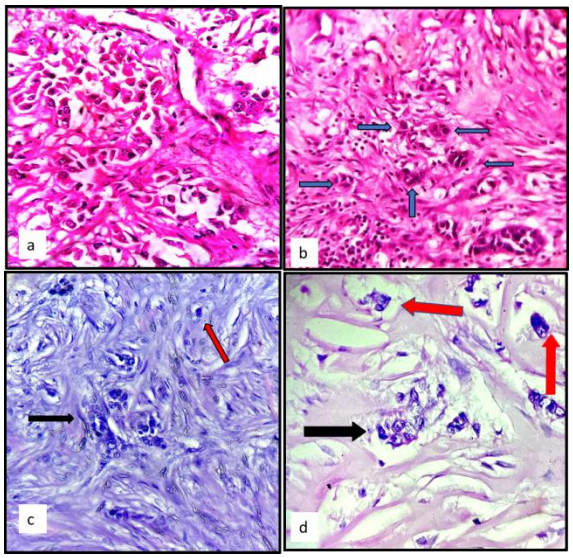

Figure 1: Hematoxylin and Eosin Stain staining. a. Tumor cells with pleomorphic nuclei with prominent nucleoli (400X). b. Tumor buds with tumor cells less than 5 cells (arrow) at invasive front of tumor (400X).c. Tumor buds with tumor cells less than 5 cells (red arrow) and poorly differentiated clusters, tumor cells more than 5(black arrow) at invasive front of tumor (400X).d. Tumor buds with tumor cells less than 5 cells (red arrow) and poorly differentiated clusters, tumor cells more than 5(black arrow) at invasive front of tumor (400X).

DISCUSSION

Tumor budding informally defined as the presence of individual cells and small clusters of tumor cells at the invasive front of carcinomas, has attracted significant recent attention, notably in the setting of colorectal carcinoma. The cells within these tumor buds that infiltrate collectively retain at least a modicum of their original epithelial identity, with clinically-relevant drivers of the process that are only now being recognized and examined.[6]

Tumor budding is a well-established independent adverse prognostic factor which may allow for stratification of patients into risk categories more meaningful than those defined by TNM staging and also potentially guide treatment decisions. Its universal acceptance as a reportable factor has been held back by lack of definitional uniformity with respect to both qualitative and quantitative aspects of tumor budding.[7,8] Tumor budding is associated with other histopathological factors known to portend a worse prognosis, namely higher tumor grade, infiltrating tumor border, the presence of lymphovascular and perineural invasion, and lymph node metastases.[9]

In this study, tumour budding were confirmed as a significant prognostic factor independent of classical or recent pathological morphologic variables like size, histological grade. Second, a high tumour budding grade, representing high invasive potential, was associated with lymph node involvement, lymphovascular invasion, apart from that poorly differentiated clusters also shows significant with lymph node status, tumour staging and with high tumour budding grade.

The findings in this study disclosed the clinical significance of PDCs in IDC-NOS. A high PDC grading reflected aggressive behavior and adverse prognosis of the tumor. This new histological parameter could be used to complement traditional histopathological prognostic factors in breast carcinoma. However, the PDC molecular features remain unclear. Further exploration is necessary to elucidate the biological significance of PDCs. However the association of high tumor budding with positive lymph nodes and lymphovascular invasion suggests that it can be considered as a poor prognostic factor. In our study we considered Lymphovascular invasion and metastatic lymph nodes as prognostic factors and did not do a survival study.

The association of high tumor budding with lymph node metastasis and lymphovascular invasion was significant in all the studies. The association of high tumor budding with necrosis was highly significant in Kumarguru et al.[10] In contrast, it was not significant in the study conducted by Gujam FJA et al.[11] Association of high tumor budding with primary tumor staging was significant in studies conducted by Liang F et al,[12] Sriwidyani NP et al,[13] and Kumarguru et al,[10] study and in our

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study. Association of high tumor budding with regional lymph node staging was significant in studies conducted by Salhia et al,[4] Kumarguru et al,[10] and present study.

Association of high tumor budding with age group distribution was not significant in studies conducted by Liang F et al,[14] Gujam FJA et al,[11] Kumarguru et al,[10] and present study. Association of high tumor budding with overall histologic grade was not significant in studies conducted by Liang F et al,[6] Gujam FJA et al,[7] Kumarguru et al,[10] and present study. In contrast, it was significant in the study conducted by Sriwidyani NP et al [Table 6].

Limitations & Future perspectives

In comparison to other studies, the number of cases was rather small. Various studies have employed various approaches to assess tumor buds and PDC. The cut off value, the number of fields counted, the strength of the objective used for counting, the stain utilized for assessing tumor buds, and the range of tumor buds were all varied, resulting in diversity in findings. This necessitates the standardization of criteria for evaluating tumor buds in order to achieve uniformity in assessment.

CONCLUSION

PDCs and tumor budding are both reproducible and important prognostic factors in invasive ductal carcinoma not otherwise defined (IDC-NOS). Good interobserver agreement is seen in PDC than tumor budding. PDC are easy to access with the help of light microscopy with such a high predictivity. More research is required, such as establishing a uniform evaluation procedure and verifying the predictive significance of PDC in larger size samples. This trait may aid in therapy and decision making and could be incorporated in the tumor reporting process for breast and colon carcinomas.

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