ORIGINAL ARTICLE

THE RELATIONSHIP OF TUMOUR BUDDING AND TUMOUR INFILTRATING LYMPHOCYTES IN DIAGNOSTIC BREAST CORE BIOPSIES WITH PATHOLOGICAL RESPONSE IN RESECTION SPECIMENS

Farhana Maryam✉, Madiha Syed, Maryam Hameed, Sajid Mushtaq, Mudassar Hussain, Kiran Imran

Shaukat Khanum Memorial Cancer Hospital and Research Center, Lahore-Pakistan

Background: Breast cancer, ranks as the second-leading global cancer-related cause of death. This article highlights prognostic significance of tumour budding and tumour infiltrating lymphocytes, emphasizing their roles in guiding treatments. Recent discoveries support their pivotal contribution in enhancing risk assessments and refining management strategies in breast cancer. **Methods:** A retrospective study of 80 in house at Shaukat Khanum Memorial Cancer Hospital diagnosed with invasive ductal carcinoma in the year 2014. Patients were categorized into two groups based on receptor status: Group 1 (ER/PR positive, Her2 negative, or ER/PR/Her2 positive) and Group 2 (Triple negative and ER/PR negative, Her2 positive). Each group had 40 cases. Pathologists evaluated tumour budding, tumour infiltrating lymphocytes, Nottingham Grading, and receptor status on core biopsies. Study aimed to determine the relationship of TB and TILs assessed on core biopsies on corresponding resection specimen in terms of pathological tumour stage and overall survival after therapy. **Results:** In Group I, TILs >50% correlated with lower pathological tumour stage, while <50% correlated with higher post- treatment tumour stage. In Group II, most had <50% TILs, but some responded well. Low TB-Status in Group I linked to higher rates of ypT0, while in Group II, high TB-Status corresponded with high PT stage, i.e., yPT1 suggesting prognostic value for TILs and TB in breast cancer response and staging. **Conclusions:** Study suggests that higher TILs percentages correlate with improved treatment response and survival in contrast to lower TILs. Similarly, lower TB-status is associated with complete treatment response (y PT0) and higher TB-status is directly related to poor treatment response and higher tumour stage.

Keywords: Tumour budding (TB); Tumour infiltrating lymphocytes (TILs); Breast cancer

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INTRODUCTION

Globally, breast cancer remains a significant health concern for women, with approximately one million new cases diagnosed annually. It stands as the most prevalent cancer among females, with an estimated 1.38 million women affected, as reported by the World Health Organization's GLOBOCAN data in 2008.[1] Invasive breast carcinoma of no special type (ductal) is the most commonly diagnosed type of breast cancer. The presence of positive axillary nodes, TNM stage, lympho-vascular invasion, and oestrogen receptor (ER) and progesterone receptor (PR) positivity are recognized prognostic factors, signifying the likelihood of recurrence or mortality associated with breast cancer.[2] Extensive research is underway to ascertain the prognostic importance of tumour budding (TB) and tumour-infiltrating lymphocytes (TILs). Tumour budding (TB) is characterized as a pathophysiological indicator of the motility of cancer cells and the initial stage in the metastatic process.[3,4] It has recently been found that the number of lymphocytes in cancer is an important prognostic factor. Immune cell infiltration, particularly anti-tumour type 1 lymphocyte infiltration, has been associated with improved prognosis in a variety of malignant tumours, including colon, ovarian, lung, and breast cancer.[4–7] Tumour-infiltrating lymphocytes (TILs) also play a crucial role in facilitating the response to chemotherapy and enhancing clinical outcomes across all subtypes of breast cancer. Immune cells play a crucial role in all stages of breast cancer development.[8] The conventional treatment protocol for managing breast cancer involves a combination of surgery, followed by radiotherapy and chemotherapy. Targeted therapy based on the immune system has the potential to serve as a therapeutic strategy to halt the advancement of breast cancer by influencing key pathways in carcinogenesis, such as the cell cycle,

angiogenesis, and metastasis.[9] Our goal is to establish the correlation between TB and TILs in diagnostic core biopsies and their correlation with the pathological response in corresponding resection specimens in terms of pathological tumour stage (pT) following neo adjuvant therapy. We also determined prognostic significance of TILs and TB in group I and group II in the terms of recurrence, metastasis and overall survival of patients.

MATERIAL AND METHODS

This research was conducted at Pathology Department of Shaukat Khanum Memorial Cancer Hospital and Research Center in Lahore, Pakistan, following approval from the Institutional Review Board (IRB). We retrieved 80 in-house patients diagnosed with Invasive Ductal Carcinoma of the breast from January to December 2014 from hospital archives. Our study included in house cases of invasive breast carcinoma of no special type (ductal) for which complete follow-up was available in hospital database. Case with other histological subtypes, lost to follow-up, equivocal immunohistochemically results for Her2neu, and scanty biopsies were excluded from our study.

Double blinded histological slide review was conducted by two consultant pathologists with significant experience in the relevant field, to assess tumour TILs, TB, tumour grading, and receptor status in core biopsies. Tumour grade was assessed utilizing the Nottingham Grading System.[10] Receptor status was determined in accordance with ASCO/CAP guidelines.[11] Keeping in view that triple negative breast cancer has poor prognosis, we categorized patients into two groups: Group 1 (ER/PR positive, Her2 negative, or ER/PR/Her2 positive) and Group 2 (Triple negative and ER/PR negative, Her2 positive) to avoid result bias. TB and TILs were assessed separately in both groups.

In breast carcinoma, TB denotes the existence of small clusters or solitary cancer cells at the forefront of tumour invasion. This occurrence is marked by the separation of tumour cells from the primary tumour mass, commonly observed at the advancing edge of invasive carcinomas.[3,4] Imai, a Japanese researcher, described this phenomenon in gastric cancer for the very first time in 1949. Pathologists in our department evaluated TB using haematoxylin and eosin (H&E) staining. Necrosis and mucinous areas were excluded from the field. In our study, an average of ten served as the cutoff for budding in ten high-power fields (HPFs). Tumours with more than 10 buds on average were designated as High-grade tumour budding" and "low-grade tumour budding" referred to cases with an average of less than 10 buds. Figure 1&2. Assessment of tumour infiltrating lymphocytes (TILs):

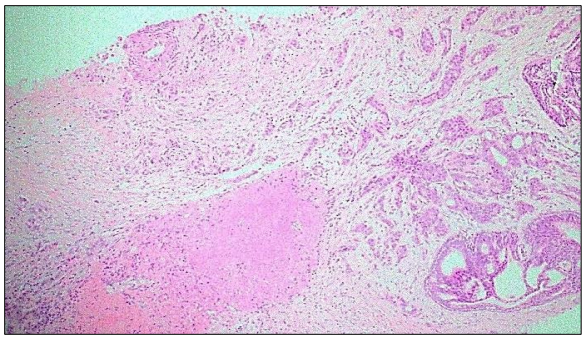

Figure-1: Tumour budding in breast cancer biopsy, 10x H&E slide shows tumour originating from main invasive tumour at 10x

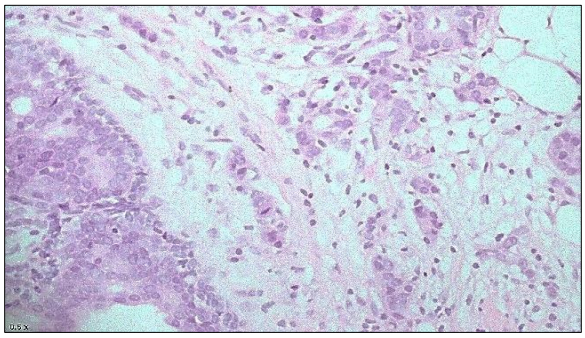

Figure-2: Tumour budding in breast cancer biopsy, H&E slide shows TB >10 at magnification, 400x

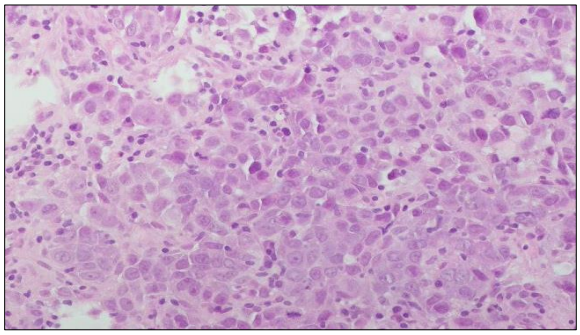

Figure-3: H&E of breast cancer biopsy shows TILS of less than 50 at magnification, ×400

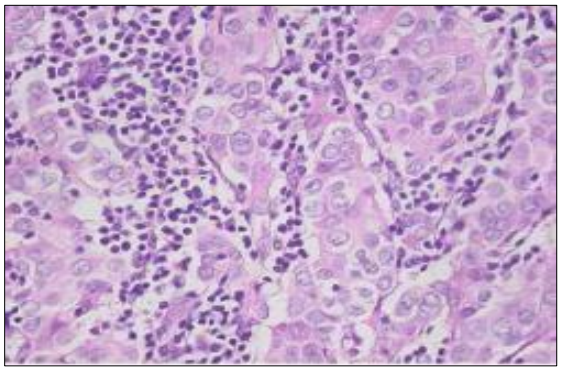

Figure 4: H&E of breast cancer biopsy shows TILS >50 at 400x magnification

TILs within breast carcinoma denote lymphocytes, like T cells and B cells that have migrated from the bloodstream into the tumour tissue. These immune cells form a crucial part of the body's defense against cancer. Breast cancers display diverse morphological variations, particularly in tumour cellularity and the composition of cancer stroma. Despite two tumours being of similar size, their absolute count of stromal lymphocytes can differ in terms of the percentage of TILs, owing to variations in stromal content concerning the tumour area.[12,13] Evaluating full-section H&E slides involved scoring the tumour stroma percentage at 5X magnification, followed by examining a single field at 10X magnification to ensure the presence of tumour cells on all sides of the image. The stromal area was then calculated as a percentage based on this assessment.

The calculation of TILs involved dividing the number of TILs by the total count of cells within the tumour tissue, then multiplying by 100. Subsequently, the TILs were categorized into two groups: >50% and <50%. A designation of >50% indicates that TILs occupy 50% of the stromal surface area, excluding 50% of the stroma plus epithelial cell area.

Each group was independently analyzed to explore the correlation between TILs and TB .The pathological tumour response on the corresponding resection specimens was determined using the College of American Pathologists (CAP) tumour protocols for breast cancer.[14] Prognosis encompasses cancer recurrence that is reappearance of cancer cells post-remission within typically five years, metastasis involving the spread of cancer cells to new areas, complicating treatment, and the overall survival of the patient, reflecting the disease's course and response to therapies.

RESULTS

Our study included 80 female patients who were further divided into two groups based on receptor studies. Each group consisted of 40 patients, spanning an age range of 26–75 years, the individuals were monitored for a period of 8 years ± 7 months. TILs and TB were assessed on core biopsies, and the pathological treatment response was evaluated separately on resection specimens in both groups.

GROUP I:

TILS:

When we assessed TILs on core biopsies, 7(17.5%) patients had TILS > 50 and 33 (82.5%) had <50. On resection specimen, out of 40 patients, 9 (22.5%) patients achieved a complete treatment response (ypT0), with 4 (12.5%) patients having <50% TILs and 5 patients having >50% TILs. The remaining (31 77.5%) patients in Group I presented with higher stages (ypT1, ypT2, ypT3) after neo-adjuvant chemotherapy.

The majority of patients in both the <50% TILs and >50% TILs groups were alive. Specifically, within the <50% TILs group, 25 (78.1%) cases were alive, while 8 (25%) patients were classified as dead. 1 patient showed recurrence however, no metastasis observed.

In the >50% TILs group, 6 (85.7%) patient was alive, 1 (14.3%) patient died and 1 (14.3%) patient showed metastasis.

TB:

When we assessed tumour budding, on core biopsies out of 40 patients 8 (20%) patients had TB more than 10 and 32 (80%) have less than 10 TB.

On resection specimen, 9(22.5%) patients showed a complete response to chemotherapy (ypt0), while 31(77.5%) patients had a higher stage after chemotherapy. Among the 9 patients who responded well to treatment, 8 (88.9%) had low TB-Status and 1 (11.1%) had high TB-Status.

Overall, 31 patients in Group 1 (77.5%) remained alive (26(65%) with low TB-Status and 5 (12.5%) with high TB- Status), and 9 (22.5%) patients died (6 (15%) with low TB-Status and 3(7.5%) with high TB-Status). In this group 1 (2.5%) patient shows recurrence with TB less than 10 and 1 (2.5%) showed metastasis with TB more than 10. The majority of patients in this group (87%) exhibited an overall survival of 8 years. A smaller percentage of patients (12%) demonstrated an overall survival of less than 4 years, and an additional 1% exhibited a 2-year overall survival. The first recurrence occurred at the 4-year mark, while the remaining patients showed no disease recurrence until the 8-year observation period. Out of the 40 patients in this group, 1 patient experienced metastasis at 4 years, while the remaining 39 patients remained free from metastasis until the 8-year endpoint.

GROUP II:

TILs:

When we assessed TILs, on core biopsies 8(20%) patients have TILS >50 and 32 (80%) had <50. On resection specimens, among them, 14(35%) patients were categorized as ypT0, with 10(25%) patients in the <50% TILs group and (10%) patients with >50% TILs.

The majority of patients in both TILs groups showed ypT stages indicating they were alive. Within the <50% TILs group, 25(71.4%) cases were alive, and 7 (20%) patients were classified as dead. 2(5%) patients showed metastasis. In the >50% TILs group, 7(87.5%) patients were alive, and 1 (12.5%) patient was classified as dead ,1 (2.5%) patient showed metastasis and 2(5%) showed recurrence.

TB:

When we assessed TB, on core biopsies out of 40 patients, 1 (2.5%) patient had TB of more than 10 and 39(97.5%) patients had less than 10 TB.

On resection specimen, 14 (35%) patients were categorized as having a complete response to

chemotherapy (ypT0), and all of them had low TB-Status. The remaining 26 (65%) patients in this group had a higher stage after treatment. Overall, 32(80%) patients in Group 2 remained alive, all of whom had low TB-Status, and 8(20%) patients died 7 (17.5%) with low TB-Status and 1 (2.5%) with high TB-Status). Three (7.5%) patients showed metastasis and 3 (7.5%) showed recurrence

majority of patients in this group (85%) exhibited an overall survival of 8 years. A small percentage of patients (9%) demonstrated an overall survival of less than 4 years, and an additional 6% exhibited a 1-year overall survival. Among the patients in this group, 3 experienced a recurrence at 3, 4, and 6 years, while the remaining 37 showed no recurrence over the 8-year period. Out of the 40 patients in this group, 3 individuals developed metastasis at 2, 2.5, and 4 years, respectively, while the remaining 37 patients did not experience metastasis throughout the 8-year observation period.

Table-1: Final Results of Group I on core biopsies

Characteristics	**Results**
Age Range	34–75 years
Tumour Grade	
Grade 1	0
Grade2	14
Grade3	26
Tumour infiltrating lymphocytes	
More Than 50	7
Less Than 50	33
Tumour Budding	
More Than 10	8
Less Than 10	32

Table-2: Final Results of Group II on core biopsies

Characteristics	**Results**
Age Range	29–60 Yrs.
Tumour Grade	
Grade 1	0
Grade2	2
Grade3	38
Tumour infiltrating Lymphocytes	
More Than 50	8
Less Than 50	32
Tumour Budding	
More Than 10	1
Less Than 10	39

Table-3: Final Results of Group I on resection specimens

Parameters	**TILs**		**TB**	
	<50	**>50**	**<10**	**>10**
Pathological response (ypT0)	N= 4	N= 5	N= 8	N= 1
Recurrence (n=1)	1	0	1	0
Metastasis (n=1)	0	1	0	1
Death (n=9)	8	1	6	3
Alive (n=31)	25	6	5	26

Table-4: Final Results of Group II on resection specimens

Parameters	**TILs**		**TB**	
	<50	**>50**	**<10**	**>10**
Pathological response (ypT0)	N= 10	N= 4	N= 14	N= 0
Recurrence (n=3)	0	2	3	0
Metastasis (n=3)	2	1	3	
Death (n=8)	7	1	7	1
Alive (n=32)	25	7	32	0

DISCUSSION

Targeted therapies that focus on the immune system have proven to be effective in treating highly immunogenic tumours such as melanoma, certain types of lung cancers, and prostate cancers and breast cancer.[15] This success has brought about a significant shift in the strategies employed for cancer treatment. Notably, the evaluation of TILs on haematoxylin and eosin (H&E) stained sections, particularly in HER-positive and triple-negative breast cancers (TNBC), has shown promising results in terms of both prognosis and prediction. Recent studies suggest that TILs could serve as a potential biomarker, offering insights into the antitumour immune response in breast cancer (BC).[4,15,16] It's worth mentioning that TB has been identified as a poor prognostic marker in oesophageal and colorectal carcinomas and lung adenocarcinoma and breast cancer.[3,4,17–19] However, the current evidence regarding TB and its role as a prognostic marker in breast cancer is limited.

The study's emphasis on exploring the interplay between TB and TILs aligns with a growing body of research that underscores the significance of these features as potential prognostic indicators in breast cancer. Notably, prior study conducted by Loi *et al.*, in 2013 and Denkert *et al*., 2018 have highlighted that elevated TILs within the tumour microenvironment are associated with improved outcomes and a more favourable response to treatment among breast cancer patients. Likewise, another study by Ueno *et al.*, in 2002 and Lugli *et al*., in 2009 shows that the presence of tumour budding, defined by small groupings of less specialized tumour cells at the leading edge of invasion, this phenomenon has been associated with unfavourable clinicopathological characteristics and a heightened likelihood of metastasis across different cancer forms, including breast cancer.

Our recent study assessed TILs and TB impact on treatment response in two groups. In Group I, TILs >50% correlated with positive responses, while <50% correlated with higher post-treatment stages. In Group II, most had <50% TILs, but some responded well. Low TB-Status in Group I linked to higher ypT-stage 0 rates, while in Group II, high TB-Status was more common in invasive tumours (ypT-stage 1),

suggesting prognostic value for TILs and TB in breast cancer response and staging

Previous study conducted by Paula *et al*.,2016 showed that the increase of immune infiltrate, particularly high levels of TILs, in TNBC is indicative of a more responsive tumour to chemotherapy but our present study shows that in group II more patients with <50 TILs showed complete treatment response. Similarly, a study conducted by C. Criscitiello *et al*., 2020 showed that breast cancer that are (ER+) and (HER2−), having high levels of Tumour- Infiltrating Lymphocytes (TILs) is significantly linked to features that indicate worse prognosis. However, our recent research indicates that having more than 50 tumour-infiltrating lymphocytes (TILs) is linked to improved responses to treatment and enhanced survival outcomes in both groups of patients.

Our study faced several limitations, primarily the inclusion of only in-house patients due to the availability of follow-up data in hospital archives. Technical factors, notably poor-quality slides with histological artifacts, emerged as a significant source of discrepancies. These artifacts were observed as a consequence of prolonged ischemic time, inadequate fixation, and issues during the processing, embedding, or microtome stages. These technical challenges were the predominant contributors to discordances in our study.

CONCLUSION

In summary, among the patients in Group I, those with TILs >50% exhibited a favourable treatment response with complete resolution, while cases with TILs <50% displayed higher post-treatment stages. In Group II, the majority of patients had TILs <50% and showed a higher ypT-stage, but there were still a few cases that responded well to treatment. Overall, these findings suggest a potential association between TILs group and treatment response, with TILs >50% showing a higher likelihood of complete response and TILs <50% associated with a potentially more advanced tumour stage

In group I Patients with "low" TB-Status tend to have a higher proportion of cases in ypT-stage 0, while the distribution in ypT-stage 1a appears more balanced between the two TB-Status categories. And in Group II, patients with "high" TB are more likely to exhibit invasive tumours (ypT-stage 1), while those with "low" TB predominantly have non-invasive tumours (ypT-stage 0) This suggests that a "low" TB is more prevalent in cases demonstrating a complete treatment response in both groups.

In terms of overall survival and TILs, the majority of both alive and deceased patients in both Group I and Group II had <50% TILs, highlighting its potential association with survival outcomes. Regarding tumour budding, alive patients in Group I showed a higher presence of high TB (83.9%), while all alive patients in Group II had low TB. Among deceased patients, high TB was associated with a lower survival rate in Group I, whereas the majority in Group II had low TB, suggesting a potential impact on overall survival, particularly in Group I.

AUTHORS' CONTRIBUTION

FM: Conceptualization of study design, data collection, data analysis, data interpretation and write-up. MS, SM: Conceptualization of study design, data collection, data analysis, data interpretation and proof reading. SM, MH: Conceptualization of study design, proof reading. KI: Data analysis and data interpretation.

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Address for Correspondence:

Farhana Maryam, Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore-Pakistan
Cell: +92 301 417 5907
Email: farhanamaryam063@gmail.com