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Original Research Article

Correlation of tumor infiltrating lymphocytes in invasive breast carcinomas: a morphological study

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ABSTRACT

Background: The aim of the study is to analyse the stromal tumor-infiltrating lymphocytes (sTILs) in invasive breast carcinomas and assess the percentage of sTILs in invasive breast carcinomas using a single H and E slide following a standardized protocol. Also, to study the correlation between sTILs and known prognostic factors.
Methods: Two year retrospective study of 190 cases using a single representative H and E slide examined by 2 observers. TILs assessed based on current recommendations from the 2014 International TILs Working Group (ITWG) for the evaluation of TILs. Histological type, histological grade, molecular subtypes using relevant IHC markers, number of lymph nodes isolated, number of lymph nodes positive with extracapsular invasion, lymphovascular invasion, perineural invasion, TNM stage and tils in percentage were documented.
Results: The collected data was analysed with IBM SPSS statistics for Windows, version 23.0. Correlation of TILS with age, SBR grade, ER, PR, Ki67, molecular subtype-showed statistical significance with an acceptable p value.
Conclusions: Many studies have supported the evidence of TILS, in different levels and density which is predictive of response to neoadjuvant chemotherapy. In certain breast carcinoma subtypes, it has a prognostic value in patients treated with adjuvant chemotherapy. From our study TILs show a correlation with few of the known prognostic factors. There is a paucity of data in Indian literature on TILs and its implications. Understanding of the role of TILs regarding could be of importance in devising novel immunotherapeutic strategies in breast cancer treatment.

Keywords: Tumour infiltrating lymphocytes, Invasive breast cancer, Prognosis

INTRODUCTION

Among women, breast carcinoma is the most prevalent cancer and the second leading cause of cancer-related deaths, surpassed only by cervical cancer.[1,2] Breast cancer arises from a multifactorial and multistep process; therefore, prevention and early diagnosis are essential for improving outcomes. The tumour microenvironment plays a significant role in the development as well as progression of malignant tumours.[3,4] TILs play an important role in mediating response to chemotherapy and have proven to be a remarkable prognostic marker in breast cancer. The TIL density in tumors is found to be a significant prognostic marker along with the other established factors.[3-5] Many studies have been done to assess the association of TILs in the neoadjuvant setting as well as in predicting pCR (pathological complete response). Evaluating TILs is an inexpensive and reproducible as well as reliable method. In addition to clinical and therapeutic factors, the host immune response may significantly influence the prognosis of cancer patients following standard treatment. Numerous studies have highlighted the importance of evaluating TILs in various malignancies, including breast, colorectal, ovarian, lung and other cancers.[6-9] Evidence from multiple studies indicates that TIL evaluation in breast cancer has significant clinical

value for predicting treatment response and prognosis, underscoring important role of immunity in influencing therapy outcomes and tumor progression.[10] As a result, in their recommendations for standardised assessment of TILs in breast cancer, The international immuno-oncology biomarker working group recommends assessing sTILs while strictly adhering to the defined outline.

TILs are distributed within two distinct regions of the tumor microenvironment: the stromal and intratumoral compartments. Although both sTILs and intratumoral TILs (iTILs) contribute to the overall TIL population, numerous studies have identified sTILs as a more reliable and reproducible parameter. This is largely because iTILs are typically less abundant, exhibit greater heterogeneity, and are often challenging to identify on hematoxylin and eosin (H and E)-stained sections.[11,12] sTILs are identified as mononuclear immune cells, predominantly lymphocytes that are found within the tumor's boundary but not directly in contact with or infiltrating the tumour cell nests.[13] sTILs are reported as the "percentage of stromal area occupied by mononuclear inflammatory cells over the total stromal area within the tumor." iTILs are lymphocytes seen within carcinoma nests that have direct cell-to-cell contact with no intervening stroma.[14-16] Recommendations published by "The ITWG 2014" for assessment of TILs on H and E slides are easy and reproducible.[3,11] In both triple-negative and HER2-positive breast tumours, high levels of sTILs have been linked to a better prognosis and response to neoadjuvant therapy.[15,17]

METHODS

This is a 2-year retrospective study of 190 cases, done in Kasturba Medical College, Manipal between September 2019 and 2021 which was approved by the institutional ethical committee. In this study, all the cases which were diagnosed on histopathology as infiltrating breast carcinoma between July 2017 and July 2019 were included. Tru-cut core biopsies and post-neoadjuvant chemotherapy specimens were excluded. Pathology archives were searched through laboratory information system (LIS) using keyword 'infiltrating breast carcinoma' and patients with this diagnosis was identified. The H and E slides were examined by 2 observers and relevant histological prognostic features such as histological type, histological grade, number of lymph nodes isolated, number of lymph nodes positive with extracapsular invasion, lymphovascular invasion, perineural invasion, TNM stage and TILs in percentage were documented. The TILs were assessed based on standardized and reproducible evaluation criteria as suggested by "ITWG 2014". Immunohistochemistry (IHC) results, if previously performed, were noted for classification into molecular subtype - ER status, PR status, HER2 status, Ki 67 status.

Statistical analysis

The collected data was analysed with IBM SPSS Statistics for Windows, Version 23.0.(Armonk, NY: IBM Corp). For categorical variables, frequency analysis and percentage analysis were utilised, while for continuous variables, the mean and standard deviation were used. The chi-square test was employed to determine the importance of categorical data. The results were tabulated and analysed using SPSS version 16. Continuous data was expressed as mean±SD. Other statistical tests such as student paired t test and Fischer exact tests were applied wherever appropriate. $P<0.05$ was considered to be significant (highly significant at $p<0.01$, significant at $0.01\leq p\leq 0.050$, no significance at $p>0.050$).

RESULTS

Out of 190 cases, 104 mastectomies and 86 lumpectomies with and without axillary clearance were included, core biopsies and post NACT specimens were excluded. Range of the age of patients was from 24-83 years. Based on the tumor size, three groups were recognized as <2 cm, 2-5 cm and >5 cm. Invasive ductal carcinoma-NST (IDC NST) was the most common histological type. The 65% of the cases were of Nottingham grade 2. Based on the IHC profile-ER, PR, Her2, Ki67-all the cases were classified into molecular subtypes. Luminal B was the most common molecular subtype. FISH testing was not done in the 18 cases of equivocal HER2 expression. The TILs were classified under recommendations by the ITWG 2014 (Figure 1-6: microscopy of TILs) (Table 1).

The correlation of TILs with known prognostic factors showed a significant correlation with age, molecular subtype ($p=0.028$) and a very significant correlation with Nottingham grade ($p=0.001$). Correlation of TILs with hormone receptors, ER and PR showed higher significance ($p=0.001$ and $p=0.0001$, respectively), however Her2 and ki67 did not show any significant correlation. Tumor size, histopathological type, LVI, PNI, ENE and TNM stage did not show any statistical significance (Table 2).

Table 1: Clinicopathological characteristics of the study population.

Parameters	Categories	N	Percentage (%)
Type of specimen, (n=190)	Modified radical mastectomy	104	54.73
	Lumpectomy	44	23.15
	Lumpectomy with axillary clearance	42	22.10
Age (in years) (n=190)	24-40	31	16.31
	41-55	75	39.47
	56-70	63	33.15
	71-86	21	11.05

Continued.

Parameters	Categories	N	Percentage (%)
Tumor size, (n=190)	<2 cm	32	18.84
	2-5 cm	125	65.78
	>5 cm	33	17.36
Histological type, (n=190)	IDC-NST	162	85.26
	Mucinous	14	7.36
	Cribriform	2	1.05
	ILC	4	2.10
	Solid papillary carcinoma	3	1.57
	Metaplastic	5	2.63
Nottingham grade, (n=190)	Grade 1	29	15.26
	Grade 2	116	61.05
	Grade 3	45	23.68
ER (n=176)*	Negative	73	41.47
	Positive	103	58.52
PR (n= 176)*	Negative	80	45.45
	Positive	96	54.54
HER2 (n=176)*	0	5	2.84
	1	80	45.45
	2	51	28.97
	3	40	22.72
Ki67, (n=176)*	<14	47	26.70
	>14	129	73.29
Molecular subtype, (n=158)**	Luminal A	29	18.35
	Luminal B	58	36.70
	HER2 enriched	33	20.88
	TNBC	38	24.05
LVI, (n=190)	Absent	121	63.68
	Present	69	36.31
PNI, (n=190)	Absent	176	92.63
	Present	14	7.36
ENE (extra nodal extension)	Absent	132	69.47
	Present	26	13.68
pT stage, (n=190)	pT1	25	13.15
	pT2	129	67.89
	pT3	32	16.84
	pT4	4	2.10
pN stage, (n=190)	pN0	64	33.68
	pN1	57	30
	pN2	27	14.21
	pN3	13	6.84
sTILs score, (n=190)	0-10%	71	37.36
	10-40%	91	47.89
	40-90%	28	14.73
sTILs group, (n=190)	A	58	30.53
	B	87	45.78
	C	45	23.68

*IHC was not done in 18 cases, **Cases with equivocal (2+) HER2 score were advised to do FISH. Out of the 51 equivocal cases, FISH was done in 33 cases, and HER2 amplification was seen in 15 cases (n=33, 45%). Scores 0 and 1+ were taken as negative, and scores of 3+ and equivocal cases after FISH with HER 2 amplification were taken as positive. FISH was not done in 18 equivocal cases (n=33).

Table 2: Correlation of TILs with various prognostic factors and its statistical significance.

Parameters	Categories	0-10%	10-40%	40-90%	P value
Age (in years)	24-40	7	16	8	0.028
	41-55	23	40	12	
	56-70	28	27	8	
	71-86	13	8	0	

Continued.

Parameters	Categories	0-10%	10-40%	40-90%	P value
Tumor size (in cm)	<2	14	10	8	0.200
	2-5	44	66	15	
	>5	13	15	5	
Histological type	IDC-NST	58	79	25	0.556
	Mucinous	6	5	3	
	Cribriform	2	0	0	
	ILC	2	2	0	
	Solid papillary carcinoma	2	1	0	
	Metaplastic	1	4	0	
Nottingham grade	Grade 1	8	44	19	0.001
	Grade 2	26	56	9	
	Grade 3	11	16	1	
ER	Negative	15	40	18	0.001
	Positive	46	47	10	
PR	Negative	16	44	20	0.0001
	Positive	45	43	8	
HER2	0 and 1	41	45	18	0.099
	2	8	7	3	
	3	12	35	7	
Ki67	<14	22	18	2	0.024
	>14	37	60	20	
Molecular subtype	Luminal A	18	11	0	0.001
	Luminal B	22	28	8	
	HER2 enriched	8	20	5	
	TNBC	5	21	12	
LVI	Absent	50	52	19	0.193
	Present	21	39	9	
PNI	Absent	65	84	27	0.695
	Present	6	7	1	
ENE	Absent	11	12	3	0.532
	Present	42	67	23	
pT stage	pT1	11	7	7	0.276
	pT2	46	66	17	
	pT3	13	15	4	
	pT4	1	3	0	
pN stage	pN0	22	29	13	0.210
	pN1	22	26	9	
	pN2	10	14	3	
	pN3	1	11	1	

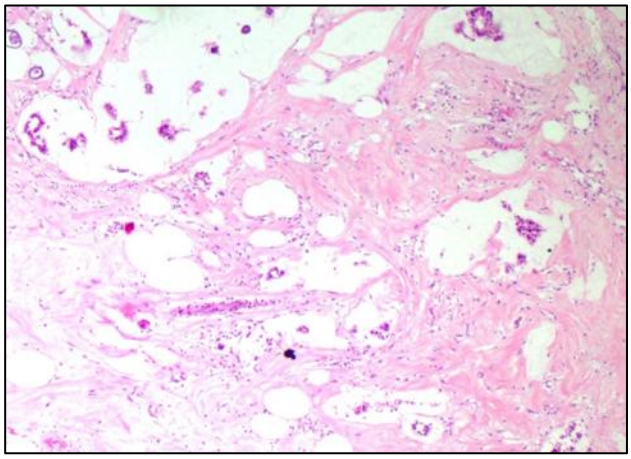

Figure 1: Microscopy: 5% TILs (H and E, 40×).

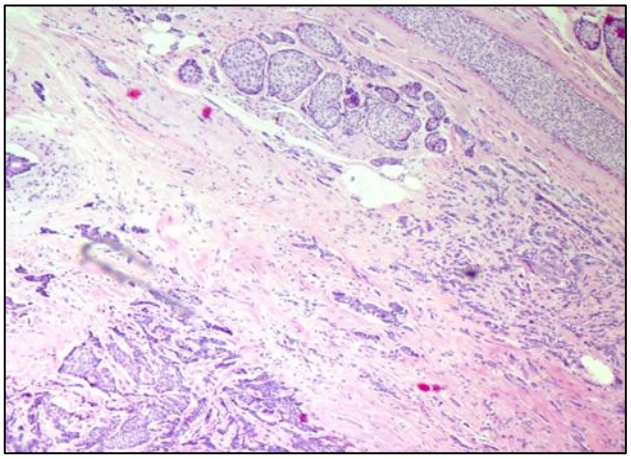

Figure 2: Microscopy: 10% TILs (H and E, 40×).

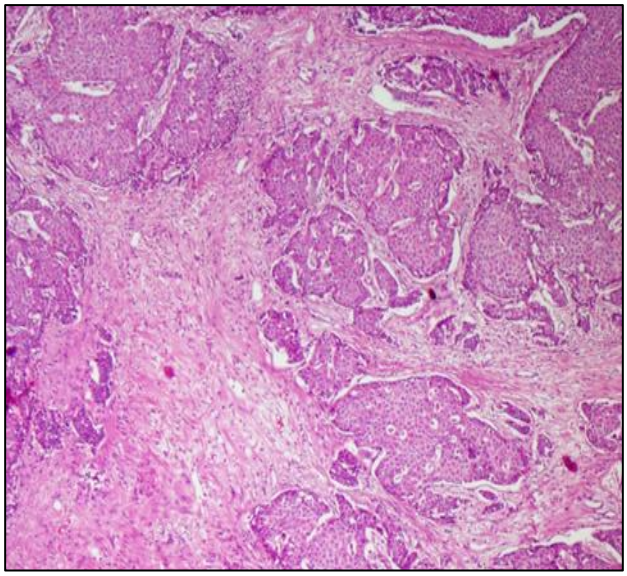

Figure 3: Microscopy: 20% TILs (H and E, 40×).

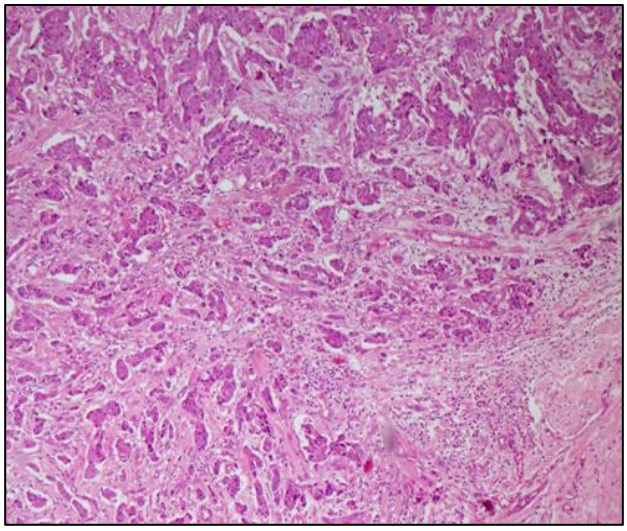

Figure 4: Microscopy: 30% TILs (H and E, 40×).

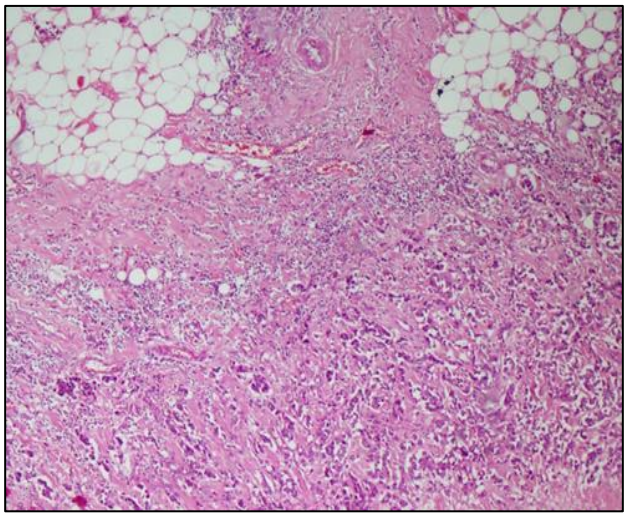

Figure 5: Microscopy: 40% TILs (H and E, 40×).

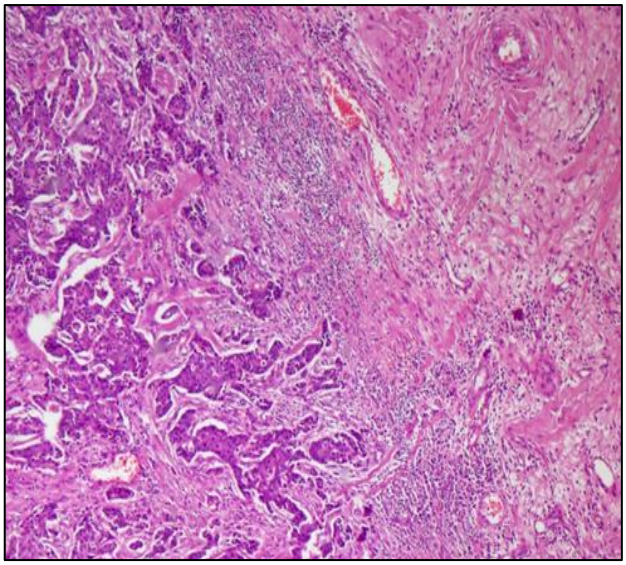

Figure 6: Microscopy: 50% TILs (H and E, 40×).

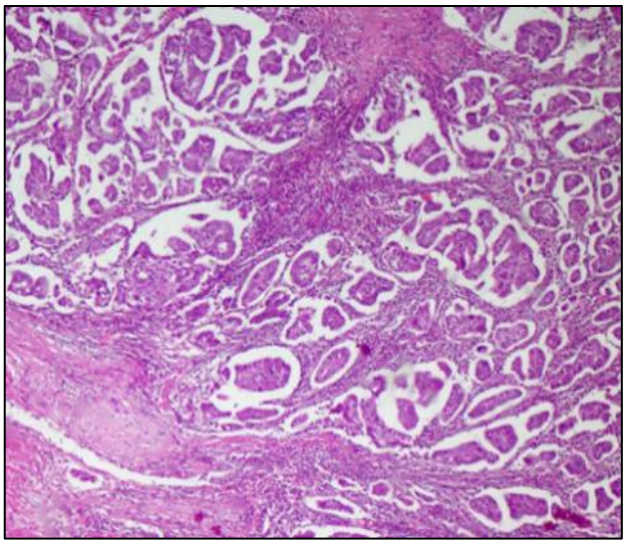

Figure 7: Microscopy: 70% TILs (H and E, 40×).

DISCUSSION

Protection of the body by the immune system relies on a tightly regulated balance between activating and inhibitory signals. The growth, proliferation, and metastatic potential of tumors are significantly determined by the complex interactions between malignant tissues and the host immune system.[19,20] Much research has been conducted since the late 1900s to support and validate the predictive and prognostic significance of TILs.[21] In post NACT (neoadjuvant chemotherapy) cases, TIL density is a predictive prognostic marker, and also plays a prognostic function in patients treated with adjuvant chemotherapy in specific breast cancer subtypes, according to multiple studies.[22] This suggests that TIL density affects treatment response and outcome in BC patients, and that assessing TIL density in clinical trial cohorts as well as in daily histopathological practise may be crucial.[23] The host-to-

tumor reaction is represented by TILs of diverse subtypes. A favourable response to treatment was seen in different malignancies which exhibited a good tumor immune infiltrate and expressed immune gene signatures in a study by Fridman et al.[24] Aaltomaa and colleagues in 1992, first reported an association between TILs and outcome in breast cancer. Since this study, many other studies have been made on TILs and researchers have very recently, validated the predictive and prognostic value of TILs based on data collected from several randomized clinical trials. In the present study, sTILs were highest in TNBC type followed by HER2 enriched type, which corroborated with the studies by Loi et al and Bjelobrk et al.[22,26]

Denkert et al were the first to notice a link between the existence of TILs and better clinical results.[14] In TNBC and HER2-positive patients receiving anthracycline-based chemotherapy, Loi et al found a linear connection between increased TIL density and a lower risk of relapse and mortality.[27] The result of the present study corroborates with other studies showing that majority of women belonged to 4th and 5th decade with higher proportion of TILs (40-90%) as well as significant correlation of TILs with age.[15,28,29] Tumor size and histopathological type did not show a significant correlation with TILS, which was in line with the other studies.[2,28-30] The present study corroborated with the results shown in studies by Denkert et al, Pujani et al, Polonia et al and Jang et al of a very significant correlation of sTILs with Nottingham histological grade. The percentage of sTILs increased with increase in grade of the tumor. The presence of increased TILs was found to be more helpful in HER2-positive cancer and TNBC than luminal subtypes.[23] The current study demonstrated increased sTILs in TNBC followed by HER2-positive cancer. Similar results were observed in previous studies. The results of the present study corroborate with those from some studies done over the past years, with a significant correlation of TILs with molecular subtypes as well as ER and PR status. Association of TILs with age also showed a significant correlation, suggesting that as age advances there was a decrease in the TIL density, and an inverse prognosis. It was also seen that maximum TILs were documented in HER2 positive and TNBC subtypes, which again is consistent with documented literature. The accomplishment of a pathological complete response (pCR) is related with a favourable long-term survival in HER2-positive and triple-negative breast cancer (TNBC).[5,31,32] An increased level of TILs were seen with increased pCR rates as reported by many studies.[31] Assessment of sTILs in breast cancer should be incorporated into daily histopathological reporting considering the prognostic significance as well as reliability of this parameter from various studies documented in literature. The recommendations by the international working group for TIL in breast cancer-2014 for evaluation of sTILs on H and E slides is easy, inexpensive and reproducible. Tramm et al, Kojima et al and Cabuk et al documented an agreeable interobserver variability in assessment of TILs in their studies.[3,33,34] Assessing the tumor microenvironment is key for devising immunotherapeutic strategies and TILs act as a readily available marker of pre-existing antitumor immunity in breast cancer.

Limitations

The present study has a relative smaller sample size. IHC (immunohistochemistry) and FISH (Fluorescent in situ hybridization) studies were not done in a few cases due to lack of resources during that time period, which made it impossible to classify these cases into molecular subtypes. This led to a minor uneven distribution of patients in the molecular subtypes, which may lead to uncertain statistical results.

CONCLUSION

The importance of TILs is gaining weight in the past few years as an important prognostic factor. The present study shows that evaluation of TILs can easily be done with minimum expertise and is reliable and reproducible following the guidelines put forth by the “ITWG 2014” .This study was done to study the correlation of TILS with the well-established prognostic factors like tumor size, age, grade, stage etc The results of this study showed a significant correlation of TILS with age, Nottingham grade, ER, PR, Ki67, molecular subtype. The host immune response, in addition to clinical and therapy characteristics, may influence the prognosis of cancer patients after routine treatment. The evaluation of TILs must be incorporated into daily histopathological reporting as indicated by many studies. TILs also play a role in predicting response to chemotherapy as well as pCR in post NACT cases. Studying TILs and its role in tumor immune response is vital in devising optimal immunotherapeutic strategies in the treatment of breast cancer. In a resource poor setting, evaluation of TILs on a single H and E slide, is an ideal inexpensive as well as reliable method.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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