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KEY WORDS: Breast cancer, Prognostic indicator, TILs - Tumor-infiltrating lymphocytes, ER: Estrogen receptor; PR: Progesterone receptor.

THE PROGNOSTIC INDICATOR OF TUMOR INFILTRATING LYMPHOCYTES IN BREAST CARCINOMA

A. Sindhu. S. Kumar	Final Year PG Student
Navya B. N	Professor
Rajesh B	Professor
Anjali Rao Kedige*	Assistant Professor *Corresponding Author

ABSTRACT

Introduction: Breast cancer is one of the common malignant tumors in women. The use of biomarkers ensures breast cancer patients receive optimal treatment. Tumor-infiltrating lymphocytes (TILs) are emerging as one of the key prognostic markers of breast cancer. **Objectives And Methodology:** Our study aims to evaluate percentage of TILs in tumor tissue, grading TILs based on the recommendations by an International TIL Working Group 2014 & compare with ER & PR status and in relation to concentration of TILs we assess prognosis of breast carcinoma for a period of 6 months. **Results:** Among 30 study subjects, TILs were of intermediate, high & lower grade in 53.3%, 40% and 6.7% cases respectively. TILs expression was statistically significant in both ER and PR, positive & negative groups ($p<0.05$). TILs were statistically highly significant as a prognostic marker in hormone negative breast cancers ($p<0.05$). **Conclusion:** Our study concludes that high TILs are correlated with favourable survival and response to treatment in breast cancer patients with ER, PR negative molecular subtype.

INTRODUCTION

In women, breast cancer is one of the most common malignant tumors with an estimated incidence of 2.3 million cases (11.7%) worldwide.[1,2] Among Indian women, breast cancer is number one among cancers; with the rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women.[3] In India, one woman is diagnosed with breast cancer every 4 minutes and one woman dies of Breast cancer every 8 minutes.[4]

The survival of the breast cancer patients, independent of therapy, can be predicted by prognostic markers which are indicators of aggressiveness, invasiveness, extent of spread of tumors, and these can be used to select patients at risk. The optimal therapy to the breast cancer patients can be ensured by the use of biomarkers. Significant role is being played by some of the established biomarkers like estrogen receptor (ER) and progesterone receptor (PR) in the selection and management of patients for endocrine therapy.[5]

In recent years, it has been shown that in breast cancer significant alterations are seen in the surrounding tumor microenvironment along with neoplastic cells, which are now recognized as a critical element for breast cancer progression, as well as potential therapeutic targets. One of the emerging key players in the tumor microenvironment are Tumor-infiltrating lymphocytes (TILs).[6] Among the TILs majority of them are T cells which can be divided into CD4+ helper cells, regulatory T cells with a CD4+, CD25+, FOXP3+ phenotype, and effector cells, such as natural killer cells and CD8+ T cells.[7,8]

Although valuable information about the tumor infiltrating lymphocyte in breast carcinoma is available, the value of TILs being a prognostic indicator in breast carcinoma is complex as there are numerous factors associated with the prognosis. TILs will help to uncover the important mechanisms of the interaction between tumor and host immunity.

According to Denkert C et al., the response to chemotherapy is dependent on the lymphocytic infiltrate as a continuous parameter in comparison of subgroups with different percentages of lymphocytes.[9] Breast cancers, if diagnosed at an early stage, can be managed in better ways.[5]

According to recent evidence, the response to therapy and improved prognosis can be predicted if TILs are present in breast cancer prior to treatment.[10] A strong association exists between extensive tumor infiltration by cytotoxic CD8+ T cells and patient survival and response to therapy.[11] And the presence of a lymphocytic infiltrate in cancer tissue is associated with the outcome.[9]

Thus, the present study was conducted with an aim to estimate the TILs related prognosis in patients with breast cancer irrespective of the molecular subtype.

OBJECTIVES

1. To evaluate the percentage of infiltrating lymphocytes in the tumor tissue
2. To study the prognosis of the breast carcinoma in relation to the concentration of TILs
3. To compare TILs with ER & PR status.

METHODOLOGY

This cross-sectional study was conducted after taking the Institutional Ethical Committee Clearance. The data was collected from histopathology laboratory of K.V.G medical college and hospital, Sullia. All histopathologically proven breast carcinoma cases reported in the year 2019 to 2021 were included in the study. The data was analyzed and correlated with hormone receptor (ER & PR) status. These patients were followed up post chemotherapy for a period of 6 months and were categorized as good and bad prognosis, good being completely cured from disease without recurrence and bad being morbid and recurred cases.

Inclusion Criteria

All histopathologically confirmed breast carcinomas irrespective of age, family history and molecular subtype were included.

Exclusion Criteria

Previously treated and recurred breast carcinoma cases were excluded

Method Of Data Collection

Data was collected based on recommendations by an International TIL Working Group 2014.

- The details of all the patients diagnosed as breast

carcinoma on histopathological examination were considered and slides were reviewed.

- Microscope with magnification of ×200–400 (ocular ×10, with objective of ×20–×40) was used for the tissue sections of 4–5 □m of thickness of formalin fixed paraffin embedded (FFPE) and stained with routine haematoxylin and eosin (H&E) stain.
- TILs were calculated in percentage for stromal component infiltration.
- TILs evaluated within borders of tumor invasion. All mononuclear cells (lymphocytes and plasma cells) were considered.
- TIL outside tumor borders, around Ductal Carcinoma in Situ, around normal lobules, at the site of previous core needle biopsies/FNAC and in areas of necrosis were not counted.
- Percentage of TILs were assessed by three pathologists separately & correlated our values and we graded them as Low (TILs: <10%), Intermediate (TILs: ≥10 and ≤40%) and High (TILs: >40%), keeping the study article by Sagado R et al as the reference. [11]
- The TILs grades were correlated with hormone receptors (ER & PR) status.

Statistical Analysis Of Data

The data was was entered in Excel sheet and was analyzed using SPSS software version-26. Categorical data was expressed in the form of frequency, proportion and graphs. Continuous/Discrete data was expressed in the form of mean and standard deviation (S.D). The data was subjected to chi-square test. P-values <0.05 were taken as statistically significant.

RESULTS

Patient And Demographic Characteristics

A total of 30 patients were included in the study with a median age of 55 years (age range, 40-75 years). Among 30 participants, 12 (40%) were aged between 51 – 60 yrs, 8 (26.7%) between 41 – 50 yrs, 5 (16.7%) between 61 – 70 yrs, 3 (10%) belonged to ≤40 yrs & 2 (6.7%) belonged to older age group (≥71) respectively.

Among the study subjects, 9 (30%) each were diagnosed with invasive breast carcinoma and invasive breast carcinoma with medullary features, 4 (13.3%) each with tubular carcinoma, and invasive ductal carcinoma, and 2 (6.66%) each were diagnosed with invasive lobular 66carcinoma and mucinous carcinoma respectively.

Relationship Between Til Expression And Pathological Characteristics

All patients were assessed for expression of stromal TILs at the site of invasion of tumor and graded as high, intermediate and low with their TIL expression being 12 (40%), 16 (53.3%) and 2 (6.7%) respectively.

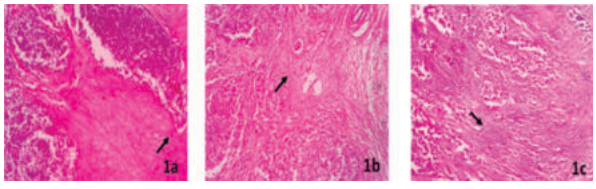

Figure 1: Percentage of stromal TILs at the site of tumour invasion. Fig 1a. Low-grade, 10%, Fig 1b. Intermediate-grade, 25%, Fig 1c. High-grade, 75%

The hormone status of all the patients was assessed for both estrogen receptor (ER) and progesterone receptors (PR). Among the 30 cases, 20 (66.7%) were hormone negative and 10 showed hormone positivity for both ER and PR.

In 10 hormone positive cases, the high, intermediate and low-grade TILs expression were seen in 1, 7 (majority of cases) and 2 cases respectively (p=0.017). This suggests that the lymphocyte infiltration could be intermediate to low grade in positive cases. Among 20 hormone negative cases, 11 and 9 cases show high and intermediate grade respectively (p=0.017) but none of the cases belong to low grade of TILs expression. Since majority of the cases show high to intermediate grade expression of TILs, we can correlate that it is one of the significant response in the surrounding tumour microenvironment. (Table 1)

Table 1: Comparison of TILs expression with ER, PR and Prognosis

Clinical variables	Response	TILs			Total	p-value
		Low grade	Intermediate grade	High grade		
ER	Positive	2	7	1	10	0.017
	Negative	0	9	11	20	*
PR	Positive	2	7	1	10	0.017
	Negative	0	9	11	20	*
Prognosis	Good	1	5	12	18	0.001
	Bad	1	8	0	9	5*

*p<0.05 - Statistically significant

3 Cases belonging to intermediate grade TIL were lost to follow up

Relationship Between Til Expression And Prognosis

Of the 30 patients followed up for prognosis, 3 patients belonging to intermediate grade TILs were lost to follow up.

We would like to emphasize through our statistics that the response to chemotherapy is dependent on the lymphocytic infiltrate as a continuous parameter, as significant number for patients, i.e. 12/12 with high TILs grade, showed good prognosis with majority of them being ER & PR negative (11/12). Among 13 intermediate grade TILs patients, that were followed up, 8/13 cases showed bad prognosis. Therefore, among the 27 cases that were followed up, 18/27 cases showed good response to chemotherapy and thus better prognosis, out of which 12/18 cases had high grade TILs (p=0.0015), among those 11/12 were negative for hormone receptor status. To infer there is significant correlation between concentration of stromal TILs with the hormone status and prognosis of the breast cancer patients. (Table 1)

Table 2: Results of univariate and multivariate survival analysis on the influence of clinic-pathological variables with TILs

Clinico-pathological variables	Univariate Analysis			Multivariate Analysis	
	Hazard ratio	CI 95%	p-value	Hazard ratio	p-value
ER Status					
Positive	Reference				
Negative	0.003	1.420 -97.154	0.023*	0.000	0.03*
PR Status					
Positive	Reference				
Negative	0.003	1.420 -97.154	0.002*	0.000	0.03*
Prognosis					
Bad	Reference				
Good	0.00	0.07 to 97.63	0.01*	0.245	0.1

*p<0.05 - Statistically significant

According to univariate and multivariate analysis when compared to hormone positive patients, the hormone negative patients showed good lymphocytic background & ultimately good prognosis. (Table 2)

Table 3: Results of multivariate survival analysis on the influence of clinic-pathological variables on grades of TILs

Clinicopathological variables	Multivariate

TILs	Likelihood ratio	CI 95%	p-value
High grade	Reference		
Intermediate Grade	17.40	6.58 to 6.89	0.003
Low grade	21.17	7.15 to 7.25	0.002

According to multivariate analysis, in comparison with high grades of TILs, low and intermediate grades show statistically significant values (p=<0.05). (Table 3)

DISCUSSION

The role of TILs has become a flair biomarker for predicting the survival in breast cancer patients. According to Gao Z et al., the assessed pooled hazard ratio (HR) values revealed that high TILs were having a favorable potential for overall survival and correlated with significantly improved disease-free survival.[12] Denkert C et al., conjectured that the release of certain tumor-associated antigens due to destruction of tumor cells by chemotherapeutic agents could set off an immune response directed against these tumor cells.[9] The study done by Gao Z et al., found similar result stating that rate of high TIL expression was significantly higher in the hormone negative group than in the positive group and reported that TIL expression was a prognostic factor in triple-negative breast cancer.[12] It is not clear that how T lymphocytes help in achieving good prognosis. However, according to studies conducted by Walser TC et al., and Ohtani H et al., the chemoattractant CXCL9, released from the T Lymphocyte is increased in tumor tissue serving the regulation of tumor growth and metastasis in animal models.[13,14] Tan W et al., reported that activation of RANK-expressing breast cancer cells through production of large amounts of RANK-L by Treg cells recruited by cancer cell promote metastasis and a worse prognosis in breast cancer is associated with high numbers of Treg.[15,6]

As our study depicted 8/13 intermediate grade TILs patients having bad prognosis, was supposedly justified by Pedoeem A et al., and Muenst S et al., that immune checkpoint molecules (programmed death-1 (PD-1) by binding to its ligands PD ligand (PD-L) 1 and PD-L2; cytotoxic T-lymphocyte antigen 4 or B- and T-lymphocyte attenuator) inhibit the function and duration of immune response by T-cell.[16,17] Blank C et al., in his study termed PD-L1 as 'molecular shield' that promotes tumor progression. Tumor cells express PD-L1, and this explains why tumor growth is rarely controlled in spite of the induction of cancer-specific T cells.[18]

According to Kurozumi S et al., survival among the ER-positive breast cancer patients was significantly better in the low-grade TIL group than in the high-grade TIL group.[19] and in such patients, a high TIL expression was a poor prognostic factor.[20] Similar to Heindl A et al., our study also showed frequent morphological TIL heterogeneity in ER-positive patients compared to ER-negative patients.[21] Thus it is understood that estrogen receptors play an important role in tumour immunosuppression.[22]

The univariate analysis using chi-square test significantly proved our study intent that TILs having effect on the prognosis of breast carcinoma patient in conjunction to their hormone status but yet in-detailed molecular studies on TILs and breast cancers are needed in order to improve the disease free survival status. Studies are needed (similar to CAR T-cell transfer therapy in lymphomas) which could help to know the ways to improve the stromal CD8-T lymphocytes prior to surgery and chemotherapy to prepare a disease free survival environment. Thus TILs are associated with longer breast cancer-specific survival, independent of other prognostic factors such as tumor grade, lymph node stage, tumor size, vascular invasion and HER2 status.

CONCLUSION

Many studies have proved that hormone positive breast cancers show good response to hormonal therapy.

Our study analysis confirmed that TILs are an ideal biomarker for favorable prognosis in hormone negative breast carcinoma. Hormone negative breast cancer has significant stromal lymphocyte response at infiltrating borders of tumour than hormone positive breast cancers. Also high TILs hormone negative carcinomas showed favorably good prognosis and response to chemotherapy in comparison with hormone positive high TILs breast carcinomas. Therefore, it is necessary to conduct more prospective clinical studies to clarify the true usefulness of TILs, independent of the other standard prognostic and predictive factors.

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