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Association Of Tumor Infiltrating Lymphocytes With Histopathological Parameters In Breast Cancer

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Abstract

Breast cancer remains a leading cause of cancer-related mortality among women worldwide. Tumor-infiltrating lymphocytes (TILs) have emerged as potential immunological markers for assessing prognosis and clinical outcomes in breast cancer. This study aims to evaluate the association between TILs and histopathological prognostic factors in primary breast cancer, following the International TILs Working Group's guidelines. A retrospective analysis was conducted on 100 breast cancer specimens collected at Sri Devaraj Urs Medical College between January 2018 and September 2023. Histopathological parameters, including tumor grade, necrosis, lymphatic invasion, vascular invasion, perineural invasion, axillary lymph node metastasis, Nottingham prognostic index (NPI), and stromal changes, were assessed alongside TILs quantification.

The results revealed a significant correlation between TILs and key prognostic factors such as tumor grade ($p=0.03$), necrosis ($p<0.0001$), NPI ($p=0.04$), and N stage ($p<0.0001$). High TIL infiltration was associated with Grade 1 tumors, lower NPI categories, and decreased nodal metastasis. Additionally, TIL levels were inversely related to tumor necrosis and advanced N stages, indicating reduced immune response in more aggressive cancers. The findings suggest that TILs can serve as a cost-effective prognostic marker in resource-limited settings and hold potential for guiding immunotherapy strategies. Limitations include the lack of molecular subtype classification and neoadjuvant/adjuvant setting analysis, warranting further research to standardize TIL evaluation in breast cancer diagnostics.

Keywords

Breast cancer, tumor-infiltrating lymphocytes (TILs), histopathological prognostic markers, Nottingham prognostic index, tumor grade, immune response, lymph node metastasis.

INTRODUCTION

One of the top causes of cancer-related mortality among women globally is breast cancer. The immune system is crucial in the development and prognosis of various cancers[1] Human studies have showed significant association between presence of subsets of lymphocytes and clinical outcomes in different solid tumors. Because of their anti-tumor response, tumor infiltrating lymphocytes are considered promising and less expensive indicators for predicting clinical outcomes in patients with breast cancer. [2]

A growing concept of research suggests that the tumor microenvironment is crucial to the development, expansion, invasion, and metastasis of tumors. Tumor infiltrating lymphocytes have become possible key biomarkers for breast cancer prognosis and prediction.[3,4]

Neoplastic transformation modifies the tissue's regular structure and triggers immunological reactions that can get rid of early cancers. Neoplastic cell transformation can evade immune regulation in conditions where elimination is insufficient. The cancer immunoediting theory, which is backed by a substantial amount of experimental data and clinical evidence, has provided the best conceptualization of this process. [4,5]

Although several studies indicate that patients of more tumor infiltrating lymphocytes have prolonged patient survival and exhibit pCRs (pathological complete response), their function and relationship to other prognostic variables in breast cancer remains debatable. [2]

OBJECTIVE

To evaluate TILs in breast cancer according to recommendations by International TILs working group.

To determine the association of TILs with other histopathological prognostic factors in primary breast cancer.

MATERIALS AND METHODS

All breast cancer specimens received in the Department of Pathology, Sri DevarajUrs Medical College from January 2018 September 2023 were included in study. The following histopathological parameters like Tumor size, type, grade, necrosis, lymphatic invasion, vascular invasion, perineural invasion, axillary lymph node metastasis, Nottingham prognostic index and stromal changes were studied.

A semiquantitive assessment of grading of necrosis was done according to the
study done by Richards CH et al [10] and was grade as

1. Absent/ None
2. Focal (<10% of tumour area)
3. Moderate (10–30%)
4. Extensive (> 30%)

The stromal TILs were classified into 4 groups such as

1. No TILs
2. Upto 10% stromal TILs
3. 20-40% stromal TILs
4. 5-90% TILs according to recommendations by an International TILS working group 2014[11] and their association with each histopathological prognostic factors were analysed.

STATISTICAL ANALYSIS

All the data collected was entered into Microsoft Excel sheet and coded. All the quantitative variables are represented by mean and standard deviation.

The categorical variables were analysed using Chi-Square as a test of significance.

Data analysis was done using SPSS 22 version.

P value <0.05 is considered statistically significant.

RESULTS

A total of 100 cases were studied. About 53% of cases were in the age group >50 years and 47% were in the age group <50 years. There was equal distribution of cases on both right and left side. The maximum number of cases were in T2 stage (55%) followed by T3 stage (31%). Infiltrating ductal carcinoma was most commonest (88%). Skin invasion and lymphovascular invasion was seen in 14% and 17% respectively. Grading of necrosis was done according to a study by Richards CH et al [10] and 28% of cases had Focal, 17% had moderate and 38% had extensive necrosis. In the study, 13% of cases had positive margins and 87% were

negative. Nearly 47% of patients were in N0 stage, 30% were in N1 stage, 14% were in N2 stage and 9% were in N3 stage. Perineural invasion was seen in 3% of the cases. Nottingham prognostic index was studied in the study subjects and 10% had NPI score between 2 to 2.4, 26% had NPI between 2.4 to 3.4, 42% had NPI between 3.4 to 5.4 and 22% of cases had NPI >5.4.

Staging of the tumor was categorised and 4% of cases were in Stage I, 2% were in Stage IA, 30% were in Stage IIA, 26% were in Stage IIB, 11% were in Stage IIIA, 8% were in Stage IIIB, 9% were in Stage IIIC and 10% were in Stage IV.

On further analysis, there was a statistically significant correlation between Grade of the tumor, lymphovascular invasion, Nottingham prognostic index and nodal metastasis with TILs.

1. **ASSOCIATION OF TUMOR GRADE WITH TILs**

TUMOR INFILTRATING LYMPHOCYTES	TUMOR GRADE			TOTAL
	GRADE 1	GRADE 2	GRADE 3	
NO INFILTRATION	1	2	3	6
LOW TILs	12	18	7	37
INTERMEDIATE TILs	14	11	3	28
HIGH TILs	19	6	4	29
TOTAL	46	37	17	100
CHI-SQUARE TEST	**P = 0.03**	**SIGNIFICANT**		

This depicts that there was an association of High tumor infiltrating lymphocytes and grade 1 breast cancer

2. **ASSOCIATION OF NECROSIS WITH TILs**

TUMOR INFILTRATING LYMPHOCYTES	**NECROSIS**				**TOTAL**
	NOTIDENTIFIED	FOCAL	MODERATE	EXTENSIVE	
NO INFILTRATION	0	0	0	6	6
LOW TILs	3	7	7	20	37

INTERMEDIATE TILs	4	7	7	10	28
HIGH TILs	10	14	3	2	29
TOTAL	17	28	17	38	100
CHI-SQUARE TEST	**P =< 0.0001**	**SIGNIFICANT**			

3. **ASSOCIATION OF NOTTINGHAM'S PROGNOSTIC INDEX WITH TILs**

TUMOR INFILTRATING LYMPHOCYTES	NOTTINGHAM'S PROGNOSTIC INDEX				TOTAL
	CATEGORY 1	CATEGORY 2	CATEGORY 3	CATEGORY 4	
NO INFILTRATION	0	1	3	2	6
LOW TILs	2	5	16	14	37
INTERMEDIATE TILs	3	8	12	5	28
HIGH TILs	5	12	11	1	29
TOTAL	10	26	42	22	100
CHI-SQUARE TEST	P = 0.04	SIGNIFICANT			

There was a significant relationship between Nottingham's prognostic index and tumor infiltrating lymphocytes

4. **ASSOCIATION OF N-STAGE WITH TILs**

TUMOR INFILTRATING LYMPHOCYTES	**N CATEGORY**				**TOTAL**
	N0	N1	N2	N3	
NO INFILTRATION	1	2	2	1	6
LOW TILs	8	12	9	8	37
INTERMEDIATE TILs	12	14	2	0	28
HIGH TILs	26	2	1	0	29
TOTAL	47	30	14	9	100
CHI-SQUARE TEST	**P = <0.0001**	**SIGNIFICANT**			

There was significant relation between N stage of disease and tumor infiltrating lymphocytes. As stage of the disease progress, the tumor infiltrating lymphocytes decreases.

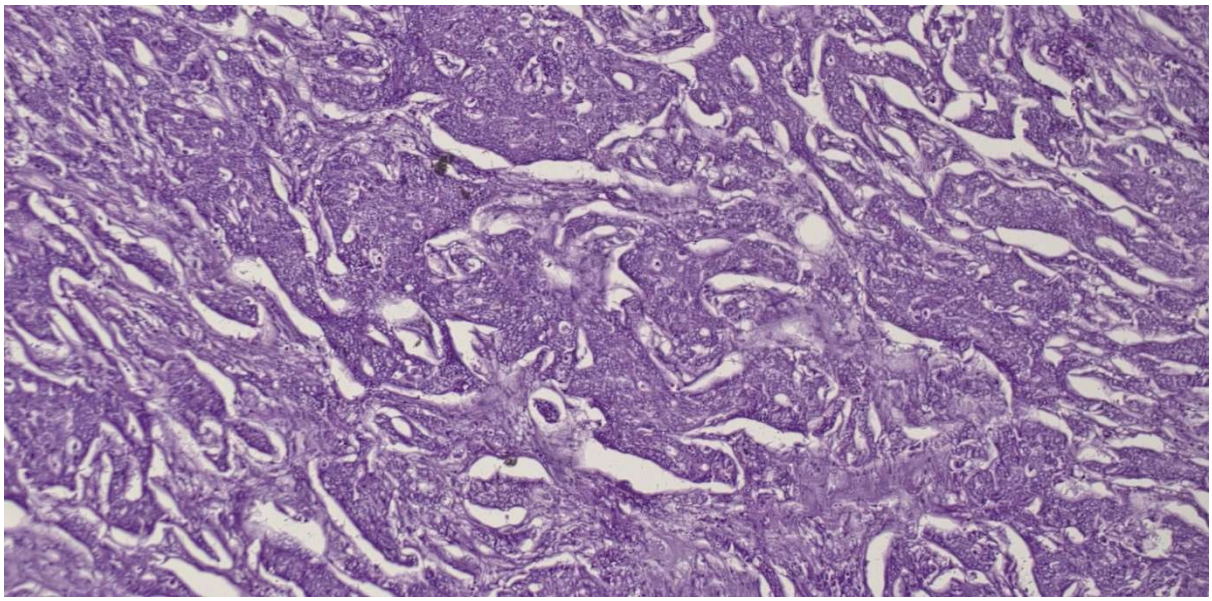

Figure 1: Microphotograph showing TILs in stroma, 0-10%(Low)

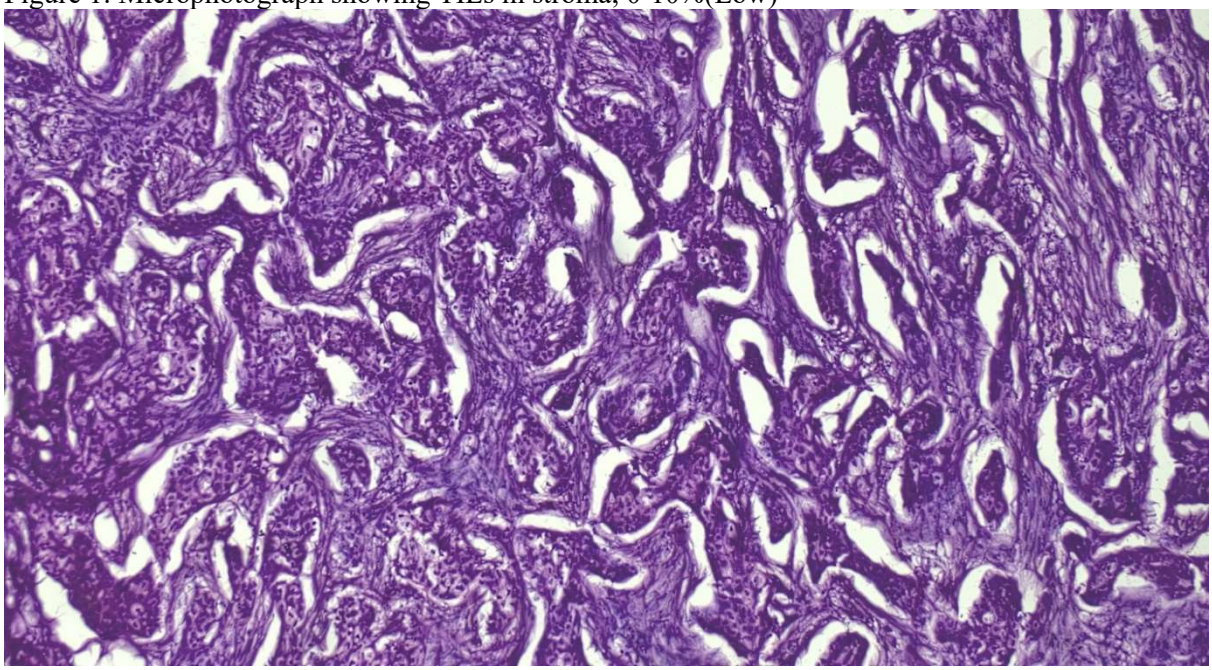

Figure 2: Microphotograph showing TILs in stroma, 10-40%(Intermediate)

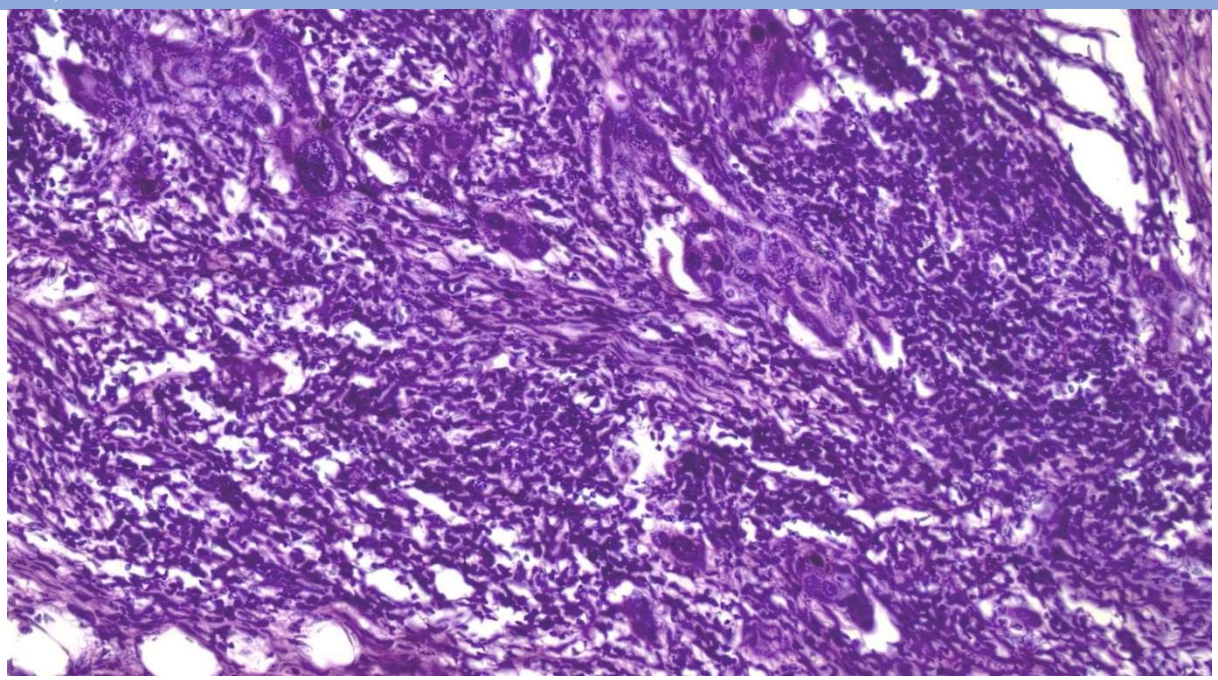

Figure 3: Microphotograph showing TILs in stroma, 40-90%(High):

DISCUSSION

While overall survival in cases of breast has improved with various therapies, recurrence and secondary metastasis remains a problem. Pathological parameters that can be used as a predictive and prognostic marker are required to make better treatment plans.[3]

Breast cancer is a heterogenous disease with different molecular subtypes and clinical outcomes[3]. An interaction between the malignant cells and the immune cells plays a pivotal role in control and elimination of cancer[9] . Several studies have examined the predictive significance of TILs in breast cancer, with varying degrees of success. The primary reason for this would be the low repeatability of the TILs assessment. According to several studies. there is a strong association between the assessment of intra-tumoral and stromal TILs, Quantification of TILS in breast cancer helps significantly assessing the aggressiveness of the tumor and also in prognosis.

In the present study ,there was a significant association between grade of tumor, necrosis, Nottingham prognostic index and axillary lymphnode metastasis and tumor infiltrating lymphocytes. A meta analysis by Gao ZH et al also showed similar data in Asian and European studies with statistically significant correlation between histological grade and lymphnode metastasis with TILs. However there was also correlation between histological type and T stage which were contrary to the present study.[3]

		Bandopadhyay A et al				Present study			
		Stromal TILs			P Value	Stromal TILs			P Value
TILs		<10%	10-40%	>40%		<10%	10-40%	>40%	

Tumor size	≤ 5	28%	14%	8%	0.030*	26%	23%	20%	0.21
	> 5	10%	28%	12%		17%	5%	9%	
Lymph node metastasis	present	20%	22%)	14%	0.608	34	16	3	<0.0001
	Absent	18%	20%	6%		9	12	26	
Grade	1	0%	2%	0%	0.309	13	14	19	0.04
	2	10%	18%	12%		20	11	6	
	3	28%	22%	8%		10	3	4	
Necrosis	Positive	26%	24%	14	0.86				<0.0001
	Negative	12%	16%	8%		10%	11%	24%	
LVI	Posit	20%	18%	6%	0.5	8%	4%	5%	0.89

	ive				01				
	Negative	18%	24%	14%		35	24	24%	
PNI	Positive	18%	22%	10%	0.951	1%	2%	0%	0.27
	Negative	20%	20%	10%		42%	26%	29%	

The frequency of high TIL levels seems to depend on the intrinsic subtype. TILs are more frequent in the aggressive subtypes of breast cancer as stated by Heppner et al.

While comparing the association of necrosis with TILs, in our study it was found that as degree of necrosis increases the TILs decreases. TILs in regions exhibiting significant central regressive hyalinization, crush artifacts, necrosis, and inflammation surrounding biopsy sites should remain unscored. A biopsy with necrosis is deemed unscorable as stated by Salgado et al.

As the N stage of the carcinoma increases there is a decrease in amount of TILs described in our study. Gao ZH et al showed no significant difference in lymphocyte predominance in lymph node metastatic and non lymph node metastatic group. But a subgroup analysis done based on region showed significant correlation in the American group, similar to our study results.

Nottingham's Prognostic index is based on size of tumor, tumor grade and lymph node metastasis. It quantifies the prognosis for the clinician and gives them an idea of what to expect and helps make a treatment plan accordingly. We have seen in this study that category 2 and 3 of Nottingham's Prognostic index (NPI) show decreased amount of TILs and there was a significant correlation between TILs and NPI. This is in keeping with the other findings of significant association of TILs with tumor grade and N stage.

	Angelico et al[11]		Present study	
	Node Positive	Node Negative	Node Positive	Node Negative
Low TILs	25.53%	12.77%	29%	8%
Intermediate TILs	Not observed	Not observed	16%	12%
High TILs	14.89%	46.89%	3%	26%
P Value	0.006		<0.0001	

The higher grade of tumor, presence of lymphovascular invasion, high score of Nottingham prognostic index

and involvement of lymphnode were associated with lesser infiltration by TILs. Considering these results of the present study less infiltration by TILs can be considered as a bad prognostic factor in breast carcinomas.

It has been demonstrated that TILs have prognostic and maybe predictive value, especially in triple-negative and overexpressed human epidermal growth factor receptor 2 breast carcinoma cases as stated by Salgado et al.

A limitation of this study is that we have not further classified breast cancer into molecular subtypes while analysing the association with TILs. Various studies have found that while TILs play a prognostic role in TNBC and Her2 positive breast cancers, it does not have a similar role in the luminal subtype. TILs are also considered to have different roles in a neoadjuvant and adjuvant settings.[6] Future studies can be planned to analyse these factors with respect to TILs, as more studies on the usefulness of TILs can help in incorporating it to all the future reports of breast cancer in a standardised form.

CONCLUSION

- Tumor-infiltrating lymphocytes (TILs) is an important immunological marker in Breast cancer. The present study showed that tumor grade, necrosis, Nottingham prognostic index and lymphnode status are associated with TILs. TILs assessment helps in prognostication in a limited resource setting. TILs also appear promising in further development of immune related novel therapeutic strategies.

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