Section: Pathology

Original Research Article

SIGNIFICANCE OF TUMOR INFILTRATING LYMPHOCYTE SCORE WITH TUMOR GRADE AND LYMPH NODE STATUS IN VARIOUS HISTOLOGICAL TYPES OF TRIPLE NEGATIVE BREAST CANCERS

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ABSTRACT

Background: Breast cancer is the 2nd most common malignancy among women in India with life time risk of 13%. They exhibit unique IHC profiles and histological subtypes that have special clinical course and outcome. Among all the breast malignancies Triple Negative Breast Carcinomas (TNBC) stand out for occurrence in younger women, having high mitotic index, higher grade, lymph node metastasis and poor survival. Recent studies have revealed the association of tumor infiltrating lymphocytes (TIL's) with disease outcome and treatment especially in TNBC's. Hence inclusion of TIL's Score or immunological grade in reports can serve as an indicator of antitumor immune response. **Aims and Objectives:** To study and evaluate histological types and grading of TNBCs, to quantify TILS scoring in various histological types of TNBCs, to study the association of histological grade and lymphnode status with TIL's in TNBCs, to evaluate the prognostic importance of TIL's scoring in TNBCs.

Materials and Methods: A retrospective analysis of all cases reported as invasive breast carcinomas in the department of pathology (i.e from June 2021 to May 2024) over a period of 3 years were collected. All triple negative cases were included in the study. Histological grading was done according to Nottingham scoring. Stromal TILS scoring was evaluated and classified as Low TILS (< 10%), Intermediate TILS (10 – 50%) and high TILS score (> 50%). The data was tabulated for statistical analysis.

Results: The total number of breast lesions over a period of 3 years were 260, among them 70 (27%) were breast malignancies. Out of 70, 31 cases (44%) belonged to triple negative breast carcinomas. The age of patients was ranging from 26 - 67 years, with the median age of 48 years. Fifty-nine percent (59%) of cases belonged to histological grade 2 and 41% of them belonged to grade 3. None of the cases belonged to grade 1 in TNBC. Thirty five percent (11 cases) belonged to low TILS, 12 cases (39%) belonged to intermediate TILS, 8 cases (26%) belonged to high TILS. In the present study histological grade and lymph node status are significantly associated with TIL's score where as tumor size is not significantly associated with TIL's score.

Conclusion: TILs represents a anti tumor T cell-mediated immunity and serves as an inexpensive, reproducible and reliable prognostic biomarker especially in TNBCs. Evaluating TIL's score in metastatic TNBCs also helps in identifying the patients who can respond to immunotherapy. Hence, incorporating TILs score into routine reporting will enable the clinician in making therapeutic decision for using PD-L1 inhibitors and in predicting the prognosis.

Keywords: Tumor, Lymphocyte Score, Lymph Node, Triple Negative Breast Cancers.

INTRODUCTION

Breast cancer is the second most common malignancy among women in India with life time risk of 13%.[1] They exhibit a wide range of morphological features, different immunohistochemical (IHC) profiles, unique histological subtypes that have special clinical course and outcome.[2] Triple negative cancers are defined as tumors that are negative for estrogen and progesterone receptors along with lack of over expression of HER2/neu when tested by immunohistochemistery or absence of its gene amplification when tested by FISH. They account for 15% of all breast cancer cases.[3] Triple negative breast cancers (TNBC) exhibit invasive ductal histology in more than 90% of cases, have high mitotic index, central necrosis, lymphocytic infiltration with pushing borders. They frequently have metaplastic elements and medullary or atypical medullary features.[4]

Among all the breast carcinomas TNBCs stand out for its occurrence in younger women, aggressive clinical course, higher grade and frequent lymph node metastasis.[5] Women with TNBC have poor survival compared with other breast cancers regardless of the stage of diagnosis.[6] They also have highest immunological potential and abundance of TILs in all the subtypes of breast carcinomas.[5]

Malignant cell and stromal cell interactions are critical during the growth and development of various types of cancers. Recent studies have revealed the association of tumor infiltrating lymphocytes (TIL's) with disease outcome and treatment especially in triple negative cases. Higher TIL's score can anticipate a favorable prognosis and response of neoadjuvant and chemotherapy.

Hence inclusion of TILS Score or immunological grade in reports can serve as an indicator of antitumor immune response of the tumor.[7]

Aims and Objectives

1. To study and evaluate histological types and grading of TNBCs.
2. To quantify TILS scoring in various histological types of TNBCs
3. To study the association of histological grade with TIL's in TNBCs
4. To study the association of lymph node status with TIL's in TNBCs
5. To evaluate the prognostic importance of TIL's scoring in TNBCs

MATERIALS AND METHODS

A retrospective analysis of all cases reported as invasive breast carcinomas in the department of pathology (i.e from June 2021 to May 2024) over a period of 3 years were collected. The cases were reviewed based on ER, PR and HER2 status.

Inclusion Criteria: The tumor samples which were showing ER-, PR- and HER2 negative status were identified as triple negative breast carcinomas (TNBCs) and included in the study.

Exclusion Criteria: Trucut biopsies and the cases treated with neoadjuvant therapy were excluded from the study.

The representative 5 mm thick H & E stained slides were reviewed based on architectural features along with clinical history. Histological grading was done according to Nottingham scoring.

For the evaluation of stromal TIL's the entire slide was scanned under low-power. Tumor hotspots were identified and focused on single high power (x400). According to International TILS working group, TIL's scoring was done within the borders of the invasive tumor. The stromal TILs were defined as mononuclear cells localized in the stromal tissue of breast carcinoma. Assuming the entire "stromal" area as 100%, the percentage of area occupied by lymphocytes in the stromal area was evaluated and classified as Low TILS (< 10%), Intermediate TILS (10 – 50%) and high TILS score (> 50%).

Data was tabulated and graphical representation was done using MS word and MS excel. Chi-square test was used to analyze the relationship between histological subtypes, histopathological grading, lymph node status, tumor size and TIL's scoring. P value (Probability that the result is true) of < 0.05 was considered as statistically significant after assuming all the rules of statistical tests.

RESULTS

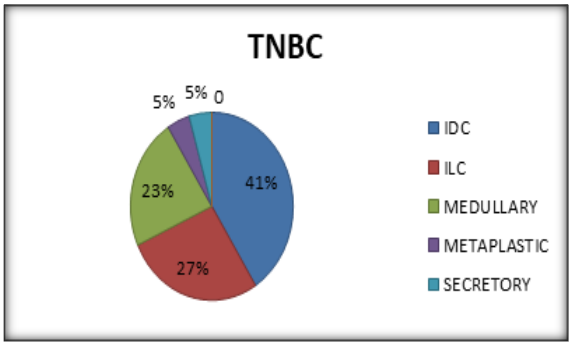

Figure 1: Histological subtypes of TNBCs

The total number of breast lesions over a period of 3 years were 260, among them 70 (27%) were breast malignancies. Out of 70, 31 cases (44%) belonged to triple negative breast carcinomas. The age of patients was ranging from 26 - 67 years, with the median age of 48 years. Fifty-nine percent (59%) of cases belonged to histological grade 2 and 41% of them belonged to grade 3. None of the cases belonged to grade 1 in TNBC. Thirty-five percent (11 cases) belonged to low TILS, 12 cases (39%) belonged to intermediate TILS, 8 cases (26%) belonged to high TILS.

Table 1: Distribution of TNBCs cases according to variables

Clinical variables	Frequency percentage (n=31)
Median age in Yrs	48
Tumor Size	
<5	25 (82%)
>5	6 (18%)
Histological subtype	
Infiltrative ductal carcinoma (IDC)	13 (41%)
Infiltrative Lobular Carcinoma (ILC)	9 (27.2%)
IDC with Medullary Pattern	7 (22.7%)
Metaplastic carcinoma	1 (4.5%)
Secretory carcinoma	1 (4.5%)
Histologic Grade	
Grade I	0
Grade II	18 (59 %)
Grade III	13 (41 %)
Lymphnode status	
Positive	14 (45%)
Negative	8 (25.8%)
Not available	9 (29%)
TIL's score	
Low	11(35%)
Intermediate	12(39%)
High	8(26%)

Table 2: TILS scoring in triple negative Infiltrative Ductal Carcinomas (IDC)

Sl. no	Age/sex	Size (cms)	Histological grade	Lymph nodes		TILS SCORE	
1	60Y/F	4.5 x 4 x 2	3	1/2	POSITIVE	< 10%	LOW
2	52Y/F	6 x 5 x 3	2	--	NA	60%	HIGH
3	54Y/F	4.5 x 3 x 2.5	2	0/19	NEGATIVE	> 50%	HIGH
4	65/F	7 x 2.5 x 2	3	5/16	POSITIVE	< 10%	LOW
5	43Y/F	7 x 3 x2.5	2	8/9	POSITIVE	10 – 20%	INTERMEDIATE
6	40Y/F	5 x 4 x 3	2	1/4	POSITIVE	70 %	HIGH
7	60Y/F	4.5 x 3 x 3	3	1/1	POSITIVE	< 10%	LOW
8	67Y/F	6 x 4 x 4	2	0/13	NEGATIVE	80%	HIGH
9	32Y/F	3 x 2 x 2	2	---	NA	30%	INTERMEDIATE
10	53/F	2.5 X 2.5 X 2	2	0/6	NEGATIVE	70%	HIGH
11	45/F	3 X 3 X 2.5	2	--	NA	40%	INTERMEDIATE
12	58/F	4.5 X 4 X 3.5	2	1/5	POSITIVE	20%	INTERMEDIATE
13	48/F	6 X 4 X 3.5	3	2/3	POSITIVE	<10%	LOW

Table 3: TILS scoring in triple negative Infiltrative lobular carcinoma (ILC)

Sl. no	Age/sex	Size (cms)	Histological grade	Lymph nodes		TILS SCORE	
1	50Y/F	3 X 2 X 2	2	--	NA	10 – 20%	INTERMEDIATE
2	45Y/F	3.5 X 3 X 2	2	8/12	POSITIVE	< 10 %	LOW
3	50Y/F	4 X 2 X 2	2	--	NA	20 %	INTERMEDIATE
4	32/F	5 X 3.5 X 3	3	0/4	NEGATIVE	<10 %	LOW
5	26/F	15 X 7 X 7	3	18/22	POSITIVE	<10 %	LOW
6	45/F	3.5 X 2.5 X 2	2	--	NA	30 %	INTERMEDIATE
7	46/F	7 X 5 X 5	3	6/10	POSITIVE	< 10 %	LOW
8	52Y/F	6.3 x 5 x 4.2	3	2/7	POSITIVE	< 10 %	LOW
9	37Y/F	3.3 x 3 x 2.9	2	0/2	NEGATIVE	40 %	INTERMEDIATE

Table 4: TILS scoring in triple negative invasive breast carcinoma of no special type with medullary pattern

Sl. no	Age/sex	Size (cms)	Histological grade	Lymph nodes		TILS SCORE	
1	32Y/F	3 X 2 X 2	3	12/12	POSITIVE	30%	INTERMEDIATE
2	35Y/F	3 X 2.2 X 1	2	0/4	NEGATIVE	70%	HIGH
3	30Y/F	3.5 X 2 X 2	3	0/11	NEGATIVE	60%	HIGH
4	33Y/F	10 X 5 X 5	3	1/1	POSITIVE	20%	INTERMEDIATE
5	45Y/F	4 X 4 X 3	3	--	NA	40%	INTERMEDIATE
6	42/F	4.4 X 4 X 3	2	0/8	NEGATIVE	80%	HIGH
7	48Y/F	5 X 4.6 X 4	3	0/1	NA	40%	INTERMEDIATE

Table 5: TILS scoring in triple negative metaplastic and secretory carcinomas

Histological type	Age/sex	Size (cms)	Histological grade	Lymph nodes		TILS SCORE	
METAPLASTIC CA	60Y/F	3.5 X 3 X 1	3	1/1	POSITIVE	< 10%	LOW
SECRETORY CA	53/F	5 X 3 X 3	2	--	NA	< 10%	LOW

Table 6: Distribution of cases in various histological types of TNBCs according to variables

Histological subtype (Total n=31)		IDC (n=13)	ILC (n=9)	Medullary (n=7)	Metaplastic (n=1)	Secretory (n=1)
Size	**< 5 cms**	7(54%)	5(56%)	5(71%)	1(100%)	--
	> 5 cms	6(46%)	4(44%)	3(29%)	--	1(100%)
Grade	**II**	9(69%)	5(55%)	2(29%)	--	1(100%)
	III	4(31%)	4(45%)	5(71%)	1(100%)	--
Lymph node status	**Positive**	7(54%)	4(45%)	2(29%)	1(100%)	--
	Negative	3(23%)	2(22%)	3(42%)	--	--
	NA	3(23%)	3(33%)	2(29%)	--	1(100%)
TIL's score	**Low**	4(31%)	5(55%)	**0**	1(100%)	1(100%)
	Intermediate	4(31%)	4(45%)	4(57%)	--	--
	High	5(38%)	**0**	3(43%)	--	--

Table 7: Association between Histological grade and TIL's score in TNBCs

	Histological Grade II	Histological Grade III	Total	P value
TIL's High	7	1	**8**	**0.006 (< 0.05)**
TIL's Intermediate	8	4	**12**	
TIL's Low	2	9	**11**	
Total	**17**	**14**	**31**	

In the present study histological grade is significantly associated with TIL's scoring with p value < 0.05.

Table 8: Association between Lymph node status and TIL's score in TNBCs

	Lymph node positive	Lymph node negative	Total	P value
TIL's High	1	6	7	**0.004 (< 0.05)**
TIL's Intermediate	4	1	**5**	
TIL's Low	9	1	**10**	
Total	**14**	**8**	**22**	

In the present study lymph node status was not available for 9 cases, hence only 22 cases were evaluated and they showed significant association with TIL's scoring with p value < 0.05.

Table 9: Association between Tumor size and TIL's score in TNBCs

	< 5 cms	> 5 cms	Total	P value
TIL's High	5	3	**8**	**> 0.05**
TIL's Intermediate	9	3	**12**	
TIL's Low	4	7	**11**	
Total	**18**	**13**	**31**	

In the present study tumor size is not significantly associated with TIL's scoring with p value > 0.05.

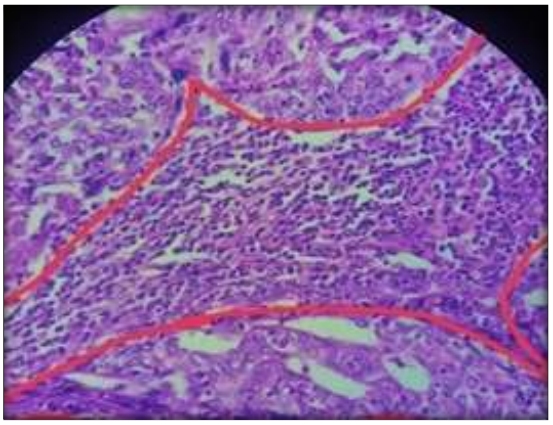

Figure 2: High TILS in IDC (H&E, X400)

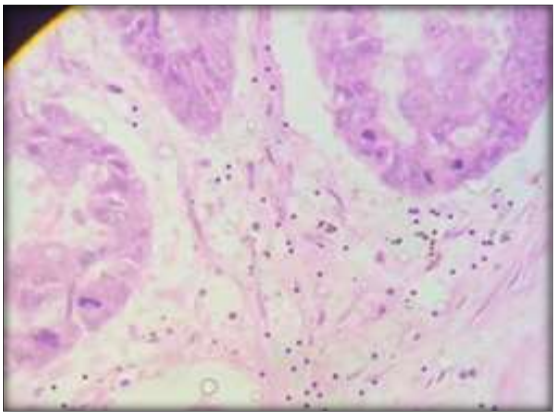

Figure 3: Intermediate TILS in IDC (H&E,X400)

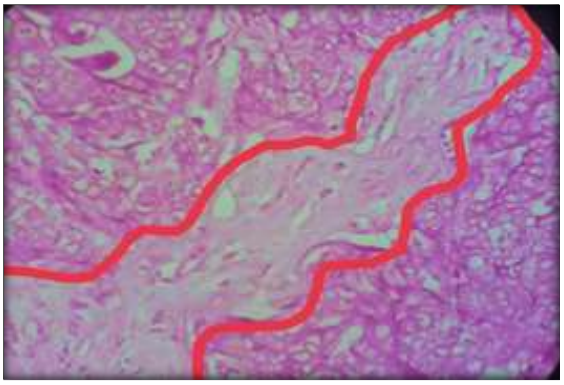

Figure 4: Low TILS in IDC (H&E, X400)

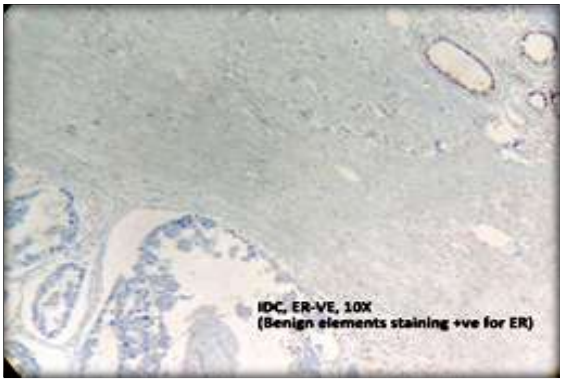

Figure 5: IDC, ER NEGATIVE (IHC, X100)

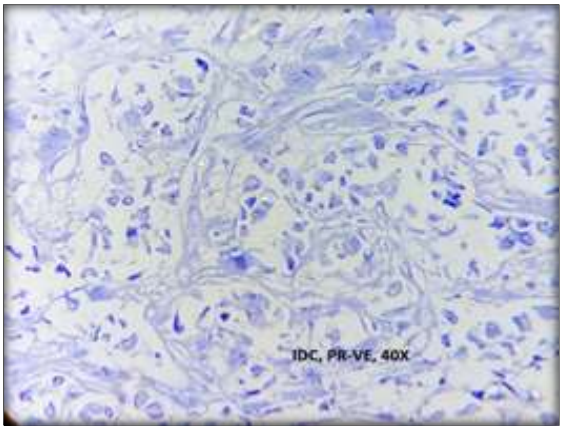

Figure 6: IDC, PR NEGATIVE (IHC, X400

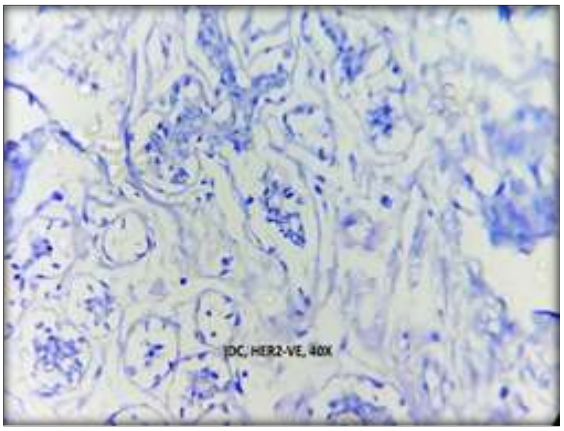

Figure 7: IDC, HER2 NEGATIVE (IHC, X400)

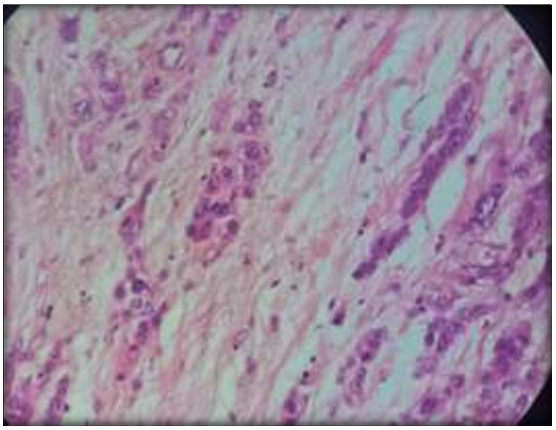

Figure 8: Low TILS in ILC (H&E, X400

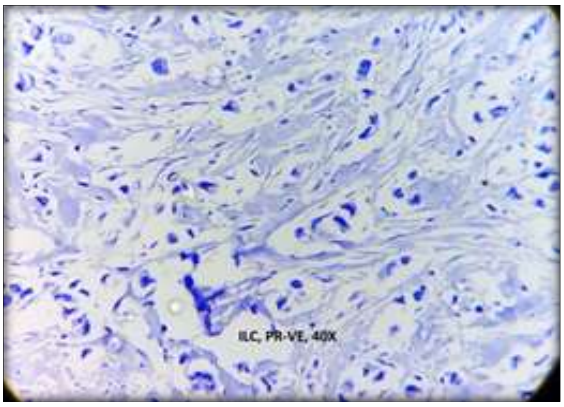

Figure 9: ILC, PR NEGATIVE (IHC, X400)

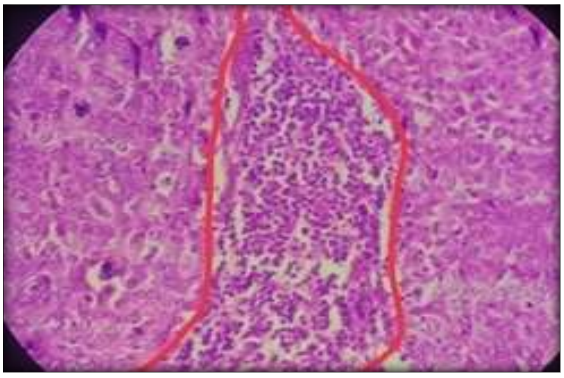

Figure 10: HIGH TILS in IDC WITH MEDULLARY PATTERN (H&E, X400)

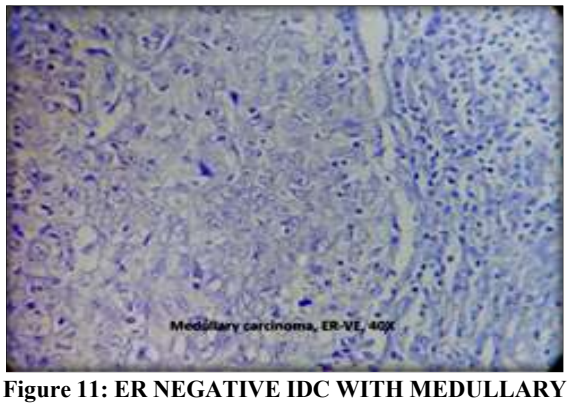

Figure 11: ER NEGATIVE IDC WITH MEDULLARY PATTERN (H&E, X400)

DISCUSSION

In patient with breast malignancies the use of immune check point blockage has revolutionized patient care and survival. The response to immunotherapy, chemotherapy and targeted therapies are predicted by the presence of TILS especially in triple negative breast cancers. TILS in the tumor microenvironment anticipate the anti tumoral host response and act as prognostic biomarker. Compared to hormone positive tumors, TNBC are frequently infiltrated by TILS. The TILs scoring in TNBC is demonstrably reproducible and correlates well with intra-epithelial TILs there by predicting the longer survival.[8]

In the study done by El Bairi et al they classified TILs into 3 categories: Immune-desert (devoid of lymphocytes), immune-excluded (lymphocytes are present in the peritumoral stroma only), and hot tumors (immune-infiltrated/inflamed).[8] International TILS working group (ITILWG) despite of suggesting TILS as continuous variable categorized TILS as <10%, 10 – 40% and > 40%. (9) In the present study we classified TILs as low (< 10 %), intermediate (10-50 %) and high (>50 %) as proposed by Hida and Ohi as this method is simple, reproducible and more applicable there by reducing the interobserver variations.[10]

According to many studies the average values of TILs in various subtypes of TNBCs was between 15 to 25%.[11] In the present study the average TILs score was 31%. Risk of relapses will be reduced by 13% in every 10% rise of TILS score in TNBC. The TILS score more than 20% is associated with excellent 5 year distant metastasis free survival in node negative TNBCs.[7]

Lower the TILs score, higher is the tumor mutation burden and genomic heterogeneity in TNBC.[8] Also larger the tumor size, positive nodes and advanced age are associated with low TILS score.[8] The significance of TILS in low grade TNBC and those with histological features having good prognosis is presently unknown.[8] Sawe et al, concluded that tumor grade and subtypes have no correlation with TILS.[12] Domingos TA observed high TILS score in basal-like immune activated molecular subtype of TNBC, intermediate in luminal androgen receptor, mesenchymal subtypes and low TILS in basal-like immune suppressed subtypes.[13] In the present study histological grade showed significant association with TIL's score, where as TIL's scoring is not significantly associated with tumor size.

TNBC displays the morphology of invasive breast malignancies with medullary features frequently.[11] WHO reclassified medullary carcinoma into invasive breast carcinoma of no special type with medullary pattern in the 5th Edition which is having high TILs component significantly contributing to the favorable outcomes.[14,15] Among 5 cases in the present study 3 cases belonged to intermediate TILS and 2 cases belonged to high TILS score.

TILS may have prognostic relevance in early metaplastic carcinomas.[16,17] In the present study we have one case of metaplastic carcinoma belonging to histological grade 3 with low TILS score.

Kurozumi et al proposed that high TILs score serve as positive prognostic marker in node negative cases than in node positive cases. Lymph node metastasis is inhibited by immune cells in the tumor micro environment. Programmed cell death protein-1 (PD-1) inhibits the activity of killer T cells. Hence PD-1 and PD-L1 inhibitors help in promoting the anti tumor immune effect by suppressing the immune check points and promoting tumor immune response.[9] TILs scoring in metastatic TNBCs helps in identifying the patients who have good chances of responding to immunotherapy.[5] Present study showed significant association between negative lymph node status and high TILs score.

CONCLUSION

TILs represents a anti tumor T cell-mediated immunity and serves as an inexpensive, reproducible and reliable prognostic biomarker especially in TNBCs. Evaluating TIL's score in metastatic TNBCs also helps in identifying the patients who can respond to immunotherapy. Hence, incorporating TILs score into routine reporting will enable the clinician in making therapeutic decision for using PD-L1 inhibitors and in predicting the prognosis.

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