Original Article

Prognostic significance of evaluation of tumor infiltrating lymphocytes in triple-negative breast cancer in residual disease postneoadjuvant chemotherapy

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Abstract

Background: Triple-negative breast cancer (TNBC) are highly heterogeneous tumors and are not eligible for hormonal therapies or human epidermal growth factor receptor type 2(HER 2)-targeted agents and they are associated with failure to achieve pathological complete response (pCR) and has unfavorable prognosis. In the residual disease (postneoadjuvant chemotherapy and surgery) of cases of TNBC, identification of parameters for risk stratification is needed for better identification of high-risk patients who require additional systemic treatments. Tumor-infiltrating lymphocytes (TIL's) are a part of the tumor microenvironment and they are indicator for monitoring immune response and they influence cancer growth, progression, and metastasis. With the success of immunotherapy in various cancers, there is an increasing interest in directly targeting the immune system in TNBC.

Material and Methods: In our study, 115 TNBC cases were taken and stromal TIL's were calculated on H and E stained slides and TIL grades (scoring according to International TIL's Working Group) were compared with clinicopathological parameters and overall survival.

Results: Statistically significant correlation was found between Stage of presentation, axillary lymph node positivity, relapse, metastasis, and TIL ($P < 0.0001$). High stage of the tumor, axillary lymph node positivity, cases of relapse, metastasis was associated with Low-grade TIL. High-grade TIL showed a good overall survival (100%) in comparison to intermediate grade TIL 2 (90%) and low-grade TIL 1 (52%).

Conclusion:TIL scoring in residual disease post neoadjuvant chemotherapy can help in the stratification of high-risk cases and can help in prognostication.

Keywords: Prognosis, triple-negative breast cancer, tumor infiltrating lymphocytes

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INTRODICTION

Neoadjuvant chemotherapy followed by surgery is the standard of care for locally advanced breast cancer and is increasingly used in the early stages of breast cancer to achieve a tumor down-staging and it improves the chance for breast conservation. Pathological complete response (pCR)

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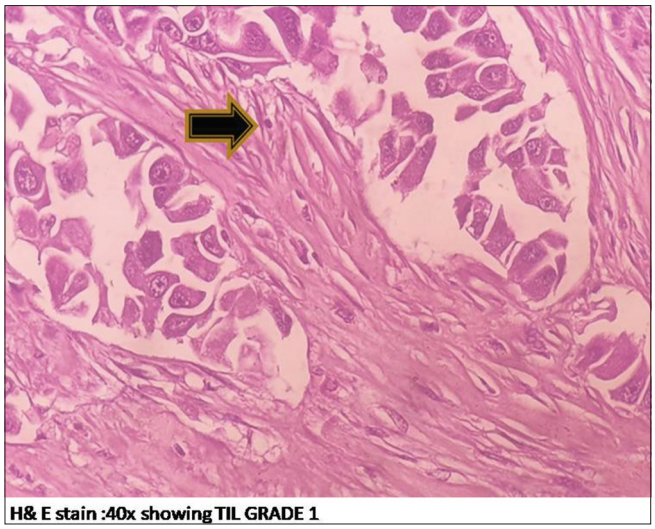

Figure 1: Tumor infiltrating lymphocyte Grade 1 (H and E, ×40)

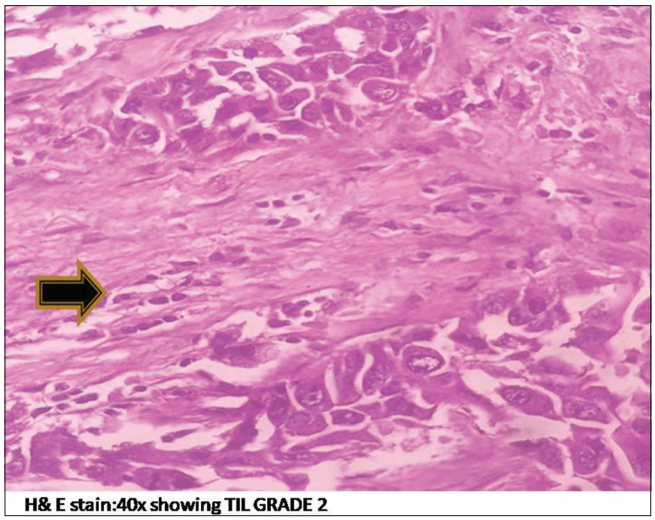

Figure 2: Tumor infiltrating lymphocyte Grade 2 (H and E, ×40)

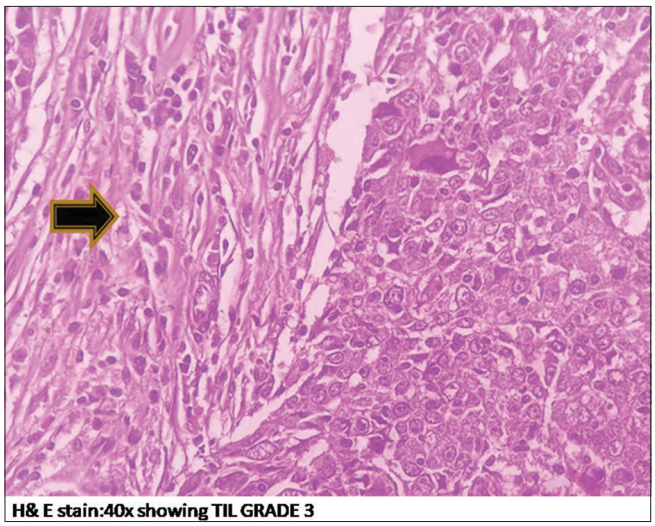

Figure 3: Tumor infiltrating lymphocyte Grade 3 (H and E, ×40)

is defined as the absence of invasive residual carcinoma in the breast and axillary lymph nodes after NACT. Only about 20% of breast cancer patients achieve pCR. Triple-negative breast cancer (TNBC) has a higher association between failure to achieve pCR and unfavorable prognosis.[1] In the residual disease (Post NACT and surgery) identification of parameters for risk stratification is needed to enable better identification of high-risk patients who require additional systemic treatments.

Tumor-infiltrating lymphocytes (TIL's) are a part of tumor microenvironment and they are an indicator for monitoring immune response and they influence cancer growth, progression and metastasis. The success of immunotherapies for melanoma and lung cancer has lead to its assessment in breast cancer.[2] TNBC is highly heterogeneous tumor and are not eligible for hormonal therapies or human epidermal growth factor receptor type 2 (HER2)-targeted agents. Due to the lack of specific targeted agents and the poor outcome of patients with TNBC, there is an increasing interest in directly targeting the immune system.[3]

Morphological evaluation of TIL's on H and E-stained sections is the preferred method to be used in daily practice as it is technically more feasible and can be routinely done. TIL's can often be found in the tumor stroma and within the tumor itself. Stromal TIL is found to be a superior and more reproducible parameter than intratumoral TIL in breast cancer especially in highly proliferative tumors like TNBC and HER2-positive cancers.[4]

A scoring system for TILs by the international TIL breast cancer working group was recommended in 2015 involving hematoxylin–eosin (HE) staining for routine practice.[4] We in our study aimed to investigate the utility of TILs using this scoring approach in post-NACT residual disease for better identification of high-risk patients who require additional systemic treatments.

Procedure

A retrospective, the single-institution study was conducted in Dr. B Borooah Cancer Institute, 115 cases of TNBC (estrogen receptor [ER], progesterone receptor [PR], and HER2 negative [ER negative PR negative and HER2 negative]) from 2015 to 2017 who received neoadjuvant chemotherapy and underwent surgery (Modified Radical Mastectomy) with residual tumor burden were included in the study., All HPE slides were reexamined for evaluation of TIL by two independent pathologists. TIL was analyzed and the percentage of Stromal TIL was reported. They were divided into 3 groups (According to the International TILS Working group):

- Low-grade TIL 1 (0%–10%) [Figure 1]
- Intermediate grade TIL 2 (11%–59%) [Figure 2]
- High grade TIL 3(≥60%) [Figure 3].

As all cases were post-NACT so TIL's were evaluated on the "residual tumor bed" which is defined as the largest cross-sectional area between residual invasive cells. TIL's were evaluated within the borders of the invasive tumor and TILs around DCIS, necrosis, normal lobules, areas of fibrosis, regressive hyalinization, and crush artifacts were excluded. All mononuclear cells (including lymphocytes and plasma cells) were scored, polymorphonuclear leukocytes are excluded.

Clinicopathological information such as age, tumor size, histological type, grade, lymphnode status, lymphovascular invasion (LVI), perineural invasion (PNI), extranodal extension (ENE), skin involvement, relapse, and metastasis status were collected and correlated with TIL. TIL was also compared with overall survival (Kaplan Meir Curve).

RESULTS

115 cases of TNBC (ER negative, PR negative, and human epidermal growth factor receptor type 2 (HER2 neu) negative] from 2015 to 2017 who received neoadjuvant chemotherapy and underwent surgery (Modified Radical Mastectomy) with residual tumor burden were included in the study. The mean age of presentation was 48 years with age ranging from 30 to 90 years. The average size of the tumor was 4.4 cm with the most common range of tumor size 2–5 cm. The most common Stage of the presentation was Stage II (56 cases) followed by Stage III (41 cases), Stage IV (16 cases) Stage I (2 cases). Statistically significant correlation was found between Stage of presentation and TIL ($P < 0.0001$). The high Stage of tumor was associated with low-grade TIL.

Histologically most of the cases were Infiltrating Duct Carcinoma (IDC) Grade III (71%) followed by IDC Grade II, Mucinous Carcinoma, Metaplastic Carcinoma. Ductal carcinoma *in situ* (DCIS) was present along with residual tumor in 9 cases of which 2 cases showed recurrence. All the cases with DCIS showed low-grade TIL in the residual tumor area. 60 cases showed axillary lymphnode positivity and out of which 56 cases showed low-grade TIL. There was a statistically significant correlation between low-grade TIL and axillary lymphnode positivity $P < 0.0001$.

ENE was seen in 7 cases out of which 6 cases showed Low-grade TIL. Relapse was seen in 23 cases and metastasis was seen in 25 cases. All the cases with relapse and metastasis had low-grade TIL. There was a statistically significant correlation between relapse, metastasis, and Low-grade TIL. However, no significant correlation was found between ENE and Low-grade TIL. The most common site of metastasis was lung (15 cases) followed by the liver (7 cases), bone, and brain in TNBC cases.

LVI was seen in 43 cases of which 37 cases showed Low-grade TIL. Skin invasion was seen in 7 cases, 6 cases showed Low-grade TIL. PNI was seen in 15 cases out of which 13 cases showed Low-grade TIL. However, no statistically significant correlation was found between LVI, skin invasion, PNI, and TIL.

Mean follow-up time was 29 months with follow-up time ranged from 12 to 48 months. TNBC showed an overall survival of 70%.The median survival time for TIL Grade I was 29 months, TIL Grade II was 45 months and TIL Grade III was 49 months. High-grade TIL showed a good overall survival (100%) in comparison to intermediate grade TIL 2 (90%) and low-grade TIL 1 (52%).

DISCUSSION

Post NACT TIL estimation is required for better identification of high-risk patients who require additional systemic treatments. Two recent studies have also indicated that higher TILs in post-NACT residual disease in TNBC is an important independent predictor of improved survival.[5,6] However, in HER2 positive disease, a study suggests an adverse prognostic role of high TILs in residual disease after NACT[7] Asano *et al*. suggest that the combination of the residual cancer burden (RCB) and TILs is a significant predictor for breast cancer recurrence after NACT[8] Recent data has showed that the presence of TILs after NACT in residual disease confers a good prognosis in an otherwise poor prognosis group.[9]

In a study by Stovgaard *et al*., the presence of TIL's in the tumor microenvironment was most prevalent in TNBC and HER2-positive breast cancer and they have been considered as a good prognostic indicator for breast cancer.[10] In another study by Li *et al*. TIL's were significantly associated with better overall survival and disease-free survival in triple-negative but not in ER–positive breast cancers.[11] Our study only TNBC cases were taken and high-grade TIL was associated with better overall survival and disease-free survival.

Morphological evaluation of TIL's on H and E-stained sections is the preferred method to be used in daily practice. Intratumoral TIL's are typically present in lower

numbers and detected in fewer cases and are difficult to observe on H and E stained slides. IHC is a must for intratumoral TIL's.[12] In a study by Adam *et al*., both stromal and intratumoral High-grade TIL were found to have good prognosis and has better cancer-free survival in TNBC patients who received adjuvant chemotherapy.[13] Loi *et al*. evaluated TILs in a series of 111 TNBC with RD after NACT. Lymphocytic infiltration in RD proved to be significantly and independently associated with both relapse-free survival and overall survival.[14] In an article by Dieci *et al*. patients with both a high RCB and low postchemotherapy TIL presented a 36% 5-year MFS.[15] In our study high TIL was associated with good overall survival.

CONCLUSION

High stage of the tumor, axillary lymphnode positivity, cases of relapse, metastasis are associated with Low-grade TIL. High-grade TIL showed a good overall survival (100%) in comparison to intermediate grade TIL 2 (90%) and low-grade TIL 1 (52%). Thus, TIL scoring in residual disease post neoadjuvant chemotherapy can help in the stratification of high-risk cases and can help in prognostication.

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Conflicts of interest
There are no conflicts of interest.

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