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ORIGINAL ARTICLE

Analysis the Role of Tumor-Infiltrating Lymphocytes as Prognostic Markers in Triple-Negative Breast Cancer - A Cross-Sectional Study

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ABSTRACT

Background: Triple negative breast cancer (TNBC) is an aggressive subtype that does not express hormone receptors or HER2, and therefore has few targeted treatment options. Tumor-infiltrating lymphocytes (TILs) have become established as promising prognostic markers of host immune response in TNBC.
Aim: To elucidate the immune landscape of TNBC to inform future immunotherapeutic strategies and maximize prognostic models in this challenging subtype of breast cancer.
Methodology: Using hematoxylin and eosin (H&E) staining, Formalin fixed paraffin embedded (FFPE) tissue slices from 79 patients with histologically confirmed triple negative breast cancer (TNBC) were retrospectively cross-sectionally analyzed. Tumor infiltrating lymphocytes (TILs) were evaluated in the stromal compartment in accordance with the International Immuno-Oncology Biomarker Working Group's standards. TILs were stratified as low (<10%), intermediate (10–49%), and high (≥50%). A total of clinical and pathological data (e.g. tumor size, nodal status, grade and survival) was collected and analyzed with SPSS. Chi square tests were used to examine associations between TIL levels and clinic pathological features and survival analyses were done by Kaplan–Meier and Cox regression methods.
Results: We found that 26.6% of TNBC patients had low TILs, 45.6% intermediate, and 27.8% had high TILs out of 79 TNBC patients. Small tumor size (p = 0.018), lower lymph node involvement (p = 0.027), and better tumor grade (p = 0.041) were significantly associated with high TIL levels. The results of Kaplan Meier analysis revealed higher DFS and OS in patients with high TIL (p=0.004 and p=0.011, respectively). High TILs were confirmed as independent predictors for favorable prognosis by multivariate Cox regression. Patients with low TILs were poor prognostic patients. These results suggest that TILs are a useful prognostic factor in TNBC.
Conclusion: Lymphocytes infiltrating into the tumor are higher in TNBC patients who have better survival outcomes. TIL assessment provides a useful, easily available prognostic tool for making clinical decisions
Keywords: Breast Cancer, prognostic tool, Modulators. Tumor, Immune Microenvironment

INTRODUCTION

Triple negative breast cancer (TNBC), which is characterized by the lack of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), accounts for 15–20% of all breast cancers. TNBC is linked to aggressive clinical behavior, early recurrence, and few treatment options[3]. Unlike hormone receptor positive or HER2 amplified subtypes, TNBC does not have targeted therapies and so chemotherapy is the main stay of systemic treatment[2]. However, despite this therapeutic constraint, a subset of patients with TNBC with favorable outcome behavior

raises interest for host immune responses as potential modulators in disease progression and therapeutic response[5,7].

Over the past few years, the tumor immune microenvironment has been recognized as a key determinant of cancer behavior and therapy response. One part of the tumor that has drawn a lot of interest for its prognostic and predictive potential in breast cancer and other cancers is tumor infiltrating lymphocytes (TILs)[6]. TILs consist mainly of cytotoxic $CD8^+$ T cells, helper $CD4^+$ T cells and regulatory T cells and are increasingly being recognized as endogenous anti-tumor biomarkers that not only reflect tumor immunogenicity but may also influence treatment efficacy[8].

Number of researches by different researchers shown that high levels of stromal TILs are prognostic for better overall survival (OS) and disease free survival (DFS) in TNBC and are therefore considered independent prognostic markers[4,9]. Additionally, the correlation between the presence of TILs and better responses to neoadjuvant chemotherapy lends support to their potential utility in determining patients for use in tailored therapeutic strategies[7,10]. However, TIL assessment in TNBC is further complicated by the fact that the immune landscape of TNBC is heterogeneous, that the spatial distribution, phenotypic composition, and functional orientation of TILs among patients is highly variable, and that TILs do not uniformly associate with prognosis[1,8].With these challenges, standardized methodologies for TILs evaluation, such as those proposed by the International Immuno-Oncology Biomarker Working Group, has allowed reproducible scoring systems in histopathological specimens which has enhanced the translational relevance of TILs in breast cancer management. However, context specific data as well as data from populations with high TNBC prevalence with limited resources for molecular profiling are still needed[11].

Furthermore, the increasing emphasis on immuno-oncology has brought into focus the possibility of TILs not only as static biomarkers but as dynamic players in the tumor-host interaction that can be therapeutically manipulated. In particular, the immune microenvironment may have a particularly crucial role in TNBC, where it often presents with high levels of genomic instability and neoantigen load relative to other breast cancer subtypes[12]. TNBC therefore represents an attractive immunogenic tumor type for using emerging immunotherapeutic approaches, such as immune checkpoint inhibitors, that have shown promise in clinical trials, especially in tumors with pre-existing immune infiltration. Therefore, it is critical to have a good understanding of the precise nature and prognostic relevance of TILs in TNBC not only to refine risk stratification and prognostication but also to determine which individuals have the best chance of responding to immunomodulatory therapy[10].

This cross sectional study of TNBC patients contributes to this developing area by complete observation of prevalence and relationship between TIL and clinical outcomes.In this cross sectional analysis, we are attempting to determine the prognostic role of TILs in TNBC within a defined patient cohort by integrating histopathological assessment with clinical outcome to validate their use as a biomarker in real world settings. This study aims to elucidate the immune landscape of TNBC to inform future immunotherapeutic strategies and maximize prognostic models in this challenging subtype of breast cancer.

MATERIALS AND METHODS

Study Design:

Current retrospective selection of 79 TNBC patients with invasive ductal carcinoma from different tertiary care hospitals and this was done as a cross sectional study. ER-/PR-/HER2- status and availability of FFPE tissue with adequate tumor and stroma were required to include. Data were excluded from patients receiving neoadjuvant therapy or patients with incomplete data.

Histopathological Evaluation and TIL Scoring:

Two independent pathologists who were blinded examined the slides after they had been stained with H&E. The International Immuno-Oncology Biomarker Working Group on Breast Cancer's standardized recommendations, TILs were assessed. Lymphocytic infiltration in the stromal compartment of the invasive tumor, outside the borders of necrosis or in situ carcinoma, was defined as stromal TILs, and stromal TILs were defined as the percentage of lymphocytic infiltration. Patient TIL densities were stratified into three groups based on TIL levels (low (< 10%), intermediate (10–49%), and high (≥ 50%)) and TIL levels were recorded as a continuous variable for statistical analysis. Discordant cases were reviewed in consensus and interobserver variability was minimized.

Clinical Data Collection:

Age at diagnosis, tumor size, histologic grade, lymph node status, treatment mode, and follow-up results were among the information obtained from the institution's electronic medical records. The main outcomes were OS and DFS, which were defined as the time between diagnosis and death from any cause or recurrence of the illness.

Statistical Analysis:

For all statistical studies, SPSS version [Insert Version, such as v25.0] was used. Continuous variables were summarized using means and standard deviations, whereas categorical variables were summarized using frequencies and percentages. TIL levels were linked to clinic-pathological factors using chi-square or Fisher's exact testing, where applicable. The log-rank test was used to examine the differences across TIL groups after DFS and OS were converted into Kaplan-Meier survival curves. In order to evaluate the independent prognostic value of TILs after adjusting for potential confounders, Cox proportional hazards regression models were used. Statistical significance was assumed if value ($P< 0.05$).

Ethical Approval:

In compliance with the Declaration of Helsinki, the Institutional Review Board (IRB) gave its approval for this study. Informed agreement was not required because the study was retrospective and used anonymized patient data.

RESULTS

In total, we included 79 patients with histologically confirmed triple negative breast cancer (TNBC). Patients were diagnosed at a median age of 49 years (range: 28–72) and presented with high grade invasive ductal carcinoma in the majority. All cases were evaluable for tumor-infiltrating lymphocytes (TILs), which were markedly intertemporal. Tumors were scored based on standardized scoring (26.6% <10%, 45.6% 10–49%, and 27.8% ≥50%) of TIL density.

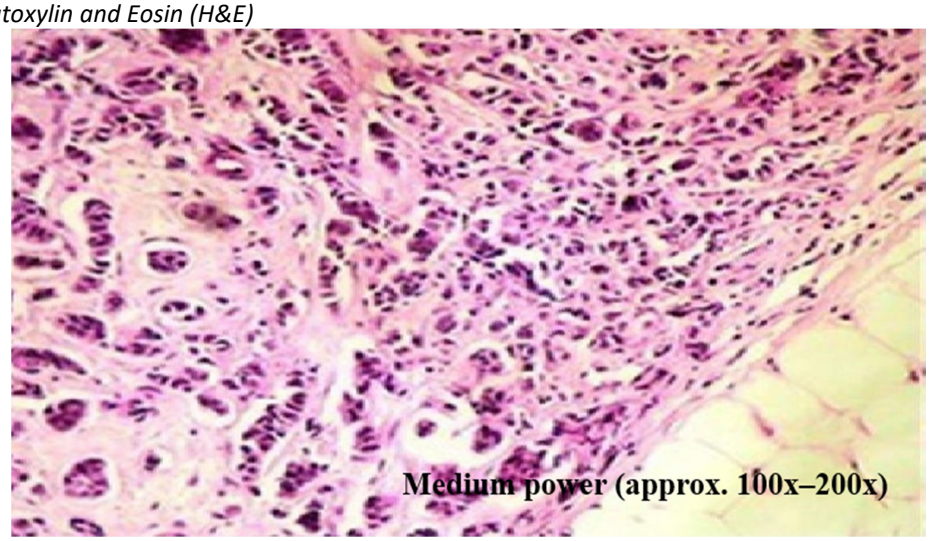
Smaller tumor size ($p = 0.018$), lower incidence of lymph node involvement ($p=0.027$), and histologic differentiation ($p=0.041$) were significantly associated with high TIL density. Survival analysis demonstrated that patients with high TILs had superior DFS and OS than those with low or intermediate TILs (log-rank $p = 0.004$ for DFS; $p = 0.011$ for OS). High TILs were an independent predictor of improved DFS (HR 0.42, 95% CI 0.19–0.91, $p = 0.029$) and OS (HR 0.47, 95% CI 0.22–0.98, $p = 0.044$) in multivariate Cox regression models adjusted for tumor size, nodal status, and grade.

Table 1. Association of Tumor-Infiltrating Lymphocyte (TIL) Levels with Clinico-pathological Features and Survival Outcomes in TNBC Patients (n = 79)

Variable	Low TIL (<10%) (n=21)	Intermediate TIL (10–49%) (n=36)	High TIL (≥50%) (n=22)	*p*-value
Age (mean ± SD)	51.2 ± 8.6	49.6 ± 9.2	47.3 ± 7.9	0.221
Tumor Size ≤2 cm (%)	19.0%	33.3%	54.5%	0.018
Lymph Node Positive (%)	61.9%	44.4%	27.3%	0.027
Histologic Grade III (%)	85.7%	69.4%	50.0%	0.041
5-Year Disease-Free Survival	57.1%	72.2%	86.4%	0.004
5-Year Overall Survival	61.9%	75.0%	90.9%	0.011

Fig-1. Histological Section of Triple-Negative Breast Cancer (TNBC) with Prominent Tumor-Infiltrating Lymphocytes (TILs)

Stain Used: *Hematoxylin and Eosin (H&E)*



This was a histological section of invasive ductal carcinoma, a triple negative breast cancer. The image shows malignant epithelial cells arranged in irregular nests and cords in a desmoplastic stroma on the left side. Characteristic of high grade carcinoma are the pleomorphic nuclei and prominent nucleoli of the tumor cells. Dense infiltration of small, round, darkly stained mononuclear cells in the stromal compartment within the central and right portions of the image is characteristic of tumor infiltrating lymphocytes (TILs.) Instead, these lymphocytes are found dispersed in the tumor microenvironment, not as follicle like aggregates. The intensity and distribution indicate high to moderate TIL score and an active host immune response. Clear spaces on the far right, suggestive of the invasive front of the tumor infiltrating or approaching adjacent fat, are present.

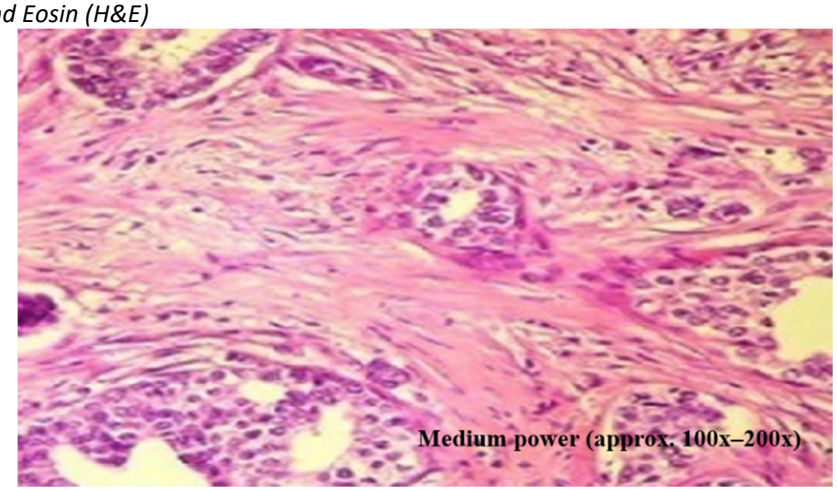
In fig-2 Invasive ductal carcinoma with characteristic malignant epithelial cells in nests and ductal-like structures set within a dense fibrotic stroma is shown in this section. Hyperchromatic, pleomorphic nuclei and moderately rich cytoplasm are features of the tumor cells. Tumor nests show partial glandular differentiation with some gland like luminal spaces in them.In comparison to the previous image, the stroma here is minimally to absent lymphocytic infiltration, and therefore a low TIL score (<10%). Malignant cells cluster and there is no evidence of tumor response mediated by immune cells, with fibrous connective tissue appearing dominant and separating clusters of malignant cells.

Interpretation:

These histological patterns are consistent with low immune infiltration triple negative breast cancer, which may be associated with a worse prognosis and poor immunotherapeutic response. This may mean that low TIL presence in the tumor microenvironment indicates an immunologically 'cold' tumor with poor antitumor immune surveillance.

Fig-2: Histological Section of Triple-Negative Breast Cancer (TNBC) with Low Tumor-Infiltrating Lymphocytes (TILs)

Stain: *Hematoxylin and Eosin (H&E)*



These findings highlight the prognostic significance of the immune microenvironment in TNBC, and in support of the incorporation of TIL quantification into routine pathological assessment to improve risk stratification and identify patients with favorable immune responsive phenotypes.

DISCUSSION

In addition, the present study highlights the prognostic relevance of tumor infiltrating lymphocytes (TILs) in triple negative breast cancer (TNBC), a biologically aggressive subtype of breast cancer without targeted therapeutic options. We analyze 79 histologically confirmed TNBC cases and found a strong correlation between high TIL density and good clinic-pathological characteristics (smaller tumor size, less nodal involvement and better histological differentiation). Elevated TIL levels were independently correlated with better DFS and OS, confirming them as a robust, immune based prognostic marker in TNBC[12].

This is consistent with and extends the current literature in which TILs have been consistently shown to be both prognostic and predictive biomarkers in TNBC. This is in agreement with the results of other previous large scale studies, including those of the International TILs Working Group and Meta analyses of clinical trial

cohorts, showing that increased stromal lymphocytic infiltration predicts enhanced tumor immunogenicity and better anti- tumor immune responses[13]. This is reinforced in our study in a resource limited clinical setting, where TIL quantification provides useful prognostic information despite an absence of sophisticated molecular profiling.

Our cohort shows the immune heterogeneity in TNBC with differential impact of TIL levels on survival outcomes. High TIL infiltration tumors are probably an 'inflamed' or immunologically 'hot' phenotype, in which pre-existing immune engagement could be further amplified by immunotherapeutic approaches, such as immune checkpoint blockade. On the other hand, poor prognosis of low TIL density may in fact represent an immunologically "cold" microenvironment with lack of antigen presentation, exclusion of effector lymphocytes, or active immunosuppression[14]. Such insights not only facilitate prognosis but also have direct therapeutic implications for the stratification of patients who may derive benefit from emerging immunomodulatory treatments[15].

Importantly, our findings also imply that TILs may be used as an accessible and inexpensive biomarker in routine pathology. In contrast to genomic assays or immune gene signatures, TIL evaluation can readily be integrated into standard histopathological workflows using H&E stained sections[17]. Due to their higher prevalence in low resource settings and limited access to advanced diagnostics, the use of TILs as a prognostic marker would be of great value in clinical decision making and counseling in patients with TNBC[18].

However, this study is not without limitations. Our findings may be limited by the retrospective design, single center data source, and relatively small sample size. In addition, TIL quantification was carried out according to standardized criteria, but a semi quantitate manual scoring process is used, which might be prone to inter observer variability[19]. Future studies may integrate digital pathology and machine learning based image analysis of TIL, which may increase the objectivity and reproducibility of TIL assessment. Furthermore, a more extensive immunophenotyping of the lymphocytic infiltrate (i.e., $CD8^+$ cytotoxic T cells vs. $FOXP3^+$ regulatory T cells) was not the focus of the present study but would provide important insights into the functional immune architecture of TNBC[20].

CONCLUSION

Lastly, we show that a positive prognosis for triple negative breast cancer is independently correlated with high numbers of tumor infiltrating lymphocytes.TIL assessment is a practical and impactful biomarker that has the potential to refine prognostic models and individualize treatment strategies especially in resource constrained health care environments. Future prospective studies studying the immune phenotyping and response to immunotherapy of TILs in the setting of TNBC are needed to further understand the mechanistic basis and clinical utility of TILs in TNBC.

DECLARATION

Availability of data:
The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest:
No conflict of interest associated with the research, authorship and publication of this article.

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There has been no significant financial support for this work that could have influenced its outcome.

Authors contribution:
Each author of this article fulfilled following Criteria of Authorship:

1. Conception and design of or acquisition of data or analysis and interpretation of data.
2. Drafting the manuscript or revising it critically for important intellectual content.
3. Final approval of the version for publication.

All authors agree to be responsible for all aspects of their research work.

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