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PROGNOSTIC SIGNIFICANCE OF TUMOR INFILTRATING LYMPHOCYTES IN RELATION TO NOTTINGHAM PROGNOSTIC INDEX IN BREAST CARCINOMA

Histopathology

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ABSTRACT

Breast cancer remains the most common malignancy affecting women worldwide, and accurate prognostic assessment is essential for guiding treatment decisions. The Nottingham Prognostic Index (NPI) is widely used for prognostic stratification based on tumor size, lymph node status, and histological grade. Recently, attention has focused on the tumor immune microenvironment, particularly tumor-infiltrating lymphocytes (TILs), which may provide additional prognostic information. This study aimed to evaluate stromal TILs in invasive breast carcinoma and analyze their association with NPI categories. This combined retrospective and prospective study included 62 cases of invasive breast carcinoma diagnosed between 2023 and 2025 at a tertiary care center. Stromal TILs were assessed on hematoxylin and eosin-stained s8697ections following International TILs Working Group recommendations and categorized as low (0–10%), intermediate (11–40%), or high (>40%). NPI scores were calculated using tumor size, lymph node status, and histological grade. Among the cases, 45.2% showed low TIL levels, while 30.6% showed high levels. Higher TIL levels were strongly associated with favorable NPI groups and showed a significant negative correlation with NPI scores. Receiver operating characteristic analysis demonstrated excellent discriminatory ability of TILs. Higher stromal TILs are associated with better prognosis and may serve as a useful adjunct in breast carcinoma evaluation.

KEYWORDS

Nottingham Prognostic Index, Prognostic biomarkers, Tumor infiltrating lymphocytes, Tumor microenvironment

INTRODUCTION

Breast cancer is the most commonly diagnosed malignancy among women worldwide and remains a leading cause of cancer-related mortality. In 2020, the World Health Organization's GLOBOCAN database reported approximately 2.3 million new cases, accounting for 11.7% of all cancer cases, and causing an estimated 685,000 deaths globally [1]. In India, the burden of breast cancers continues to rise, with increasing incidence reported across both urban and rural populations [2]. Breast cancer is a biologically heterogeneous disease exhibiting wide variation in morphology, molecular characteristics, clinical behaviour, and response to therapy, which necessitates accurate prognostic stratification [3].

Prognostication in breast cancer traditionally relies on established clinicopathological parameters such as tumor size, lymph node status, histological grade, and hormone receptor expression [4]. Among composite prognostic tools, the Nottingham Prognostic Index (NPI) is one of the most widely validated and routinely used systems for estimating survival and guiding clinical decision-making [5].

Despite the continued utility of Nottingham Prognostic Index, increasing evidence suggests that tumor behaviour is not solely determined by intrinsic characteristics of malignant epithelial cells but is also profoundly influenced by the surrounding tumor microenvironment [6]. The tumor microenvironment comprises immune cells, stromal cells, extracellular matrix components, and signaling molecules that collectively regulate tumor growth, invasion, and metastasis [7]. Among immune components, tumor-infiltrating lymphocytes (TILs) have emerged as a key biomarker reflecting the host anti-tumor immune response and has been shown to possess prognostic significance in breast cancer [8].

Tumor-infiltrating lymphocytes are lymphoid cells that migrate from the peripheral circulation into tumor tissue and accumulate within the tumor stroma and tumor nests [9]. High levels of stromal TILs have been consistently associated with improved disease-free survival and overall survival in breast cancer [10]. Stromal TILs have also demonstrated predictive value for response to chemotherapy and targeted therapies [11].

To standardize evaluation, the International TILs Working Group proposed a reproducible method for assessing stromal TILs on routine hematoxylin and eosin stained sections [12]. This method recommends reporting the percentage of stromal area occupied by mononuclear inflammatory cells within the borders of invasive carcinoma [13]. The simplicity and reproducibility of this approach make TIL assessment feasible in routine histopathology laboratories [14].

Although both Nottingham Prognostic Index (NPI) and tumor infiltrating lymphocytes (TILs) have been individually studied as prognostic indicators, fewer studies have explored their relationship within the same patient cohort. Understanding how the host immune response correlates with established measures of tumor aggressiveness may provide additional insight into prognostic stratification. In this context, the present study was undertaken to assess stromal TIL levels in breast carcinoma and to evaluate their association with clinicopathological features and the Nottingham Prognostic Index, using routinely available histopathological parameters.

METHODS

Study design , Study setting and Study population

This observational study employed a combined retrospective and prospective design to assess the prognostic relevance of stromal tumor-infiltrating lymphocytes (TILs) in invasive breast carcinoma. The study was conducted in the Department of Pathology at a tertiary care referral center, between January 2023 and March 2025. A total of 62 histopathologically confirmed cases of invasive breast carcinoma were included. Cases were selected based on the availability of adequate formalin-fixed paraffin-embedded tumor tissue for TIL assessment, complete clinicopathological data necessary for calculation of the Nottingham Prognostic Index, and sufficient pathological material for histological evaluation. Patients with pure ductal carcinoma in situ, those who received neoadjuvant chemotherapy, core needle biopsy or lumpectomy specimens, and stromal-predominant breast tumors were excluded from the study.

Resected specimens were fixed in 10% neutral buffered formalin, routinely processed, and embedded in paraffin. Sections of 4–5 µm thickness were prepared and stained with hematoxylin and eosin for microscopic examination.

Assesment of Stromal tumor-infiltrating lymphocytes

Stromal TILs were evaluated on hematoxylin and eosin-stained sections according to the recommendations of the International TILs Working Group (2014). Assessment was restricted to the stromal

compartment within the borders of the invasive tumor, and stromal TILs were defined as the proportion of mononuclear inflammatory cells present within the intratumoral stromal area. The percentage of stromal TILs was recorded as a continuous variable.

Calculation of Nottingham Prognostic Index (NPI)

Tumors were graded using the Modified Scarff-Bloom-Richardson (Nottingham) grading system, in which tubule formation, nuclear pleomorphism, and mitotic count were individually scored from 1 to 3, and the aggregate score was used to assign Grade I, II, or III. Lymph node status was categorized based on the number of metastatic lymph nodes, with score 1 assigned for node-negative cases, score 2 for one to three positive nodes, and score 3 for four or more positive nodes. The Nottingham Prognostic Index was calculated using the standard formula: NPI = (0.2 × tumor size in cm) + histological grade + lymph node score. Patients were stratified into prognostic groups based on NPI score as good (<3.4), moderate (3.41–5.4), and poor (>5.4) prognosis.

STATISTICALANALYSIS

Performed using the Statistical Package for the Social Sciences (SPSS) software, version 47.0. Associations between stromal tumor-infiltrating lymphocyte percentages and Nottingham Prognostic Index categories were evaluated using the chi-square test. Correlation between continuous variables was assessed using Spearman's rank correlation coefficient. Receiver operating characteristic curve analysis was performed to determine the predictive performance of TIL levels in identifying adverse prognostic groups. A *p value* < 0.05 was considered statistically significant.

The study protocol was approved by the Institutional Ethics Committee of Osmania Medical College. For the prospective component, informed consent was obtained from patients in accordance with institutional guidelines, and confidentiality of patient data was maintained throughout the study

RESULTS

A total Sixty-two cases of histopathologically confirmed breast carcinoma were included in the study. The mean age was 54.44 ± 12.31 years (median: 52.5 years), and 96.8% of patients were female. Invasive ductal carcinoma (no special type) was the predominant histological subtype (88.7%), followed by invasive lobular carcinoma (8.1%). Tumor size ranged from 1 to 17 cm (mean: 4.27 ± 2.64 cm), with 67.7% of tumors measuring between 2 and 5 cm. According to the Modified Scarff Bloom Richardson grading system, 53.2% of tumors were Grade II, 40.3% were Grade I, and 6.5% were Grade III. Lymph node metastasis was identified in 48.4% of cases, while 51.6% were node-negative.

The mean stromal tumor-infiltrating lymphocyte (TIL) percentage was 25.60 ± 23.96%. Based on category distribution, low TIL levels were observed in 45.2% of cases, intermediate levels in 24.2%, and high TIL levels in 30.6%. The mean Nottingham Prognostic Index (NPI) value was 4.47 ± 1.57, with 37.1% of cases classified as good prognosis, 24.2% as moderate prognosis, and 38.7% as poor prognosis.

The association between stromal TIL category and MSBR grade was analysed using the Chi-square test. No statistically significant association was observed between TIL category and histological grade (p = 0.618) (Table 1).

A highly significant association was observed between stromal TIL category and NPI prognostic group ($\chi^2 = 53.737$, $p < 0.001$; Cramer's V = 0.855). High TIL tumors were predominantly associated with the good prognostic group, whereas low TIL tumors were largely seen in the poor prognostic group (Table 2).

Table 1: Association between stromal tumor-infiltrating lymphocyte (TIL) category and Modified Scarff Bloom Richardson (MSBR) histological grade in breast carcinoma (n = 62)

TIL category	Grade I	Grade II	Grade III	Total
High	6	12	1	19
Intermediate	6	7	2	15
Low	13	14	1	28
Total	**25**	**33**	**4**	**62**

Table 2: Association between stromal tumor-infiltrating lymphocyte (TIL) category and Nottingham Prognostic Index (NPI) prognostic group in breast carcinoma (n = 62)

TIL category	Good	Moderate	Poor	Total
High	19	0	0	19
Intermediate	2	13	0	15
Low	2	2	24	28
Total	**23**	**15**	**24**	**62**

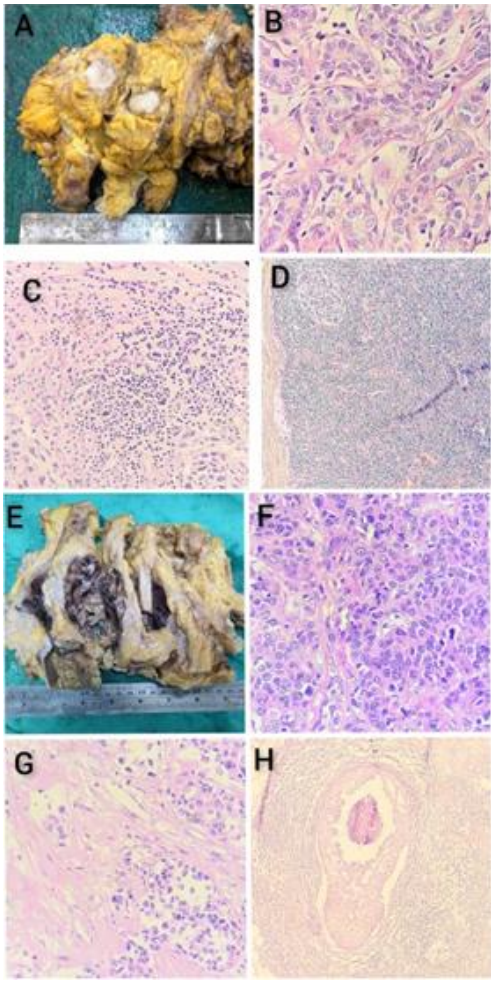
Comparison of NPI values across TIL categories showed significant differences (Kruskal–Wallis test, $p < 0.001$). Tumors with low TIL levels demonstrated higher NPI values, while tumors with high TIL levels showed lower NPI values.

Spearman correlation analysis demonstrated a strong negative correlation between stromal TIL percentage and NPI value ($\rho = -0.838$, $p < 0.001$). Receiver operating characteristic analysis showed excellent discrimination of the poor prognostic NPI group by stromal TIL percentage (AUC = 0.964).

Representative histopathological images illustrating the relationship between stromal tumor-infiltrating lymphocyte (TIL) density and prognostic status are shown in Figures 1 . Figure 1 (A-D) demonstrates a case with high stromal TILs associated with a low Nottingham Prognostic Index (NPI) corresponding to the good prognostic group, whereas Figure 1 (E-H) shows a case with low stromal TILs associated with a high NPI corresponding to the poor prognostic group.

Figure 1

A-D. Low Nottingham Prognostic Index with high stromal tumor-infiltrating lymphocytes: Representative case showing low Nottingham Prognostic Index (NPI) with high stromal tumor-infiltrating lymphocytes (TILs). (A) Gross specimen demonstrating a tumor measuring 2.1 × 2 × 1.8 cm. (B) Histologic features showing well-formed tubules (>75%, score 1), moderate nuclear pleomorphism (score 2), and mitotic count of 3/10 high-power fields (score 1), giving a Modified Bloom–Richardson Score (MBRS) of 4/9 (Grade I). (C) Stromal TILs >40%, corresponding to the high TIL category. (D) Absence of lymph node metastasis. The calculated NPI was 2.42, corresponding to the good prognostic group.



E-H. High Nottingham Prognostic Index with low stromal tumor-infiltrating lymphocytes: Representative case showing high Nottingham Prognostic Index (NPI) with low stromal tumor-infiltrating lymphocytes (TILs). (E) Gross specimen demonstrating a tumor measuring 7 × 5 × 4.8 cm. (F) Histologic features showing poorly formed tubules (<10%, score 3), marked nuclear pleomorphism (score 3), and mitotic count of 12/10 high-power fields (score 2), giving a Modified Bloom–Richardson Score (MBRS) of 8/9 (Grade III). (G) Stromal TILs <10%, corresponding to the low TIL category. (H) Lymph node metastasis present (2/16 nodes). The calculated NPI was 6.4, corresponding to the poor prognostic group.

DISCUSSION

Breast carcinoma remains one of the most frequently studied malignancies worldwide because of its high incidence and considerable biological heterogeneity. Variations in tumor size, histological grade, nodal status and molecular characteristics contribute to differences in disease behaviour and clinical outcomes. In recent years, increasing attention has been directed toward the tumor microenvironment, particularly immune cells infiltrating the tumor stroma. Tumor-infiltrating lymphocytes (TILs) have emerged as important indicators of host immune response and may provide additional prognostic information beyond conventional clinicopathological parameters. The present study evaluated clinicopathological features, stromal TILs and the Nottingham Prognostic Index (NPI) in 62 histopathologically confirmed cases of breast carcinoma.

The clinicopathological profile observed in this cohort was broadly consistent with patterns reported in several Indian and international studies. Most patients were in the fifth to sixth decades of life, which corresponds to the commonly reported age distribution of breast carcinoma in India [15]. Tumor size demonstrated considerable variation, with most tumors measuring between 2–5 cm. Similar, findings have been reported in studies from developing countries where organized screening programmes are limited and patients frequently present after symptom onset [1]. Histologically, invasive ductal carcinoma of no special type was the predominant subtype in this study, consistent with global pathological distributions of breast cancer [16]. . Histological grading showed predominance of Grade II tumors, a pattern commonly reported in several clinicopathological series [17]

A major focus of the present study was the evaluation of stromal TILs as an indicator of the tumor immune microenvironment. Stromal TIL assessment provides a practical histopathological method for evaluating immune activity within tumors using routine hematoxylin and eosin–stained sections, as recommended by the International TILs Working Group [12]. In the present cohort, stromal TIL levels demonstrated considerable variability, with tumors showing low, intermediate and high infiltration categories.

Importantly, stromal TIL category showed a strong association with NPI prognostic groups. Tumors with higher levels of stromal lymphocytic infiltration were predominantly associated with favourable prognostic categories, whereas tumors with low TIL levels were more frequently observed in poorer NPI groups. Continuous variable analysis also demonstrated a strong inverse correlation between stromal TIL percentage and NPI value, indicating that higher immune infiltration is associated with better prognostic index scores.

These findings are consistent with previous studies reporting the prognostic significance of TILs in breast carcinoma. Denkert et al. reported that increased stromal TIL levels were associated with improved prognosis and better response to systemic therapy in breast cancer [18]. Similarly, Loi et al. demonstrated that higher TIL levels were significantly associated with improved survival outcomes, particularly in aggressive breast cancer subtypes such as triple-negative breast cancer [19]. Comparable observations have also been reported in Indian studies evaluating stromal tumor-infiltrating lymphocytes in breast carcinoma, where increased TIL levels were associated with favourable clinicopathological parameters and improved prognostic indicators [20]. The biological explanation for this association lies in the presence of cytotoxic lymphocytes that recognize tumor-associated antigens and contribute to tumor cell destruction. Conversely, tumors with minimal immune infiltration may represent immune-evasive phenotypes that permit continued tumor progression.

However, certain limitations of the present study should be acknowledged. The study was conducted on a relatively small cohort of 62 cases from a single tertiary care centre, which may limit the generalizability of the findings. In addition, molecular subtype analysis and long-term survival data were not included. Future studies incorporating larger patient cohorts and survival analysis may further clarify the prognostic significance of stromal immune infiltration in breast carcinoma.

The present study adds to the growing evidence suggesting that stromal tumor-infiltrating lymphocytes have important prognostic relevance in breast carcinoma and may serve as a readily assessable histopathological marker. The findings should nevertheless be interpreted within the context of certain study characteristics. The analysis was based on a cohort of 62 cases from a single tertiary care centre, representing an institutional patient population. In addition, molecular subtype evaluation and long-term survival data were not included in the present analysis. Future studies involving larger and more diverse patient populations, along with incorporation of molecular profiling and outcome data, may further clarify the prognostic role of stromal immune infiltration in breast carcinoma.

CONCLUSION

Overall, the findings of the present study highlight the importance of stromal tumor-infiltrating lymphocytes as a reflection of host immune response in breast carcinoma. The strong association between stromal TIL levels and Nottingham Prognostic Index categories suggests that immune infiltration within the tumor microenvironment may complement established prognostic indicators. As stromal TIL assessment can be performed on routine histopathological sections without additional cost or specialized testing, it may serve as a practical and cost-effective prognostic marker in routine diagnostic practice, particularly in resource-limited settings.

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