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Original Research Article

Histopathological and Immunohistochemical Correlation of Triple-Negative Breast Cancer with Clinical Outcomes

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Abstract:

Background: The aggressive subtype of breast cancer known as triple-negative breast cancer (TNBC) is distinguished by the lack of expression of the HER2 gene, the progesterone receptor (PR), and the estrogen receptor (ER). Because of its high likelihood of recurrence, lack of targeted medicines, and restricted treatment options, it is linked to a poor prognosis.

Aim: To evaluate the correlation between histopathological and immunohistochemical features of TNBC with clinical outcomes, and to identify key prognostic indicators that could guide therapeutic decisions.

Methods: At IGIMS, Patna, 125 patients with histologically proven TNBC participated in 24-month prospective observational research. Clinical information, immunohistochemical markers (Ki-67, p53, CK5/6), lymphovascular invasion, and histological grading were all documented. In order to evaluate survival, metastasis, and recurrence, patients were routinely monitored. SPSS version 23.0 was used for the statistical analysis, and $p < 0.05$ was chosen as the significance level.

Results: Invasive ductal carcinoma was the most common histological type (89.6%), and 62.4% of tumors were grade III. High Ki-67 expression (>20%) was noted in 72.8% of cases, p53 positivity in 63.2%, and CK5/6 positivity in 46.4%. Disease-free survival (DFS) at 2 years was 61.6%, and the overall survival rate was 86.4%. Significant associations were observed between DFS and high Ki-67 index ($p = 0.002$), grade III tumors ($p = 0.001$), and lymph node positivity ($p < 0.001$). CK5/6 and p53 expression did not show significant correlation with clinical outcomes.

Conclusion: TNBC is predominantly a high-grade, high-proliferation breast cancer with poor clinical outcomes. Important prognostic variables include lymph node involvement, Ki-67 index, and histological grade. These results highlight the necessity of strong multimodal treatment plans and early identification.

Recommendations: Routine assessment of Ki-67 and nodal status should be incorporated into TNBC workups to better stratify patient risk. To investigate the possible role of additional biomarkers and immunotherapy in the treatment of TNBC, long-term follow-up and additional research are advised.

Keywords: Triple-negative breast cancer, Ki-67, histopathology, immunohistochemistry,

prognosis.

Introduction

Human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) expression are all absent in triple-negative breast cancer (TNBC), a unique and aggressive subtype of breast cancer. TNBC is more common in younger women and those with BRCA1 mutations, and it accounts for roughly 10–15% of all instances of breast cancer [1]. TNBC is particularly difficult to treat due to the lack of targeted hormonal treatments, which frequently results in worse prognoses than other subtypes of breast cancer [2].

Histopathologically, TNBC is predominantly invasive ductal carcinoma of no special type (IDC-NST), but it encompasses a heterogeneous group of tumors, including medullary and metaplastic carcinomas [3]. High histological grade, increased mitotic index, and frequent lymphovascular invasion are common features, contributing to its aggressive clinical behavior [4]. Immunohistochemically, TNBC often exhibits high proliferation indices, such as elevated Ki-67 levels, and may express basal markers like cytokeratin 5/6 and epidermal growth factor receptor (EGFR), aiding in its identification and subclassification [5].

Recent developments have brought attention to the role that tumor-infiltrating lymphocytes (TILs) play in TNBC. An active immunological milieu is suggested by the correlation between elevated TIL levels and increased survival rates and better responses to chemotherapy [6]. Moreover, programmed death-ligand 1 (PD-L1) expression has become a promising predictor of immunotherapy responsiveness, especially with drugs like pembrolizumab [7].

Treatment options for TNBC have expanded with the introduction of immunotherapy. Pembrolizumab added to neoadjuvant chemotherapy dramatically increased pathological complete response rates and event-free survival in patients with early-stage TNBC, according to the KEYNOTE-522 study [8]. Immunotherapy has been included to the conventional treatment procedures for high-risk TNBC as a result of these findings.

The prognosis for TNBC is still uncertain despite these developments, as there is a greater chance of metastasis and early recurrence, especially in the first three years after diagnosis. Compared to other subtypes of breast cancer, TNBC has a poorer five-year survival rate of about 77%. Therefore, ongoing research into the histopathological and immunohistochemical characteristics of TNBC is crucial for developing more effective, personalized treatment strategies. To evaluate the correlation between histopathological and immunohistochemical features of TNBC with clinical outcomes, and to identify key prognostic indicators that could guide therapeutic decisions.

Methodology

Study Design

This research was observational, analytical, and prospective.

Study Setting

The research was conducted in the Department of Pathology at Indira Gandhi Institute of Medical Sciences (IGIMS), Patna, a tertiary care teaching hospital. All diagnostic and follow-up procedures were carried out in coordination with the Department of Surgery and Oncology.

Study Participants

The study included 125 female patients who had been diagnosed with triple-negative breast cancer. Over the course of 24 months, these individuals were selected from among those who presented to the inpatient and outpatient departments. The lack of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) in immunohistochemistry was used to establish the diagnosis of TNBC.

Inclusion Criteria

- Invasive breast cancer cases with histological confirmation.
- Immunohistochemistry results for ER, PR, and HER2 are negative.
- Patients who are at least eighteen years old.
- Patients who agreed to follow-up procedures and provided informed permission.

Exclusion Criteria

- Patients with prior history of breast malignancy or other malignancies.
- Cases with incomplete clinical, histopathological, or immunohistochemical data.
- Patients lost to follow-up or who withdrew consent at any stage of the study.
- Male patients with breast cancer.

Bias

Measures were taken to reduce bias in information and selection. Standardized protocols were followed for sample collection, processing, immunohistochemical staining, and reporting. Blinded analysis was done by two independent pathologists to reduce observer bias.

Data Collection

Using a pre-made case record form, demographic, clinical, and pathological data were gathered. Age, tumor size, histological grade, lymph node status, tumor subtype, and therapy information were among the data. Clinical outcomes were recorded during regular follow-up visits, including recurrence, metastasis, and survival status.

Procedure

Standard histopathological procedures were followed in the processing of all surgical and core needle biopsy specimens. Slides stained with hematoxylin and eosin (H&E) were analyzed for nodal involvement, lymphovascular invasion, tumor type, and grade. To verify triple-negative status, ER, PR, and HER2 immunohistochemical staining was carried out. Additional markers such as Ki-67, p53, and CK5/6 were evaluated for research purposes. Follow-up of patients was done at 3-month intervals to assess disease progression or recurrence.

Statistical Analysis

SPSS version 23.0 was used to enter and analyze the data. The clinicopathological and demographic features were compiled using descriptive statistics. The Student's t-test or ANOVA, when applicable, were used to compare continuous data, while the Chi-square test or Fisher's exact test was used to compare categorical variables. The Kaplan-Meier method was used to analyze survival, and the log-rank test was used to evaluate differences in survival. Statistical significance was defined as a p-value of less than 0.05.

Results

Out of 125 patients diagnosed with (TNBC), the **mean age** was **48.2 ± 10.6 years**, with the majority of patients (n=72, **57.6%**) falling in the **41–60 years** age group. A total of **96 patients (76.8%)** presented with a **palpable breast lump**, and **29 patients (23.2%)** reported nipple discharge, pain, or skin changes. The **right breast** was affected in 68 cases (54.4%) and the **left breast** in 57 cases (45.6%).

Table 1: Distribution of Patients by Age Group and Clinical Presentation

Parameter	Number of Patients (n=125)	Percentage (%)
Age Group (years)		
< 40	28	22.4
41–60	72	57.6
> 60	25	20.0
Clinical Presentation		
Palpable lump	96	76.8
Nipple discharge	15	12.0
Pain	9	7.2
Skin/nipple changes	5	4.0
Laterality		
Right breast	68	54.4
Left breast	57	45.6

Histopathological Findings

Invasive ductal carcinoma (IDC) was the predominant histological subtype, observed in **112 patients (89.6%)**. **Histological grade III** tumors were most frequent (n=78, **62.4%**), followed by grade II (n=40, **32.0%**) and grade I (n=7, **5.6%**). Lymphovascular invasion was seen in **44 patients (35.2%)**, and axillary lymph node involvement was noted in **83 patients (66.4%)**.

Table 2: Histopathological Features

Feature	Number of Patients (n=125)	Percentage (%)
Histological Type		
Invasive ductal carcinoma	112	89.6
Metaplastic carcinoma	9	7.2
Medullary carcinoma	4	3.2
Histological Grade		
Grade I	7	5.6
Grade II	40	32.0
Grade III	78	62.4
Lymphovascular Invasion	44	35.2
Lymph Node Involvement	83	66.4

Immunohistochemical Profile

All patients were negative for ER, PR, and HER2. Additional markers were analyzed: **Ki-67 index > 20%** was found in **91 cases (72.8%)**, and **p53 positivity** was noted in **79 cases (63.2%)**. Basal markers such as **CK5/6** were positive in **58 cases (46.4%)**, indicating a basal-like phenotype.

Table 3: Immunohistochemical Markers in TNBC

Marker	Positive Cases (n=125)	Percentage (%)
Ki-67 > 20%	91	72.8
p53 Positive	79	63.2
CK5/6 Positive	58	46.4

Clinical Outcomes and Correlation Analysis

At the end of the 24-month follow-up period:

- **Disease-free survival (DFS)** was achieved in **77 patients (61.6%)**
- **Local recurrence** occurred in **19 patients (15.2%)**
- **Distant metastasis** developed in **29 patients (23.2%)**
- The **overall mortality rate** was **13.6% (n=17)**

Patients with **Ki-67 > 20%**, **grade III tumors**, and **positive lymph node status** had significantly higher recurrence and poorer survival outcomes ($p < 0.05$).

Table 4: Correlation of Clinicopathological Features with Disease-Free Survival

Feature	DFS Achieved (n=77)	DFS Not Achieved (n=48)	p-value
Histological Grade III	34	44	0.001*
Ki-67 > 20%	42	49	0.002*
Lymph Node Positive	35	48	<0.001*
p53 Positive	50	29	0.08
CK5/6 Positive	30	28	0.54

*Statistically significant

Survival Analysis

Patients with lower histological grade, no lymph node involvement, and low Ki-67 (<20%) had improved overall and disease-free survival, according to Kaplan-Meier survival curves. The survival difference depending on lymph node status ($p < 0.001$) and Ki-67 score ($p = 0.001$) was statistically significant, according to the log-rank test.

Summary of Key Findings

- Poor clinical results in TNBC are highly correlated with lymph node metastases, high histological grade, and increased Ki-67.
- There was no statistically significant association between survival and CK5/6 positive.
- Overall survival was 86.4%, and the 2-year DFS rate was 61.6%.

Discussion

This prospective study involving 125 patients with (TNBC) over a 24-month period revealed significant insights into the clinicopathological and immunohistochemical characteristics and their association with clinical outcomes.

With a mean age of 48.2, the majority of patients were middle-aged. The right breast was significantly more frequently afflicted, and the majority showed up with a palpable lump. The most common histological subtype was invasive ductal carcinoma, and a significant percentage of tumors (62.4%) displayed grade III differentiation, which denotes aggressive activity. Lymphovascular invasion and axillary lymph node involvement were present in more than one-third and two-thirds of patients, respectively—both known poor prognostic indicators.

Immunohistochemical analysis showed that a significant proportion of patients (72.8%) had a high Ki-67 proliferation index (>20%), and 63.2% expressed p53, supporting the aggressive nature of TNBC. Nearly half of the cases were positive for CK5/6, suggesting a basal-like phenotype in a substantial subset of patients, although this did not show a significant correlation with survival outcomes.

Clinical outcome analysis demonstrated that 61.6% of patients remained disease-free at the end of two years, while 23.2% developed distant metastases, and 13.6% succumbed to the disease. A statistically significant correlation was found between high Ki-67 index, grade III tumors, lymph node positivity, and poorer disease-free survival. These findings reinforce the

prognostic value of histological grade, proliferation index, and nodal status in TNBC. Conversely, CK5/6 and p53 expression, although prevalent, did not show a statistically significant impact on survival, suggesting they may have limited prognostic utility in isolation.

Recent studies have emphasized the significant prognostic and predictive value of histopathological and immunohistochemical features in (TNBC). One comprehensive study classified TNBC tumors into molecular subtypes using gene expression and found distinct histopathological correlates: basal-like immune-activated (BLIA) tumors had the highest tumor-infiltrating lymphocytes (TILs), while luminal androgen receptor (LAR) subtypes displayed apocrine differentiation and lower mitotic scores. This suggests that TILs and apocrine features are easily assessable markers that can help infer molecular subtype and likely therapeutic response, especially in resource-limited settings [9].

A large multicenter cohort from Mexico evaluated immunohistochemical markers like CD8, AR, and DCLK1. High CD8 expression and increased stromal TILs were significantly associated with improved disease-free and overall survival. In contrast, AR and DCLK1 expressions showed no significant prognostic value on their own, underlining the dominance of immune markers in outcome prediction [10].

Artificial intelligence tools have shown promise in predicting treatment responses using histopathological images. An AI model trained on H&E and multiplex immunohistochemistry (IHC) images (PD-L1, CD8+, CD163+) achieved high accuracy in predicting neoadjuvant chemotherapy outcomes, particularly for HER2+ and TNBC subtypes, outperforming manual pathology-derived features [11].

An immunophenotypic study identified that TNBC tumors negative for both SOX10 and AR had significantly poorer prognosis. This dual negativity, along with high American Joint Committee on Cancer (AJCC) stage and absence of chemotherapy, independently predicted adverse outcomes, supporting the inclusion of SOX10/AR profiling in risk stratification [12].

Whole slide imaging (WSI) analysis revealed that collagenous stromal features were linked with reduced pathologic complete response and worse clinical outcomes. When combined with clinical and pathological data, WSI features enabled improved risk prediction through computational models [13]. Another cohort analyzing AR and basal-like markers (EGFR, CK5/6, CK14, CK17) in TNBC patients undergoing neoadjuvant chemotherapy found that none of these IHC markers had significant correlation with survival. Instead, residual cancer burden and lymph node ratio emerged as dominant predictors, highlighting the importance of staging and surgical outcomes over baseline biomarkers [14].

A Dutch study reported that histological subtypes within TNBC (e.g., metaplastic and lobular carcinoma) were associated with worse relapse-free and overall survival compared to carcinoma of no special type (NST), indicating the prognostic value of subtype classification via histological review [15]. Further, a retrospective analysis confirmed that increased TIL concentration was associated with high proliferative index (Ki-67) and significantly better survival. TIL evaluation during routine pathology could thus enhance clinical decision-making in TNBC, especially among older patients [16].

Conclusion

In conclusion, this study highlights that TNBC is characterized by aggressive histopathological features and poor clinical outcomes. The findings underscore the importance of integrating histopathological grading, Ki-67 index, and lymph node status into routine diagnostic and prognostic assessments to stratify risk and

guide treatment strategies in TNBC patients.

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