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Triple-negative (ER, PgR, HER-2/neu) breast cancer in Indian women

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Abstract: The aim of our study was to analyze triple-negative (TN) breast cancer, which is defined as being negative for the estrogen receptor (ER), the progesterone receptor (PgR), and the human epidermal growth factor receptor 2 (HER-2/neu) and which represents a subset of breast cancer with different biologic behavior. We investigated the clinicopathological characteristics and prognostic indicators of lymph node-negative TN breast cancer. Medical records were reviewed from patients with node-negative breast cancer who underwent curative surgery at Grant Medical College and Sir JJ Group of Hospitals, Mumbai, India, from May 2007 to October 2010. Clinicopathological variables and clinical outcomes were evaluated. Among 683 patients included, 136 had TN breast cancer and 529 had non-TN breast cancer. TN breast cancer correlated with younger age (<35 years, $P = 0.003$) and a higher histopathologic and nuclear grade ($P < 0.001$). It also correlated with a molecular profile associated with biological aggressiveness: negative for Bcl-2 expression ($P < 0.001$), positive for the epidermal growth factor receptor ($P = 0.003$), and a high level of p53 ($P < 0.001$) and Ki-67 expression ($P < 0.00$). The relapse rates during the follow-up period (median 56.8 months) were 14.7% for TN breast cancer and 6.6% for non-TN breast cancer ($P = 0.004$). Relapse-free survival (RFS) was significantly shorter among patients with TN breast cancer compared with those with non-TN breast cancer: 3.5-year RFS rate 85.5% versus 94.2%, respectively; $P = 0.001$. On multivariate analysis, young age, close resection margin, and triple negativity were independent predictors of shorter RFS. TN breast cancer had a higher relapse rate and more aggressive clinicopathological characteristics than non-TN in node-negative breast cancer. Thus, TN breast cancer should be integrated into risk factor analysis for node-negative breast cancer.

Keywords: TN breast cancer, hormone receptors, ER, PgR, HER-2/neu, Ki-67

Introduction

Breast cancer is the most common cancer in women in the world.[1] Although its incidence appears to be leveling off in Western countries, after decades of increasing, it is still high and continues to increase in certain countries where it initially had a low incidence.[2]

Early detection of breast cancer and the use of aggressive multimodal treatment have successfully resulted in a decrease in mortality from the disease. Prognostic and predictive factors have been widely used in treatment decisions. These factors include the extent of axillary lymph node involvement, histopathologic grade, age of the patient, status of hormone receptors (HRs), and human epidermal growth factor receptor-2 (HER-2/neu), and involvement of lymphatic or microvascular spaces. Recent studies suggest that breast cancer is a heterogeneous disease and that patients with the same diagnostic and clinical prognostic profile can have markedly different clinical

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outcomes. Therefore, further understanding of the biology of the disease is needed to improve treatment outcome and reduce mortality.[2] Gene expression profiling has identified five subtypes of breast cancer (luminal A, luminal B, normal breast-like, HER-2/neu-overexpression, and basal-like), each of which has a different prognosis.[3–5] The basal-like and HER-2/neu+ subtypes have shorter relapse-free survival (RFS) and overall survival (OS) than the luminal tumors.[3,6]

Basal-like breast cancers are often called triple-negative (TN) breast cancer, defined as estrogen receptor (ER)-negative, progesterone receptor (PgR)-negative (ie, HR-negative), and HER-2/neu-negative. Approximately 80%–90% of TN phenotypic breast cancers are deemed to be basal-like when appropriately tested for immunohistochemical cancer biomarkers and gene expression. Moreover, there is a consistent trend across studies confirming unfavorable clinical outcomes associated with the TN phenotype or basal-like breast cancer.[4,7]

Previous studies in Western countries show that TN breast cancer has aggressive clinical and pathologic features, including onset at a young age, advanced stage at diagnosis, high histopathologic and nuclear grade, high mitotic index, higher frequency of unfavorable histopathology, and more distant recurrence.[8–11] In addition, evidence indicates that the prevalence and clinical outcomes of TN breast cancer differ among races.[11] Bauer et al[8] have reported that TN breast cancer is more prevalent among non-Hispanic black women compared with other ethnic groups, who when affected with this subtype had the worst survival. Carey et al[11] also reported that basal-like breast tumors occurred at a higher prevalence among African American women compared with other racial groups. However, there are limited studies of the prevalence, characteristics, and prognosis of TN breast cancer in Asian populations. A recent study of Korean patients indicated that the basal-like subtype, which is positive for one or more of the basal markers and negative for HRs and HER-2/neu, was not associated with a poor prognosis. This study also showed that the survival rate associated with the basal-like subtype does not differ from that of other subtypes, with the exception of the HER2/neu-overexpressing subtype, which has the worst survival rate.[12] In contrast, a recent study of breast cancer patients receiving neoadjuvant chemotherapy showed that TN breast cancer was associated with shorter survival than other subtypes, even though it was associated with a higher response rate.[13]

ERs and PgRs are steroid receptors localizing to the nucleus. The ER and PgR status of a tumor impacts on the disease-free survival (DFS) interval in lymph node-positive groups of patients besides predicating the response to endocrine therapy (more specifically to the antiestrogenic tamoxifen) or in patient selection for alternative first-line treatment. ER and PgR positivity is denoted by nuclear staining brown of both the invasive and in situ components of the breast cancer. Positive ER and PgR results were further qualified using a rapid semiquantitative H score ranging from 0 to 8, which takes into account both the intensity of staining and the proportion of tumor cells staining positive for ERs and PgRs with appropriate cut-off values for treatment of advanced disease. The score for proportion staining multiplied by the score for staining intensity is equal to the score.[8]

Human epidermal growth factor receptor 2 (HER-2/neu) is one among a family of transmembrane cell surface glycoprotein receptors with intrinsic tyrosine kinase activity that helps regulate normal cell growth, division, and survival. HER-2/neu is an independent prognostic marker of clinical outcome in node-positive patients, and an HER-2/neu-positive result is both a marker of aggressive disease with propensity for recurrence and a target for treatment using humanized monoclonal anti-HER-2/neu antibody trastuzumab (Herceptin), which gives substantial clinical benefit in patients with metastatic breast cancer. (CAP recommended grading of the immunohistochemical staining for HER-2/neu overexpression.[12]

The present study was designed to investigate the clinicopathological characteristics and prognostic significance of TN breast cancer in Indian women.

Materials and methods

Patients who were diagnosed with breast cancer and underwent curative surgery at Grant Medical College and Sir JJ Group of Hospitals, Mumbai, India, from May 2007 to October 2010 were included in the study. The inclusion criteria were i) breast cancer with negative lymph nodes on pathological examination and ii) available results of immunohistochemistry (IHC) for HRs and HER-2/neu. Patients who received adjuvant trastuzumab (n = 1) or neoadjuvant chemotherapy (n = 2) were excluded. We retrospectively evaluated each patient's clinicopathological features, molecular biomarkers, and clinical outcome. The study protocol was approved by the Institutional Ethics Committee of Grant Medical College and Sir JJ Group of Hospitals, Mumbai, India (No IEC/Pharm/36/07). Expressions of ER, PgR, and HER-2/neu were analyzed in specimens of invasive duct breast cancer tissue of Indian women during modified radical mastectomy.

Protocol for immunohistochemistry

IHC was used to test for the expression of the following molecular markers: ER, PgR, HER-2/neu, p53, Ki-67, B-cell lymphoma 2 (Bcl2), and epidermal growth factor receptor (EGFR). The routinely formalin-fixed, paraffin-embedded tissue blocks were sectioned at 4 μm thickness and then used for IHC. Tissue sections were deparaffinized in xylene, rehydrated with graded ethanol, and immersed in Tris-buffered saline (TBS). After an antigen-retrieval process, primary antibodies were used as previously described.[14,15]

The tissue sample was fixed in fixative 10% neutral buffered formalin for 12–24 hours. The tissue sample was processed in an autoprocesser, tissue with paraffin wax was embedded on an embedding station, and the paraffin blocks were cut by microtome at 4 μ sections and dried overnight at 37°C. Prior to antibody staining, the slides were pretreated with microwave irradiation to unmask binding epitopes. After blocking endogenous peroxide activity with a 3% solution of hydrogen peroxide in methanol for 30 minutes, the slides were immersed in 200 mL of 10 mM citric acid (pH 6.0) for 5 minutes at 100 W and four 5-minute cycles at 50 W. After topping up the buffer with distilled water, this step was repeated. The slides were then left to stand for 10 minutes in buffer at room temperature before being washed thoroughly in tap water.

After three washes in TBS, the slides were incubated with a 1:25 dilution of mouse anti- ER α monoclonal primary antibody (clone: 1D5; M7047; DakoCytomation, Denmark), 1:25 dilution of mouse anti-PgR monoclonal primary antibody (clone: PgR 636; M3569; DakoCytomation, Denmark), 1:25 dilution of mouse anti-HER-2/neu monoclonal primary antibody (clone: CB11; NCL-L-CB11; Visionbiosystems Asia Pacific), 1:800 Ki-67 (clone MIB-1 DakoCytomation, Denmark), 1:50 Bcl-2 (clone 124), and 1:50 EGFR (clone H11) in TBS for 1 hour at room temperature. After three more washes in TBS, secondary antibody (K0355; DakoCytomation, Denmark) biotinylated goat antibody (LINK) to mouse/rabbit immunoglobulin, dilute antibody (1:100) in TBS was applied for 1 hour at room temperature. After an additional three washes, streptavidin–biotin/horse radish peroxidase complex (Enzyme Label) (K0355; DakoCytomation, Denmark) dilute antibody (1:50) in TBS was applied for 1 hour at room temperature. After an additional three washes, the staining was visualized by adding diaminobenzidine (DAB kit; K3467; DakoCytomation, Denmark) for 5 minutes at room temperature. The slides were washed well in tap water and counterstained with Harris's hematoxylin for 10 seconds to 1 minute and then dehydrated, cleared, and mounted in Distrene Plasticiser Xylene (DPX). Positive and negative controls were performed with each batch of slides. Surgical specimens from the same patient were stained on the same run.

Positive and negative controls

Positive control tissue was used for ER. Normal breast epithelial cell nuclei are often used as an internal control with PgR. Uterine cervix tissue with stromal cells and basal squamous epithelial cells should show a distinct nuclear reaction with minimal cytoplasm staining for HER-2/neu. Human tumour control is Paget's disease, which almost invariably shows gene amplification and 3+ protein overexpression for p53, breast cancer tissue, Ki-67, lymphoid tissue in tonsils, Bcl2, and tonsil lymphoid tissue EGFR. Placental tissues were used as positive controls. Negative controls included sections of breast tumor tissue incubated overnight in the respective blocking sera instead of the primary antibodies, following NordiQC IHC quality controls 2007.

Scoring methods

Scoring for proportion staining was as follows: 0 denotes no nuclear staining, 1 denotes <1% nuclei staining, 2 denotes 1%–10% nuclei staining, 3 denotes 11%–33% nuclei staining, 4 denotes 34%–66% nuclei staining, and 5 denotes 67%–100% nuclei staining. Scoring for staining intensity was as follows: 0 denotes no staining, 1 denotes weak staining, 2 denotes moderate staining, and 3 denotes strong staining. The score for proportion staining multiplied by the score for staining intensity is equal to the score. Score 0 indicates that endocrine treatments or tamoxifen will definitely not work and such patients should receive an alternative first-line treatment. Score 2–3 indicates a 20% chance of response to endocrine treatment. Score 4–6 indicates a 50% chance of response to endocrine treatment. Score 7–8 indicates a good (75%) chance of response to endocrine treatment. 0 score is negative, which denotes no staining seen or staining seen in less than 10% of tumor cells. Score 1+ is negative, which denotes that a faint/barely perceptible membrane staining is detected in more than 10% of tumor cells but that the cells are stained in only part of the membrane. Score 2+ shows a borderline or weakly positive result, which denotes that weak to moderate complete membrane staining is seen in more than 10% of tumor cells. Score 3+ is strongly positive, which denotes that strong complete membrane staining is seen in more than 30% of tumor cells. True HER-2/neu positivity is shown by crisp brown-colored membrane staining in at least 30% of the invasive tumor. Score 3 is two steps higher than HER-2/neu expression in surrounding benign breast parenchyma.

Tissue HER-2/neu receptor overexpression and/or HER-2/neu gene amplification is essential for treatment with anti-HER-2/neu monoclonal antibody (trastuzumab), ie, Herceptin, which has significant clinical benefit in patients with metastatic breast cancer. Scores 0 and 1 are unequivocally negative with no further intervention recommended.

Statistical analysis

The comparisons of clinicopathological variables and patterns of relapse between TN breast cancer and non-TN breast cancer were made using Pearson's χ^2 test or Fisher's exact test as appropriate. Two-sided *P* values of <0.05 were considered statistically significant. The associations between molecular markers and clinicopathological variables, including TN breast cancer and RFS, were analyzed by Kaplan–Meier plots and log-rank tests. RFS was calculated from the date of surgery to the first detection of disease recurrence. Multivariate analyses were carried out using the Cox regression model. A significance level of 0.05 was used for covariate entry. SPSS Version 16 (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

A total of 1135 patients were diagnosed with breast cancer and underwent curative surgery. Of those, 683 patients were included in the study. The demographic and clinical characteristics of the patients are summarized in Table 1. Four hundred and nine patients (59.9%) were ER-positive, 273 patients (40.1%) were PgR-positive, 175 patients (25.6%) were HER-2/neu-positive, and 123 patients (18%) were HER-2/neu-undetermined.[10] One hundred and thirty-six patients (19.9%) had TN breast cancer identified as ER-negative, PR-negative, and HER-2/neu-negative (Figure1A, 1B, and 1C), and 529 patients (77.5%) had non-TN breast cancer indentified as HR-positive or HER-2/neu-positive. We excluded 18 patients (2.6%) classified as HR-negative and HER-2/neu-undetermined from further analyses comparing TN breast cancer and non-TN breast cancer. Two hundred and eighty-four (41.6%) patients underwent breast-conserving surgery, and 237 patients among them received adjuvant radiotherapy to the lesion. Four hundred and eighteen (61.2%) patients received adjuvant systemic chemotherapy. The median duration of follow-up was 56.8 months (range 1–89.1 months). Fifty-eight (8.5%) patients had relapse of disease during the follow-up period.

We compared the clinicopathological features of TN breast cancer with those of non-TN breast cancer (Table 2 and 3). One hundred and thirty-six (19.9%) patients had TN breast cancer and 529 (77.5%) had non-TN breast cancer.

Table 1 Demographic and clinical characteristics in breast cancer of Indian women

Variables	No. of patients (n = 683) (%)
Sex	
Male	1 (0.1)
Female	682 (99.9)
Age (years)	
Median (range)	47 (22–84)
<35	51 (7.5)
≥35	632 (92.5)
Type of surgery	
Breast-conserving surgery	284 (41.6)
Mastectomy	399 (58.4)
Histology	
Invasive ductal carcinoma	605 (88.5)
Invasive mucinous carcinoma	23 (3.4)
Invasive papillary carcinoma	17 (2.5)
Invasive lobular carcinoma	8 (1.2)
Metaplastic carcinoma	7 (1.0)
Medullary carcinoma	5 (0.7)
Tubular carcinoma	5 (0.7)
Invasive cribriform carcinoma	5 (0.7)
Invasive micropapillary carcinoma	3 (0.4)
Mixed invasive lobular and ductal carcinoma	3 (0.4)
Invasive apocrine carcinoma	2 (0.3)
Pathological tumor size	
<2 cm	399 (58.5)
≥2 cm	284 (41.5)
Adjuvant chemotherapy	
None	262 (38.4)
Yes	418 (61.2)
Unknown	3 (0.4)

Compared with non-TN breast cancer, TN breast cancer correlated with younger age (below 35 years, $P = 0.003$), higher histopathologic and nuclear grade ($P < 0.001$ and $P < 0.001$, respectively), negative staining for Bcl2 expression ($P < 0.001$), positive staining for EGFR ($P = 0.003$), and high levels of p53 ($P < 0.001$) and Ki-67 expression ($P < 0.001$) (Table 2). Although more patients with TN breast cancer had received adjuvant chemotherapy than those with non-TN breast cancer ($P < 0.001$), a greater percentage of those with TN breast cancer relapsed during the follow-up period (14.7% vs 6.6%, respectively; $P = 0.004$) (Table 3).

We analyzed the association between clinicopathological variables and RFS. The results of univariate analyses are summarized in Table 4. Younger age (below 35 years), breast-conserving surgery, tumor size larger than 2 cm, the presence of endovascular or lymphatic tumor emboli, a close resection margin (<3 mm), ER negativity, TN breast cancer, negative staining for Bcl-2 expression, and high levels of Ki-67 expression correlated with shorter RFS.

Table 2 Comparison between triple-negative (TN) and non-TN breast cancer

Variables	No. of TN patients (%) (n = 136)	No. of non-TN (%) (n = 529)	*P* value[a]
Age (years)			0.003
<35	18 (13.2)	31 (5.9)	
≥35	118 (86.8)	498 (94.1)	
p53			<0.001
<25%	71 (52.2)	425 (80.3)	
≥25%	65 (47.8)	94 (17.8)	
Unknown	0 (0)	10 (1.9)	
Ki-67			<0.001
<20%	85 (62.5)	495 (93.6)	
≥20%	51 (37.5)	21 (4.0)	
Unknown	0 (0)	13 (2.5)	
Bcl2			<0.001
(–)	90 (66.2)	108 (20.8)	
(+)	43 (31.6)	409 (77.3)	
Unknown	3 (2.2)	12 (2.3)	
Epidermal growth factor receptor			0.003
(–)	65 (47.8)	275 (52)	
(+)	10 (7.4)	10 (1.9)	
Unknown	61 (44.9)	244 (52.0)	

Note: [a]Based on Pearson's χ^2 test (using Fisher's exact test if N ≤ 5).

On multivariate analysis, younger age (hazard ratio [HR] 2.880; 95% confidence interval [CI] 1.396–5.939, $P = 0.004$), a close resection margin within 3 mm (HR 4.495; 95% CI 1.011–19.986, $P = 0.048$), and TN breast cancer (HR 2.382; 95% CI 1.351–4.199, $P = 0.003$) were independently associated with shorter RFS and surgery type, resection margin, and chemotherapy, as shown in Table 3.

Four-year RFS rates in patients with TN breast cancer and non-TN breast cancer were 85.5% and 94.2%, respectively ($P = 0.001$) (Figure 2). Eighteen patients (90.0%) with relapsed TN breast cancer had their relapses within 3 years after surgery, whereas 19 patients (57.3%) with relapsed non-TN breast cancer had relapses within 3 years after surgery ($P = 0.007$) (Figure 3). The distribution of the sites of recurrence (distant, locoregional, or contralateral breast) was not statistically different between TN and non-TN breast cancer ($P = 0.968$). TN breast cancer patients who were younger had shorter RFS than those without these features ($P = 0.028$). Patients with TN disease also had shorter RFS than patients who were HR-positive ($P < 0.001$) or HR-negative/HER-2/neu-positive ($P = 0.384$) (Figure 4). According to HR and HER-2/neu status, the number of TN patients was 136 (19.9%), 4-year RFS rate was 85.5%, and HR was 2.445. HR-negative and HER-2/neu rates were undetermined for 18 patients (2.6%).

Discussion

Molecular classification of breast cancer has revealed the heterogeneity of the disease with respect to prognosis and response to therapy. Among the subgroups of breast cancer, TN breast cancer is particularly feared because it is associated with a poor clinical outcome and it has no specific systemic treatment.[9,16] However, clinical data on TN breast cancer in Asian populations are limited. Thus, we investigated the clinicopathological features and the prognostic indicators of lymph node-negative TN breast cancer in Indian women.

In the present study, 19.9% (136/683) of the included patients had TN breast cancer. Carey et al[11] found that the prevalence of the TN subtype among patients with breast cancer in the US was 26.4%; among non-African American patients with breast cancer this prevalence was 23%. Bauer et al[8] reported that in the US the prevalence of TN breast cancer among patients with all forms of breast cancer was 12.4% and that this prevalence was highest among non-Hispanic black patients with breast cancer, at 24.6%. Previous studies among Asian women have reported more than 30% of breast cancer with the TN subtype.[12,13] Although the prevalence of TN breast cancer in our study (19.9%) was lower than in these other studies, the prevalence among Indian may not actually be lower. The lower prevalence in our study may be the result of including only node-negative patients in combination with the association of TN breast cancer with advanced stage and thus node-positive status.

In the current study, TN breast cancer was associated with younger age, higher histological and nuclear grade, negative staining for Bcl-2, positive staining for EGFR, and high levels of p53 and Ki-67 expression.[37] These characteristics are known to be cancer biomarkers of biologic aggressiveness and poor prognosis in breast cancer.[17–20] Our observation that TN breast cancer has a shorter RFS than non-TN breast cancer in lymph-node negative cancer is consistent with most other studies.[7–10,14] We also found that TN breast cancer was an independent prognostic factor for shorter RFS. These results indicate that the prognosis of TN breast cancer in Indian populations does not differ from that in other countries.

The published data incorporating 5892 cases strongly support the prognostic role of Bcl2 as assessed by IHC in breast cancer, showing that it is associated with both DFS and OS (pooled HR estimates of 1.66 and 1.64, respectively). These effects were slightly attenuated but still significant in multivariate analyses (adjusted HRs of 1.58 and 1.37 for DFS and OS, respectively), showing that this effect is independent of lymph node status, tumor size, and tumor grade, as well as a range of other biological variables on multivariate analysis.[20]

Table 3 Independent prognostic factors influencing relapse-free survival (multivariate analysis)

Variables	Hazard ratio[a]	95% confidence interval	*P* value
Age (<35 years)	2.880	1.396–5.939	0.004
Close resection margin <3 mm	4.495	1.011–19.986	0.048
TN	2.382	1.351–4.199	0.003
Variables	**No. of TN patients (%) (n = 136)**	**No. of non-TN patients (%) (n = 529)**	***P* value[b]**
Histology			
Invasive ductal carcinoma	122 (89.7%)	467 (88.3)	
Invasive mucinous carcinoma	0 (0)	23 (4.3)	
Invasive papillary carcinoma	2 (1.5)	15 (2.8)	
Invasive lobular carcinoma	0 (0)	8 (1.5)	
Metaplastic carcinoma	4 (2.9)	2 (0.4)	
Medullary carcinoma	5 (3.7)	0 (0)	
Tubular carcinoma	0 (0)	5 (0.9)	
Invasive cribriform carcinoma	1 (0.7)	4 (0.8)	
Invasive micropapillary carcinoma	0 (0)	3 (0.6)	
Invasive apocrine carcinoma	1 (0.7)	0 (0)	
Mixed invasive lobular and ductal carcinoma	1 (0.7)	2 (0.4)	
Endovascular or lymphatic tumor emboli			0.203
(–)	113 (83.1)	462 (87.3)	
(+)	22 (16.2)	64 (12.1)	
Unknown	1 (0.7)	3 (0.6)	
Close resection margin			0.589
<3 mm	0 (0)	5 (0.9)	
≥3 mm	136 (100)	524 (99.1)	
Histological grade			<0.001
1 or 2	41 (30.1)	331 (62.6)	
3	79 (58.1)	97 (18.3)	
Unknown	16 (11.8)	101 (19.1)	
Nuclear grade			<0.001
1 or 2	49 (36.0)	366 (69.2)	
3	83 (61.0)	108 (20.4)	
Unknown	4 (2.9)	55 (10.4)	
Adjuvant chemotherapy			<0.001
None	19 (14.0)	241 (45.6)	
Yes	117 (86.0)	285 (53.9)	
Unknown	0 (0)	3 (0.6)	
Relapse			0.004
None	116 (85.3)	494 (93.4)	
Yes	20 (14.7)	35 (6.6)	

Notes: [a]Adjusted for age, type of surgery, tumor size, endovascular or lymphatic tumor emboli, status of resection margin, triple-negative (TN) breast cancer, B-cell lymphoma 2, and Ki-6. [b]Based on Pearson's χ^2 test (using Fisher's exact test if $N \leq 5$).

The number of studies included in the meta-analysis with different treatment endpoints was too small to perform a meaningful analysis of its predictive role. However, there is an emerging consensus that Bcl2 plays a key role in determining response to endocrine therapy and chemotherapy.[21] Bcl2 is an estrogen-responsive gene, and many clinical studies have shown an association with a favorable response to endocrine therapy.[21] Bcl2 is a component of the 21-gene signature used to predict recurrence in tamoxifen-treated ER-positive, node-negative breast cancer. Its role is being evaluated prospectively in the TAILORx (Trial Assigning Individualized Options for Treatment (Rx)) trial.[22]

Bcl2 is an independent indicator of favorable prognosis for all types of early-stage breast cancer. This study establishes the rationale for the introduction of Bcl2 IHC to improve prognostic stratification. Further work is now needed to ascertain the exact way to apply Bcl2 testing for risk stratification and to standardize Bcl2 IHC for this application.

Breast cancer is heterogeneous, and the existing prognostic classifiers are limited in accuracy, leading to unnecessary treatment of numerous women. Bcl2, an antiapoptotic protein, has been proposed as a prognostic marker, but this effect is considered to relate to ER status. This study aimed to test the clinical validity of Bcl2 as an independent prognostic marker.[23]

Table 4 Correlation of clinicopathological variables and molecular biomarkers with relapse-free survival (RFS) (univariate analysis)

Variables	No. of patients (n = 683) (%)	4-year RFS rate (%)	Hazard ratio	95% confidence interval	*P* value[a]
Age (years)					
<35	51 (7.5)	78.6	3.19	1.611–6.318	0.0004
≥35	632 (92.5)	93.2			
Type of surgery					
Breast-conserving surgery	284 (41.6)	90	0.589	0.351–0.986	0.0427
Mastectomy	399 (58.4)	93.7			
Pathological tumor size					
<2 cm	399 (58.5)	93	0.595	0.355–0.996	0.0225
≥2 cm	284 (41.5)	89.5			
Endovascular or lymphatic tumor emboli					
(–)	589 (86.2)	93	0.483	0.260–0.896	0.018
(+)	90 (13.2)	86.1			
Unknown	4 (0.6)				
Close resection margin					
<3 mm	678 (99.3)	60	5.729	1.396–23.512	0.006
≥3 mm	5 (0.7)	92.4			
Histological grade					
1 or 2	379 (55.5)	92.8	0.658	0.376–1.150	0.1411
3	184 (26.9)	87.9			
Unknown	120 (17.6)				
Nuclear grade					
1 or 2	421 (61.6)	93	0.663	0.389–1.128	0.127
3	202 (29.6)	88.5			
Unknown	60 (8.8)				
Estrogen receptor					
(–)	274 (40.1)	87.4	2.609	1.535–4.435	0.0002
(+)	409 (59.9)	95.4			
Progesterone receptor					
(–)	410 (60.0)	90.1	1.755	0.986–3.123	0.054
(+)	273 (40.0)	95.3			
HER-2/neu					
Positive	175 (25.6)	92.5	1.015	0.559–1.840	0.712
Undetermined	123 (18)	93	0.793	0.316–1.728	
Negative	385 (56.4)	91.9			
Groups according to HR and HER-2/neu status					
Triple-negative	136 (19.9)	85.5	2.445	1.411–4.236	0.001
Non-triple-negative	529 (77.5)	94.2			
HR-negative and HER-2/neu-undetermined	18 (2.6)				
p53					
<25%	502 (73.5)	92.7	0.729	0.417–1.275	0.2686
≥25%	171 (25.0)	90.8			
Unknown	10 (1.5)				
Ki-67					
<20%	596 (87.3)	93.3	0.422	0.223–0.799	0.0064
≥20%	74 (10.8)	83.2			
Unknown	13 (1.9)				
B-cell lymphoma 2					
(–)	209 (30.6)	87.1	2.249	1.338–3.781	0.0017
(+)	459 (67.2)	94.5			
Unknown	15 (2.2)				
Epidermal growth factor receptor					
(–)	348 (51.0)	92.4	1.726	0.234–12.726	0 .5855
(+)	22 (3.2)	94.7			
Unknown	313 (45.8)				

(*Continued*)

Table 4 (*Continued*)

Variables	No. of patients (n = 683) (%)	4-year RFS rate (%)	Hazard ratio	95% confidence interval	*P* value[a]
Adjuvant chemotherapy					
None	262 (38.4)	94.3	0.645	0.366–1.135	0.1285
Yes	418 (61.2)	90.8			
Unknown	3 (0.4)				

Note: [a]Based on log-rank test.
Abbreviations: HER-2, human epidermal growth factor receptor 2; HR, hormone receptor.

In the current study, most of the relapses in TN breast cancer occurred within the first 3 years, in contrast to non-TN breast cancer. This finding reflects the aggressiveness of TN breast cancer and is consistent with previously reported results, such as those from the study by Dent et al.[9] They reported that the risk of recurrence declined rapidly after 4 years and that no recurrences occurred after 8 years. Rakha et al[24] reported that the only prognostic cancer biomarker among the TN breast cancer in the lymph node-negative subgroup was the basal phenotype, defined as the expression of CK5/6 or CK14.[33] These results suggest the possibility of subclassifications of TN breast cancer and the necessity for further study. Patients with TN breast cancer had shorter RFS than patients who were HR-positive or HR-negative/HER-2/neu-positive. Considering the high proportion of HER-2/neu-positive patients among HR-negative patients (39.5%) in this study and the expected efficacy of adjuvant trastuzumab, it is reasonable to separate TN breast cancer from HR-negative breast cancer when planning treatment.[25,26]

The current study has a number of limitations. Some patient records lacked the results of immunohistochemical analyses for biologic cancer biomarkers other than HR and HER-2/neu. The result of HER-2/neu fluorescence in situ hybridization in the primary tumor was not available in the majority of patients. In the present study, HER-2/neu 0 or 1+ was classified as HER-2/neu-negative for clarifying TN breast cancer, although a previous study showed that the clinical outcome of TN breast cancer was not significantly different whether HER-2/neu-2+ patients were classified as HER-2/neu-negative or HER-2/neu-positive.[10,28,29] Eighteen (14.6%) HR-negative patients were classified as HER-2/neu-undetermined. There is a lack of consensus regarding the definition of basal-like breast cancer and TN breast cancer. However, in spite of different classifications, there is a consistent result across all studies suggesting the aggressive clinicopathological and biologic features of TN breast cancer and basal-like breast cancer.[3,10] Another limitation is the short duration of follow-up, which makes the OS analysis unfeasible. In conclusion, TN breast cancer, defined by negative HR and HER-2/neu status, was associated with more aggressive clinicopathological features and molecular biomarkers and with shorter RFS. We confirmed that TN breast cancer was a significant prognostic factor in lymph node-negative breast cancer in Indian women. Thus, identifying this

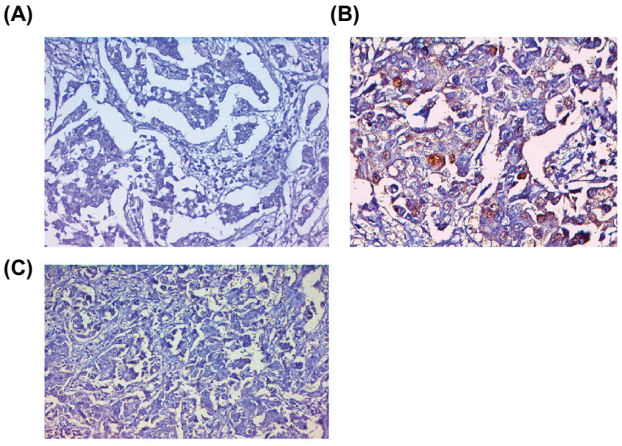


Figure 1 Immunohistochemical determination of ER using 1D5 antibody, a negative nuclear staining of tumor cells (**A**), PgR using PgR 636 antibody, negative nuclear staining of tumor cells (**B**), and (**C**) HER-2/neu antibody CB11, negative membrane staining of tumor cells in infiltrating duct breast cancer.

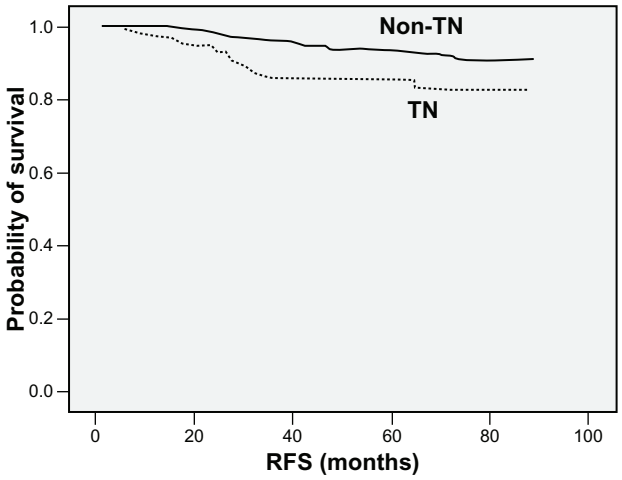


Figure 2 Kaplan–Meier plot of RFS according to triple negative (TN) phenotype.

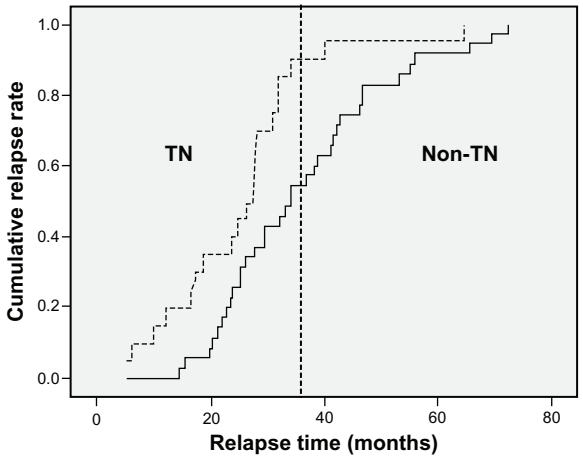


Figure 3 Kaplan–Meier plot of cumulative relapse rate among patients with relapses. TN = triple negative breast cancer in Indian women.

subtype should be integrated into risk factor analysis for node-negative breast cancer.

Positive lymph node metastases (LNMs) are already been treated aggressively with adjuvant therapy. In negative LNMs there is a subset that needs special attention. In negative LNMs, study of biomarkers of tumor becomes more important because it leads to decisions on adjuvant therapy and thereby chances of prolonged survival. TN breast cancer was associated with more aggressive clinicopathological features and molecular biomarkers and with shorter RFS. TN breast cancer was a significant prognostic factor in lymph node-negative breast cancer in Indian women.

Lymph node status was determined on the basis of analysis of hematoxylin and eosin-stained sections from each block of serially sectioned lymph nodes removed during surgery.

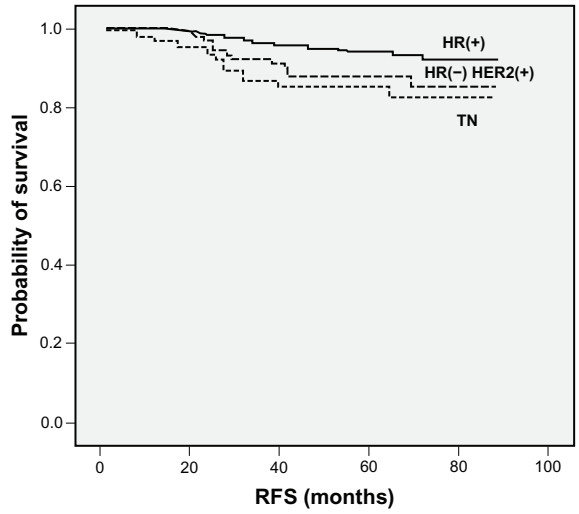


Figure 4 Kaplan–Meier plots of RFS according to HR and HER-2/neu status.

The involved nodal ratios, also referred to as the proportion of involved nodes, were defined as the number of involved nodes/the number of dissected nodes. The involved nodal ratio was evaluated with the various cut-off percentage levels. The pathologic tumor stage was assessed according to the sixth edition of the American Joint Committee on Cancer staging system.[27] The pathologic complete response (pCR) was defined as the complete disappearance of invasive carcinoma in both breast and axillary lymph nodes after three cycles of chemotherapy. Residual ductal carcinoma in situ was included in the pCR category.

LNM of breast cancer is a prognostic factor of the greatest importance, and data on its status have a great impact on decision making regarding postoperative adjuvant therapy. The General Rules for Clinical and Pathological Recording of Breast Cancer[17] classify LNM into n0, n1α, n1β, n2, etc. Excluding the complexity relating to parasternal lymph nodes within this classification, n0 corresponds to no LNM, n1α to metastasis to ≤3 axillary nodes, n1β to metastasis to ≥4 axillary nodes, n2 to metastasis to subclavicular lymph nodes, and n3 to metastasis to supraclavicular lymph nodes. More specifically, the distinction between n1α and n1β is the number of positive nodes. Miura and Hiratsuka et al[31] reported that the greater the number of positive nodes, the poorer the prognosis of breast cancer. Veronesi et al[32] proposed the classification of LNM by the level of invasion (levels I, II, and III), and, during the 1960s, Auchincloss stated the importance of the metastatic site as well as the number of positive nodes.[33] The location number in the General Rules is a classification involving both the number of positive nodes and the metastatic level or location.[17]

The study revealed a significant difference in survival between location numbers, ie, n0, n1α, n1β, and n2, confirming the validity of the location number as a prognostic factor. With regard to the number of positive nodes, comparisons of x and $x + 1$ positive nodes showed no significant difference in survival between patients with one and two positive nodes, but there was a significant difference between those with two and three positive nodes. Although patients with three and four positive nodes showed a slight difference ($P = 0.056$) in survival, it was not as great as that observed between those with two and three positive nodes. No significant difference in survival was found between patients with four and five positive nodes, but the difference between those with nine and 10 positive nodes was significant.[34]

Lately, some studies have reported that the phonotypical and molecular features of BRCA1-associated breast cancers are

sporadically shared by TN breast cancers.[26,29–35] These findings suggest that the defect in the DNA-repair pathways characteristic of BRCA1-related cancers may also occur in TN breast cancers and that this molecular defect may be more specifically targeted.[30,32–37] On the basis of previous data, further studies are needed to define breast cancer subtypes in greater detail and to develop and assess specifically targeted therapies.

Conclusion

TN breast cancer is associated with more aggressive clinicopathological features and molecular biomarkers and with shorter RFS. TN breast cancer is a significant prognostic factor in lymph node-negative breast cancer in Indian women.

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Disclosure

The authors report no conflicts of interest in this work.

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