RESEARCH ARTICLE

Survival of Triple Negative versus Triple Positive Breast Cancers: Comparison and Contrast

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Abstract

Background: Triple negative (TN) and triple positive (TP) breast cancers both are aggressive types but TN generally has a shorter survival. Objectives: To compare the clinical characteristics and treatment outcomes for patients with TN versus TP breast cancer and to assess various prognostic factors affecting overall survival. Materials and Methods: A retrospective audit of 85 breast cancer patients was conducted in the Department of Radiation Oncology and Medical Oncology on patients from 2006 to 2013 for whom IHC for ER, PgR and Her-2 neu were available. The patients were stratified into: ER-, PR- and Her-2 neu- (Arm A, n=47) and ER+, PgR+ and Her-2 neu+ (Arm B, n=38). Results: TN subtype had higher numbers of premenopausal and advanced stage patients as compared to TP subtype. The locoregional recurrence (LRR) and distant metastatic rate was also higher in TN subtype but there was no definite pattern in both the arms. Among the prognostic factors, patients with premenopausal status and advanced stage in TN breast cancer had inferior survival (P=0.07) whereas for those with postmenopausal status and early stage there was no survival difference between the two arms. Conclusions: TN subtype tends to be more aggressive in terms of younger age and advanced stage at presentation, higher tumour grade, LRR and metastasis, suggesting need for future research efforts on providing aggressive treatment to these patients. We could attribute better outcome for TP subtype to receptor positivity enabling role of hormonal treatment and targeted therapy, although less number of patients received targeted therapy.

Keywords: Breast neoplasms - ErbB-2 - incidence - local recurrence - receptor - retrospective studies

Asian Pac J Cancer Prev, **17 (8)**, 3911-3916

Introduction

Presently a basic understanding of the current molecular classification of breast cancers is of paramount importance as these tests helps us to predict the benefit of specific therapies such as Her-2 neu targeted therapy and chemotherapy (Allison, 2012). Triple negative breast cancer (TNBC) is a unique subgroup constituting 10-20% of all breast cancers, seen more often in younger patients with poor prognosis (Carey et al., 2006; Morris et al., 2007). TNBC has higher response rate than luminals, but with a shorter disease-free survival (DFS) and overall survival (OS) (Hudis and Gianni et al., 2011). While triple positive breast cancer (TPBC) patients also have higher tumour grade, larger tumour size, exhibits worse prognosis (Ades et al., 2014) with a distinct profile of response to hormonal therapy and chemotherapy in view of Her-2 neu overexpression. Keeping this in mind that both triple negative (TN) and triple positive (TP) subtypes have aggressive behaviour we undertook this study to compare and contrast the clinical profile and treatment outcome of TN versus TP molecular subtypes of breast cancer and to analyse the various prognostic factors affecting overall survival.

Materials and Methods

This retrospective study comprised of patients with a histopathological diagnosis of breast cancer falling under the molecular subtype of either triple negative or triple positive before the initiation of cancer directed therapy presenting to the Department of Radiation Oncology and Medical Oncology from 2006 to 2013 in Christian Medical College & Hospital, Ludhiana. As per institutional policy, all patients were evaluated by a multidisciplinary team and treatment decision was finalized in the weekly tumour board meeting. The demographic, clinical, pathological, treatment and follow-up details of patients were included in the study if they fulfilled the following inclusion and exclusion criteria.

Inclusion criteria: *i*). Age above 18 years. *ii*). Paraffin blocks available for immunohistochemistry (IHC) examination of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor

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receptor 2 (Her-2 neu) status.

Exclusion criteria: i). Stage IV at presentation (AJCC staging). ii). Second primary malignancy.

The details of 85 patients were found eligible for analysis in this study. They were stratified into two arms and analysed individually.

Arm A (Triple negative): Patients with immunohistochemical staining negative for estrogen receptor (ER-), progesterone receptor (PgR-) and Her-2 neu negative.

Arm B (Triple positive): Patients with immunohistochemical staining positive for estrogen receptor (ER+), progesterone receptor (PgR+) and Her-2 neu positive.

All these patients had undergone a pre-treatment evaluation comprising of comprehensive history, physical examination, complete blood counts, biochemical tests and staging workup as appropriate. Patients were staged according to the 2006 & 2010 American Joint Committee on Cancer (AJCC) staging classification respectively based on the time period of presentation. Tissue for IHC staining for ER, PgR and Her-2 neu protein were obtained by trucut biopsy of the breast lump.

Immunohistochemistry analysis

Immunohistochemical staining was carried out on 4 μ sections using 'NovoLink polymer detection system' from Leica Biosystems, Newcastle upon Tyne, United Kingdom. Staining was performed using primary antibodies against Estrogen receptor (ER, clone 6F11, Leica Biosystems), Progesterone receptor (PgR, clone 16, Leica Biosystems) and Her-2 neu (clone CB11, Leica Biosystems). ER and PgR were reported as positive when ≥ 1% tumour cells showed nuclear positivity. Her-2 neu was scored 0 when there was no staining or membrane staining in < 10% tumour cells; 1+ when faint or barely perceptible membrane staining was observed in >10% tumour cells and the cells were stained in only part of their membrane; 2+ when weak to moderate complete membrane staining was observed in >10% tumour cells; 3+ when strong complete membrane staining was observed in > 10% tumour cells. Score of 0 and 1+ were considered negative, 2+ as weakly positive and 3+ as strongly positive. Cases with 2+ staining on IHC were subjected to analysis by fluorescence in situ hybridization (FISH) which was considered as gold standard.

Treatment plan

Surgery: All patients presenting with early stage breast cancer, underwent upfront modified radical mastectomy (MRM) or breast conservation therapy (BCT), followed by adjuvant chemotherapy (CT) and External Beam Radiotherapy (EBRT) while those with locally advanced breast cancer, received neoadjuvant CT (max upto 6 – 8 cycles), reassessed for tumour response and underwent MRM with adjuvant CT followed by adjuvant EBRT.

Radiation therapy

Radiation treatment was delivered with either Co-60 teletherapy unit or 6 MV linear accelerator. A total dose of 50 Gy in 25 fractions over a period of 5 weeks was delivered to 78.7% and 97.4% patients in the arm A and arm B respectively, to the chest wall and draining nodal areas.

Hormonal therapy

Hormone receptor positive patients received hormonal therapy with either SERMs- Tab. Tamoxifen or aromatase inhibitor- Tab. Letrozole or Anastrozole. Tamoxifen was given to premenopausal women for 5 years and Letrozole / Anastrozole was prescribed to post-menopausal women either as first-line hormonal therapy or after 5 years of Tamoxifen.

Targeted therapy

Although there were 38 patients tested positive for Her-2 neu status, only 3 patients received targeted therapy in the form of adjuvant Trastuzumab (for 1 year) due to financial constraints.

Follow-up

Post-treatment evaluations were performed every month for the first year, then every 2 months for the second year and 3 monthly for the third year and annually thereafter. Imaging (Chest x-ray and USG abdomen and / or CECT chest and abdomen) was done every 6 months.

Statistical analysis

In the descriptive analysis, continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as count (percentages). Univariate analysis comparing the arm A (triple negative) and arm B (triple positive) was performed using independent unpaired t-test for normally distributed variables. For categorical variables Chi-Square test was used. Fisher's exact test was used when there was one or more of cells with an expected frequency <5. Overall survival (OS) was estimated by the Kaplan-Meier method. OS was calculated from the date of the start of primary therapy till death due to any cause or last follow-up. A two-tailed P value <0.05 was considered significant. All statistical analysis were performed using SPSS, version 21.0. Armonk, NY: IBM corp.

Results

Table 1 illustrates the patients demographic, clinical, pathological and treatment variables for each group. There was no statistically significant difference in terms of baseline variables, including age, disease stage, tumour histology, surgery and site of metastasis in both the arms. The mean age of patients in arm A and arm B patients was 48.79±11.67 years (range=28–75 years) and 49.63±9.67 years (range=30–72 years) respectively. Median follow-up was 48 months (range: 13 - 84 months). Among the patients with TNBC, most of the patients (65.96%) presented with poorly differentiated tumours (grade III) while among those with TPBC, 34.21% had poorly differentiated tumours (P=0.004).

Locoregional recurrence and distant metastasis

After a median follow-up of 48 months, 12.8% and

8% patients had locoregional recurrence in TN and TP arms, respectively (P=0.72). Further, we also found that the incidence of distant metastasis was 30% and 15.8% in arm A and B respectively (P = 0.08). However, it did not reach statistically significant level. The risk of developing locoregional recurrence and distant metastasis was found to be similar over the period of follow-up for both the arms.

We then analysed the various prognostic factors namely age, menopausal status, tumour grade and stage at presentation affecting overall survival for both the molecular subtypes. In the univariate analysis, we found that factors such as age, menopausal status and stage of the disease were found to have impact on OS in both the molecular subtypes, although not statistically significant while tumour grade had no impact on OS. Patients presenting with early stage breast cancer had no difference in OS in both the arms (Figure 1) whereas for those with advanced stage, OS was found to be worse for TN patients as compared to TP patients (Figure 2), although it did not reach statistical significance (P = 0.07). Our data also suggested that young premenopausal patients with TNBC had shorter OS (Figure 3) as compared to TPBC patients while there was no such difference in postmenopausal patients (Figure 4) in both the molecular subtypes (P=0.08).

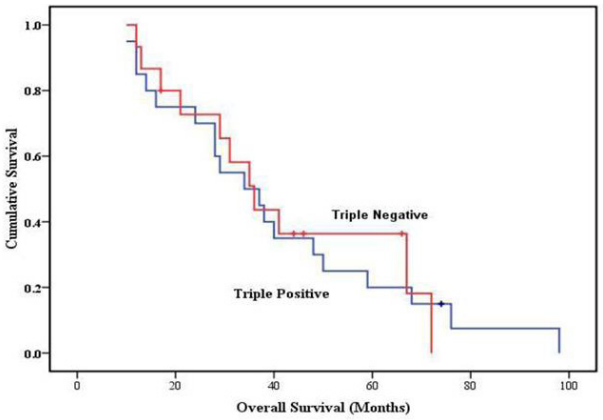

Figure 1. Early Stage Disease Overall Survival

Table 1. Distribution of Baseline Variables between the Two Arms

Variables	Arm A (n = 47)	Arm B (n = 38)	P-value
Mean age (range)	48.79 ± 11.67 years (28 – 75)	49.63 ± 9.67 years (30 – 72)	0.72***
Age (years)			0.109*
< 50	28 (59.57%)	16 (42.11%)	
≥ 50	19 (40.43%)	22 (57.89%)	
Menopausal status			0.029*
Premenopausal	28 (59.57%)	13 (34.21%)	
Postmenopausal	19 (40.43%)	25 (65.79%)	
T-stage			0.009*
T1-2	14 (29.79%)	21 (55.26%)	
T3	16 (34%)	6 (15.79%)	
T4	17 (36.17%)	11 (28.95%)	
N-stage			0.224*
N0	10 (21.28%)	5 (13.16%)	
N1	15 (31.91%)	19 (50%)	
N2-3	22 (46.81%)	14 (36.84%)	
Stage Group			0.262*
I & II	19 (40.43%)	20 (52.63%)	
Brain only III	28 (59.57%)	18 (47.37%)	
Histopathological Grade			0.004*
Brain only I & II	16 (34.04%)	25 (65.79%)	
Brain only III	31 (65.96%)	13 (34.21%)	
Tumour Histology			0.595**
Brain only Ductal	42 (89.36%)	35 (92.11%)	
Brain only Lobular	2 (4.26%)	2 (5.26%)	
Brain only Medullary	1 (2.13%)	-	
Brain only Metaplastic	2 (4.26%)	-	
Brain only Mucinous	-	1 (2.63%)	
Chemotherapy Regimen			0.011*
Brain only FAC / AC / FEC	11 (23.40%)	21 (55.26%)	
Brain only AC Taxanes	27 (57.45%)	13 (34.21%)	
Brain only TAC	9 (19.15%)	4 (10.53%)	
Surgery Type			0.244**
Brain only BCS	3 (6.38%)	5 (13.16%)	
Brain only Mastectomy	44 (93.62%)	33 (86.84%)	
Site of metastasis			0.808**
Visceral only	5 (10.64%)	2 (5.26%)	
Bone only	7 (14.88%)	4 (10.53%)	
Brain only	3 (6.38%)	0	

FAC, 5-Fluorouracil, Adriamycin, Cyclophosphamide; AC, Adriamycin, Cyclophosphamide; FEC, 5-Fluorouracil, Epirubicin, Cyclophosphamide; TAC, Docetaxel, Adriamycin, Cyclophosphamide; BCS, Breast conserving surgery. *chi-square test; **fisher's exact test; ***unpaired t-test

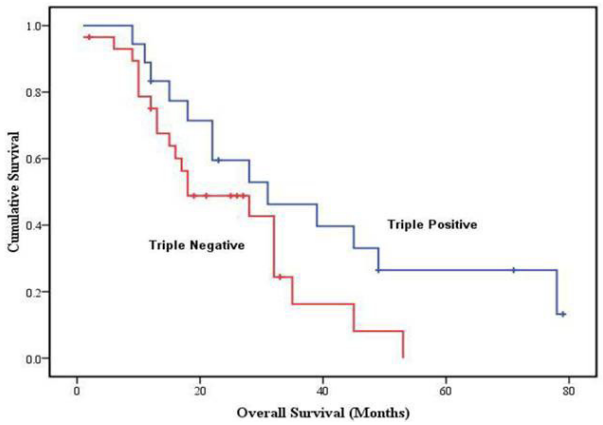

Figure 2. Advanced Stage Disease Overall Survival

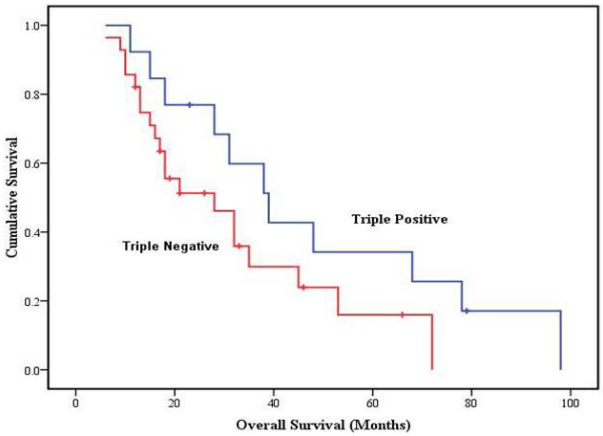

Figure 3. Overall Survival for Premenopausal Cases

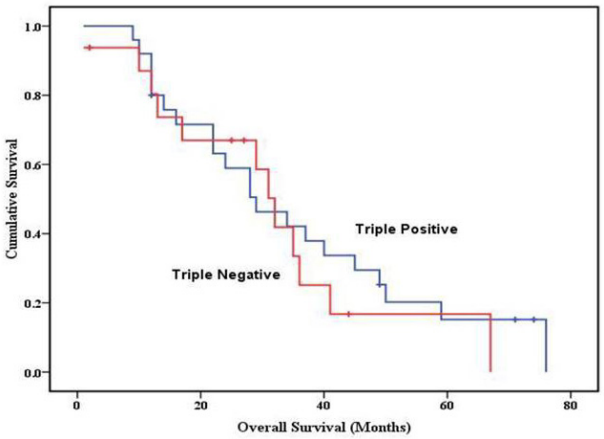

Figure 4. Overall Survival for Postmenopausal Cases

Discussion

Breast cancer is a heterogeneous and complex disease with regard to biological behaviour, response to treatment and prognosis (Sorlie et al., 2001; Sandhu et al., 2010). This prognostic information for each individual patient is based on the analysis of biological markers in the primary tumour including ER, PgR, Her-2 neu and Ki-67 (Goldhirsch et al., 2009) together with age, tumour size, histological grade and lymph node involvement. Due to this, there has been an increasing clinical interest in distinguishing the triple positive from triple negative cancers because TP cancers derive benefit from hormonal therapy and targeted therapy while to target TNBC patients, there are still limited therapeutic options. TNBC represent a unique subgroup of breast cancers with heterogeneous clinical presentation, clinical behaviour, histological grade, and response to therapy ((Brouckaert et al., 2012) as compared to TPBC. Because of this, there has been a great enthusiasm in distinguishing breast cancer into various intrinsic molecular subtypes with gene expression profiles in order to highlight the similarities and differences in prognosis between TP and TN molecular subtypes.

Our study revealed that TNBC patients present at a younger age (P=0.109) with advanced stage of disease (P=0.262) and predominantly higher tumour grade (P=0.004) as compared to TPBC patients. These findings matched with that of Jack et al. (Jack et al., 2013) in which they reported that the diagnosis of TNBC is more likely to be in younger women, those living in deprived areas, and with a more advanced stage of disease. An observational study by Sajid et al. (Sajid et al., 2014) showed that TNBC was noted in 65.88% of patients with age<50 years similar to 59.57% in our study.

It is important to note that hormone receptor status has been found to change the rate of locoregional recurrence and distant metastasis. Our data showed that patients with TP subtype were less likely to develop locoregional recurrence (8% *vs* 12.8%; P=0.72) and distant metastasis (15.8% *vs* 30%; P=0.08) after surgery than the TN subtype similar to as reported by Dent and colleagues (Dent et al., 2007) in a cohort of 1061 patients with breast cancer, an increased risk of distant recurrence following diagnosis was noted among patients with TNBC tumours compared with other subtypes (hazard ratio [HR]=2.6; 95% confidence interval [CI], 2.0-3.5; P<0.0001). We could attribute the higher incidence of development of locoregional recurrence and metastatic disease in TNBC to the aggressive nature of the disease. Studies suggest that ER - negative tumours mostly recur within 5 years of diagnosis, whereas ER - positive tumours recur later but after 15 years, the incidence of recurrence becomes similar for both the receptor types (Ahmed, 2013). Our study failed to demonstrate any definite pattern of locoregional recurrence and distant metastasis during follow-up, which was specific to either of the arms. The possible explanation for this could be the short follow-up period with less number of patients.

Several studies have demonstrated that the molecular subtypes of breast carcinoma vary in prognosis, with worse clinical outcomes traditionally seen among the two hormone receptor negative subgroups (ER- and / or Her-2 neu+) compared to luminal subgroups (ER+) (Sorlie et al., 2001; Sorlie et al., 2003; Ovcaricek et al., 2011). Our findings showed that superior survival rates for the TPBC subtype than TNBC subtype (P=0.07), even though only 3 (7.9%) patients received targeted therapy. These results corroborated with Onitilo et al. (Onitilo et al., 2009) who reported that ER+ / PgR+, Her-2 neu+ subtype had statistically equivalent survival to the reference ER+ / PgR+, Her-2 neu- subtype, and in practice, both types have better prognostic and therapeutic connotations while the TN subtype (ER- / PgR-, Her-2 neu-) had the worst overall survival (hazard ratio,1.8; 95% CI, 1.06-3.2), and

disease-free survival (hazard ratio, 1.5; 95% CI, 0.8-3.0).

Approximately half of Her-2 neu positive breast cancers are hormone receptor (HR) positive. In TPBC, Her-2 neu positive status brings special challenge to the treatment due to its aggressive nature. Trastuzumab, an anti-Her-2 neu targeted therapy, has proven to be a powerful treatment option in clinical practice leading to improvement in survival and an increase in sensitivity to chemotherapeutic agents (Yin et al., 2011). Majority of our patients eligible for Her-2 neu directed therapy were unable to receive it because of medical contraindication, financial constraints and lack of insurance cover. Inspite of this, TP patients benefitted from endocrinal therapy leading to a better OS as compared to TN patients. Recent ASCO guidelines recommend the use of combination therapy with Her-2 neu blocking agents and endocrinal treatment for advanced breast cancer patients (Giordano et al., 2014) although Her-2 neu positivity is associated with a reduced benefit from endocrine therapy (Vici et al., 2015). Contrary to this, our findings revealed that majority of the patients with TP subtype, even without trastuzumab, showed a better OS compared to TN subtype (P=0.07) suggesting hormone receptor status to be an important prognostic factor in breast carcinoma patients.

On evaluating potential factors that may be associated with survival, univariate analysis indicated that young age, premenopausal status, and advanced stage of the disease were found to be associated with shorter OS in the TN arm as compared to TP arm. Tumour grade was not associated with a difference in OS in both the molecular subtypes. Our findings are consistent with the results published by Lara-Medina et al. (2011) on TNBC in Hispanic patients in which younger age (P<0.001), premenopausal status (P=0.04), high histologic grade (P<0.001), and advanced disease (P<0.001) were associated independently with TNBC. Osako and colleagues (Osako et al., 2008) studied premenopausal status as an unfavourable prognostic factor in TNBC patients. They concluded that premenopausal women with TNBC have poor prognosis.

Though our study provides interesting information regarding the similarities and differences between TNBC and TPBC, it is not devoid of limitations including its retrospective design, short follow-up, less number of patients and unmatched study groups. Despite these limitations, our results are in accordance with those of published literature and point towards the aggressive nature of TNBC as well as superior outcome of TPBC patients even without the use of targeted therapy.

To the best of our knowledge, no previous studies comparing the two aggressive types i.e., TN and TP breast carcinoma have been conducted. Our data strongly adds an important layer of information in concordance with the literature that TNBC is more aggressive in terms of presentation at a younger age, advanced stage, higher tumour grade, increased likelihood of locoregional recurrence and metastasis compared to TPBC subtype. However, larger and longer studies are needed to validate the data. Further, the advances in knowledge concerning TNBC over the last few years led to a clear and urgent medical need to understand the behaviour and to develop more effective treatment options as a major focus of breast cancer research. It is the need of the hour to design additional aggressive therapeutic strategies to target these difficult to treat patients.

Thus, we conclude that our present work highlights 2 key observations: First, TNBC has poor prognosis presenting at young age with advanced stage and high grade tumours, a propensity for higher locoregional relapse and distant metastasis than TPBC. This observation indicates that there is still room for future research efforts on providing aggressive treatment to TNBC patients. In addition, prognostic factors such as young age, premenopausal status and advanced stage of the disease have negative impact on these patients. Second, TPBC patients did fairly well even without trastuzumab and thus it is reasonable to suggest that in near future more patients will receive adjuvant trastuzumab therapy thereby enhancing the favourable impact on survival as compared to TNBC patients.

Acknowledgements

We wish to acknowledge our Radiation Oncology staff for all the help in data collection.

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