RESEARCH ARTICLE

# Prognostic Significance of HER-2/neu and Survival of Breast Cancer Patients Attending a Specialized Breast Clinic in Kolkata, Eastern India

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## Abstract

**Introduction: The worldwide incidence of breast cancer has increased rapidly in recent years. The scenario of Eastern India is also showing the same trend. It is necessary to study the utility of HER-2/neu as a prognostic factor in breast cancer survival. However, there have not been detailed studies in this respect with the breast cancer patients of Eastern India. Thus this study was conducted. Materials and Methods: In this hospital-based study 86 breast cancer patients attending a breast clinic of a reputed institute of Eastern India and having invasive ductal carcinomas were observed for a period of 5 years after surgery. Associations between 5 years observed survival and status of ER, PR and HER-2/neu of the patients were critically evaluated. Results: There was statistically significant association between survival pattern for 5 years and the HER-2/neu status (p=0.00001). Better survival was observed for the patients with HER-2/neu negative tumors 67(100%) compared to HER-2/neu positive tumors 7(36.8%). Conclusion: There is strong interaction between survival and HER-2/neu expression of breast cancer patients. Thus the patients with HER-2/neu positive tumors need to be treated aggressively.**

**Keywords:** Breast cancer – prognosis - HER-2/neu – survival - follow-up-Eastern India

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## Introduction

Breast cancer (BC) is the most common leading female cancer in the world (Ferlay et al., 2000: 2008). In eastern India, BC is the most frequently reported cancer (22.7%) in females and the age-specific incidence rate is 25.1 per 100,000 populations (Sen et al., 2002). Several studies reported that there are various prognostic markers in survival of BC (Borg et al., 1990; Pia et al., 2006; Tovey et al., 2009; Pankaj et al., 2010).

Human Epidermal Growth Factor Receptor 2 (HER-2) individually associated with poor survival in BC (Caroline et al., 2003). But nothing has been proved conclusively to be the best in survival of breast cancer in India. Prognosis of breast cancer can be depended on the risk of aggressiveness, faster death or recurrence and shortened disease-free survival (DFS) or overall survival (OS). DFS and OS are commonly used in breast cancer prognosis. Prognosis of breast cancer is depended on clinical factors, histopathological parameters, hormone receptors and molecular based markers. Clinical factors include stage of the disease, menopausal status, age, menarche, lactation, symptoms and others. Histopathological parameters are such as tumor size, grade, lymph node metastasis and Nottingham Prognostic Index (NPI). Hormone receptors are estrogen receptor (ER) and progesterone receptor (PR). Molecular based markers are like oncogenes (e.g., myc), proto-oncogenes (e.g., HER-2/neu), tumor suppressor genes (e.g., p-53), proliferative markers (e.g., Ki 67), apoptosis genes (e.g., Bcl-2) and cell cycle regulators (e.g., cyclins) (Cooke et al., 2001; Graciela et al., 2006). HER-2/neu oncoprotein is a member of the erbB/ epidermal growth factor receptor (EGFR)/ class I family of receptor tyrosine kinase activity, activation of genes involved cell growth, which is related with shortened survival, more aggressiveness and poor prognostic marker (Slamon et al., 1987; Tsuda et al., 1989).

Evaluation of ER, PR and HER-2/neu expression by immunohistochemistry (IHC) is routinely performed in breast carcinomas. ER, PR and HER-2 are associated with recurrence which poor clinical outcome (Paul et al., 2009). Over expression of HER-2 is prognostic maker of tumor aggressiveness and responsiveness to adjuvant therapy. The aim of our study was to usefulness of HER-2/neu as prognostic significance in breast cancer survival. This study aimed to find any association, if any, between

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the survival and the parameters such as menstrual status, tumor Size (T-size), histological grade, lymph node metastasis, NPI, ER, PR and HER-2/neu status in breast cancer and was conducted for the first time with the BC patients of Eastern India. The study was undertaken in the Institute of Post Graduate Medical Education & Research (IPGME&R) and Seth Sukhlal Karnani Memorial Hospital (SSKM), Kolkata, India.

## Materials and Methods

In this study 86 BC patients having invasive ductal carcinoma who were registered as well as operated at the Breast Clinic in IPGME&R and SSKM Hospital, Kolkata, India in 2005 were followed up for a period of five years. Information of these patients was maintained in Comprehensive Breast Clinic Service & Breast Cancer Research Unit of this institute. All patients were treated with standard therapeutic protocol like surgery followed by hormone therapy/chemotherapy/radiotherapy as appropriate. Most common regime of intravenous chemotherapy was FAC regime. It consisted of Inj. 5 F.U. 600 mg/m$^2$, Inj. DOXORUBICIN 60 mg/m$^2$ and Inj. CYCLOPHOSPHAMIDE 600 mg/m$^2$ per cycle. Total 6 cycles of chemotherapy was given with a gap of 3 weeks between 2 cycles. Hormone therapy commonly given was TAMOXIFEN 20 mg for 5 years. LETROZOLE 2.5 mg was given to some post menopausal patients for 5 years. After completion of treatment, patients were being followed up for a total period of five years. Patients were followed up monthly for the first three months and then once in four months for the rest of the period. Survival period was calculated from the date of surgery to the date of last contact of the patients. The period from the date of surgery to date of death from any cause was considered as the overall survival and to the date of recurrence of disease was considered as the disease free survival.

NPI values are calculated on the basis of the formula as (Belle et al., 2011)

NPI=Tumor size x 0.2+Lymph node metastasis (1= no node, 2=1 to 3 nodes positive, 3=4 or more nodes positive +Grade (I=1, II=2 or III=3)

*Histology and Immunohistochemistry*

The specimen was evaluated by standard Grossing and histopathology method. The tumor size, nodal status, histopatholgical subtype and grade were noted. For immunohistochemistry, paraffin sections of tumors were deparaffinized and hydrated by successive washes with xylene, 100%, 70%, 50% ethanol for 5 minutes each. Antigen retrieval buffer accomplished with diluted antigen retrieval buffer and dipped with TRIS buffer. Peroxidase was blocked with 3% hydrogen peroxide. Subsequently, slides were washed in TRIS buffer, incubated with 10% normal animal serum followed by the primary antibody (rabbit anti-ER antibody or rabbit anti-PR antibody or rabbit anti-c-erbB2; HER-2/neu) and incubated 45 minutes at RT. The slides were then incubated with biotinylated secondary antibody for 45 min, followed by DAB Chromogen (followed Lica kit). Counterstaining was done with hematoxylin. Sections were dehydrated by washing sequentially with 70% ethanol, 100% ethanol, and xylene. Cover slips were mounted on slides using Paramount. Digital images of stained and unstained cells were obtained using an Olympus microscope equipped with a SPOT digital camera.

*Statistical Analysis*

Descriptive statistical methods were used to summarize the different information of the patients. The period of survival had been calculated from the date of surgery of the patients. Chi-square test was used to find the degree of association and test of proportion was also used to find the significant differences between different proportions. The Kaplan-Meier method was used to estimate the survival and log-rank test was used to compare the pattern of survival in different groups. The p-value of $\leq 0.05$ was considered as statistically significant. All the statistical calculations and the corresponding p-values were calculated with the help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC).

## Results

The mean±standard error (s.e.) age of the patients was 50.90±1.04 years and median age of the patients was 50 years.

As per Table 1 the observed 5 year survival for pre-menopausal patients 39(97.5%) was significantly higher compared to post-menopausal patients 35(76.1%) (p=0.001).

There was a statistically significant gradually decrease in observed survival with the increase of clinical stage of the disease as at Stage-I 9(100%), Stage-II 22(95.7%), Stage-III 41(83.7%) and Stage-IV 2(40%) (p=0.006).

There was a gradually decrease in observed survival with the increase of the tumor size. 21(75%) patients survived for 5 years with large size (≥4 cm) tumors. 47(90.4%) and 6(100%) survived for 5 years with tumor size (2-3.99 cm) and (<2 cm) respectively (p=0.09).

8(100%), 12(100%) and 54(81.8%) patients survived for 5 years with Grade-I, Grade-II and Grade-III (Grade as per modified Bloom and Richardson grading) respectively (p=0.12).

In node negative tumors, 14(100%) patients had observed survival for 5 years whereas 27(100%), 28(87.5%), and 5(38.5%) survived for 5 years in 1-3, 4-9 and >9 lymph node metastasis respectively which was found statistically significant (p=0.0001).

The better 5 year survival for 15(100%) patients with low NPI (<5.4) was observed compared to 59(79.7%) patients with high NPI (≥5.4) (p=0.19).

In ER positive tumor, 48(91.7%) patients survived for five years compared to ER negative tumors of 26(70.3%) which was statistically significant (p=0.0007). Thus better survival pattern had been observed for patients with ER positive tumors. The exactly same pattern of survival had been observed for patients with PR positive tumors.

There was statistically significant association between survival pattern for 5 years and with status of HER-2 /neu (p=0.00001). Better survival pattern had been observed for

**Table 1. Clinicopathological Details According to Observed 5 Years Survival**

| Clinicopathological Details | | Overall Survival | | Death | | p-value |
|---|---|---|---|---|---|---|
| | | # | % | # | % | |
| Menopausal Status | Premenopausal | 39 | 97.5 | 1 | 2.5 | 0.001* |
| | Postmenopausal | 35 | 76.1 | 11 | 23.9 | |
| Stage | I | 9 | 100 | 0 | 0 | 0.006* |
| | II | 22 | 95.7 | 1 | 4.3 | |
| | III | 41 | 83.7 | 8 | 16.3 | |
| | IV | 2 | 40 | 3 | 60 | |
| Tumor Size (cm) | 0-1.99 | 6 | 100 | 0 | 0 | 0.09 |
| | 2-3.99 | 47 | 90.4 | 5 | 9.6 | |
| | ≥4 | 21 | 75 | 7 | 25 | |
| Grade | I | 8 | 100 | 0 | 0 | 0.12 |
| | II | 12 | 100 | 0 | 0 | |
| | III | 54 | 81.8 | 12 | 18.2 | |
| Lymph Node Status | No Node | 14 | 100 | 0 | 0 | 0.0001* |
| | 1-3 | 27 | 100 | 0 | 0 | |
| | 4-9 | 28 | 87.5 | 4 | 12.5 | |
| | ≥10 | 5 | 38.5 | 8 | 61.5 | |
| NPI | < 5.4 | 15 | 100 | 0 | 0 | 0.191 |
| | ≥ 5.4 | 59 | 79.7 | 12 | 16.9 | |
| ER | Positive | 48 | 91.7 | 1 | 8.3 | 0.0007* |
| | Negative | 26 | 70.3 | 11 | 29.7 | |
| PR | Positive | 48 | 91.7 | 1 | 8.3 | 0.0007* |
| | Negative | 26 | 70.3 | 11 | 29.7 | |
| HER-2/neu | Positive | 7 | 36.8 | 12 | 63.2 | 0.00001* |
| | Negative | 67 | 100 | 0 | 0 | |

#Number of patients; *Statistically Significant

**Table 2. Clinicopathological Details According to HER-2/neu Status**

| Clinicopathological Details | | HER-2/neu (+) | | HER-2/neu (-) | | p |
|---|---|---|---|---|---|---|
| | | # | % | # | % | value |
| Menopausal Status | Premenopausal | 3 | 7.5 | 37 | 92.5 | 0.005* |
| | Postmenopausal | 16 | 34.8 | 30 | 65.2 | |
| Stage | I | 0 | 0 | 9 | 100 | 0.00001* |
| | II | 1 | 4.3 | 22 | 95.7 | |
| | III | 13 | 26.5 | 36 | 73.5 | |
| | IV | 5 | 100 | 0 | 0 | |
| Tumor Size (cm) | 0-1.99 | 0 | 0 | 6 | 100 | 0.006* |
| | 2-3.99 | 6 | 11.5 | 46 | 88.5 | |
| | ≥4 | 13 | 46.4 | 15 | 53.6 | |
| Grade | I | 0 | 0 | 8 | 100 | 0.87 |
| | II | 0 | 0 | 12 | 100 | |
| | III | 19 | 28.8 | 47 | 71.2 | 0.0248* |
| Lymph Node Status | No Node | 0 | 0 | 14 | 100 | 0.00001* |
| | 1-3 | 1 | 3.7 | 26 | 96.3 | |
| | 4-9 | 7 | 21.9 | 25 | 78.1 | |
| | ≥10 | 11 | 84.6 | 2 | 15.4 | |
| NPI | <5.4 | 0 | 0 | 15 | 100 | 0.05* |
| | ≥5.4 | 19 | 26.8 | 52 | 73.2 | |
| ER | Positive | 1 | 2 | 48 | 98 | 0.000002* |
| | Negative | 18 | 48.6 | 19 | 51.4 | |
| PR | Positive | 1 | 2 | 48 | 98 | 0.000002* |
| | Negative | 18 | 48.6 | 19 | 51.4 | |
| Survival; | OS: DFS | 4 | 6 | 63 | 94 | 0.00001* |
| | Relapse | 3 | 42.9 | 4 | 57.1 | |
| | Death | 12 | 100 | 0 | 0 | |

#Number of patients; *Statistically Significant

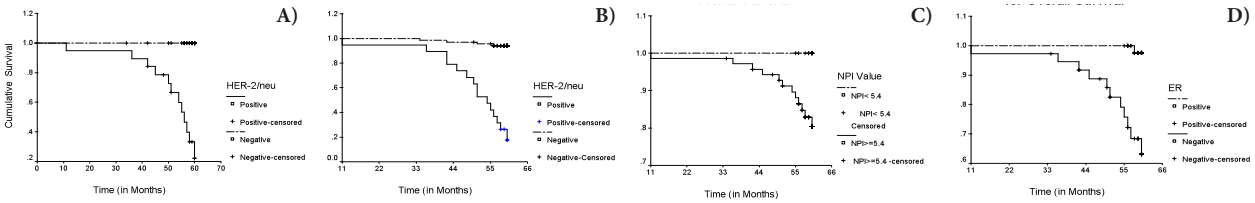


**Figure 1. A) Survival Curves (Kaplan-Meier).** A) Overall Survival, B) Disease Free, C and D) Overall Survival.

the patients with HER-2/neu negative tumors 67(100%) compared to HER-2/neu positive tumors 7(36.8%).

As per Table 2 for HER-2/neu negative tumors pre-menopausal patients 37(92.5%) was significantly higher than post-menopausal patients 30 (65.2%) (p=0.005). Similarly, patients with Stage-I 9(100%) was significantly higher than Stage-II 22(95.7%), Stage-III 36(73.5%) and Stage-IV 0 (0.0%) (p=0.00001) for HER-2/neu negative tumors. Patients with tumor size (<2 cm) 6(100%) was significantly than tumor size of 2-3.99 cm 46(88.5%) and tumor size (>4 cm) 15 (53.6%) for HER-2/neu negative tumors (p=0.006). Patients with Grade-I 8(100%) was significantly higher than Grade-III 47(71.2%) (p=0.0248). But there was no significant difference between Grade-I and Grade-II for HER-2 negative tumors (p=0.87). Patients with lymph node negative tumors 14(100%) was significantly higher than 1-3 lymph nodes 26(96.3%), 4-9 lymph nodes 25(78.1%) and >9 lymph nodes metastasis 2(15.4%) for HER-2/neu negative tumors (p=0.00001).

For HER-2/neu negative tumors patients with low NPI (<5.4) 15(100%) was significantly higher than with high NPI (≥5.4) 52(73.2%) (p=0.05). In HER-2/neu negative tumors, 48(98%) patients with ER positive is significantly higher than 19(51.4%) with ER negative (p=0.000002). The exactly same pattern of PR status had been observed for patients with HER-2/neu negative tumors.

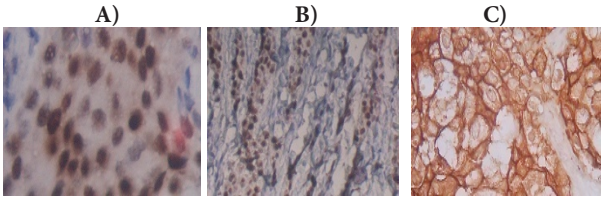


**Figure 2. Immunohistochemistry.** A) Strong ER expression in IDC. B) Strong PR expression in IDC. C) HER-2/neu strong staining in IDC.

Figure 1 describes the overall survival pattern of the BC patients with HER-2/neu positive and negative. As the Log Rank test the overall 5-year survival of the BC patients with HER-2/neu negative is statistically significantly better than HER-2/neu positive (p=0.00001). Also the DFS pattern is shown and found to be better for HER-2/neu compared to HER-2/neu positive (p=0.00001). For overall survival pattern of the BC patients as per their NPI values no significant difference was found (p=0.084). Figure 1D shows the overall survival pattern of the BC patients as per their ER status and patients with ER positive had better survival pattern compared to ER negative (p=0.0001). Figure 2 illustrates ER, PR and HER-2/neu positive staining by IHC.

## Discussion

HER-2 positive tumor had high risk of breast cancer than HER-2 negative tumor (Shaheenah et al., 2010). HER-2 marker had predicting prognosis and outcome of breast cancer (Payne et al., 2008). HER-2 status was shown a significant predictor of disease-free survival in node –negative small size tumors (Rakkhit et al., 2009). High risk of relapse for patients who had HER-2 positive with node negative or small size tumor (Ana et al., 2010). In node negative tumors amplification of HER-2 was found a good predictor of both overall survival and relapse (Cooke et al., 2001). HER-2 gene amplification was showed 20%-30% in primary breast carcinoma and was associated with concurrent HER-2 receptor overexpression. HER-2 status was superior to all other prognostic marker in lymph node positive patients (Slamon et al., 1987: 1989). Prognostic index can be depended on stage of the disease and histological grade of BC (Donald et al., 1991).

Tumor size increased shortened survival and lymph node involvement increased (Christinel et al., 1989). For large tumor size (>4 cm) poor survival pattern and larger proportion of HER-2 positivity had been observed. In high grade tumor (grade-III), we found the poor survival outcome in BC patient. There was strong association between HER-2/neu positive and ER negativity. HER-2 positive tumor cell was poor prognostic outcome in Stage-I, Stage-II and Stage-III in breast cancer (PiaWu¨lfing et al., 2006). Poor survival pattern had been observed in Stage-III and Stage-IV compared to Stage-I and Stage-II. HER-2/neu positivity had been observed in Stage-III and Stage-IV. SM Tovey et al suggested that HER-2/neu positive breast cancer patients are poor survival outcomes in low grade and node- negative tumors (Tovey et al., 2009). Our results also suggest that HER-2/neu positive patients showed a poor survival pattern. Gullick WJ et al demonstrated that HER-2/neu expression is associated with high grade tumor, ER negative tumor and poor prognostic outcome (Gullick WJ et al., 1998). We have also found the same result. All the HER-2 positive cases are found in Grade-III and poor survival pattern had been observed in the same grade. Amplification of HER-2/neu predicts poor survival in node –positive tumors in breast cancer (Borg et al., 1990). Chia et al suggested that in ER-negative patients, HER-2/neu status was good prognostic marker in node negative patients (Chia et al., 2008). For HER-2/neu positive showed higher number of lymph node metastasis. Also higher number of lymph node metastasis showed a poor survival pattern. NPI is good prognostic marker of survival in breast cancer (André et al., 2011).

NPI is a reliable index to predict overall survival of breast cancer patients over five years. NPI<5.4 is associated with good prognosis (about 80% survival over 5 years) while (NPI≥5.4) has less than 50% five year survival rate. High NPI values (≥5.4) showed poor observed survival compared to low NPI values (<5.4). Also proportion of patients with HER-2/neu positive was higher for high NPI values. No patient had been found with HER-2/neu positive for low NPI values. Less proportion of recurrence of disease occurred for HER-2/neu positive cases. Thus higher proportion of disease free survival had been observed in HER-2/neu negative cases. Considering all the findings prognostic significance of HER-2/neu and its survival of breast cancer patients had been observed in this study.

In conclusion, being one of the pioneer studies on breast cancer survival of eastern India this study provided some idea of pattern of survival of breast cancer. HER-2/neu overexpression was associated with large tumor size, high grade, positive lymph node metastasis and high NPI which is poor prognostic outcome of the disease. We concluded that HER-2/neu expression was significantly correlated with advance stage, poor DFS, high relapse rate and death. Role of HER-2/neu status in oncogenic transformation is very important, many studies have investigated that overexpression of HER-2/neu is poor disease out come. Our studies have indicated that overexpression of HER-2/neu is reduced DFS and OS compared with patients whose tumors do not overexpressed HER-2/neu. HER-2/neu expression is directly correlated with ER negativity and also associated with higher NPI value which is poor prognostic outcome. Our study demonstrated that high NPI group patients have shortened DFS, reduced OS and enhanced aggressiveness. Furthermore, according to our data, HER-2/neu seems to modulate angiogenesis and is involved in the development and progression of cancer. HER-2/neu over expression implies aggressive tumor biology in breast cancer & it can predict tumors likely to have poor prognosis poor overall survival. Patients with HER-2/neu positive tumors need to be treated aggressively. We also conclude that inhibition HER-2/neu overexpression may decrease tumor progression in patients and may block breast carcinogenesis, reducing the incidence of breast carcinoma in patients at high risk. This study demonstrates that overexpression of HER-2/neu is individually associated with poor survival in breast cancer.

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