10.1053.paor.2001.0313 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Significance of Immunohistochemical c-ErbB-2 Product Localisation Pattern for Prognosis in Human Breast Cancer

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Breast cancer is an increasingly important cause of illness and death among women. In recent years several novel prognostic determinants of breast cancer have been identified, including c-ErbB-2. In this study, expression of c-ErbB-2 in breast carcinoma was correlated with axillary lymph node metastases and disease outcome. The expression of c-ErbB-2 oncoprotein was analysed in 315 tumor specimens of infiltrating ductal carcinoma of breast. They were categorized according to the modified Bloom and Richardson criteria into three histological grades. These patients also had axillary lymph nodes sampling. The expression of c-ErbB-2 oncoprotein was analysed immunohistochemically. Over expression of c-ErbB-2 were observed in 39.36% tumors. Axillary lymph node metastasis had significant correlation with intensified positivity of c-ErbB-2. C-ErbB-2 positive patients did show resistance to chemotherapy when compared for recurrence and distant metastases following surgery ($p < 0.05$). At a median follow-up of 48 months in c-ErbB-2 positive patients, the overall survival was 3.0 years and disease free survival was 2.5 years. c-ErbB-2 negative tumor patients showed a far better survival. In this group the overall survival was 4.44 years and the disease free survival was 3.78 years. These findings reinforce the view that c-ErbB-2 immunohistochemical detection is of help in detecting a subgroup of breast carcinoma patients who are at high risk. This may also be of particular relevance in decisions regarding adjuvant chemotherapy to these patients. (Pathology Oncology Research Vol 7, No 3, 190–196, 2001)

Keywords: c-ErbB-2, breast cancer, prognosis, drug resistance, nodal metastases

Introduction

C-ErbB-2, which is also known, as HER-2/neu gene is the human counterpart of the rat neu oncogene originally identified in ethylnitrosourea induced rat neuro-glioblastomas. The 185-kD c-ErbB-2 gene product is similar in overall organisation and primary aminoacids sequence to the EGFR. The c-ErbB-2 protooncogenes and transformed genes differ by a single point mutation, which changes a valine to a glutamic acid in the transmembrane region. This mutation is associated with enhancement of an intrinsic protein tyrosine kinase activity and increased oncogenic potential of c-ErbB-2 gene product. The transforming potential of the gene is also related to levels of protein expression. Over expression of the c-ErbB-2 protein is found in 15-40% of breast carcinomas. This is caused by c-ErbB-2 gene amplification in most cases.[1]

Interestingly it has been reported recently that breast carcinoma which overexpressed c-ErbB-2 had significantly longer disease free survival and overall survival when treated with a high dose regime of adjuvant chemotherapy. This concluded that over expression of c-ErbB-2 may be a useful marker to identify patients most likely to benefit from high doses of adjuvant chemotherapy.[2]

Gene amplification results in increased synthesis of the encoded protein. Because the gene product is ultimately responsible for the biological activity of the gene, it appears reasonable that direct measurement of protein, for example, by Western blotting or by immunohistochemical methods

Received: March 29, 2001; *revised:* May 14, 2001; *accepted:* June 20, 2001;

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 1219-4956/01/030190+07 $ 12.00/0

would be as relevant clinically as the measurement of the number of gene copies. Immunohistochemical study has an advantage over Western blotting that the random dilution effect of stroma and non-neoplastic tissues is eliminated.

With the approval of genetically engineered monoclonal antibody trastuzumab (Herceptin) in Sept. 1998 by the food and drug administration agency (FDA, USA) for the treatment of patients with metastatic breast cancer whose tumors overexpressed c-ErbB-2 protein, the importance of detection of c-ErbB-2 has become increasingly essential. This was for the first time in the field of breast carcinoma that a therapy was developed from an understanding of the biology of the disease. Trastuzumab is a monoclonal antibody directed against a normal cell surface protein called HER-2/neu, a receptor homologous to the receptor for epidermal growth factor. In breast carcinoma the gene for this protein, HER-2/neu/c-ErB2 is structurally normal, but in 15-40% of breast carcinoma the gene is greatly amplified and HER-2 protein is correspondingly overexpressed.[3]

Many studies during the last 15 years have shown that amplification of c-ErbB-2 along with over expression of HER-2 is a predictor of aggressive clinical behaviour independent of other known prognostic factors for breast cancer. It correlates with shorter overall survival independent of nodal status. When considered together with another independent prognostic indicator, such as nuclear grade, the prognostic value of this marker is greatly enhanced. For instance, high grade, node-positive HER-2 overexpressing breast carcinoma has an extremely poor response to conventional adjuvant chemotherapy.[3]

The main objective of this study was to assess the independent and interdependent prognostic value of c-ErbB-2 in carcinoma of breast in our female population.

Materials and Methods

A sample of 315 patients with histologically proven diagnosis of invasive ductal carcinomas (IDC) of breast with lymph nodes sampling from Jan 1992 to Dec 1997 were included in the study. Based on available information we assumed a difference of 1.5 years in survival time of patients with c-ErbB-2 positive and c-ErbB-2 negative. Our sample size of 315 was expected to detect this difference with a power of at least 90% at a 5% level of significance.

Morphological variables like age, grade, carcinoma type, vascular / lymphatic invasion, lymph node status & tumor size were recorded. Other variables like menopausal status, parity, distant metastasis; treatment protocol and survival details were retrieved from their medical records. Tissue fixation was done in 10% buffered formalin and processing was done by routine method through alcohol, xylene. After processing, the tissues were embedded in paraffin using the Histocenter 2 from Shandon. 5µm thick sectioning was done by Microtome AS 325 from Shandon. The same breast tumor paraffin blocks were used to make further sections for immunohistochemistry. The sections were cut and picked on poly-L-Lysine coated slides. Expression of c-ErbB-2 protein was evaluated using Rabbit Anti-Human c-ErbB-2 oncoprotein polyclonal antibody c-ErbB-2 (DAKO, Denmark), diluted at 1/25, following pre-treatment of sections in a microwave oven (5x3 min. at 630 W) using PAP technique. A breast carcinoma section expressing c-ErbB-2 was used as a positive control. Same case omitting the primary antibody was used as a negative control with each staining procedure.

Based on College of American Pathologists (CAP) recommendation c-erbB2 was scored as either positive or negative when >10% cells were staining. The reason being that it is a common observation including ours that whenever C-erbB2 is expressed, it is expressed rather homogenously on almost all tumor cells. Likewise when it is negative, it is clearly negative, therefore the common scoring system on a scale of 1-3 i.e., 1+ (<10 % cells positive)., 2+ (10-50% cells positive) and 3+ (>50% cells positive) was not used. Intensity of staining i.e., partial or complete membrane staining as well as thickness of membrane staining was taken into account and differed as +, ++, +++. (*Figure 1a,b*)

Intracytoplasmic staining for c-ErbB-2 in absence of membrane staining was disregarded. Slides were scored on a double-headed microscope (Olympus BX50) separately by two senior histopathologists. Immunostaining was repeated on equivocal cases and consensus was achieved between the two pathologists in all cases.

Statistical analysis

Our main interest was to estimate the survival time for breast cancer patients and look into the relationship between survival time and their prognostic variables. The death of the patients was considered as an event. The data was examined carefully, and decisions were made that how a variable is going to be analysed. Either a continuous variable could be analysed as such, or categorised according to cut off levels, which are biologically plausible.

The Kaplan Meier estimator is an important tool for analysing censored data. The Survival curves, the mean (Standard error for mean), median Survival time (Standard error for median) along with the 25th and 75th percentiles were estimated for each prognostic variable using this method.

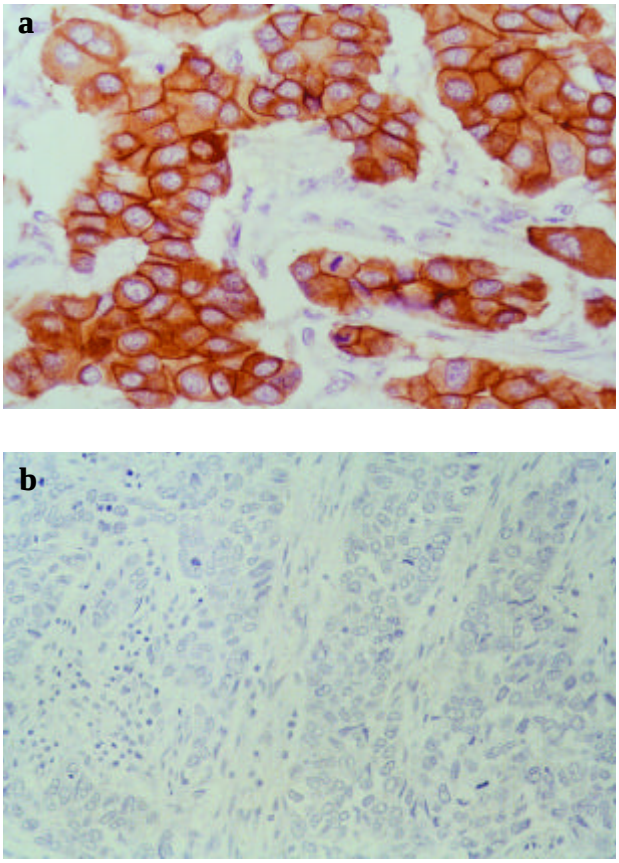
Univariate analysis was done to examine the relationship of each prognostic factor with the survival time using the Cox proportional hazard model or Log rank test. For qualitative variables, if more than two categories existed, and then dummy variables were introduced. Hazard ratios along with there 95% CI were used to describe the relationship between each prognostic variable and the outcome variable.

Multivariate analysis was done to identify a subset of prognostic variables that relate significantly to the hazard, and consequently the survival of the patient. The model fitting was aimed to fit the most parsimonious model, which was biologically able to explain the data. The multivariate analysis also helped us to control for the confounding and study effect modification. An adjusted hazard ratio along with there 95% CI was used to describe the relationship between the set of prognostic variables and the outcome variable.

Results

Descriptive Analysis

Table 1 provides the descriptive statistics about the sample. Analysis was done on a total number of 315 observations, with 36.2% survived till the end of this study i.e. May 1999. Four censored observations as they died due to causes other than breast cancer. The mean and median survival times were calculated using the Kaplan Meier technique. Since in our country carcinomas of breast occur at a relatively younger age (approx. 10 years earlier than the western world) with the incidence more common in the reproductive age group, we dichotomised age at a cut off level of 49 years thus 51% of the subjects were in the reproductive age group, with a mean survival time of 3.35 years (standard error {SE}= 0.13) in contrast to the 49% in the post-menopausal age with a mean survival time of 3.17 years (SE=0.14). On an average 25% of the subjects in the pre-menopausal group are surviving more than 4.67 years, in contrast to the 4.16 years, in the postmenopausal group. The median survival time was also better among the pre-menopausal group, with 50% of the subjects surviving more than 3.58 years, in contrast to the 3.00 years median survival time for post-menopausal group.



Figurer 1. (a) Infiltrating ductal carcinoma breast stained with a polyclonal antibody against c-erb B2/Her-2 by Immunoperoxidase technique. Note very strong membrane staining (+++). Cytoplasmic staining which may also seen in this tumor is not taken into account. x40; (b) Infiltrating ductal carcinoma breast stained with the same antibody as above. Tumor cells are negative for c-erb B2/her2 expression. x20

Histological grading showed a median survival time of 3.17 years and 3.33 years for the subjects with grade 1 and grade II, with corresponding mean survival time of 3.11 years and 3.41 years (SE=0.22), while those with grade III tumors had median survival times of 2.67 years (mean 2.91). When the prognostic marker c-ErbB-2 was absent, the mean and median survival time was found to be significantly better. Mean overall survival in C-ErbB-2 negative patients was 4.44 years compared to when this markers was positive. For staging we used the TNM (tumor, node, metastasis) classification. A better survival for the subjects in an early stage is seen, with a mean survival of 3.45 years (SE=0.24) & median survival time 3.58 years for stage 1 in comparison to the mean survival of 2.86 years (SE=0.28) and median survival time 3.08 years for stage IV. The presence of metastatic lesions in any organ of the body was negatively associated with the prognosis as median survival time for subjects with metastasis was 2.42 years (mean 2.86) when compared to the median survival time for subjects without metastasis 3.58 years (mean 3.5). Similarly with increasing tumor size, the prognosis appears to worsen, with better mean and median survival times among subjects with smaller lesions. Controlling all potential contributors, the effect of c-ErbB-2 on survival time was still significant.

Clinical, histopathological and immunohistochemical characteristics

The histopathological characteristics of the tumors are listed in *Table 2.* All were IDC. Regarding histological grade, 45 (14%) of the tumor were well differentiated, 214 (68%) were moderate, and 56 (18%) were poorly differentiated carcinomas. According to size, tumors were divided into three categories of <2 cm 68 (22%), 2-5 cm 175 (56%) and > 5 cm 72 (23%) in diameter. Positive axillary lymph nodes status was observed in 170 (54%), while negative axillary lymph node status was observed in 145 (46%) subjects.

c-ErbB-2 protein over expression was observed in 124 (39.36%) patients out of 315 cases. Its relationship to histopathological and other immunohistochemical characteristics is shown in *Table 2*. The difference in c-ErbB-2 expression between patients aged <49 years and >49 years was not statistically significant (p= value 0.4368).

c-ErbB-2 over-expression was significantly correlated with histological differentiation, (p 0.0361), tumor size (p 0.0158), and axillary lymph nodes metastases (p 0.0216). Brain and lung metastases were also seen with c-ErbB-2 positive cases with a p value of 0.0235 and 0.0001 respectively. 36 (29%) out of 124 c-ErbB-2 positive cases showed ER/PgR positivity, a positive but non-significant inverse relationship between c-ErbB-2 over expression and ER content was observed (p 0.0575).

Vascular/lymphatic invasion was identified in 61 (49%) of c-ErbB-2 positive cases. There was no significant difference with a p value of 0.3168.

Overall there was a predominance of stage II, 53 (43%) with c-ErbB-2 positivity, followed by stage III 44 (35%), than stage I 18 (15%) and stage IV 9 (7%). In case of negative C-ErbB-2 there was a predominance of stage III 84 (44%) followed by stage II 61 (32%), than stage IV 26 (14%), and stage I 20 (10%). There was an inverse correlation with a p value of 0.0123.

Table 1. **Descriptive analysis showing the summary survival data for prognostic factors associated with survival in patients with breast carcinoma**

Variables		*Total cases n (%)*	*Median survival time (SE)*	*Mean survival time (SE)*
Age	< 49 years	161 (51.11)	3.58 (.18)	3.35 (.13)
	≥ 49 years	154 (48.99)	3.00 (.17)	3.17 (.14)
Grades	I	45 (14.28)	3.17 (.25)	3.11 (.22)
	II	214 (67.93)	3.33 (.16)	3.41 (.13)
	III	56 (17.77)	2.67 (.27)	2.91 (.23)
Vasc. / Lymph invasion	Negative	165 (52.3)	3.33 (.16)	3.27 (.13)
	Positive	150 (47.7)	3.00 (.17)	3.24 (.14)
C-erb-B2	Negative	191 (60.6)	4.36 (.18)	4.44 (.13)
	Positive	124 (39.3)	3.33 (.16)	3.00 (.13)
Stages	Stage I	38 (12.00)	3.58 (.27)	3.45 (.24)
	Stage II	114 (36.20)	3.75 (.39)	3.65 (.16)
	Stage III	128 (40.63)	2.50 (.12)	2.89 (.14)
	Stage IV	35 (11.11)	3.08 (.36)	2.86 (.28)
Family history	No	267 (84.7)	3.25 (.13)	3.22 (.11)
	Yes	48 (15.3)	3.50 (.28)	3.43 (.26)
Hormonal therapy	None	114 (36%)	2.92 (.19)	2.88 (.16)
	Yes	201 (64%)	3.58 (.27)	3.47 (.12)
Chemo-therapy	None	80 (25.4)	3.00 (.17)	3.11 (.15)
	Yes	235 (74.6)	3.33 (.16)	3.32 (.12)
Metastasis	None	211 (67%)	3.58 (.14)	3.50 (.13)
	Yes	104 (33%)	2.42 (.13)	2.86 (.15)
Tumor size	< 2 cms.	68 (21.58)	3.50 (.18)	3.26 (.17)
	2-5 CMS.	175 (55.55)	3.33 (.16)	3.32 (.13)
	> 5 CMS.	72 (22.85)	3.00 (.17)	3.06 (.21)

Survival analysis

After a median follow-up of 48 months (range 3 to 73 months), the overall survival of breast cancer patients amounted to 63%. In univariate as well as multivariate analyses c-ErbB-2 over expression had a significant influence on survival (*Table 3, 4*). Overall survival rates amounted to 72% and 38% in patients with negative and positive c-ErbB-2 protein over expression in tumors. c-ErbB-2 positivity, when compared with the overall survival, is statistically highly significant with a p value of 0.020. At a median follow-up of 48 months, the overall survival was 3.0 years and disease free survival 2.5 years. c-ErbB-2 negative tumor patients showed a far better survival with the overall survival of 4.44 years and disease free survival of 3.78 years. By univariate analysis c-ErbB-2 showed significant correlation with axillary lymph nodes positivity (*Table 4*), tumor size larger than 2 cm and ER/PgR negativity.

Axillary lymph node negativity and c-ErbB-2 positivity was seen in 58 (47%) cases, with a mean tumor size of 3.2 cm, 40% cases were negative for ER/PgR, with an overall survival of 3.15 years and disease free survival of 2.6 years. Whereas axillary lymph nodes positivity and c-ErbB-2 positivity was seen in 66 (53%) cases, with a mean tumor size of 4.5 cm, 55% cases were negative for ER/PgR, with an overall survival of 2.8 years and disease free survival of 2.4 years. Statistically there was a significant correlation with a p value of 0.0016.

In multivariate analysis, the independent prognostic factors for breast cancer patients were tumor size, axillary lymph nodes involvement, histological grade, c-ErbB-2 over expression and ER /PgR status.

Logistic regression analysis and Kaplan-Meier Survival analysis was applied to see the affect of c-ErbB-2 over-expression on resistance to adjuvant chemotherapy with

respect to recurrence and distant metastases. c-ErbB-2 positive patients did show resistance to chemotherapy when looked for recurrence and distant metastases following surgery with a p value of 0.0204

Discussion

Breast cancer is a heterogeneous group of tumors. The necessity of reliable prognostic markers in breast cancer management and prognosis is evident. There is generally no accepted natural history of breast cancer. Several biological variables are known to reflect the biology of breast carcinoma, e.g. axillary nodal status, tumor size, nuclear grade, ER and PgR content, S-phase fraction, DNA ploidy, oncogene amplification etc. Variables, which provide substantial prognostic information, are useful to guide the choice of treatment of the individual patient. At present it is agreed that spread of tumor to the axillary nodes is the most reliable predictor of survival and relapse, together with tumor size and the presence or absence of distant metastases.

Breast carcinoma is characterised by a multitude of genetic abnormalities involving both oncogenes and tumor suppressor genes. The significance of amplified oncogene in breast cancer has been the subject of debate. c-ErbB-2 is the gene known to show the highest frequency of amplification, and has in several reports been associated with breast carcinoma progression and clinically aggressive behavior.[4] c-ErbB-2 is also amplified in breast carcinoma in situ.[5]

The frequency of C-ErbB-2 gene over-expression in the cohort of patients reported in our study (39.36%) is consistent with that of previously reported by others (10%-40%). In our patients we found significant association between C-ErbB-2 over-expression and other biological parameters like histological grade, tumor size and axillary lymph nodes, as reported by others in this region.[6,7]

According to tumor subsets, by mean of univariate analysis C-ErbB-2 was recognised as a significant prognostic factor for axillary lymph nodes positivity tumor size larger than 2 cm and ER/ PgR negativity (*Table 3*).

Table 2. **c-ErbB-2 over-expression and histological characteristics.**

Parameters	*Total No. of patients*	*c-ErbB-2 protein over-expression*		*P value*
		Negative (191)	*positive (124)*	
Grade				
I	45	10 (23%)	35 (77%)	0.0361
II	214	141 (66%)	73 (34%)	
III	56	42 (75%)	14 (25%)	
Tumor size				
≤ 2 cm.	68	45 (66%)	23 (34%)	0.0158
2-5 cm.	175	118 (67%)	57 (33%)	
> 5 cm.	72	59 (82%)	13 (18%)	
Axillary lymph nodes				
Negative	143	85 (59%)	58 (40%)	0.0216
Positive	172	106 (62%)	66 (38%)	
ER / PgR negative	130	72 (55%)	58 (45%)	0.0575
ER / PgR positive	89	53 (60%)	36 (40%)	
Only ER positive	96	66 (69%)	30 (31%)	
Distant metastases				
Brain	38	17 (45%)	21 (55%)	0.0235
Lung	29	03 (10%)	26 (90%)	0.0001
Bone	42	29 (69%)	13 (31%)	0.7562

Table 3. **Independent variables related to prognosis (Cox multivariate analysis).**

Variable	*Coefficient*	*Standard error*	*P value*	*Hazard ratio*
Axillary lymph nodes positive (0 / 1-3 / 4 +)	0.6369	0.117	0.0001	1.891
Tumor size	0.5357	0.119	0.0001	1.709
Grade (I, II, III)	0.7352	0.242	0.0002	2.086
C-ErbB-2 (0 / +)	0.2841	0.134	0.034	1.329
ER / PgR negative / ER / PgR positive or ER positive	0.2897	0.144	0.039	0.7418

In multivariate analysis, the independent prognostic factors for breast cancer patients were tumor size, axillary lymph node involvement, histological grade, c-ErbB-2 over expression and ER/PgR content. Concerning tumor subsets, however, c-ErbB-2 over expression acquired significance only in the prognostically worse group showing positive axillary lymph nodes, larger tumor size, and ER/ PgR negativity.

Some investigators think that c-ErbB-2 oncoprotein expression is not correlated significantly with axillary lymph node metastases.[5,8,9,10,11] It has been suggested that measuring c-ErbB-2 oncoprotein may produce a powerful prognostic factor, providing additional and independent predictive information on both the interval to relapse and overall survival in patients with node-positive or node negative breast carcinoma. Other investigators reported that amplification of c-ErbB-2 was associated with poor

prognosis only in patients with axillary lymph node metastases.[12,13,14] c-ErbB-2 over-expression, usually measured by immunohistochemistry, has been shown to be an independent prognostic factor in several studies on either node-positive and node-negative patients, or studies on all patients in multivariate models.[12,13,15-19,20] The biological activity of c-ErbB-2 is conditioned not only by its abundance but also by the availability of autocrine and paracrine growth factors in the tumor and the presence of three other related receptor tyrosine kinases that are necessary to couple peptide hormones to signalling by c-ErbB-2.

Table 4. **Prognostic value of c-ErbB-2 protein over-expression in tumor subset analysis (multivariate p value/Hazard rates, 95% confidence limits are only given if c-ErbB-2 remained an independent factor in Cox's analysis)**

Factor	*C-ErbB-2 protein over expression*	
	Univariate analysis P value	*Multivariate analysis (p value)/Hazard rate (95% confidence limit)*
Grade		
I	0.0480	
II	NS	
III	NS	
Tumor size		
< 2cm	NS	0.0008 / 1.491 (1.13-1.97)
>2 cm	0.016	
Axillary lymph nodes		
Negative	NS	0.0361 / 1.405 (1.04-1.91)
Positive	0.0004	
ER / PgR positive	0.0711	
ER / PgR negative	NS	0.0371 / 1.496 (1.03-2.18)
ER positive	0.0392	

We studied the c-ErbB-2 oncoprotein, which encodes a glycoprotein similar in structure but distinctly different from the epidermal growth factor receptor.[21] Immunohistochemical study of paraffin-embedded tissue is easier and has a great potential for widespread clinical application than the DNA analysis using Southern blotting which required detecting over-expression of oncogene. However, opinions differ on the relationship between c-ErbB-2 oncoprotein and prognosis. Some investigators believe that the amplification or expression of c-ErbB-2 oncoprotein is an important prognostic factor for breast carcinoma.[9,15,22,23] Others report that the presence of c-ErbB-2 oncoprotein is not associated significantly with prognosis.[5,10,11,24] Differences in the number of entered patients, selection of patients, unequal follow-up periods, or therapeutic regimens may cause these discrepancies. In our study, overall survival and disease free survival were significantly shorter and poorer for patients with c-ErbB-2 oncoprotein expression than for those without such expression.

As reported by Bernard[25] Sharma[26] and Shimizu[27] c-ErbB-2 positive tumors show resistance to adjuvant chemotherapy, we have also evaluated c-ErbB-2. c-ErbB-2 positive patients did show resistance to chemotherapy when compared for recurrence and distant metastases following surgery. The mechanisms responsible for drug resistance with c-ErbB-2 over-expression are unclear but seem to be independent of the multi-drug resistance-1 system. It is thought that c-ErbB-2 signalling might alter the sensitivity to chemotherapeutic agents by acting on genes controlling drug-activating enzymes.[25,27] Although more c-ErbB-2 positive cases diagnosed were in stage II, compared to C-ErbB-2 negative cases, a good proportion of which was in stage III, still poor overall survival and disease free survival in c-ErbB-2 positive cases may be due to the resistance of these tumors to adjuvant chemotherapy.

Above findings suggest that it would be worthwhile to detect the over-expression of c-ErbB-2 in all breast carcinoma patients with or without axillary lymph nodes positivity.

Studies of the humanised c-ErbB-2 (HER-2 /neu) monoclonal antibody trastuzumab (Herceptin) have demonstrated a potent inhibitory activity of the antibody against tumor cell lines over expressing the c-ErbB-2 protein and also increased the sensitivity of experimental tumors to chemotherapy, possibly by lowering the threshold for cells to undergo apoptosis following drug exposure.

Recently, two large multi-centre trials have reported objective responses in 16% of patients (8% complete) in a group of patients with c-ErbB-2 over-expressing tumors and who had received prior combination chemotherapy[18] and improved response rates when combined with other chemotherapeutic agents.[19] Another Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/neu-overexpressing metastatic breast cancer reveals objective response with the treatment.[28]

Patients suitable for new therapy require selection by demonstration of over expression of c-ErbB-2 protein by immunohistochemistry. Hopefully, in the near future c-ErbB-2 screening will become a routine in all breast cancer patients. However how many of these may potentially benefit from this novel therapy due to very high cost of such treatment remain a big question mark for developing countries like Pakistan.

Acknowledgement

This study was supported by "Dean's research award", The Aga Khan University and a generous grant from 'Pakistan Science Foundation', Islamabad, Pakistan.

References

1. *Guido S, Georg F, Joachim T:* Fluorescence in Situ Hybridization for detecting C-ErbB-2 amplification in breast tumor fine needle aspiration biopsies. Acta Cytologica 40:164-173, 1996.
2. *Muss HB, Thor AD, Berry DA:* Cerb-B-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. N Engl J Med 330:1260-1266, 1994.
3. *William C:* More than one way to look for HER-2. Cap Today 13:31-54, 1999.
4. *Perren TJ:* C-ErbB-2 oncogene as a prognostic marker in breast cancer. Br J Cancer 63:328-332, 1991.
5. *van de Vijver MJ, Peterse JL, Mooi WJ:* Neu-protein over expression in breast cancer. Association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. N Engl J Med 319:1239-1245, 1988.
6. *Aryandono-T, Harijadi, Ghozali-A:* Correlation of clinical, pathological status, hormone receptor and C-ErbB-2 oncoprotein in breast cancer patients. Gan-To-Kagaku-Ryoho Suppl 2:600-606, 2000.
7. *Looi-LM, Cheah-PL:* C-ErbB-2 oncoprotein amplification in infiltrating ductal carcinoma of breast relates to high histological grade and loss of oestrogen receptor protein. Malays J Pathol. 20:19-23, 1998.
8. *Wright C, Angus B, Nicholson S:* Expression of c-ErbB-2- oncoprotein: a prognostic indicator in human breast cancer. Cancer Res 49:2087-2090, 1989.
9. *Walker RA, Gullick WJ, Varley JM:* An evaluation of immunoreactivity for C-ErbB-2 protein as a marker of poor short-term prognosis in breast cancer. Br J Cancer 60:426-429, 1989.
10. *Barnes DM, Lammie GA, Millis RR:* An immunohistochemical evaluation of C-ErbB-2 gene expression in human breast carcinoma. Br J Cancer 58:448-452, 1988.
11. *Gusterson BA, Gelber RD, Goldhirsch A:* Prognostic significance of C-ErbB-2 expression in breast cancer. J Clin Oncol 10:1046-1049, 1992.
12. *Tandon AK, Clark GM, Chamness GC:* HER-2/neu oncogene protein and prognosis in breast cancer. J Clin Onco 1989; 17:1120-1128, 1989.
13. *Tsuda H, Hirohashi S, Shimasto Y:* Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units, hst-1 / int-2 and C-ErbB-2 / ear-1. Cancer Res 49:3104-3110, 1989.
14. *Borg A, Tandon AK, Sigurdsson H:* HER-2/ neu amplification predicts poor survival in node-negative breast cancer. Cancer Res 50:4332-4337, 1990.
15. *Slamon DJ, Clark GM, Wong SG:* Human breast cancer: Correlation of relapse and survival with amplification of HER-2/neu oncogene. Science 235:177-183, 1987.
16. *Wright C, Angus B, Nicholson S:* Expression of c-ErbB-2- oncoprotein: a prognostic indicator in human breast cancer. Cancer Res 49:2087-2090, 1989.
17. *Gullick WL, Wright C, Barnes D:* C-ErbB-2 protein overexpression in breast carcinoma is a risk factor in patients with involved and uninvolved lymph nodes. Br J Cancer 63:434-438, 1991.
18. *Cobleigh MA, Vogel CL, Tripathy D:* Efficacy and safety of herceptin as a single agent in 222 women with HER-2 over expression who relapsed following chemotherapy for metastatic breast cancer. ASCO, Los Angeles California 1998; Abstract 376.
19. *Slamon D, Leyland-Jones B, Shak S:* Addition of herceptin to first line chemotherapy for HER-2 over expression metastatic breast cancer markedly increases anticancer activity; A randomised multinational controlled phase III trial. ASCO, Los Angeles California 1998; Abstract 377.
20. *Jakic-Razumovic-J, Petrovecki-M, Uzarevic-B:* Mutual predictive value of c-ErbB-2 over expression and various prognostic factors in ductal invasive breast carcinoma. Tumori 86:30-36, 2000.
21. *King CR, Kraus MH, Aaronson SA:* Amplification of a v-erbB related gene in human mammary carcinoma. Science 229:974-976, 1985.
22. *Berger MS, Locher GW, Saurer S:* Correlation of C-ErbB-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. Cancer Res 48:1238-1243, 1985.
23. *Slamon DJ, Godolphin W, Lones LA:* Studies of HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 244:707-712, 1989.
24. *Ali IU, Campbell G, Lidereau R:* Amplification of C-ErbB-2 and aggressive human breast tumors. Science 235:1795-1798, 1988.
25. *Bernard T, Jacques B:* Prognostic significance of HER-2 /neu oncoprotein expression in node-positive breast cancer. Cancer 73:2359-2365, 1994.
26. *Sharma BK, Ray A, Kaur S:* Immunohistochemical co-expression of c-ErbB-2/Neu oncoprotein, altered tumor suppressor (p53) protein, EGF-R and EMA in histological subtypes of infiltrating duct carcinoma of the breast. Indian J Exp Biol 37: 223-227, 1999.
27. *Shimizu C, Fukutomi T, Tsuda H*: c-ErbB-2 protein over expression and p53 immunoreaction in primary and recurrent breast cancer tissues. J Surg Oncol 73:17-20, 2000.
28. *Baselga J, Tripathy D, Mendelsohn J:* Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/neu-overexpressing metastatic breast cancer. Semin Oncol 26 (Suppl 12): 78-83, 1992.