Correlation of HER-2/neu Protein Overexpression with Other Prognostic and Predictive Factors in Invasive Ductal Breast Cancer

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Abstract. *Background: The objective of our study was to investigate the association between Her-2/neu status and other clinicopathological characteristics of ductal breast carcinoma. Patients and Methods: A total of 120 cases of breast carcinoma were included in this study. The immunohistochemical staining for HER-2/neu, hormone receptors, p53 and Ki-67 were evaluated. Results: HER-2/neu protein overexpression was present in 4 out of 63 T1 lesions, in 13 out of 44 T2 lesions, in 3 out of 7 T3 lesions, and in 3 out of 6 T4 lesions. Protein overexpression was found in 10 out of 21 grade III tumors and 13 out of 72 grade II tumors. Overexpression was not detected in grade I tumors. Of the 23 Her-2/Neu-positive cases, ER- and PR-negative status was detected in 61% and 69%, respectively. Her-2 protein overexpression was seen in 23 out of 93 high Ki-67 tumors, whereas overexpression was not detected in low Ki-67 cases. Conclusion: Statistically significant correlation was found between HER-2/neu protein overexpression and large tumour size, high histological grade, ER and PR negativity, and high Ki-67 proliferative index.*

Breast cancer is a major concern worldwide and is one of the highest causes of death (1). The increasing incidence and significant breast cancer mortality highlight the need for new therapeutic development, especially targeted treatment. A humanized monoclonal antibody, trastuzumab (Herceptin™), targeting the HER-2/neu gene product is a prime example of this new class of treatment (2-6).

The HER-2 gene encodes a 185 kDa transmembrane phosphoglycoprotein with tyrosine kinase activity and is a member of the human epidermal growth factor receptor gene family. Her-2/neu (c-erbB-2) gene amplification, which usually results in overexpression of the encoded transmembrane protein, occurs in approximately 15% to 30% of invasive breast cancers (2-6). Many studies have shown that HER-2/neu overexpression is an adverse prognostic factor (2, 7, 8). The overexpression of Her-2/neu protein and amplification of the Her-2/neu gene is also associated with poor prognostic tumour characteristics, such as high histological grade, high proliferative index, negative or lower oestrogen receptor (ER) expression, lymphoid infiltration and p53 mutation (9-12). Her-2/neu protein overexpression is associated with lower response to methotrexate-based treatment regimens and hormone response modulators, such as tamoxifen, and higher response to doxorubicin-based regimens (13-15).

A major challenge in the success of Herceptin™ treatment is patient selection (3, 9, 16). Several techniques are available for the assessment of Her-2 status in patients with breast cancer (17, 18). The most practical to perform in the routine practice of pathology are immunohistochemistry (ICH) to assess HER-2 protein overexpression, fluorescence *in situ* hybridization (FISH) to assess gene amplification, and chromogenic *in situ* hybridization (CISH) which also allows detection of gene amplification. Studies have been proposing the use of an algorithm to optimize and simplify the assessment of HER-2/neu status, suggesting that FISH or CISH should be reserved for those cases with 2+ staining on IHC (5, 9, 19, 20).

The objective of our study was to better understand the relationship between Her-2 status and ER and PR status, p53 protein expression, Ki-67 expression, histological grade, tumor size, lymph node status and patient age in invasive ductal breast carcinomas.

Patients and Methods

We studied 120 patients with ductal invasive breast carcinoma who underwent total mastectomy or lumpectomy with axillary dissection between 2000 and 2003, at the Institute of Oncology, Sremska Kamenica. Breast cancer specimens were rewiewed using morphological and immunohistochemical criteria according to the

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Table I. *Clinicopathological characteristics breast carcinoma specimens.*

Clinicopathological features (n=120)		
	N	%
Age (years)		
<50	28	(23%)
>=50	92	(77%)
Tumor size		
T1b	16	(13%)
T1c	47	(39%)
T2	44	(37%)
T3	7	(6%)
T4	6	(5%)
Tumor grade		
HG 1	27	(23%)
HG 2	72	(59%)
HG 3	21	(18%)
Lymph node		
negative	65	(54%)
positive	55	(46%)
HER-2/neu		
negative	97	(80%)
positive	23	(20%)
ER		
negative	31	(26%)
positive	89	(74%)
PR		
negative	40	(33%)
positive	80	(67%)
Ki-67		
negative	27	(23%)
positive	93	(77%)
p53		
negative	93	(77%)
positive	27	(23%)

HER-2/neu was defined as negative when the DAKO score was 0, 1+ or 2+, and positive when 3+.

Table II. *Association between HER-2/neu protein overexpression and pathological characteristics in infiltrating ductal breast carcinoma specimens.*

Clinicopathological features	HER-2/neu negative n=97	HER-2/Neu positive n=23	*p* Value
Age (years)	59.44±12.90	57.48±12.43	NS
Lymph node			
N0	54	11	NS
N1+N2	43	12	
Tumor size			
T1b	14	2	$p=0.001$
T1c	45	2	
T2	31	13	
T3	4	3	
T4	3	3	
Tumor grade			
G1	27	0	$p<0.0001$
G2	59	13	
G3	11	10	
ER			
negative	17	14	$p=0.0001$
positive	80	9	
PR			
negative	24	16	$p=0.0001$
positive	73	7	
Ki-67			
negative	27	0	$p=0.009$
positive	70	23	
p53			
negative	79	14	NS
positive	18	9	

WHO classification of breast cancer (21). The histological grading was performed using the modified criteria of Bloom and Richardson, as described by Elston and Ellis (22).

All immunohistochemical studies of HER-2/neu, ER/PR, p53, and Ki-67 were performed on 4-mm sections of formalin-fixed, paraffin-embedded tissues after an antigen retrieval procedure, as described in our previous study (23). IHC staining for ER, PR, p53, Ki-67 and HER-2/neu was carried out according to the LSAB2 method using DAKO (DakoCytomation, Glostrup, Denmark), N-series primary monoclonal antibodies. For each run of staining, a positive and negative control slide were also prepared.

Her-2/neu was scored on a 0 to 3 scale according to the criteria set by Dako, with minor modification (24). Positive IHC staining (HER-2/neu protein overexpression) was defined as intermediate or strong membrane staining in more than 10% of the tumor cell population, whereas weak staining, and faint membrane staining in more than 10% of the tumor cells, as well as membrane staining in less than 10% of the tumor cell population and cytoplasmic staining were considered to be negative. Fluorescence *in situ* hybridization (FISH) was not performed on the weak positive cases (score 2) in this study. ER/PR, p53 and Ki-67 positivity was defined as nuclear staining in more than 10% of tumor cells (10, 25). The percentage of positive cells was semiquantified manually.

Student's *t*-test was used for comparison of mean tumor size and mean patient age for each category of cases. The chi-square test was used to examine the categorical variables and the association between HER-2/neu status and other clinicopathological variables. For continuous variables, correlations were computed by means of the Spearman rank-correlation coefficient. The results were considered statistically significant if the p value was <0.05.

Results

Table I summarises the clinicopathological features of all 120 women with operable ductal invasive breast carcinoma.

Overall HER-2 protein overexpression was seen in 23 cases (20%). The mean age of patients with positive Her-2/neu expression was 57.48 (SD 12.43) years, which is 2 years younger than those who lacked Her-2 expression (Table II), but this difference was not statistically significant. HER-2 protein overexpression was present in 4 out of 63 T1 lesions (6%), in 13 out of 44 T2 lesions (29%), in 3 out of 7 T3

lesions (43%), and in 3 out of 6 T4 lesions (50%), ($p=0.001$, Chi-square test). Tumors with strong Her-2 expression tended to be larger than those lacking expression (scores 0 and 1), with mean sizes of 3.61 cm (SD 1.68) and 1.88 cm (SD 0.65), respectively ($p=0.000013$, Student's *t*-test). Of the Her-2-positive cases, 52% had lymph node metastases, as opposed to 44% among the Her-2- negative cases, but this difference was not statistically significant. Protein overexpression was seen in 10 out of 21 grade III tumors (48%) and in 13 out of 72 grade II tumors (18%). Overexpression was not detected in grade I tumors ($p<0.0001$, Chi-square test). A positive correlation was detected between the grade of the tumors and Her-2 protein overexpression ($r=0.41$, $p=0.000004$). Of the 23 Her-2/neu-positive cases, ER- and PR-negative status was detected in 61% and 69%, respectively. On the other hand, ER- and PR-negative carcinomas were Her-2/neu positive in 45% and 37%, respectively. In this study, Her-2/neu protein overexpression was associated with a statistically significant higher rate of ER- and PR-negative status ($p=0.0001$, Chi-square test). A negative correlation between Her-2 expression and ER and PR was noted ($r=–0.37$, $p=0.000029$ and $r=–0.29$ respectively, $p=0.0014$). Of the Her-2-positive cases, 39% had p53 protein overexpression, as opposed to 18% among the Her-2-negative cases, but this difference was not statistically significant. Her-2 protein overexpression was seen in 23 out of 93 high Ki-67 proliferative index tumors (25%), whereous overexpression was not detected in low Ki-67 proliferatine index cases. In this study, a statistically significant relationship was established between Her-2/neu protein overexpression and Ki-67-positive status ($p=0.009$).

In summary, in this study of 120 cases of infiltrating ductal breast carcinoma in which Her-2/neu protein overexpression by IHC was performed, a statistically significant association was established between Her-2/neu protein overexpression and large tumor size, high histological grade, apsence of ER and PR receptors, and overexpression of Ki-67.

Disscusion

Human breast tumours are diverse in their natural history and in their responsiveness to treatments. Classical clinical and morphological prognostic factors, such as axillary node status, tumor size, histological grade and type, vascular space invasion, and patient age have a well-established role in the management of breast-cancer-bearing patients. However, they do not allow patients to be stratified for appropriate therapy on an individual basis. The advances in the understanding of the molecular and genetic alteration underlying breast cancer development and progression have resulted in the identification of a great number of cell biological markers, such as steroid hormone receptors, oncogene or supressor-gene mutations and/or expression, growth factors, and cell-cycle associated molecules which would be of value in individualizing therapy (26, 27).

In this study, we found that 23 (20%) out of 120 cases were Her-2-positive. Although there is a wide variation in Her-2 overexpression and amplification, our figure appears to be within the commonly accepted rate of 15% to 30% (3, 6). While it has been suggested that Her-2/neu gene amplification is more common in younger patients (28-30), this study failed to reveal a significant relationship between Her-2/neu protein overexpression and patient age. Ariga *et al.* (10) and Prati *et al.* (11) also found no association of Her-2/neu status with patient age.

Our study, as well as others, did not find a significant association between Her-2/neu overexpression and presence of positive axillary lymph nodes (11, 13, 28, 31), although this has been suggested.

Tumor size is one of the most useful predictors of tumor behavior in breast cancer. Our results show a tendency of Her-2 overexpression to be more associated with larger tumor size ($p<0.01$). The higher rates of Her-2 overexpression in larger tumors size have also been documented in some previous studies (32). On the other hand, Her-2/neu overexpression was not found to be associated with large tumor size by some other studies (11-13).

Her-2 amplification/overexpression in different histological grades of female breast cancer has traditionally been a subject of interest. In a study of Hoff *et al.* (33), infiltrating ductal carcinomas were significantly more likely to show Her-2/neu amplification than infiltrating lobular carcinomas ($p<0.005$), and higher grade tumors were more likely to demonstrate Her-2/neu amplification than lower grade ductal carcinomas ($p<0.001$). Similarly, other studies have also reported that histologically high grade tumors are associated with an increased rate of Her-2/neu positivity (11-13, 28, 31, 34). In our study, the majority of Her-2/neu protein overexpressing infiltrating ductal carcinomas were of high histological grade ($p<0.01$).

Estrogen and progesterone receptor determination are established procedures in the routine management of patients with breast cancer, primarily as predictive factors for response to therapeutic and adjuvant hormonal therapy (21, 35). Previous studies have shown Her-2/neu-positive tumors to have a higher association with ER and PR negativity (11-13, 28, 36). In our study, Her-2/neu protein overexpression was associated with a statistically significant higher rate of ER- and PR-negative status ($p<0.01$). Our study, as some others, also demonstrates that ER-positive cases can have Her-2/neu overexpression/amplification. ER-positive, Her-2/neu-positive tumors have a poorer disease-free and overall survival than ER-positive, Her-2/neu-negative tumors, suggesting that Her-2/neu overexpression/ amplification may be a better predictor

of response to tamoxifen therapy than ER status alone (10). Huang *et al.* reported that in women with ER-positive tumours, the expression of PR affects the likelihood of Her-2/neu overexpression, and it may be that women with ER+PR- tumours should be targeted with more aggressive treatment than those with ER+PR+ tumours (12). An interesting article that analyzed the association between Her-2/neu and hormone receptor status in breast cancer showed that the amplification/overexpression of this oncogene was associated with lower ER/PR levels since they were analyzed as continuous rather than dichotomous variables. The autors reported that whenever tumors were positive for both hormone receptors and Her-2/neu, the levels of ER/PR were significantly lower than those with nonelevated Her-2/neu expression (37). The recent understanding of the molecular basis of breast cancer growth and progression led to the identification of tumor subtypes with potentially different biological behavior: luminal, Her-2 overexpressing and basal-like breast carcinomas. The tumors of the basal subtype express the basal cytokeratins and are histologically poorly differentiated, ER-negative/PR-negative/Her-2-negative. This group presents a therapeutic challenge for the oncologist. Siziopikou *et al.* (38) reported that the majority of the "triple negative" patients have basal subtype tumors with high EGFR expression and that these tumors may be the subgroup of breast carcinomas that could potentially benefit the most from novel EGFR-targeted therapeutic strategies (39).

In female breast carcinoma, several studies have shown the MIB-1 labeling index to be an excellent independent prognostic marker that can be used to stratify patients into good and poor prognostic groups (40-42). Trihia *et al.* (43) have shown MIB-1-positive breast cancers to be associated with Her-2/neu positivity in the lymph node-positive cohort. In our study, a statistically significant relationship was established between Her-2/neu protein overexpression and Ki-67 (MIB-1) positive status ($p<0.01$).

There are studies indicating that coexistence of Her-2/neu overexpression as shown by IHC and accumulation of p53 protein is a strong prognostic marker in node-negative breast cancer, even though p53 by itself does not have any independent prognostic value (44, 45). The data on Her-2/neu overexpression and p53 mutation are conflicting. While Her-2/neu overexpression was not found to be associated with the presence of p53 by some studies (46), including ours, significant association between the two has also been reported (12, 45).

Conclusion

Her-2/neu status in breast cancer is important because it provides valuable prognostic, predictive and therapeutic information. The association of Her-2/neu with additional prognostic factors has always been of interest. In this study of 120 cases of infiltrating breast carcinomas in which Her-2/neu expression was shown by IHC, there was a significantly higher rate of Her-2/neu protein overexpression in tumors with large size, high histological grade, negative ER and PR status, and Ki-67 overexpression. Our study did not find associations of Her-2 /neu status with the following: patient age, lymphonodal status or p53 protein expression. Some of these variables failed to show a strong association with Her-2/neu status, maybe due to the sample size. Our findings may confirm that cases with 2+ staining by IHC should be confirmed by FISH or CISH.

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