Original Article

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HER-2/Neu and Hormone Receptor Analysis in Breast Carcinomas and Their Association with Clinicopathologic Parameters

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ABSTRACT

Objective: Invasive breast carcinomas of no special type (IC-NST) are the heterogeneous tumours showing distinct prognostic features even in patients with similar clinicopathological characteristics. To date, many clinicopathological data have been analyzed to make a guess about prognosis and to determine treatment modality. In this study, HER-2/neu status was analyzed by using both immunohistochemical (IHC) and fluorescence in situ hybridization (FISH) methods, and its correlations with hormone receptor status and clinicopathological parameters were investigated.

Materials and Methods: The study was included 112 female patients with diagnosis of IC-NST. FISH for HER-2/neu was applied in only primary tumour tissues, while IHC analyses for HER-2/neu, estrogen (ER) and progesterone receptors (PR) were applied on both primary and metastatic lymph node foci. The results were compared with appropriate statistical methods.

Results: Our rates of HER-2/neu overexpression and gene amplification in the overall study group were 22.3 and 25%, respectively. In the metastatic group, these rates were higher than those of the overall study group (34% and 40%, respectively). Gene amplification rate of the axilla positive group was 40%, while this rate in non-metastatic group was 6.7% (p=0.015). Overexpression and amplification results were compliant (χ^2=77,591, p<0.001). The concordance rates in HER-2/neu negative and overexpression groups were 95.3% and 88%, respectively. Our false negativity rate was 4.7%. While 36% of score 3+ cases were ER positive, 67.1% of HER-2/neu negative cases showed ER positivity (p=0.01). The increase of gene amplification rate in ER negative cases over 50 years age was more than two times and statistically significant (p=0.014).

Conclusion: The concordance rates between the results of IHC and FISH in the HER-2 negative and the overexpression categories were compatible with the literature and lower than the literature, respectively. In the case of ER negativity, the patient's age over 50 years was associated with a higher rate of gene amplification.

Keywords: Breast, invasive carcinoma, FISH, HER-2/neu, hormone receptors, immunohistochemistry

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Introduction

Invasive breast carcinomas of no special type (IC-NST) are a group of tumours with heterogeneous clinical and biologic characteristics. To date, various biological and clinical parameters have been intensively investigated in the determination of prognosis and treatment planning. Some of these parameters are pathological stage, degree of histological differentiation, levels of estrogen receptor (ER), progesterone receptor (PR), and HER-2/neu (c-erbB2) which is a member of epidermal growth factor receptor family. HER-2/neu overexpression/amplification is associated with unfavorable histopathological parameters and poor prognosis. Hormone receptors and HER-2/neu are investigated by immunohistocemical (IHC) methods. HER-2/neu analysis in breast carcinomas provides prognostic data. Besides, this analysis allows evaluation of indication for treatment with trastuzumab which is a monoclonal antibody developed against HER-2/neu receptor (1, 2). HER-2/neu status is evaluated by using IHC and fluorescence in situ hybridization (FISH) methods (1, 2). These methods are nowadays routinely used in many centers.

In the literature, we noticed that there was the limited number of case series that HER-2/neu and hormone receptor (HR) expressions were evaluated in combination and have comparatively analyzed both in the primary tumour and metastatic axillary lymph node tissues. Our aim in this study were determined; a) to analyze the concordance between IHC and FISH results for HER-2/neu; b) to

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investigate the correlation between the results of HER-2/neu analysis in primary and metastatic tumour foci; c) to reveal the correlations among HER-2/neu status, HR expressions and clinicopathological parameters.

Materials and Methods

One hundred twelve cases with IC-NST diagnosed between the years 2000 and 2011 were included in the study. After approval by Tokat Gaziosmanpasa University Clinical Research Ethics Committee, medical reports retrieved from archival files. Paraffine blocks and slides belonged to the cases who had undergone modified radical mastectomy with axillary dissection (n=65) or only excisional breast biopsy (n=47) were reviewed. IHC analyses were performed on primary and metastatic lymph node tumour tissue samples. In our department, tissue fixation procedure is applied for 24 hours with 10% buffered formaldehyde solution. Clinicopathological data and information about the age, gender, stage, histological differentiation (grade), and size of the tumour, status of the axillary lymph node, and number of metastatic lymph nodes were retrieved from pathology reports. In the age analysis, the patients were evaluated according to mean age and threshold age (50 years). The cases were classified as patients aged ≥50, and <50 years. Tumours were also analyzed based on their longest diameters as ≤20 mm, 21-50 mm, and ≥51 mm. The cases with axillary metastasis were classified according to the number (1-3, and ≥4) of metastatic lymph nodes. IHC and FISH analyses were performed on 4 µm-thick paraffin sections. HER-2/neu (clone; e2-4001+3B5, mouse monoclonal, dilution; 1/400, antigen retrieval; citrate, incubation period; 30 minutes Thermo Fisher Scientific, Fremont, USA), ER (clone; SP1, rabbit monoclonal, dilution; 1/100, antigen retrieval; citrate, incubation period; 30 minutes, Thermo Fisher Scientific, Fremont, USA) and PR (clone; SP2, rabbit monoclonal, dilution; 1/100, antigen retrieval; citrate, incubation period; 30 minutes Thermo Fisher Scientific, Fremont, USA) were analyzed using IHC. IHC staining was carried out by fully automated immunohistochemical staining device of Leica Bond-Max (Leica Biosystems, Nussloch, Germany). HER-2/neu status in the primary tumour tissues was also evaluated using FISH. IHC analyses were performed on both primary tumour tissues and metastatic lymph node tissue samples. Due to the technical reasons, the immunohistochemical analyses for HER-2/neu and HR on metastatic lymph nodes of 2 cases could not been performed. For each antibody, appropriate negative and positive controls were used.

The local ethics committee approved the study protocol and provided all the necessary ethical permissions. The study is a retrospective analysis. But written informed consent forms were not obtained from patients who participated in this study.

Evaluation of Immunohistochemical Stains

Whole section area of tumors was evaluated for all antibodies in IHC analyses. Threshold value for ER and PR positivities was accepted as 10% (3). For HER-2/neu, membranous staining was accepted as significant, and the original standardized immunohistochemical testing algorithm recommended by the Modified 2013 ASCO/CAP Guidelines on HER2 Testing in Breast Cancer was used for the evaluation of HER-2/neu expression (4). According to this modified guideline, score 0; no membrane staining or incomplete membrane staining in <10% of invasive tumour cells, score 1+; faint/barely perceptible or weak incomplete membrane staining in >10% of invasive tumour cells, score 2+; strong complete membrane staining in ≤10% of invasive tumour cells or weak/moderate complete membrane staining in >10% of invasive tumour cells and score 3+; strong complete homogenous membrane staining in >10% of invasive tumour cells (Figure 1).

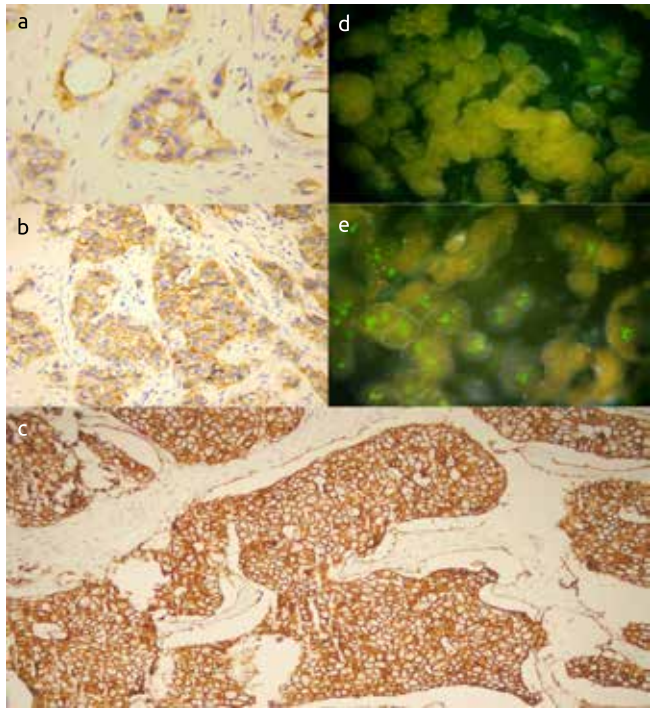

Figure 1. a-e. High power appearance of score 1+ expression. Partial/weak membranous HER-2/neu immunostaining of tumour cells (DAB, X40) (a). Complet/moderate membranous immunostaining of tumour cells in a case with score 2+ expression for HER-2/neu (DAB, X20) (b). Complet/strong membranous immunostaining of tumour cells in a case with score 3+ expression for HER-2/neu (DAB, X20) (c). Normal HER-2/neu signal acitivity (green signals) in a patient with breast carcinoma. Orange signals represent centromere 17 (original magnification X1000) (d). HER-2/neu gene amplification in another case. Increased green signals point out HER-2/neu gene amplification (original magnification X1000) (e)

Scores 0 and 1+ expressions are considered as negative. Score 3+ expression was accepted as positive and score 2+ as "equivocal". To determine HER-2/neu gene status precisely, FISH analysis is recommended for cases in the equivocal category. One of the objectives of our study was to investigate the concordance between the results of IHC and FISH, irrespective of the results of the IHC analysis. FISH analysis was performed on all primary tumour specimens. For HER-2/neu, FISH analysis was realized in paraffin sections fixated with buffered formaldehyde. In the analysis, ZytoVision brand Zytolight SPEC HER2/CEN17 dual color probe kit (ZytoVision, Bremerhaven, Germany) was used.

FISH Evaluation

Using suitable filter sets (DAPI, FITC, Texas Red, TRITC, and Triple filters), green and orange-yellow hybridization signals which represented HER-2/neu gene and chromosome 17 centromere (CEP 17) were observed, respectively. In cells which do not display HER-2/neu amplification, 2 green signals which represent 2 alleles of HER-2 gene and 2 orange-yellow signals which signify chromosome 17 centromeres are observed. To assert the presence of amplification, the ratio between green and orange-yellow signals should be ≥2. In each prima-

Table 1. Clinicopathological characteristics of the overall study group (n=112)

Mean age	56, 36 (34-88)
Age threshold	**n (%)**
<50	40 (36)
>50	70 (64)
Out of the analysis	2
Surgical method	**n (%)**
Tumor resection/excision with axillary dissection	65(58)
Tumor resection/excision without axillary dissection	47(42)
Tumor diameter (mm)	
<20	28 (25.5)
21-50	71 (64.5)
>51	11 (10)
Out of the analysis	2
Histological grade of Bloom-Richardson	**n (%)**
I	24 (21.5)
II	64 (57)
III	24 (21.5)
Axillary lymph node status (65 cases)	**n (%)**
Positive	50 (77)
Negative	15 (23)
Number of positive lymph nodes	**n (%)**
1-3	17 (44)
≥4	33 (66)
ER	**n (%)**
Positive	68 (60.7)
Negative	44 (39.3)
PR	**n (%)**
Positive	42 (37.5)
Negative	70 (62.5)
Her-2/neu expression (IHC)	**n (%)**
Score 0	69 (61.6)
Score 1+	16 (14.3)
Score 2+	2 (1.8)
Score 3+	25 (22.3)
Her-2/neu gene amplification (FISH)	**n (%)**
Positive	28 (25)
Negative	84 (75)

mm: millimeter; HR: hormone receptors; ER: estrogen receptor; PR: progesterone receptor; IHC: immunohistochemistry; FISH: fluorescence in situ hybridization

ry tumour sample, at least 60 distinctly separated nuclei with optimal morphology were counted and evaluated (2). If the ratio between the number of green signals and orange-yellow signals is 2 or more, then a gene amplification is considered as present. Increased number of green signals in separate dots or their accumulations as small clusters was accepted as a significant positive signal. Evaluable significant signals within intact nuclei should appear as separate, distinct signals (Figure 1). Since chromosome 17 polysomy can lead to false-positive results, it should be taken into consideration during identification, and evaluation of this entity. If in more than 6% of the tumour cells, presence of ≥3 orange-yellow CEP17 signals was detected, then this condition was defined as chromosome 17 polysomy (2). Reliability of hybridization results was ensured in comparative evaluation of staining intensities of sections prepared from the cases with those of positive and negative controls provided with the kit.

Statistical Analysis

In comparisons of means of numerical values (when sample size/number of groups=2) t-test for independent samples was used. Still in comparisons of numerical values, when sample size/number of groups was ≥3, One Way ANOVA was used. When significant differences were found between groups, in pairwise comparisons between groups, Tukey HSD Post-hoc test was used. However, in comparisons between qualitative variables chi-square tests were used. Correlations detected at p values of ≤0.05 were considered as statistically significant. Statistical analysis was performed by using Statistical Packages for the Social Sciences (SPSS) version 19 commercial software (IBM Corp.; Armonk, NY, USA).

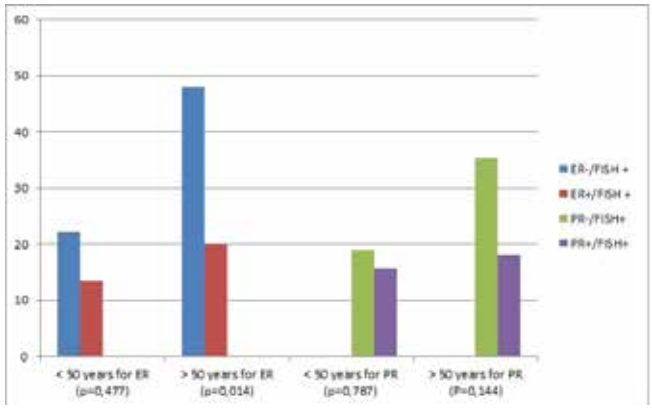

Figure 2. Gene amplification rates in cases aged >50 and <50 years of age according to ER and PR status in the overall study group

Results

The mean age of the overall study group was 56.4 years (age range; 34-88). Since the ages of two cases could not be obtained, age analysis was realized in 110 cases. Because of the same reason, 2 cases were excluded from the analysis of tumour size. The mean diameter of the tumours was 31.8 mm (range: 7-100 mm). Clinicopathological characteristics of the study group are given in Table 1. Primary tumour size was related to histological grade. As tumour size increased, degree of its differentiation decreased (p=0.019). Any correlation between the mean age and the other parameters was not seen in the overall study group.

In the overall study group, the rates of HER-2/neu overexpression (score 3+) and gene amplification were 22.3% and 25%, respectively.

Table 2. Comparison of IHC and FISH results for HER-2/neu in the overall study and axilla positive groups

HER-2/neu scores of primary tumor foci (overall study group)	FISH (+)	FISH (-)	Total	Statistic
Score 0	2 (2.9%)	67 (97.1%)	69 (61.6%)	
Score 1+	2 (12.5%)	14 (87.5%)	16 (14.3%)	x^2=78.229
Score 2+	2 (100%)	0	2 (1.8%)	p<0.001
Score 3+	22 (88%)	3 (12%)	25 (22.3%)	
Total	28 (25%)	84 (75%)	112 (100%)	
HER-2/neu scores of metastatic lymph node tumor foci	**FISH (+)**	**FISH (-)**	**Total**	**Statistics**
Score 0	6 (20%)	24 (80%)	30 (62.5%)	
Score 1+	3 (50%)	3 (50%)	6 (12.5%)	x^2=18.994
Score 2+	3 (75%)	1 (25%)	4 (8.3%)	p<0.001
Score 3+	8 (100%)	0	8 (16.7%)	
Total	20 (41.7%)	28 (58.3%)	48 (100%)	

IHC: immunohistochemistry; FISH: fluorescence in situ hybridization

Chromosome 17 polysomy was not determined in any of our cases. ER and PR positivity rates were 60.7% and 37.5%, respectively.

Grade, gene amplification and ER expression correlated with each other. Gene amplification increased in parallel with histological grade. Gene amplification rates in grade I, II and III cases were 4.2%, 34.4% and 20.8%, respectively (χ^2=8.778, p=0.012). Although there was no statistically significance, grade II and III tumours showed higher HER-2/neu overexpression rates than that of grade I tumours (χ^2=8.554, p=0.073).

In the analysis of the cases with axillary dissection (65 cases), a significant correlation was detected between axillary status and gene amplification. Gene amplification was seen in 40% (20 cases) of axilla positive cases, while only 6.7% (1 case) of non-metastatic cases showed gene amplification (χ^2=5.862, p=0.015).

In the primary tumour tissues, a strong correlation existed between HER-2/neu expression and amplification (χ^2=78.229, p<0.001). The 88% (22 cases) of score 3+ cases (25 cases) were FISH positive, while 95.3% of HER-2/neu negative cases (score 0/1+ cases) were FISH negative. There were only 2 cases in the equivocal category (score 2+), and both showed gene amplification (Table 2).

HER-2/neu expression and amplification were correlated with ER status (χ^2=9.131, p<0.01, χ^2=4.991, p<0.025, respectively). ER positive cases were accumulated within HER-2/neu negative and nonamplified categories. The rates of ER positive cases in these categories were 83.8% and 82.4%, respectively. Such a relationship for PR status was not determined. The 97.7% of ER negative cases was PR negative, while 60.3% of ER positive cases was PR positive (χ^2=38.371, p<0.001).

When gene amplification status was analysed based on HR status in consideration of 50 years age as a threshold value, a statistically significant correlation for ER status was detected in the cases over 50 years old. The 48% of ER negative cases over 50 years of age was HER-2/neu amplified cases while only 20% of ER positive cases in the same age group showed gene amplification (χ^2=6000, p=0.014). Gene amplification in the cases of younger than 50 years was detected in 13.6% and 18.2% of ER positive and ER negative cases, respectively. (χ^2=0.505, p=0.477). FISH positivity was detected in 35.4% (22 cases) and 19% of PR negative cases aged >50 and <50 years, respectively. In PR positive cases, the corresponding rates were 18.2%, and 15.8%, respectively (χ^2=2.134, p=0.144, χ^2=0.073, and p=0.787, respectively) (Figure 2) In generally, HR negative cases aged over 50 years demonstrated higher rates of gene amplification when compared with HR negative cases younger than 50 years of age.

In the analyses of HER-2/neu gene and HR expressions in metastatic lymph node foci, the following data were obtained;

Lymph node metastasis was detected in 50 of 65 cases who had undergone axillary dissection. Since paraffin blocks of 2 out of 50 cases did not exist, IHC analyses were realized on the metastatic lymph nodes of 48 cases. The results of IHC analyses on metastatic lymph node foci were shown in table 3.

A concordance was detected between HER-2/neu expressions of metastatic lymph node foci and primary foci. Majority of the cases which were score 0 and 1+ for primary tumour foci were also score 0 and 1+ at their metastatic lymph node foci. The score distribution at metastatic foci of the cases that are score 3+ for primary tumour foci was as follows; 17.6% (3 cases) for score 0, 17.6% (3 cases) for score 1+, 17.6% (3 cases) for score 2+ and 47.1% (8 cases) for score 3+ (χ^2=28.690, p=0.001) (Table 4).

A significant correlation existed between HER-2/neu expression at metastatic foci and the corresponding primary tumours' gene amplification. The distribution of gene amplification rates according to IHC scores was follows; 20% of score 0 cases, 50% of score 1+ cases, 75% of score 2+ cases and 100% of score 3+ cases were FISH positive (χ^2=18.994, p<0.001) (Table 2).

Table 3. The status of HER-2/neu gene and HR in the axilla positive group (n=50)

ER	**n (%)**
Positive	24 (50)
Negative	24 (50)
Out of analysis	2
PR	**n (%)**
Positive	12 (25)
Negative	36 (75)
Out of analysis	2
HER-2/ne expression (IHC)	**n (%)**
0	30 (62.5)
1+	6 (12.5)
2+	4 (8.3)
3+	8 (16.7)
Out of analysis	2
HER-2/neu gene amplification (FISH) on primary tumors	**n (%)**
Positive	20 (40)
Negative	30 (60)

HR: hormone receptors; ER: estrogen receptor; PR: progesterone receptor; IHC: immunohistochemistry; FISH: fluorescence in situ hybridization

A significant and inverse correlation existed between HER-2/neu expressions and ER positivity at metastatic foci. While 61.1% of score 0/1+ cases were ER positive, only 12.5% of score 3+ cases were ER positive (χ^2=7.278, p=0.026).

A positive correlation was existed between ER and PR expressions on metastatic lymph node foci. Half of ER positive cases was also PR positive while all of ER negative cases was PR negative (χ^2=16.000, p<0.001).

Discussion and Conclusion

In breast cancers, in additional to conventional clinicopathological parameters, analysis of HER-2/neu receptor gene which has a therapeutic role has been gained the importance recently. Therefore, it is very important to correctly identify patients with tumour showing HER-2/neu overexpression/gene amplification. Early studies suggested that as many as 30% of breast cancers had HER-2/neu overexpression (2). The rates of false positivity and false negativity in these studies were reached to 19% and 10%, respectively (2). After these reports, following revisions of guideline and refinement of test performance parameters such as tissue handling, methodology and quality assurance measures, more recent publications indicate that the rates of HER-2/neu positivity is between 13% and 20%. In these publications, the rates of false positivity and false negativity are reduced to less than 6% and less than 2%, respectively (2).

In the present study, our rates of overexpression and gene amplification in the overall study group were 22.3% and 25%, respectively. Our rate of HER-2/neu overexpression was slightly higher than those of the recent literature. The overexpression and amplification rates in the axilla positive group were 34% and 40%, respectively. The high HER-2/neu positivity rate of the overall study group may be stemming from the fact that nearly half of the overall study group consists of the patients with axillary metastasis.

According to the actual ASCO/CAP guideline, it is recommended to attain a concordance of 95% between IHC and ISH (in situ hybridization) (4). Although our concordance rate in HER-2/neu negative group was compatible with the guideline, our concordance rate in score 3+ cases was lower than that of the guideline. In the overall study group, 3 cases with score 3+ tumour were nonamplified cases. Although IHC assays were repeated on the same and different tumour blocks, the results were not changed. FISH analyses could not be repeated. Since FISH analyses could not be reperformed, our ability to comment was limited in this condition. Even so, we may think that this discrepancy in score 3+ cases is due to HER-2/neu heterogeneity in these cases' tumours. HER-2/neu heterogeneity was reported in 11%-40% of breast cancers (2). It is emphasized that heterogeneity was more frequently seen in HER-2/neu positive tumours (2). There is a limited data in the literature how HER-2/neu heterogeneity affects the outcome of trastuzumab treatment in breast cancers (5, 6). In the overall study group, although score 3+ cases showed low concordance rates according to FISH results, a significant correlation existed between the results of IHC and FISH assays (p<0.001).

In the multi-centre study of Tuzlalı et al. (1), HER-2/neu negative cases (score 0/1+) from 9 different centres were analyzed by SISH (silver in situ hybridization). Rates of SISH positivity varied between 0 and 10.48% in their study. The false negativity rates for two centers were seemed obviously high. Tuzlalı et al. (1) suggested that these high false negativity rates may be derived from a defect in any step of their IHC procedures. Although the upper limit of 5% false negativity, ASCO/CAP guideline suggested that percentage of the false negative tests as close to 0% as possible should be aimed by laboratories (1). Our false negativity rate was 4.7%. Although this rate was below the recommended upper limit, it was close to this limit. We think that the factors such as fixation, tissue processing and clone of primary antibody in the preanalytic-analytic periods played a major role in the emergence of a false negativity rate close to the upper limit.

When HER-2/neu gene status was analyzed on axillary metastatic lymph node foci, a remarkable condition was noticed. Any score of HER-2/neu expression of metastatic foci was strongly correlated with gene amplification. Score 0, 1+, 2+ and 3+ expressions of metastatic foci corresponded to FISH positive primary tumours in the rates of 20%, 50%, 75% and 100%, respectively (p<0.001). A similar distribution was also determined between HER-2/neu expressions of primary and metastatic tumour foci (p=0.001). These results pointed out that metastatic tumour foci had more HER-2/neu heterogeneity according to primary tumours. In addition, these outcomes may also indicate that HER-2/neu gene is not effective alone in the identification of the metastatic clone in the primary tumour. In the analysis of 65 cases that underwent axillary dissections, gene amplification in axilla positive cases was markedly higher than that of non-metastatic cases (40% vs 6.7%) (p=0.015). A similar correlation for HER-2/neu overexpression was not seen (34% vs 20%) (p=0.479). This phenomenon may indicate that factors other than gene amplification might be also responsible for overexpression of HER-2/neu receptor. These results

Table 4. Comparison of HER-2/neu IHC scores of primary tumor foci and metastatic lymph node tumor foci

HER-2/neu scores of primary tumor foci (overall study group)	HER-2/neu scores of metastatic lymph node tumor foci					
	Score 0	Score 1+	Score 2+	Score 3+	Total	Statistic
Score 0	21 (87.5%)	3 (12.5%)	0	0	24 (50%)	
Score 1+	5 (83.3%)	0	1 (16.7%)	0	6 (12.5%)	
Score 2+	1 (100%)	0	0	0	1 (2.1%)	x^2=28.690
Score 3+	3 (17.6%)	3 (17.6%)	3 (17.6%)	8 (47.1%)	17 (35.4%)	p=0.001
Total	30 (62.5%)	6 (12.5%)	4 (8.3%)	8 (16.7%)	48 (100%)	

IHC: immunohistochemistry

may explain the reason why some cases with HER-2/neu gene amplification benefit from trastuzumab therapy, while the remaining cases do not. In the literature overall response rate to trastuzumab therapy was reportedly varied between 18 and 50 percent (7).

The associations between HER-2/neu status and risk of recurrence/metastasis, shorter life expectancy have been reported in the literature (8). According to the results of many studies, high proliferative index, tumour grade, HR negativity, p53 positivity and axillary metastasis have been associated with overexpression/amplification of HER-2/neu gene (9-12). In the present study, HER-2/neu overexpression was not associated with axillary metastasis (p=0.479), while gene amplification correlated with axillary metastasis (p=0.015). Some studies (12) have reported the relationships that are similar with ours results between gene amplification and axillary metastasis, while others could not find such a correlation (9, 13, 14). In our study, tumour grade did not correlate with HER-2/neu expression, but it displayed a statistically significant correlation with gene amplification (p=0.012). Tumour grade increased in parallel with enhanced gene amplification. In studies of Prati et al. (9) and Ariga et al. (10), gene amplification rates increased in direct proportion with increases in tumour grades. Many other studies have also pointed out to strong correlations between gene overexpression/amplification and tumour grade (13, 15, 16). Prognostic impact of tumour size has been reported by Lee et al. (17). They suggested that HER-2/neu overexpression was correlated with tumour size. However, some studies did not find such a correlation between HER-2/neu and tumour size (10). In our study, any correlation between HER-2/neu status and tumour size was not observed.

HER-2/neu is generally inversely correlated with HR status. We also observed this inverse correlation on both primary tumour foci and metastatic lymph node tumour foci. This correlation is in parallel with unfavourable prognostic impact of the HER-2/neu gene. Amplification/overexpression of HER-2/neu is generally associated with HR negativity. This phenomenon explains why HER-2/neu positive tumours are refractory to hormonal therapy. In the present study, significant correlations between HER-2/neu amplification/expression and ER status were also determined. Such a significant correlation for PR expression was not seen. ER status is determinative for the indication of postoperative tamoxifen therapy. In ER positive patients, response rates to tamoxifen therapy ranges between 40%-70% (17, 18). Factors determining response rates to chemotherapeutic agents as trastuzumab or tamoxifen have not been fully elucidated yet. Overexpression/amplification of HER-2/neu gene herald resistance to methotrexate and tamoxifen and an improved response to doxorubicine. In advanced stage breast cancers, HER-2/neu positivity is important in the indication for trastuzumab therapy (19). HR expression is not generally seen in score 3+ cases (20). It has been suggested that estrogen bound to HER-2/neu receptor inhibits this gene (21). It has been demonstrated that this correlation is age-dependent and during the premenopausal period such a correlation was not observed (22, 23). Score 3+/HR positive cases reportedly demonstrated lower response rates to hormonal therapies when compared with HER-2/neu negative/HR positive cases, and resistance to therapy was seen in very advanced ages (22-26). Score 3+/HR positive combination is more frequently seen in young patients, and it has been suggested that HER-2/neu overexpression did not affect response to hormonal therapy in this age group (22, 23). In the literature, the rates of ER positivity in HER-2/neu positive cases changed between 6.8% and 50% (10, 19, 22-25, 27-30). In our rates of ER positivity in the cases with HER-2/neu overexpression and amplification were 36% and 42.9%, respectively. However, the PR positivity rates were lower than these rates (20% and 25%, respectively). In the literature, rates of ER and PR positivity changed between 46% to 68% (17, 30-34) and 42% to 58% (30, 33, 34) respectively. Our ER positivity rate (60.7%) was consistent with the literature, while our PR positivity rate (37.5%) was relatively low according to the literature. Gene amplification rate in HR negative cases >50 years of age was detected to be at least two times higher than in HR negative cases <50 years of age (Figure 2). The increase of gene amplification rate in ER negative cases over 50 years age was more than two times and statistically significant (p=0.014).

In conclusion, we understand that the HER-2/neu gene status alone, and with other parameters, may have significant correlations with clinical and prognostic aspects. In addition to evaluating HER-2/neu gene status with accurate and valid methods, it may be more useful to evaluate the gene status with other clinicopathologic parameters.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziosmanpaşa University Clinical Research (09-GEKTIP-002).

Informed Consent: Informed consent was not taken due to retrospective design of the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - R.D.K., F.M., A.M., A.B.İ., A.D.; Design - R.D.K., F.M.; Supervision - R.D.K., F.M.; Resources - R.D.K., F.M.; Materials - R.D.K., A.M.; Data Collection and/or Processing - R.D.K., F.M., F.A.D.,

A.B.İ.; Analysis and/or Interpretation - R.D.K., İ.E., F.M., A.M.; Literature Search - R.D.K., F.M.; Writing Manuscript - R.D.K., F.M.; Critical Review - R.D.K., F.M., A.M., A.B.İ., F.A.D.

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