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Concurrent expression of immunhistochemical parameters in breast cancer patients; clinical implications and consistency with Bloom-Richardson system

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Abstract

We sought to determine prognostic importance of expression p53, c-erbB-2 (also known HER2/neu or HER2), estrogen receptor and progesterone receptor in breast cancer patients by investigating their relationship with histopathological and clinical parameters. We also investigated whether different parameters other than Bloom-Richardson grading system might be used in classification of breast cancer patients on the basis of concurrent expression of immunhistochemical parameters. Seventy-one invasive ductal carcinoma patients were included. We studied immunhistochemical parameters including, estrogen receptor, progesteron receptor, p53 and c-erbB-2. Specimens that were archived in pathology department were re-assessed to determine necrosis, lymph and blood vessel invasion, perineural invasion, peritumoral inflamatory reaction characteristics. Bloom-Richardson grading system was applied for each specimen. Multivariate discriminant analysis was performed to test the relationship between Bloom-Richardson system and immunhistochemical parameters. Mean age was 50.79 ± 11.92. Forty-eight patients (67.6%) were estrogen receptor positive, 34 (47.9%) were progesterone receptor positive, 38 (53.5%) were p53 positive and 46 (64.8%) were c-erbB-2 positive. Necrosis was less common and peritumoral inflammatory reaction was more common among estrogen receptor positive patients. According to the discriminant analysis, 52.1% of patients with concurrent expression of ER, PR, p53 and c-erbB-2 were correctly classified according to overall Bloom-Richardson grade, 49.3% were correctly classified according to nuclear pleomorphism score and 77.5% were correctly classified according to mitotic count. Prognostic classification of patients could be done on the basis of mitotic characteristics of the tumor. Further study is warranted to establish the standard threshold for mitotic count for breast tumors of different types.

Keywords: breast cancer, immunhistochemical markers, Bloom-Richardson grading system, mitotic caunt

1. Introduction

Breast cancer is the most common cancer among women worldwide and the second most common cause of cancer-related deaths, following lung cancer. About 1.000.000 new cases are detected per year. Although widespread mammography usage enabled the early detection of most lesions, no significant decrease in mortality has been achieved (1). A precise determination of populations at risk for developing breast cancer is almost impossible since the disease has multifactorial pathogenesis (2, 3). Status of hormone receptors, proliferative activity, inactivation of tumor suppressor genes, and overexpression of oncogenes are the prognostic factors that are interrelated with each other (4, 5) in disease pathogenesis.

In this study, we sought to determine the prognostic importance of expressions of tumor suppressor gene p53, a protooncogene c-erbB-2, and the status of estrogen and progesterone receptors in breast cancer patients by investigating their relationship with histopathological and clinical parameters. We also investigated whether different parameters other than the Bloom-Richardson grading system might be used to classify breast cancer patients based on the concurrent expression of immunohistochemical parameters.

2. Materials and Methods

2.1. Study design and patients

A retrospective archive study was performed in the radiation oncology and pathology departments of Ondokuz Mayıs University Hospital. Patients who received medical care in the radiation oncology department for the treatment of breast carcinoma between 1999 and 2003 were detected. Of these, 71 patients who were diagnosed, based on a histopathologic study, with invasive ductal carcinoma and those in whom we studied immunohistochemical parameters including estrogen receptor (ER), progesterone receptor (PR), p53 and c-erbB-2 were considered eligible for the study. Patient demographics, clinical data, preoperative mammographs, and TNM stages were recorded for analysis. Hemotoxyilen-eosin stained specimens that were stored in the pathology archive were re-assessed by the investigators to determine necrosis, lymph and blood vessel invasion, perineural invasion, and peritumoral inflammatory reaction characteristics. Bloom-Richardson grading system was applied for each specimen. All histomorphological assessments were performed using a Leica

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HMLB45 microscope. The microscope had an area diameter of 0.50 mm. Scoring for the mitotic count was as follows; 0-7 mitoses: 1 point, 8-14 mitoses: 2 points, 15 and more mitoses: 3 points. The number of metastatic lymph nodes, tumor diameter in metastatic nodes, the status of perinodal infiltration, and preoperative mammographs were used to perform TNM staging.

2.2. Re-assessment of specimens

The archived specimens were re-assessed for ER, PR, p53, and c-erbB-2 independently from pathology reports. The percentage of expressing cells for ER, PR, and p53 was determined by counting 300 tumor cells under x40 magnification. Positive staining <10% was considered as (-) staining, and >10% was considered as (+) staining. C-erbB-2 positivity was defined as membrane staining. According to the staining pattern, when no staining was observed, or membrane staining was present in <10% of tumor cells, it was defined as (-); when there was faint membrane staining in >10% of tumor cells and only part of the membrane was stained, it was defined as (+); when there was weak to moderate complete membrane staining in >10% of tumor cells, it was defined as (++); when there was strong complete membrane staining in 10% of tumor cells, it was defined as (+++). These categories were dichotomized as follows; (-) and (+) cases were defined as (-), and (++) and (+++) cases were defined as (+).

2.3. Statistical analysis

All statistical analyses were performed using SPSS (SPSS, Inc. Chicago, IL. USA) packaged software. Visual histograms and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test) were used to determine normal distribution. Continuous variables were defined by the mean ± standard deviations. ROC analysis was used for testing the sensitivity and specificity of the 10% threshold for the expression of immunohistochemical parameters. The chi-square test was used to test the relationship between immunohistochemical parameters and menopause status, tumor size, lymph node involvement, grade, staging systems, and histological features. Pearson correlation was used to test the relationship between age and immunohistochemical parameters. A p-value less than 0.05 was considered as statistical significance. Multivariate discriminant analysis was performed to test the relationship between the Bloom-Richardson system and immunohistochemical parameters. Cases were divided into two categories, whether the mitotic count was less or greater than 13 mitoses per 10 high-power fields.

3. Results

The mean age was 50.79 ± 11.92 (ranging from 27 to 76). Forty-eight patients (67.6%) were ER positive, 34 patients (47.9%) were PR positive, 38 patients (53.5%) were p53 positive, and 46 patients (64.8%) were c-erbB-2 positive. Out of 71 patients, 61 underwent modified radical mastectomy (MRM) + axillary lymph node dissection (ALND), 5 underwent simple mastectomy + axillary sampling, 3 underwent quadrantectomy + axillary sampling, and 2 patients underwent lumpectomy + axillary sampling. Breast cancer is located on the left side in 36 patients (51%) and on the right side in the remaining. There was no significant correlation between age and expression of ER (r=0.082, p=0.497), PR (r=-0.099, p=0.413), p53 (r=-0.14, p= 0.245) ve c-erbB-2 (r=-0.210, p=0.079).

Table 1. Distribution of menopause status, tumor size groups, axillary lymph node metastasis and tumor characteristics among cases with positive expression of imunohistochemistry parameters.

Variable	ER positive (n=48/71)	PR positive (n=34/71)	p53 positive (n=38/71)	c-erbB-2 positive (n=46/71)
Menopause status				
Premenopause (n=29)	21 (72)	18 (62)	16 (55)	20 (69)
Postmenopause (n=42)	27 (64)	16 (38)	22 (52)	26 (62)
P value	0.607	0.057	1.000	0.618
Tumor size				
<2 cm (n=9)	7 (78)	4 (44)	4 (44)	5 (56)
2-5cm (n=46)	32 (70)	23 (50)	24 (52)	28 (61)
>5 cm (n=16)	9 (56)	7 (44)	10 (63)	13 (81)
P value	0.485	0.889	0.654	0.280
Axillary lymph node metastasis				
0 (n=26)	14 (54)	9 (35)	13 (50)	16 (62)
1-3 (n=22)	18 (82)	14 (64)	13 (59)	14 (64)
4-9 (n=16)	12 (75)	9 (56)	9 (56)	11 (69)
10 ve ↑(n=7)	4 (57)	2 (29)	3 (43)	5 (71)
Perineural invasion (12/71)				
Yes	9 (75)	5 (42)	9 (75)	6 (50)
No	3 (25)	7 (48)	2 (25)	6 (50)
P value	0.739	0.756	0.123	0.322
Blood vessel invasion (5/71)				
Yes	5 (100)	3 (60)	1 (20)	3 (60)
No	0 (0)	2 (40)	4 (80)	2 (40)
P value	0.167	0.665	0.176	1.000
Lymphatic invasion (11/71)				
Yes	9 (82)	7 (64)	7 (64)	7 (64)
No	2 (18)	4 (36)	4 (36)	4 (36)

P value	0.484	0.332	0.527	1.000
Necrosis (20/71)				
Yes	7 (35)	7 (35)	11 (55)	13 (65)
No	13 (65)	13 (65)	9 (45)	7 (35)
P value	**0.001**	0.197	1.000	1.000
Peritumoral inflammatory reaction (40/71)				
Yes	23 (58)	16 (40)	23 (58)	30 (75)
No	17 (42)	24 (60)	17 (42)	10 (25)
P value	**0.045**	0.156	0.480	0.049

Menopause status, increasing tumor size and the number of axillary lymph node metastases, perineural invasion, blood vessel invasion, lymphatic invasion, necrosis, and peritumoral inflammatory reactions were not significantly associated with expression of any markers, except necrosis was less common, and the inflammatory peritumoral response was more common among ER positive patients (Table 1).

Table 2. Distribution of Bloom Richardson grades among cases with positive expression of imunhistochemistry parameters

Bloom Richardson Grading System	ER positive (n=48/71)	PR positive (n=34/71)	p53 positive (n=38/71)	c-erbB-2 positive(n=46/71)
Grades				
I (n=11)	43.7 ± 27%	20.4 ± 74%	10.6 ± 21%	26.3 ± 30%
II (n=45)	47.6 ± 34%	29.1 ± 32%	23.2 ± 29%	47.4 ± 38%
III (n=15)	10.67 ± 25%	10.1 ± 22%	31.2 ± 36%	73.3 ± 25%
p values	0.001	0.094	0.227	p=0.004

According to the Bloom-Richardson grading, 11 patients (16%) were grade 1, 45 patients (63%) were grade 2, and 15 patients (21%) were grade 3. The rate of ER expression was significantly decreasing (p=0.001), and c-erbB-2 expression was significantly increasing with increasing tumor grade (p=0.030). The distribution of expression percentages among three different grades is given in table 2. According to the discriminant analysis, 52.1% of patients with concurrent expression of ER, PR, p53, and c-erbB-2 were correctly classified according to overall Bloom-Richardson grade (table 3), 49.3% were correctly classified according to nuclear pleomorphism score, and 77.5% were correctly classified according to mitotic count where cut-off value for the mitotic count was taken as 13 mitotic figures seen in 10 high power fields (table 4).

Table 3. Assessment of Bloom Richardson grade and discriminant grade

Bloom-Richard Grade	Discriminant grade			Total
	Grade I	Grade II	Grade III	
Grade I	**8 (72.7%)**	1 (9.1%)	2 (18.2%)	11
Grade II	18 (40%)	**18 (40%)**	9 (20%)	45
Grade III	2 (13.3%)	2 (13.3%)	**11 (73.4%)**	15

According to the discriminant analysis, 52.1% of patients with concurrent expression of ER, PR, p53 and c-erbB-2 were correctly classified according to overall Bloom-Richardson grade

Table 4. Assessment of mitotic count and discriminant grade

Mitotic grade	Discriminant grade		Total
	13 and lower	14 and higher	
13 and lower	**36 (75%)**	12 (25%)	48
14 and higher	4 (17%)	**19 (83%)**	23

According to the discriminant analysis, 77.5% of patients with concurrent expression of ER, PR, p53 and c-erbB-2 were correctly classified according to mitotic count grade

4. Discussion

Most studies investigated the relationship between clinical prognosis and expression of immunohistochemistry and prognosis markers, including tumor grade, DNA ploidy, S-phase analysis, and microscopic microvessel intensity, in order to explain and predict the clinical progress of breast cancer (5-8). According to the American Pathologist Consensus Statement in 1999, tumor size, histological grade, histological type, and hormone receptor status were considered the most useful categories in the clinical progress and management of breast cancer patients (9).

In studies where prognostic and survival effects of ER and PR status were investigated, it was suggested that the status of these hormone receptors was not adequate alone to predict the prognosis and early relapse and determine which patients would benefit from endocrine therapy (10). Chia et al. (9) found in their 10-year follow-up study of 1187 patients with non-metastatic breast cancer that 5-year survival was better in patients with ER expression and those with unknown receptor status than in those without ER expression, whereas 10-year survival was similar between groups. The incidence of ER expression was reported to range from 55 to 72%, whereas the

incidence of PR expression was reported to range from 33 to 70% (11). Our findings regarding ER (67.6%) and PR (47.6%) expressions seem compatible with those previously reported.

Overexpression of p53 and c-erbB-2 are known to be associated with the aggressive clinical course (2, 7, 12-17). Gretarsdottir et al. (13) found in their series of 193 breast cancer patients that patients with p53 expression tended to have a slightly worse prognosis, and these tumors were more resistant to therapy than those without p53 expression. In immunohistochemical studies, it was found that 16 to 58% of breast tumors had positive p53 expression (13, 18), whereas other studies reported different rates for positive p53 expression (5-7). Characteristics of the study group, type of antibody used, and subjective differences in assessment of positivity were reported as the potential causes of the inconsistency in results that were reported from different studies (19). Our finding that 53.5% of patients had p53 expression seems compatible with those previously reported.

Samur et al. (16) were the first who describe that c-erbB-2 expression was a poor prognostic factor for survival. Studies have also shown that c-erbB-2 overexpression was associated with an increased mortality risk (7). A scale that ranged between negative and 3+ (excessively positive) has been described for c-erbB-2 immunohistochemical staining (16). Excessive expression was reported to be ranging from 10-30% (2-4, 7, 14, and 16). Compatible with these findings, in our study, c-erbB-2 expression was found at 64.8%, and excessive expression was found at 50.7%.

The relationship between patient age and menopausal status and immunohistochemistry markers have been evaluated in various studies. Zavagno et al. (20) found in their series of 1226 breast cancer patients that tumors of patients under 40 years of age had more aggressive behavior than those of patients over 75 years of age. Rodrigues et al. (15) reported that younger patients had higher ER, PR, and p53 expressions, whereas there was no significant association between age and c-erbB-2 expression. Samur et al. (16) reported in a series of 169 patients that there was no significant association between age and c-erbB-2 expression. We found no significant correlation between age and expression of any immunohistochemistry marker.

Mc Guive et al. (22) found that ER expression was associated with a longer duration of disease-free survival in stage II postmenopausal patients. In general, ER expression was reported to be lower in premenopausal women (1). Samur et al. (16) found no significant association between menopause status and c-erbB-2 expression. We found no significant relationship between menopause status and the expression of any immunohistochemistry marker.

Most studies reported that tumor size was an important prognostic factor in patients without lymph node metastasis (9). Chia et al. (9) reported that tumor size and grade might be important determinants for predicting the clinical course and individualizing the therapy. One study of 767 patients who did not have lymph node metastasis and were not receiving systemic adjuvant chemotherapy reported that 27% of patients with tumors >10 cm had disease relapse. We found no significant correlation between tumor size and the expression of any of the immunohistochemistry markers. This may be due to differences in patient populations.

Patients who do not have axillary lymph node metastasis have a better prognosis, and lymph node status has been suggested to be the most important factor in predicting disease-free survival (5, 14, and 21). Although ER, PR, p53, and c-erbB-2 expressions have been considered as important predictors of outcome, their significance is controversial in patients who do not have lymph node metastasis. Reed et al. (14) reported that p53 and c-erbB-2 expressions were associated with worse prognosis in this group of patients. So et al. (21) found that the expression of p53 and c-erbB-2 did not have prognostic value in this group of patients.

Axillary lymph node status and immunohistochemical markers expression relation were also evaluated in various studies. Gretarsdottir et al. (13) found no difference between patients with and without lymph node metastasis regarding the presence of the p53 mutation. Ilhan et al. (23) reported that they couldn't find an association between nodal involvement and molecular subtypes of breast cancer. Consistent with those findings, we found no significant association between ER, PR, or p53 expression and axillary lymph node status.

In a series of 1500 women, Jafarimojarrad et al. (24) evaluated various conventional prognostic factors, including; ER, PR, p53, Cathepsin D, c-erbB-2, bcl-2, Ki-67, and p21 expression and they also assessed invasion parameters including; perineural, blood vessel and lymphatic invasion. Among these parameters, the only significant association was between ER expression and the presence of perineural invasion, whereas blood vessel invasion and lymphatic invasion were not associated with any conventional parameters. This is an interesting finding since ER expression has not been known to be associated with worse prognostic features. We found no significant association between perineural invasion and any immunohistochemistry markers. Also, consistent with these findings, we found no significant association between blood vessel or lymphatic invasion and ER, PR p53, or c-erbB-2 expression.

Some studies reported that increasing peritumoral inflammatory cell infiltration was associated with decreasing hormone receptor expression (25, 11). Another study reported that c-erbB-2 overexpression was also associated with this feature. We found that both ER expression and c-erbB-2 expression were significantly associated with peritumoral inflammatory cell infiltration. Fisher et al. (26) reported that ER expression was higher in tumors without necrosis. We found a significant association between ER expression and

tumor necrosis, whereas there was no significant association between tumor necrosis and expression of PR, p53, or c-erbB-2.

Bloom-Richardson grading is the most commonly used histologic scale worldwide (3, 25). Inter-observer variability is an important problem in applying this system. Technical factors, such as the fixation method, also influence its reliability (11, 25). It may also be related to the fact that tubal formation and nuclear properties are subjective features that are used in grading tumors. Another important point is that this system classifies most patients in grade II (25, 27). This large category is also poorly reproducible (27).

Reed et al. (14) reported in their study of 613 patients that histological tumor grade and tumor diameter were the most important prognostic factors in patients without lymph node metastasis. They found that increasing tumor grade was associated with decreasing ER and PR expressions and increasing p53 and c-erbB-2 expressions. They also reported that they obtained better prognostic groups when they stratified the histological groups as grade I, II, or III in survival analysis. In our study majority of patients were grade II (63%). We sought to determine whether the accumulation in group II was about the biological properties of the tumor or whether it was due to the incapability of the grading system to discriminate the cases truthfully. Therefore we performed a discriminant analysis to achieve a more sophisticated prediction of tumor grade based on the concurrent expression of ER, PR p53, and c-erbB2. We found that Blood-Richardson grade II showed the lowest consistency (40%) with discriminant classification. This finding supports the fact that various prognostic factors are incompatible with each other in Bloom-Richardson grade II cases.

The discriminant analysis we performed based on concurrent expression of ER, PR, p53, and c-erbB2 revealed that discriminant classes showed 52.1% compatibility with Bloom-Richardson grades. When we performed the analysis based on nuclear scores, we found that the compatibility was lower (49.3%).

Mitotic count is an important feature in the Bloom-Richardson scoring system (28). Chang et al. reported that this is a good single parameter for survival prediction (29). Buhmeida et al. (30) reported that the mean cut-off values of the mitotic activity index (mitotic figures/10 hpf) and the standardized mitotic index (mitotic figures/mm²) could be applied to distinguish breast cancer patients into groups with favorable and less favorable prognosis. Parham et al. found that grading or indexing by the presence or absence of mitotic activity or necrosis better predicted the clinical course than the Bloom-Richardson system (25). Baak et al. found that mitotic activity was a strong prognostic factor in patients without lymph node metastasis when the cut-off level for the mitotic activity index was set at 10 (25). Our study performed a discriminant analysis based on the concurrent expression of ER, PR p53, and c-erbB2, with a cut-off level for the mitotic count set at 13. We found that discriminant classes were 77.5% compatible with Bloom-Richardson grades.

Since concurrent expression of ER, PR p53, and c-erbB2 was highly compatible with grading by mitotic count, we suggest that prognostic classification of patients could be done based on the mitotic characteristics of the tumor. Further study may be justified to establish the standard threshold for the mitotic count for breast tumors of different types that allows performing a dichotomous classification

Conflict of interest

The authors declared no conflict of interest.

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The study was conducted at Ondokuz Mayıs University department of pathology

Authors' contributions

Concept: D.Y., F.K., Design: D.Y., F.K., Data Collection or Processing: D.Y., F.K., Analysis or Interpretation: D.Y., F.K., Literature Search: D.Y., Writing: D.Y.

Ethical Statement

Ethics committee approval is not required for this study.

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