p53, c-erbB-2 expression and steroid hormone receptors in breast carcinoma: correlations with histopathological parameters

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Summary

Objective: The aim of the study was to evaluate the p53 tumor suppressor gene, c-erbB-2 and steroid hormone receptor expression in breast carcinoma by immunohistochemistry and to correlate them with different histopathologic parameters.

Materials and Methods: p53, c-erbB-2, steroid hormone receptors and their correlation with age, tumor size, histological grade, axillary lymph-node status, and menopausal status were investigated in 65 breast carcinoma cases. All markers were measured immunohistochemically on paraffin sections. Association between estrogen receptor (ER), progesterone receptor (PgR), p53 and c-erbB-2 expression and clinicopathologic variables were assessed by the chi-square test for qualitative parameters.

Results: c-erbB-2 staining was found in 35.3% of breast carcinomas and was associated with ductal subtype and age under 35 ($p = 0.022$, $p = 0.003$, respectively); p53 staining was seen in 27.6% of cases and was associated with high histological grade and postmenapausal status ($p = 0.038$, $p = 0.002$, respectively). Progesterone receptor expression was associated with positive axillary status ($p = 0.003$).

Conclusion: We concluded that expressions of c-erbB-2, p53, ER, and PgR may be used in the evaluation of breast carcinoma. Therefore the present study suggests that p53 expression is a marker of high histological grade in postmenopausal status, and that c-erbB-2 expression is associated with histologically ductal subtype.

Key words: Breast carcinoma; p53; c-erbB-2; Estrogen receptor; Progesterone receptor.

Introduction

Breast cancer is one of the most common cancers among women. Calculations have estimated a lifetime risk of more than seven percent for women in the Western world. Despite better diagnostic methods and development of various treatment regimens during the last ten years, the mortality of the disease is still high [1]. It is therefore important to establish reliable and reproducible prognostic tests that will help in selecting optimal treatment for each case [2]. In the past few years most decisions concerning the prognosis and management of breast cancer patients have been made on the basis of traditional factors such as tumor size, histological grading, nodal status and histopathological features [3].

c-erbB-2 and p53 are two molecular markers that have been the focus of investigation in patients with breast carcinoma. They are easily assessable and independent in predicting clinical outcome and should have a beneficial impact on cancer treatment. However, most of the published data have relied on immunohistochemical detection of the proteins as a surrogate marker of underlying genetic alterations, a detection method that often gives variable results due to technical factors [4].

The association between p53 and c-erbB-2 overexpression relation to estrogen receptor (ER) status in ductal breast carcinoma is still unclear. However many reports have shown that p53 and c-erb-B-2 protein overexpression may be an indicator of poor prognosis in breast cancer, particullary if axillary node metastases are present [5].

We investigated the importance of the overexpression of c-erbB-2, p53, ER and progesterone receptor (PgR) in breast carcinoma to assess the relationship between immunoreactivity and histopathologic variables such as age, tumor size, histological grade and type, axillary status, clinical stage, and menopausal status.

Material and Methods

This study was conducted on 65 cases of breast carcinoma. Paraffin blocks of tumor samples from 65 patients with operable breast cancer treated at our institution from 2000 to 2002 were obtained. The mean age of the patients was 54.1 years (range 28 to 86 years).

The tumors were graded according to the grading system by Bloom and Richardson based on 1-3 scoring of tubule formation, nuclear pleomorphism and mitotic counts [6]. The clinical stages (TNM classification) [7] were: Stage I in 13, Stage II in 38, Stage III in nine, and Stage IV in five.

The distribution of clinical, histological, and prognostic features of the patients is shown in Table 1. Samples of resected breast tissue and dissected axillary nodes were fixed in 10% neutral buffered formaline for 24 hours. Samples were then embedded in paraffin blocks using the standard method and cut with microtome into 4 μm thick sections. One section was examined histologically after hematoxylin and eosin staining to confirm malignancy. The other sections were used to examine ER, PgR, p53 and c-erbB-2 expression.

Routine avidin-biotin complex immunohistochemical techniques [8] were used for the assessment of all antibodies includ-

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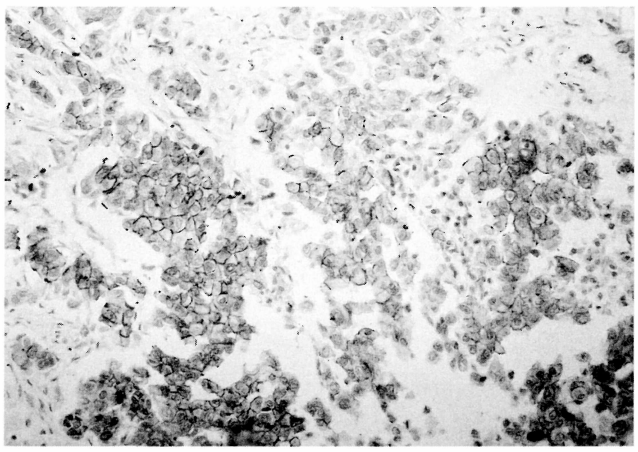

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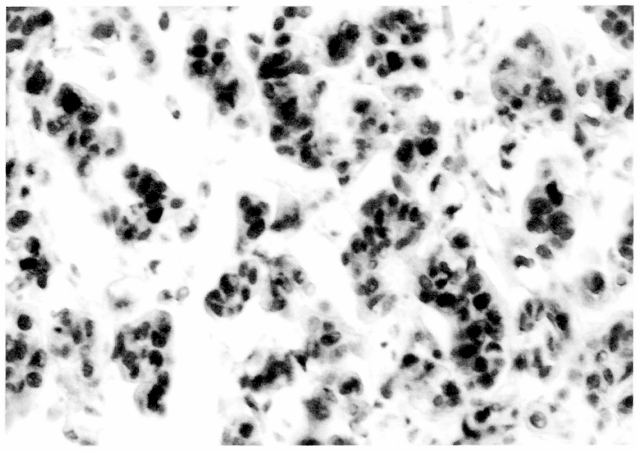

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Figure 1. — c-erbB-2 membranous staining in primary invasive ductal breast carcinoma (Immunoperoxidase, original magnification x 200).
Figure 2. — p53 nuclear staining in primary invasive ductal breast carcinoma (Immunoperoxidase, original magnification x 200).

ing ER (Dako), PgR (DAKO), p53 (Neo Marker) and c-erbB-2 (DAKO) reactivity. The complex for each reaction was visualized using 3-amino-9ethylcarbazole (AEC), counter-stained with hematoxylin, and examined under light microscope.

Tumors were registered as showing p53, c-erbB-2, ER, PgR overexpression if more than 10% of the tumor cells were positive.

Statistical analysis

All statistical analyses were performed on a personal computer with the SPSS 7.5 PC program. To compare categorical variations, the chi-square test (Fisher's Exact Test) was used; p values of p = 0.05 or less were considered statistically significant.

Table 1. — *Distribution of different prognostic factors in patients with breast carcinoma.*

Features		No. of cases (%)	X^2	p
Age	< 35	18 (27.6%)	12.938	< 0.0001
	> 35 *	47 (72.3%)		
Diameter	< 2 cm	14 (21.5%)	6.862	= 0.032
	2-5 cm	20 (30.7%)		
	> 5 cm*	31 (47.6%)		
Axillary status	Negative	21 (32.3%)	8.138	= 0.004
	Positive*	44 (67.6%)		
Histological grade	1	7 (10.7%)	15.723	< 0.0001
	2	32 (49.2%)		
	3	26 (40%)		
Histological type	Ductal*	51 (78.4%)	59.662	< 0.0001
	Lobular	8 (12.3%)		
	Other	6 (9.2%)		
Clinical Stage	I	13 (20%)	40.785	< 0.0001
	II*	38 (58.4%)		
	II	9 (13.8%)		
	IV	5 (7.6%)		
ER	Negative	18 (27.6%)	12.938	< 0.0001
	Positive*	47 (72.3%)		
PgR	Negative	22 (33.8%)	6.785	= 0.009
	Positive*	43 (66.1%)		
Menopausal Status	Premenopausal*	42 (64.6%)	5.554	= 0018
	Postmenopausal	23 (35.3%)		

*When compared with > 35 and < 35 years of age, < 2 cm and > 5 cm, negative and positive, axillary status histological grade 2 and others, histological type-ductal and others, clinical Stage II and others, ER positive and negative, PgR positive and negative, premenopausal and postmenopausal status.

Results

Immunostaining for p53 appeared as a distinct homogeneous nuclear staining in tumor cells. p53 staining was positive in 18 of 65 (27.6%) breast carcinomas. p53 staining was constantly nuclear (Figure 1). c-erbB-2 staining was considered specific only when membranous (Figure 2). c-erbB-2 staining was positive in 23 of 65 breast carcinomas (35.3%). The expression of ER correlated with the expression of PgR (p = 0.021) and correlated inversely with p53 (p = 0.0042). The chi-square test between clinical, histological, and prognostic features and ER, PgR, p53 and c-erbB-2 are shown in Table 2.

Complete patient data including age, menopausal status, tumor size, nodal status, clinical stage, tumor type, and histological grade are shown in Table 1. There was no correlation between tumor size and clinical stage in relation to ER, PgR, p53 and c-erbB-2.

Overexpression of c-erbB-2 was found in the patients under 35 years of age and ductal subtype (p = 0.003 and p = 0.022), respectively. Positive hormone receptors were found significantly more in low histological grade tumors (p = 0.03 for ER and p = 0.02 for PgR).

Significant associations were observed between PgR expression and axillary lymph node metastasis (p = 0.003), but no association was found between the immunoreactivity of the other three markers and nodal metastasis.

The histologic subtype of more than 70% of the cancers in this study was ductal carcinoma. There was no correlation between expression of ER and p53 and histologic subtypes (Table 2). However, as reported in Table 2, expression of c-erbB-2 and PgR was statistically associated with ductal subtype (p = 0.022 and p = 0.005), respectively. p53 positivity in the invasive component was statistically associated with high histological grade (p = 0.038). A trend of association was seen with c-erbB-2 immunoreactivity, but it did not reach statistical significance (p = 0.085); 28.5% positive for histological grade 1 patients, 56.2% positive for grade 2 patients and 73%

Table 2. — . *Correlation of ER, PgR, p53 and c-erbB-2 positivity with clinicopathological parameters.*

Clinicopathological parameters	n	No. of cases with expression ER%	p-value (X^2)	PgR (%)	p-value	p53 (%)	p-value	c-erbB-2	p-value (X^2)
Age									
< 35	18	11 (61.1%)	(NS)**	11 (61.1)	(NS)	7 (38.8)	(NS)	15 (83.3)*	0.003
> 35	47	36 (76.5)	0.212	32 (68)	0.595	25 (53.1)	0.226	20 (42.5)	(8.709)
Diameter									
< 2 cm	14	11 (78.5)	(NS)	9 (64.2)	(NS)	3 (16.6)	(NS)	5 (35.7)	(NS)
2-5 cm	20	16 (80)	0.406	17 (85)	0.083	5 (25)	0.063	11 (55)	0.445
> 5 cm	31	20 (64.5)	(1.805)	17 (54.8)	(4.967)	16 (51.6)	(5.536)	17 (54.8)	(1.618)
Axillary status									
Positive	21	15 (71.4)	(NS)	19 (90.4)*	0.003	6 (27.2)	(NS)	9 (42.8)	(NS)
Negative	44	32 (72.7)	0.568	24 (54.5)	(8.196)	11 (25)	0.491	24 (54.5)	0.269
Histological grade									
1	7	4 (57.1)	(NS)	5 (71.4)	(NS)	1 (14.2)*	0.038	2 (28.5)	(NS)
2	32	21 (65.6)	0.175	22 (68.7)	0.806	14 (43.7)	(6.519)	18 (56.2)	0.085
3	26	22 (84.6)	(3.485)	16 (61.5)	(0.431)	17 (65.3)		19 (73)	(4.921)
Histological type									
Ductal	51	37 (72.5)	(NS)	39 (74.6)*	0.005	18 (35.2)	(NS)	24 (47)*	0.022
Lobular	8	6 (75)	0.915	3 (37.5)	(11.920)	1 (12.5)	0.316	1 (12.5)	(7.621)
Other	6	4 (66.6)	(0.126)	1 (16.6)		1 (16.6)	(2.304)	0 (0)	
Clinical stage									
I	13	9 (69.2)	(NS)	9 (69.2)	(NS)	4 (30.7)	(NS)	8 (61.5)	(NS)
II	38	28 (73.6)	0.894	25 (65.7)	0.986	13 (46.4)	0.225	15 (53.5)	0.509
III	9	7 (77.7)	(0.610)	6 (66.6)	(0.143)	3 (33.3)	(4.365)	4 (44.4)	(2.317)
IV	5	3 (60)		3 (60)		4 (80)		3 (60)	
Menopausal status									
Premenopausal	42	29 (69)	(NS)	28 (66.6)	(NS)	15 (35.7)	(NS)	22 (52.3)	(NS)
Postmenopausal	23	18 (78.2)	0.427	15 (65.2)	0.906	18 (78.2)*	0.002	11 (47.8)	0.463
			(0.630)		(0.014)		(10.764)		(0.123)

*Significant; **(NS): Non significant.

for grade 3 patients. p53 positivity was statistically associated with postmenopausal status ($p = 0.002$). No association was found between c-erbB-2 positivity and ER/PgR or between p53 positivity and ER/PgR. However c-erbB-2 positivity and p53 positivity were statistically associated weakly ($p < 0.1$).

Discussion

Breast cancer continues to frustrate oncologists worldwide. Prognosis of breast cancer varies considerably from patient to patient, and even a very small malignant lesion at the limits of detection by mammography or palpation may have metastatic potential [9].

Overexpression of the p53 and c-erbB-2 oncogenes are the two most common genetic abnormalities associated with breast cancer. c-erbB-2 oncogene encodes for a 185 kD membrane protein related to the protein kinase family which is closely related in structure to, but biologically distinct from, the epidermal growth factor receptor (EGFR). Genomic DNA amplification of the c-erbB-2 oncogene is the most common mechanism of activation of the gene, leading to overexpression of the c-erbB-2 protein. c-erbB-2 gene amplification and p185 overexpression in breast tumors have been studied in many reports. p185 immunoreactivity is localized to the cell membrane [10].

One of the best known tumor suppressor genes is the p53 gene. It is located on chromosome 17 and encodes for a nuclear phosphoprotein that binds to DNA, preventing progression of the cell from the G1 to the S-phase in the cell cycle. Wild-type p53 protein has a short half-life and is usually undetectable. Alterations in the p53 gene result in a p53 gene-product protein with a prolonged half-life that accumulates in the nucleus [11]. p53 protein has been investigated immunohistochemically in most human neoplasms, including breast, lung, bladder, ovarian and colorectal cancers [12, 13].

In this study we analyzed immunohistochemically the expression of p53, c-erbB-2, and steroid hormone receptors in patients with breast carcinomas to assess the relationship between histopathologic variables such as age, tumor size, histological grade and type, axillary status, clinical stage, and menopausal status.

The primary tumor was stained by using immunohistochemical procedures. Products of p53, c-erbB-2 genes, ER, and PgR were studied immunuhistochemically in 65 patients.

In breast carcinomas, estimates of the frequency of p53 mutations range from 35 to 50% [12, 13]. Our results agree with those of Yokota *et al.* [14] who reported p53 immunreactivity as 27.1% in their cases. Berry *et al.* [15] observed positive immunohistochemical results in 46.7% of invasive breast carcinomas. However, in this study, p53 positive cases were found as 27.6%. The percentage of positive cases reported in other studies was related to the number of antibodies and kind of tissue used. Correlations between histopathological criteria and immunohistochemical results were made.

The type of breast cancer occurring in young women has been and still remains the subject of much contro-

versy. Some reports have shown a worse prognosis in younger groups [16]. It could be suggested that breast carcinomas in younger women are biologically different from those in older women: several findings may favor that hypothesis, including increased incidence of high grade tumors and increased rates of aneuploid tumors in younger women [17].

The synthesis of ER and PgR is under estrogen control. However, it is not sufficient to accurately predict prognosis and response to endocrine therapy. There are few reports on the relationship between PgR status and lymph node metastasis [7, 18]. Rudas *et al.* [19] reported a strong correlation between ER and PgR status and tumor differentiation. In this study, it was found that PgR expression increased with lymph node metastasis and ductal subtype (Table 2). Pinder *et al.* [13] reported that no correlation was observed between ER and PgR and tumor differentiation. Thus, the relationships between PgR and histopathological features including the degree of tumor differentiation and lymph node metastasis have been a matter of controversy. However, it is agreed that ER-negative and PgR-negative tumors are more likely to have a higher grade of malignancy. Barnes *et al.* [20] found that the p53 protein alteration/overexpression is correlated with hormone receptor negativity, high histologic grade and overall prognosis.

Altered p53 expression has been reported by Cattoretti *et al.* [21] to be significantly associated with lack of estrogen receptor, but not with tumor size, grade, or nodal involvement. Our findings also supported the views of other researchers that tumor size was not correlated with p53 positivity. However, a high p53 positivity with an increasing tumor size has been reported by some researchers [22]. Our results agree with those of Ostrowsky *et al.* [8] who found an association between p53 expression and lack of estrogen receptor expression and postmenopausal age, and a statistically significant association with high tumor grade.

c-erbB-2 protein immunuhistochemical overexpression has usually been reported in 10% to 40% of breast cancers [23]. Berry *et al.* [15] reported that c-erbB-2 overexpression was found in 21.0% cases. Korkolis *et al.* [24] found c-erbB-2 overexpression in 46.1% of tumors. We found 35.3% of c-erbB-2 positive cases in our study. Correlations of c-erbB-2 and other prognostic factors have shown that only stage, node and mitotic index had significant correlations [25]. We did not find similar associations. In this study, overexpression of c-erbB-2 was found to be associated with patients less than 35 years of age and ductal subtype.

Estimation of c-erbB-2, p53 and ER status seems to be a powerful tool to discriminate between different phenotypes of breast carcinoma. c-erbB-2 and p53 oncoproteins have been recognized as independent molecular markers of aggressive tumor behavior and the prognostic significance remains in the node-negative as well as node-positive breast cancers [26].

In conclusion, we found that overexpression of p53 and c-erbB-2 protein in breast carcinoma may be correlated with high grade tumor and may thus affect the prognosis of patients with breast cancer. The presence of c-erbB-2 and p53 oncogenes may provide useful information in the histological diagnosis of breast cancer. Prospective studies on this issue can add information about the biological behavior of malignant breast tumors and guidedance in the future treatment of breast carcinoma.

References

[1] Haerslev T., Jaacobsen G.K., Zedeler K.: "Correlation of growth fraction by Ki-67 and proliferating cell nuclear antigen (PCNA) immunohistochemistry with histopathological parameters and prognosis in primary breast carcinomas". *Breast Cancer Res. Treat.*, 1996, *37*, 101.

[2] Ellis G.K., Gown A.M.: "New applications of monoclonal antibodies to the diagnosis and prognosis of breast cancer". *Pathol. Annu.*, 1990, *25*, 193.

[3] Caleffi M., Teague M.W., Jensen R.A., Vnenck Jones C.L.: "p53 gene mutations and steroid receptor status in breast cancer. Clinicopathologic correlations and prognostic assessment". *Cancer,* 1994, *73,* 2147.

[4] Ho G.H., Calvano J.E., Bisogna M., Borgen P.I., Rosen P.P., Tan L.K. *et al.*: "In microdissected ductal carcinoma in situ, HER-2/neu amplification, but not p53 mutation, is associated with high nuclear grade and comedo histology". *Cancer,* 2000, *89,* 2153.

[5] Bebenek M., Bar J.K., Harlozinska A., Sedlaczek P.: "Prospective studies of p53 and c-erbB-2 expression in relation to clinicopathological parameters of human ductal breast cancer in the second stage of clinical advancement". *Anticancer Res.,* 1998, *18,* 619.

[6] Bloom H.J.G., Richardson W.W.: "Histological grading and prognosis in breast cancer". *Br. J. Cancer,* 1957, *11,* 3641.

[7] Ohsumi S., Kasahara M., Hayashi K., Murakami M., Kuroda M., Miemoto H. *et al.*: "Cyto-biological analysis of the breast cancer". *Fujita Gakuen Igakkashi* (in Japanese), 1994, *18*, 217.

[8] Ostrowsky J.L., Sawan A., Henry L.: "p53 expresssion in human breast cancer related to survival and prognostic factors: An immunohistochemical study". *J. Pathol.*, 1991, *164*, 75.

[9] Kesari A.L., Chellam V.G., Nair P.P., Ahmed I., Madhavan J., Thomas P.A. *et al.*: "p53 tumor suppressor protein and tissue proliferative fraction in infiltrating duct carcinoma". *J. Surg. Oncol.*, 1997, *65,* 159.

[10] Dawkins H.J., Robbins P.D., Smith K.L., Sarna M., Harvey J.M., Sterrett G.F. *et al.*: "What's new in breast cancer? Molecular perspectives of cancer development and the role of the oncogene c-erbB-2 in prognosis and disease". *Pathol. Res. Pract.*, 1993, *189,* 1233.

[11] Shi Y.F., Xie X., Zhao C.L., Ye F., Lu S.M., Hor J.J. *et al.*: "Lack of mutation in tumour-suppressor gene p53 in gestational trophoblastic tumours". *Br. J. Cancer,* 1996, *73,* 1216.

[12] Takikawa Y., Noguchi M., Kitagawa H., Thomas M.: "Immmunohistochemical detection of p53 and c-erbB-2 proteins: prognostic significance in operable breast cancer". *Breast Cancer,* 1994, *1*, 17.

[13] Pinder S.E., Wencyk P.M., Naylor H.E., Bell J.A., Elston C.W., Robertson J.F.R. *et al.*: "The assesment of multiple variables on breast carcinoma fine needle aspiration (FNA) cytology specimens: metod, preliminary results and prognostic associations". *Cytopathology,* 1995, *6,* 316.

[14] Yokota T., Imamura M., Teshima S., Suzuki H., Tezuka F., Kikuchi S. *et al.*: "c-erbB-2, p53 protein expression and steroid hormone receptors in breast carcinomas: an immunohistochemical study". *Anticancer Res.,* 1999, *19,* 4007.

[15] Berry D.A., Muss H.B., Thor A.D., Dressler L., Liu E.T., Broadwater G. *et al.*: "HER-2/neu and p53 expression versus tamoxifen resistance in estrogen receptor-positive, node-positive breast cancer". *J. Clin. Oncol.*, 2000, *18*, 3471.

[16] Chung M., Chang H.R., Bland K.I. *et al.*: "Younger women with breast carcinoma have a poorer prognosis than older women". *Cancer,* 1996, *77*, 97.

[17] Winchester D.P., Osteen R.T., Menck H.R.: "The National Cancer Data Base report on breast carcinoma characteristics and outcome in relation to age". *Cancer,* 1996, *7,* 1838.

[18] Barbareschi M., Leonardi E., Mauri F.A., Serio G., Dalla Palma P.: "p53 and c-erbB-2 protein expression in breast carcinomas. An immunohistochemical study including correlations with receptor status, proliferation markers, and clinical stage in human breast cancer". *Am. J. Clin. Pathol.,* 1992, *98,* 408.

[19] Rudas M., Neumayer R., Gnant M.F., Mittelbock M., Jakesz R., Reiner A.: "p53 protein expression, cell proliferation and steroid hormone receptors in ductal and lobular in situ carcinomas of the breast". *Eur. J. Cancer,* 1997, *33,* 39.

[20] Barnes D.M., Dublin E.A., Fisher C.J., Levison D.A., Millis R.R.: "Immunohistochemical detection of p53 protein in mammary carcinoma: an important new independent indicator of prognosis?". *Hum. Pathol.*, 1993, *24,* 469.

[21] Cattoretti G., Rilke F., Andreola S., D'Amato L., Delia D.: "p53 expression in breast cancer". *Int. J. Cancer,* 1988, *41*, 178.

[22] Bhargava V., Thor A., Deng G., Ljung B.M., Moore D.H. 2nd, Waldman F. *et al.*: "The association of p53 immunopositivity with tumor proliferation and other prognostic indicators in breast cancer". *Mod. Pathol.,* 1994, *7*, 361.

[23] Quenel N., Wafflart J., Bonichon F., De Mascarel I., Trojani M., Durand M. et al.: "The prognostic value of c-erbB2 in primary breast carcinomas: a study on 942 cases". *Breast Cancer Res. Treat.*, 1995, *35*, 283.

[24] Korkolis D., Ardavanis A., Yotis J., Kyroudi A., Gorgoulis V., Kittas C.: "HER-2/neu overexpression in breast cancer: an immunohistochemical study including correlations with clinicopathologic parameters, p53 oncoprotein and cathepsin-D". *Anticancer Res.,* 2001, *21*, 2207.

[25] 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,* 2000, *27,* 600.

[26] Tsutsui S., Ohno S., Murakami S., Hachitanda Y., Oda S.: "Prognostic value of c-erbB2 expression in breast cancer". *J. Surg. Oncol.,* 2002, *79*, 216.

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