Hormone Receptors: Association with Prognostic Factors for Breast Cancer

Receptores Hormonais: Associação com Fatores Prognósticos para Câncer de Mama

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ABSTRACT

Positivity for hormonal receptors (HR) in breast cancer patients is associated both with a better prognosis, and with variables predictive of favorable prognosis. A study was designed to assess predictors of positivity of estrogen (ER) and progesterone receptors (PR). Data from 306 patientes with infiltrating ductal carcinomas who were consecutively diagnosed and treated over a period of 20 months were included. Selected variables related to patients and tumors (micro and macroscopic characteristics as well as immunohistochemically-determined tumor markers) were studied. Bivariate analysis showed that some of variables were associated ($p \leq 0.05$) with the HR positivity: age $\geq$ 60 years, post-menopause, age at menarche (> 11 years), tumor size (< 4,0 cm), histological grade (low), nuclear pleomorphism (low), number of mitoses (low), MIB-1 (negative) and p53 (negative). Unconditional logistic regression revealed that the following variables were independent predictive factors of positivity of ER: age $\geq$ 60 years ($p < 0,001$), histological grade I ($p < 0,05$), positive PR ($p < 0,001$) and negative p53 ($p < 0,05$). For PR, two models were evaluated: a) age $\geq$ 60 years ($p < 0,05$), age at menarche > 11 years ($p < 0,05$) and histological grades I and II ($p < 0,05$); b) histological grades I and II ($p<0,05$) and positive ER ($p< 0,001$). In this study, only age at diagnosis, histological grade, PR and p53 were independents predictors of positivity of ER. Age at diagnosis, age at menarche and histological grade (or histological grade and ER) predicted a positive PR.

Key words: breast neoplasms; hormonal receptors; carcinoma infiltrating duct; tumor markers biological; logistic regression; histological grade; nuclear pleomorphism grade.

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RESUMO

Os tumores de mama positivos para receptores de estrogênio (RE) e/ou de progesterona (RP), além de apresentarem um prognóstico mais favorável, mostram associações com outras variáveis de bom prognóstico. A partir de um estudo com 306 carcinomas ductais infiltrantes de mama, foram construidos modelos preditivos para a positividade dos RE e dos RP. Foram estudadas variáveis relacionadas às pacientes e ao tumor (características macro e microscópicas e marcadores tumorais processados por imuno-histoquímica). Na análise bivariada, algumas variáveis se associaram estatisticamente com a positividade dos RE e RP, porém, na regressão logística não condicional somente as seguintes variáveis foram fatores preditivos independentes: idade da paciente (RE e RP), idade da menarca (RP), grau histológico (RE e RP), RP (RE) e p53 (RE). De acordo com os resultados deste estudo as variáveis idade da paciente ao diagnóstico, grau histológico, RP e p53 foram fatores preditivos para a positividade dos RE, enquanto que a idade da paciente, a idade da menarca e o grau histológico o foram para os RP.

Palavras-chave: neoplasias mamárias; carcinoma de ductos infiltrante; grau histológico; grau nuclear ou pleomorfismo nuclear; receptores hormonais; marcadores biológicos de tumor; regressão logística.

INTRODUÇÃO

According to Rosen (1997), hormonal receptors (HR) are proteins that bind to circulating hormones, mediating their cellular effects. The most extensively studied receptors in breast cancer are estrogen receptors (ER) and progesterone receptors (PR)[1].

According to Patino *et al*. (1988), some two-thirds of breast cancers are HR-positive[2], but this figure varies from one author to another. For example, while Zeng & Xu (1991)[3] found positive rates of 72% for ER and 54% for PR, Elliott *et al.* (1994) found 60% and 44%, respectively[4].

Most authors show a positive association between the presence of a HR (ER and/or PR) and a favorable prognosis, for both disease-free and overall survival[2,5-13].

The literature also includes several studies showing associations between the presence of a HR (ER and/or PR) and other indicators of good prognosis: white patients[7]; post-menopausal patients[1,5,7,11,14]; small tumor size[13,15]; low histological grade[6,13-15]; low nuclear grade[6,14,15]; and low mitotic activity[14].

The findings are conflicting with regard to tumor markers. While some studies show associations between positive HR (ER and/or PR) and absence of markers[16-19], others have failed to find such associations[20-23].

All these findings have important treatment implications, since the value of HR (ER and PR) studies in predicting response to hormonal treatment for advanced breast cancer is well-founded: positive response rate is 77% for ER/PR-positive tumors; 46% for ER-negative PR-positive tumors; 27% for ER-positive PR-negative tumors; and 11% for ER/PR-negative tumors[24].

Considering the relative scarcity of research in Brazil on HR patterns in women with breast cancer, this study focused on the construction of predictive models for HR in hospitalized patients with breast cancer and their association with selected variables.

METHODOLOGY AND DATA SOURCES

All malignant breast tumors (incident cases) submitted to surgery with lymph node dissection by the Mastology Service of the Cancer Hospital/Brazilian National Cancer Institute (HC/INCA), the largest public reference hospital for cancer in the city of Rio de Janeiro, Brazil, from January 1, 1995, through August 31, 1996, were obtained. A respective slide review was carried out twice at the Anatomo-Pathology Service by two pathologists (authors ALAE and LMMCR)

without consulting any other patient data.

Following a review of all available slides, with a total of 398 tumors diagnosed previously as malignant, 306 were reclassified as infiltrating ductal carcinoma (IDC), and only this histological type was analyzed in this study, considering both its higher prevalence and worse prognosis[25-27].

Macroscopic and microscopic study characteristics were the following: type of surgical specimen (quadrantectomy; segmentectomy; simple mastectomy; Patey mastectomy; radical mastectomy; other); tumor size (largest diameter in centimeters); formation of tubules (≥ 75%; 10 to 74%; < 10%); nuclear pleomorphism (mild; moderate; intense); number of mitoses per ten high-power fields, hpf (< 10/hpf; 11 to 20/hpf; ≥ 20/hpf); histological grade as proposed by Bloom & Richardson (1957)[28] and later modified by Elston & Ellis (1991)[29] (well-differentiated or grade I; moderately differentiated or grade II; poorly differentiated or grade III); vascular and or lymphatic invasion; multicentricity; skin and/or nipple involvement; total number of lymph nodes examined; total number of lymph nodes involved by neoplasia; presence of perinodal fat infiltration; and surgical limits condition.

Recovery of antigen in the immunohistochemical preparations was performed by moist heat (pressure cooker). Method of detection was the peroxidase-antiperoxidase reaction (PAP), based on Sternberger *et al.* (1970)[30] and adapted by Santos *et al.* (1995)[31]. The preparations were also analyzed at the same time by the two above-mentioned pathologists. Assessment of stained neoplastic cell distribution was performed, and only those moderately or intensely stained were considered positive; weakly stained cells were considered negative. The cut-off used to separate positive and negative stains was 10% of stained cells (this criterion is used by various authors in the specialized literature[32-35]). Other cut-off points used to quantify cell staining were: (+) 10 to 25%; (++) 25 to 75%; (+++) > 75%. The immunoreactivity was localized in the nucleus for ER, PR, MIB-1, PCNA, and p53, in the cell membrane for c-*erb*B-2, and in intracytoplasmic granules for catepsina-D.

Data obtained from patient files were the following: age at first diagnosis; time between initial symptoms and first consultation at the HC/INCA; age at menarche; age at menopause for patients having reached menopause naturally or by surgery; number of pregnancies, number of births and spontaneous or induced abortions; age at first at-term delivery for patients having given childbirth; family history of breast cancer; degree of affinity for family history of breast cancer (mother; daughter; sister; grandmother; aunt; cousin; other); family history of other types of cancer; anatomical site of family cancer (ovary; endometrium; colon; other).

Databank organization was performed with EPI-INFO software version 6.04 (U.S. Department of Health and Human Services and Public Health Service and Centers for Disease Control, USA). Bivariate analysis included crude odds ratios (OR) with 95% confidence intervals (CI) aimed to estimate the degree of association between the study variables and the HRs; chi-square test (c^2) of linear trend for ordinal variables; chi-square test of independence (c^2) to evaluate null hypothesis of the observed associations; Mantel-Haenszel OR (OR_{MH}) and 95% confidence intervals, following stratification of selected variables. Further, unconditional logistic regression was developed for the construction of parsimonious models in the determination of hormonal receptors (ER and PR) as outcome in patients with breast IDC, using EGRET software (Epidemiological GRaphics, Estimation, and Testing, version 0.26.6, 1985–1991, SERC & CYTEL).

Independent variables tested in the multivariate analysis (logistic regression) were chosen following the previous stages (bivariate and stratified analyses) as well as biological criteria evaluated by the authors. Potential confounders and variables showing significant p-values (at 5%) for presence of interaction were selected for logistic regression, as well as variables of biological interest.

RESULTS

In this study the positivity for ER and PR was, respectively, 55,2% and 41,8%.

The main associations observed between the selected variables and ER and PR are

presented, respectively, in Tables 1 and 2. Firstly, a gradual and linear increase of OR between ER-positivity and age was observed ($p < 0.0001$) in Table 1, where the lower the level of ER positivity, the higher the observed OR. The same is shown in Table 2 for PR.

A statistically significant positive association was also found between ER and menopausal status (pre- *vs.* post-menopause), [OR = 2.39 (95% CI = 1.38-4.16); p = 0.0008]. The association observed between PR and this same variable was small and not statistically significant (p = 0.37).

Using patient's age as the main predictive factor for the study outcomes, ER and PR, [ER *vs.* patient's age: OR_{crude} = 2.78 (95% CI = 1.67–4.64); PR *vs.* patient's age: OR_{crude} = 1.75 (95% CI =1.06-2.87)], stratification was performed for associations between ER and patient's age (Table 3) and PR and patient's age (Table 4) with the other variables described above.

Candidate variables for inclusion in the model were those which the stratified analysis suggested to be potential confounders and/or interaction variables (menarche, tumor size, histological grade, number of mitoses, PR, and p53 for ER outcome; and menarche, ER, and number of mitoses for PR outcome). For PR outcome, histological grade was also included, since according to the literature, high histological grade tumors tend to be PR-negative[6,13,14].

Since interaction between age and number of mitoses is biologically implausible, we opted not to include an interaction term between these variables.

The test models contained only the following variables: (a) for the ER outcome: patient's age, tumor size, number of mitoses, histological grade, menarche, PR (negative *vs.* positive), p53 (positive *vs.* negative); (b) for the PR outcome: patient's age, menarche, number of mitoses, histological grade, and ER (negative *vs.* positive).

For modeling ER outcome, after several attempts including and excluding variables according to their isolated and model's statistical significance, we opted for that containing the following variables: PR (negative *vs.* positive), patient's age (< 60 years *vs.* ≥ 60 years), p53 (positive *vs.* negative), and histological grade (I *vs.* II *vs.* III) ($p < 0.001$) (Chart 1.1).

After similar procedures for modeling the PR outcome, we opted for two models that were considered acceptable: a) one containing the variables histological grade (I *vs.* II *vs.* III), patient's age (< 60 years *vs.* ≥ 60 years), and menarche (≤ 11 years *vs.* > 11 years) (p-value < 0.001); the variable ER (negative *vs.* positive) did not reach statistical significance in this model (Chart 1.2.1); b) and a smaller model, containing only the variables ER (negative *vs.* positive) and histological grade (I *vs.* II *vs.* III) (p-value < 0.001), which did not accept any other variable with a significant p-value (Chart 1.2.2).

DISCUSSION

According to the literature, both ER and PR positivity are highly associated with patient's age at diagnosis: tumor HR-positivity increases significantly with age, that is, positivity is greater in women 60 aged years or older, as well as in post-menopause[1]. In this study, both ER and PR were significantly associated ($p < 0.05$) with patient's age (< 60 years *vs.* ≥ 60 years). When the association was studied between different levels of positivity for HR (+++ *vs.* ++ *vs.* + *vs.* negative) and patient's age (< 60 years *vs.* ≥ 60 years), we observed a chi-square with a high linear trend and significant p-value ($p < 0.01$), for both ER and PR. Estrogen receptors was also significantly associated with menopausal status ($p < 0.001$), but PR was not.

The current study also found a statistically significant association between PR (negative) and early menarche (≤ 11 years) ($p < 0.05$), a result which disagreed with Rosen (1997)[1], who failed to find this association. The association found for this study between ER (negative) and early menarche was statistically borderline (p = 0.09).

In agreement with the literature, our bivariate analysis showed statistically significant associations between ER- and PR-positive tumors and other good prognostic indicators, such as: tumor size shorter than 4.0 cm ($p < 0.005$)[13]; low histological grade (p = 0.01); low nuclear grade ($p < 0.01$)[1,6,13,14]; and reduced mitotic activity ($p < 0.001$)[14].

Stierer *et al*. (1993)[14] and MacGrogan *et*

Table 1 - Associations between selected variables and estrogen receptors (bivariate analysis), among breast cancer patients, Rio de Janeiro, 1995-1996.

ER	age < 60 years	%	age ≥ 60 years	%	OR	95% CI
+++	7	4.1%	20	15.4%	1.00	(reference)
++	33	19.3%	45	34.6%	2.10	0.79-5.53
+	35	20.5%	24	18.5%	4.17	1.52-11.39
Negative	96	56.1%	41	31.5%	6.69	2.63-17.04
Total	171	100%	130	100%		
			c^2=27.16	(p<0.0001)	c^2trend=26.80	(p<0.0001)
ER	pre-menopausal	%	post-menopausal	%	OR	95% CI
Negative	52	60.5%	80	39.0%	2.39	1.38-4.16
Positive	34	39.5%	125	61.0%		
Total	86	100%	205	100%		
			c^2=11.24	(p=0.0008)		
ER	menarche ≤11 years	%	menarche >11 years	%	OR	95% CI
Negative	18	34.6%	115	47.7%	0.58	0.29-1.14
Positive	34	65.4%	126	52.3%		
Total	52	100%	241	100%		
			c^2=2.96	(p=0.09)		
ER	tumor ≥4.0 cm	%	tumor < 4.0 cm	%	OR	95% CI
Negative	78	53.4%	49	34.0%	2.22	1.34-3.69
Positive	68	46.6%	95	66.0%		
Total	146	100%	144	100%		
			c^2=11.08	(p=0.0009)		
Histological grade	ER-	%	ER+	%	OR	95% CI
Grade I	20	14.6%	51	30.2%	1.00	(reference)
Grade II	60	43.8%	100	59.2%	1.53	0.83-2.81
Grade III	57	41.6%	18	10.7%	8.08	3.85-16.93
Total	137	100%	169	100%		
			c^2=40.92	(p<0.0001)	c^2trend=34.24	(p<0.0001)
Tubular formation	ER-	%	ER+	%	OR	95% CI
≥75%	7	5.1%	12	7.1%	1.00	(reference)
10 a 74%	33	24.1%	61	36.1%	0.93	0.33-2.58
<10%	97	70.8%	96	56.8%	1.73	0.65-4.59
Total	137	100%	169	100%		
			c^2=6.38	(p=0.04)	c^2trend=5.22	(p=0.02)
Nuclear pleomorfism	ER-	%	ER+	%	OR	95% CI
Mild	4	2.9%	8	4.7%	1.00	(reference)
Moderate	71	51.8%	134	79.3%	1.06	0.31-3.64
Intense	62	45.3%	27	16.0%	4.59	1.27-16.56
Total	137	100%	169	100%		
			c^2=31.46	(p<0.0001)	c^2trend=27.33	(p<0.0001)
Mitoses number	ER-	%	ER+	%	OR	95% CI
≤10/hpf	50	36.5%	111	65.7%	1.00	(reference)
11-20/hpf	51	37.2%	48	28.4%	2.36	1.41-3.95
>20/hpf	36	26.3%	10	5.9%	7.99	3.68-17.37
Total	137	100%	169	100%		
			c^2=34.93	(p<0.0001)	c^2trend=34.59	(p<0.0001)
ER	PR -	%	PR +	%	OR	95% CI
Negative	125	70.2%	12	9.4%	22.8	11.1-48.0
Positive	53	29.8%	116	90.6%		
Total	178	100%	128	100%		
			c^2=111.50	(p<0.0001)		
ER	MIB +	%	MIB -	%	OR	95% CI
Negative	90	49.5%	47	37.9%	1.60	0.98-2.63
Positive	92	50.5%	77	62.1%		
Total	182	100%	124	100%		
			c^2=3.98	(p=0.05)		
ER	p53 +	%	p53 -	%	OR	95% CI
Negative	40	74.1%	97	38.5%	4.57	2.25-9.39
Positive	14	25.9%	155	61.5%		
Total	54	100%	252	100%		
			c^2=22.77	(p<0.0001)		

c^2 trend: c^2 of linear trend

Table 2 - Associations between selected variables and progesterone receptors (bivariate analysis), among breast cancer patients, Rio de Janeiro, 1995-1996.

PR	age <60 years	%	age ³60 years	%	OR	95% CI
+++	5	2.9%	8	6.2%	1.00	(reference)
++	24	14.0%	27	20.8%	1.42	0.41-4.94
+	32	18.7%	29	22.3%	1.77	0.52-6.01
negative	110	64.3%	66	50.8%	2.67	0.84-8.49
total	171	100%	130	100%		
			c^2=6.55	(p=0.09)	c^2 trend=6.43	(p=0.01)
PR	menarche <11 years	%	menarche >11 years	%	OR	95% CI
negative	24	46.2%	149	61.8%	0.53	0.28-1.02
positive	28	53.8%	92	38.2%		
total	52	100%	241	100%		
			c^2=4.34	(p=0.04)		
PR	tumor ³4.0 cm	%	tumor <4.0 cm	%	OR	95% CI
negative	95	65.1%	69	47.9%	2.02	1.22-3.36
positive	51	34.9%	75	52.1%		
total	146	100%	144	100%		
			c^2=8.68	(p=0.003)		
Histological grade	ER-	%	ER+	%	OR	95% CI
Grade I	29	16.3%	42	32.8%	1.00	(reference)
Grade II	88	49.4%	72	56.3%	1.77	1.00-3.12
Grade III	61	34.3%	14	10.9%	6.31	2.98-13.35
Total	178	100%	128	100%		
			c^2=25.96	(p<0.0001)	c^2 trend=24.71	(p<0.0001)
Tubular formation	PR-	%	PR+	%	OR	95% CI
³75%	7	3.9%	12	9.4%	1.00	(reference)
10 a 74%	49	27.5%	45	35.2%	1.87	0.68-5.16
<10%	122	68.5%	71	55.5%	2.95	1.11-7.83
Total	178	100%	128	100%		
			c^2=6.98	(p=0.03)	c^2 trend=6.88	(p=0.009)
Nuclear pleomorphism	PR-	%	PR+	%	OR	95% CI
mild	6	3.4%	6	4.7%	1.00	(reference)
moderate	108	60.7%	97	75.8%	1.11	0.35-3.57
intense	64	36.0%	25	19.5%	2.56	0.75-8.69
total	178	100%	128	100%		
			c^2 =9.77	(p=0.008)	c^2 trend=8.76	(p=0.003)
Mitoses number	PR-	%	PR+	%	OR	95% CI
≤ 10/hpf	76	42.7%	85	66.4%	1.00	(reference)
11-20/hpf	63	35.4%	36	28.1%	1.96	1.17-3.27
> 20/hpf	39	21.9%	7	5.5%	6.23	2.63-14.75
total	178	100%	128	100%		
			c^2 =22.56	(p<0.0001)	c^2 trend=22.35	(p<0.0001)
PR	p53 +	%	p53 -	%	OR	95% CI
negative	42	77.8%	136	54.0%	2.99	1.43-6.35
positive	12	22.2%	116	46.0%		
total	54	100%	252	100%		
			c^2=10.36	(p=0.001)		

c^2 trend: c^2 of linear trend

al (1996)[13] showed that the presence of hormonal receptors (ER and PR) were not associated with nodal status and so did we in our study.

According to Rosen (1997)[1], most PR-positive tumors are also positive for ER, and our study found an OR of 22.80 between both (95% CI = 11.10-48.00; $p < 0.0001$).

In disagreement with the literature, which mentions strong and statistically significant associations between hormonal receptors and c-*erb*B-2[18,19,36-38], our study failed to find such associations.

Querzoli *et al.* (1996)[39], Seshadri *et al.* (1996)[40], and Sundlad *et al.* (1996)[41] found inverse associations between MIB-1 and hormone receptors (ER and PR), while Pinder *et al.* (1995) found no such association (MIB-1 and ER)[42]. In the current study, only ER was inversely and significantly associated with MIB-1 ($p = 0.05$); our study also found no significant association between PCNA and HR, unlike Siitonen *et al.* (1993)[43] and Gasparini *et al.* (1992)[44].

In disagreement with Henry *et al.* (1990)[45] and Eng Tan *et al.* (1994)[46], we found no association between increased expression of catepsin-D and estrogen receptors.

Most studies, including ours, found statistically significant inverse associations between ER and PR and p53 ($p < 0.001$). Barbareschi (1996)[47], in a bibliographical review, found associations between p53 and ER in 8 out of 11 studies and between p53 and PR in 7 out of 9, where such associations had been investigated. A literature review showed an association between p53 and ER in several studies[48-56] and no such association in only one[57]. Association between p53 and PR was also present in some studies[49,52,55,57,58].

In summary, the results of bivariate analysis in the current investigation agree with the literature as to the association between positive HR and the variables patient's age (60 years or over), menopausal status (post-menopause), tumor size (smaller than 4.0cm), histological grade (low), nuclear pleomorphism (low), mitotic activity (low), MIB-1 (negative), and p53 (negative), while they disagree in relation to the variables age at menarche, c-*erb*B-2, catepsin-D, and PCNA.

Using a multivariate aproach, only patient's age, histological grade, PR, and p53 were significantly associated with ER (Chart 1.1). For the PR outcome, independent factors included patient's age, age at menarche, and histological grade in one model (Chart 1.2.1), and only histological grade and ER in another (Chart 1.2.2).

As mentioned above, study of hormonal receptors is important for predicting both breast cancer prognosis and response to hormone therapy. Although it was not this study's aim (since patient follow-up was not carried out), one can suggest that the group of patients with ER- and PR-positive tumors probably has a better prognosis, since positivity for both (ER and PR) was associated with variables also related to a better prognosis.

CONCLUSIONS

According to this study, the variables patient's age, histological grade, PR-positivity and p53 were significantly associated with presence of ER, while the variables patient's age, age at menarche, and histological grade (or histological grade and ER-positivity) were significantly associated with the presence of PR in women with infiltrating ductal carcinoma of the breast.

REFERENCES

1. Rosen PP. Breast Pathology., Philadelphia: Lippincott-Raven, 1997:295-320.
2. Patino JF, Cavanzo C, Francisco J. Receptores de estrogeno en cancer mamario: demostracion inmuno-histoquimica con anticuerpos monoclonales / Estrogen receptors in breast cancer: immunohistochemical demonstration with monoclonal antibodies [Abstract]. Cirugía (Bogotá) 1988;3:5-8.
3. Zeng QF, Xu JH. A study on the relationship among estrogen receptor, progesterone receptor and glucose-6-phosphate dehydrogenase activity in primary breast cancer. [Abstract]. Chung-hua Ping Li Hsueh Tsa Chih 1991; 20:107-9.
4. Elliott RL, Head JF, Mccoy JL. Comparison of estrogen and progesterone receptor status to lymphocyte immunity against tumor antigens in breast cancer patients.. Breast Cancer Res Treat 1994;30:299-304.
5. Thorpe SM. Prognostic value of steroid hormone

receptors: multivariate analysis of systemically untreated patients with node negative primary breast cancer. Cancer Res 1987;47:6125-33.

6. Osborne CK. Prognostic factors in breast cancer. Principles & Practice of Oncology 1990;4:1-11.
7. Pertschuk LP, Kim DS, Nayer K, et al. Immunocytochemical estrogen and progestin receptor assays in breast cancer with monoclonal antibodies: histopathologic,, demographic, and biochemical correlations and relationship to endocrine response and survival. Cancer 1990;66:1663-70.
8. Reiner A, Neumeister B, Spona J, Reiner G, Schemper M, Jakesz R. Immunocytochemical localization of estrogen and progesterone receptor and prognosis in human primary breast cancer. Cancer Res 1990;50:7057-61.
9. Hendricks JB, Wilkinson EJ. Comparison of two antibodies for evaluation of estrogen receptors in paraffin-embedded tumors [Aabstract]. Modern Pathol 1993;6:765-70,
10. Kelsey JL, Horn-Ross PL. Breast cancer: magnitude of the problem and descriptive epidemiology. Epidemiol Rev 1993;15:7-16.
11. Mansour EG, Ravdin PM, Dressler L. Prognostic factors in early breast carcinoma. Cancer 1994;74:381-400.
12. Beckmann MW, Niederacher D, Massenkeil G, et al. Expression analyses of epidermal growth factor receptor and HER-2/*neu*: no advantage of prediction of recurrece or survival in breast cancer patients. Oncology 1996;53:441-7.
13. Macgrogan G, Soubeyran I, De Mascarel I, et al. Immunohistochemical detection of progesterone receptors in breast invasive ductal carcinomas: a correlative study of 942 cases. Appl Immunohistochem 1996;4:219-27.
14. Stierer M, Rosen H, Weber R, Hanak H, Spona J, Tücheler H. Imunohistochemical and biochemical measurement of estrogen and progesterone receptors in primary breast cancer: correlation of histopathology and prognostic factors. Ann Surg 1993;218:13-21.
15. Sundblad AS, Ahn C, Mehta P, Caprarulo L, Battifora H. Determinación inmunohistoquímica de receptores hormonales en cáncer de mama: estudio retrospectivo en 322 casos [Abstract]. Medicina (B Aires) 1992;52:333-40.
16. Chariyalertsak S, Cheirsilpa A, Sugano K, Ohkura H. Immunohistochemical detection of c-*erb*B-2 oncoprotein in patients with breast cancer [Abstract]. J Med Assoc Thai 1992; 79:715-21.
17. Carlomagno C, Perrone F, Gallo C, et al. c-*erb*B-2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph-node metastases. J Clin Oncol 1996;14:2702-8.
18. Keshgegian AA. ErbB-2 oncoprotein overexpression in breast carcinoma: inverse correlation with biochemically and immunohistochemically, determined hormone receptors. Breast Cancer Res Treat 1995;35: 201-10.
19. Quénel N, Wafflart J, Bonichon F, et al. The prognostic value of c-*erb*B-2 in primary breast carcinoma: a study on 942 cases. Breast Cancer Res Treat 1995;35:283-91.
20. Pierce LJ, Merino MJ, D'angelo T, et al. Is c-*erb*B-2 a predictor for recurrent disease in early stage breast cancer? [Abstract]. Int J Radiat Oncol Biol Phys 1994;28:395-403.
21. Horiguchi J, Iino Y, Takei H, Yokoe T, Ishida T, Morishita Y. Immunohistochemical study on the expression of c-*erb*B-2 oncoprotein in breast cancer. Oncology 1994;51:47-51.
22. Osaki A, Toi M, Yamada H, Kawami H, Kuroi K, Toge T. Prognostic significance of expression of c-*erb*B-2 oncoprotein in breast cancer patients [Abstract]. Gan to Kagaku Ryoho 1991;18:1181-5.
23. Mccann AH, Dervan PA, O'regan M, et al. Prognostic significance of c-*erb*B-2 and estrogen receptor status in human breast cancer. Cancer Res 1991;51:3296-303.
24. Osborne CK, Yochmowitz MG, Knight WA, Mcguire WL. The value of estrogen and progesterone receptors in the treatment of breast cancer. Cancer 1980;46:2884-8.
25. Pereira H, Pinder SE, Sibbering DM, et al. Pathological prognostic factors in breast cancer. IV: should you be typer or a grader? A comparative study of two histological prognostic features in operable breast carcinoma. Histopathology 1995;27:219-26.
26. Pinder SE, Ellis IO, Elston CW. Prognostic factors in primary breast carcinoma. J Clin Pathol 1995;48:981-3.
27. Elston CW, Ellis IO. Prognostic factors in breast cancer. In: Annual meeting of The United States and Canadian Academy of Pathology [Handout]. 1996:1-73.
28. Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer: a study of 1049 cases of wich 359 have been followed for 15 years. Br J Cancer 1957;11:359-77.
29. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of

histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991;19:403-10.

30. Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG. The unlabeled antibody-enzyme method of immunohistochemistry: preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-anti-peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 1970;18:315-23.
31. Santos RTM, Wakamatsu A, Kanamura CT, Nonogaki S. Procedimentos imuno-histoquímicos e de hibridização in situ. In: Manual de imuno-histoquímica com menções à técnica de hibridização molecular. São Paulo: Sociedade Brasileira de Patologia, 1995:91-9.
32. Hanna WM, Kahn HJ, Chapman JAW. The correlation of Ki-67 growth factor and ERICA in breast cancer. Mod Pathol 1992;5:220-3.
33. Isola J, Weitz S, Visakorpi T, et al. Cathepsin D expression detected by immunohistochemistry has independent prognostic value in axillary node-negative breast cancer. J Clin Oncol 1993;11:36-43.
34. Derossi DR, Bacchi CE. Cathepsina D em carcinoma de mama: correlação com grau histológico e receptor de estrógeno. J Bras Patol 1995;31:100-5.
35. Rosen PP, Lesser ML, Arroyo CD, Cranor M, Borgen P, Norton L. p53 in node-negative breast carcinoma: an immunohistochemical study of epidemiologic risk factors, histologic features, and prognosis. J Clin Oncol 1995;13:821-30.
36. Allred DC, Clark GM, Molina R, et al. Overexpression of HER-2/neu and its relationship with other prognostic factors change during the progression of in situ to invasive breast cancer. Hum Pathol 1992;23:974-9.
37. Lipponen HJ, Aaltomaa S, Syrjanen S, Syrjanen K. c-*erb*B-2 oncogen related to p53 expression, cell proliferation ad prognosis i breast cancer [Abstract]. Anticancer Res, 1993;13:1147-52.
38. Têtu B, Brisson J. Prognostic significance of HER-2/*neu* oncoprotein expression in node-positive breast cancer: the influence of the pattern of immunostaining and adjuvant therapy. Cancer 1994;73:2359-65.
39. Querzoli P, Albonico G, Ferretti S, et al. MIB-1 proliferative activity in invasive breast cancer measured by image analysis. J Clin Pathol 1996;49:926-30.
40. Seshadri R, Leong AS, Mccaul K, Firgaira FA, Setlur V, Horsfall DJ. Relationship between p53 gene abnormalities and other tumour characteristics in breast-cancer prognosis. Int J Cancer 1996;69:135-41.
41. Sundblad AS, Ahn C, Battifora H. Immunohistochemical detection of bcl-2 and MIB-1 / Ki-67 in breast cancer: retrospective analysis of 238 cases [Abstract]. Medicina (B Aires) 1996;56:252-8.
42. Pinder SE, Wencyk P, Sibbering DM, et al. Assessment of the new proliferation marker MIB-1 in breast carcinoma using image analysis: associations with other prognostic factors and survival. Br J Cancer 1995;71:146-9.
43. Siitonen SM, Kallioniemi OP, Isola JJ. Proliferating cell nuclear antigen immuno-histochemistry using monoclonal antibody 19A2 and a new antigen retrieval technique has prognostic impact in archival paraffin-embedded node-negative breast cancer. Am J Pathol 1993;142:1081-89.
44. Gasparini G, Meli S, Pozza F, Cazzavillan S, Bevilacqua P. PC-10 antibody to proliferating cell nuclear antigen (PCNA) is not related to prognosis in human breast carcinoma [Abstract]. Growth Regul 1992;2:145-50.
45. Henry JA, Mccarthy AL, Angus B, et al. Prognostic significance of the estrogen-regulated protein, cathepsin D, in breast cancer: an immunohistochemical study. Cancer 1990;65:265-71.
46. Eng Tan P, Benz CC, Dollbaum C, et al. Prognostic value of cathepsin D expression in breast cancer: immunohistochemical assessment and correlation with radiometric assay. Ann Oncol 1994;5:329-36.
47. Barbareschi M. Prognostic value of the immunohistochemical expression of p53 in breast carcinoma: a review of the literature involving over 9.000 patients. Appl immunohistochem 1996;4:106-16.
48. Iwaya K, Tsuda H, Hiraide H, et al. Nuclear p53 immunoreaction associated with poor prognosis of breast cancer [Abstract]. Jpn J Cancer Res 1991;82:835-40.
49. Barbareschi M, Leonardi E, Mauri FA, Serio G, Palma PD. p53 and c-*erb*B-2 protein expression in breast carcinomas: an immunohistochemical study including correlations with receptor status, proliferating markers, and clinical stage in human breast cancer. Am J Clin Pathol 1992;98:408-18.
50. Hanzal E, Gitsch G, Kohlberger P, Dadak C, Miechowiecka N, Breitenecker G. Immunohistochemical detection of mutant p53-suppressor gene product in patients with

breast cancer: influence on metastasis-free survival [Abstract]. Anticancer Res 1992;12:2325-9.

51. Isola J, Visakorpi T, Holli K, Kallioniemi OP. Association of overexpression of tumor suppressor protein p53 with rapid cell proliferation and poor prognosis in node-negative breast cancer patients. J Natl Cancer Inst 1992;84:1109-14.
52. Marks JR, Humphrey PA, Wu K, et al. Overexpression of p53 and HER-2/neu proteins as prognostic markers in early stage breast cancer. Ann Surg 1994;219:332-41.
53. Castiglioni T, Elsner B, Curutchet HP, Mostesions M, Debonis D. Análisis imunohistoquímico de p53 y c-*erb*B-2 en el carcinoma de mama / Imunohistochemical analysis of p53 and c-*erb*B-2 in breast cancer [Abstract]. Medicina (B Aires) 1995;55:415-20.
54. Haerslev T, Jacobsen GK. An immunohistochemical study of p53 with correlations to histopathological parameters, c-*erb*B-2, proliferating cell nuclear antigen, and prognosis. Hum Pathol 1995;26:295-301.
55. Sirvent JJ, Salvadó MT, Santafé M, Martínez S, Brunet J, Alvaro T, Palacios J. p53 in breast cancer. Its relation to histological grade, lymph-node status, hormone receptors, cell-proliferation fraction (ki-67) and c-*erb*B-2: immunohistochemical study of 153 cases. Histol Histopathol 1995;10:531-9.
56. Wakasugi E, Kobayashi T, Tamaki Y, et al. p21 (waf1/Cip1) and p53 protein expression in breast cancer. Am J Clin Pathol 1997;107:684-91.
57. Davidoff AM, Herndon JE, Glover NS, et al. Relation between p53 overexpression and established prognostic factors in breast cancer. Surgery 1991;110:259-64.
58. Martinazzi M, Crivelli F, Zampatti C, Martinazzi S. Relationship between p53 expression and other prognostic factors in human breast carcinoma: an immunohistochemical study. Am J Clin Pathol 1993; 100:213-7.