© Med Sci Monit, 2004; 10(12): BR462-467
PMID: 15567977

WWW.MEDSCIMONIT.COM
Basic Research

Received: 2004.02.09
Accepted: 2004.10.28
Published: 2004.12.01

Reassessing tumor markers in local recurrences of breast cancer: a new insight

José Luiz Pedrini[1ABDCEF], Mariana Pedrini[1BCDE], Ricardo Francalacci Savaris[2ACDEF], Luciana Machado[3BDE], Melina Grudzinski[1BCEF], Cláudio Galleano Zettler[3ABDE]

[1] Unity of Mastology of Hospital Nossa Senhora da Conceição, Ministério da Saúde, Brazil
[2] Department of Obstetrics and Gynecology, UFRGS, Brazil
[3] Department of Pathology of Fundação Faculdade Federal Ciências Médicas de Porto Alegre, RS, Brazil

Source of support: Departmental sources.

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Background: It is believed that a local recurrence of a primitive breast cancer has the same prognostic factor profile as its primary breast cancer tumor.

Material/Methods: We compared the immunohistochemical expressions of the tumor suppressor protein p53, estrogen receptor (ER), c-erbB2, and E-cadherin in 57 primary invasive breast cancers and in their respective LRs. The McNemar test and the kappa index were used for statistical analysis.

Results: In 30 patients (52.6%) the expression of at least one of these markers was different between the primary and locally recurrent tumors. No significant difference was observed between variations in the positive and negative expressions in the primary tumor and local recurrence in cerbB2 (kappa=0.86), E-cadherin (kappa=0.55), and p53 (kappa=0.7). However, the ER presented a low kappa index (kappa=0.26, p>0.05).

Conclusions: ER expression should be reviewed in local recurrent breast cancer. This relevant change in ER expression is likely to change the current clinical practice in breast cancer evaluation and treatment.

key words: **breast cancer • p53 • c-erbB2 • E-cadherin • estrogen receptor • local recurrence**

Full-text PDF: http://www.MedSciMonit.com/pub/vol_10/no_12/4992.pdf

Word count: 3011
Tables: 3
Figures: –
References: 36

Author's address: José Luiz Pedrini, Rua Thomas Gonzaga, 430/16, Porto Alegre – RS, Brazil, 91340-480, e-mail: josepedrini@terra.com.br

Background

Breast cancer is the most common form of malignancy occurring in women around the world. In 2003, more than 41,610 new cases (46% of all cancer sites) were diagnosed and 9,335 deaths from breast cancer (10.4%) occurred in Brazil [1]. African American females have a higher mortality rate due to breast cancer [2]. Surgery is the main modality of the local treatment for breast cancer. On the other hand, many women will benefit from hormone therapy. To choose the best adjuvant therapy, the tumor markers are analyzed in the primary tumor for prognostic and predictive purposes. During the 1980's, much work was done with assays on steroid receptor in breast cancer [3–7] . More recently, tumor markers were assessed by immunohistochemistry [6,8–10]. Some authors found a high concordance, ranging from 71% to 85%, between estrogen receptor (ER) expression in primary breast carcinoma and its local, regional recurrence, or metastasis. Based on these works, some authors believe that the same profile of the tumor marker in the primary tumor (PT) will be expressed in their local recurrence (LR) [3,4,10–13]. Therefore, the analysis of tumor markers in ductal invasive breast cancer is not commonly reassessed in the LR.

Nevertheless, other authors found discordance between PTs and their metastatic recurrence [5–7]. This apparently contradiction could arise from the different methods of assessing tumor markers, i.e. cytosol assays or immunohistochemistry, or due to the heterogeneity within a breast tumor mass. Variations in steroid receptor may result from tumoral subpopulations and/or differences in tumor cellularity of the cancer [14]. Most recent decisions for breast cancer treatment are based on prognostic and predictive factors. Among these tumor markers, p53, E-cadherin, estrogen receptor, and c-erbB2 have been used [13,15]. Estrogen receptor, p53, and C-erbB2 are related to cell proliferation [16–18], while E-cadherin is related to cell adhesion [19,20]. Four and 20% of primary tumors will have a local recurrence within 10 years [21].

After searching the literature (MEDLINE 1966–2004), only two small studies were found comparing the expression of estrogen receptor in the PT and in its LR, but in a small sample (6 and 14 cases) [3,11]. Others investigated the expressions of estrogen and progesterone receptors, HER2/neu, and p53 in ductal carcinoma *in situ* treated with breast-conserving therapy (BCT) and in its local or distant recurrence [13]. These authors found some differences in the tumor markers expressed in the PT and its LR, although not statistically significant.

To our knowledge, this is the first study with a larger sample size to investigate tumor marker expression in PT and in its LR in invasive ductal carcinoma of the breast. If the LR changes its tumor marker expression, this work will be added to the literature that supports the results that have shown discordance between steroid receptor in the primary breast carcinoma and in its recurrence.

The aim of this study was to determine the expressions of p53, E-cadherin, estrogen receptor, and c-erbB2 in patients with primary breast cancer tumor and in its respective local recurrence.

Material and Methods

Patients

Fifty-seven cases of histologically confirmed local recurrence of invasive breast cancer diagnosed between 1995 and 2000 were retrieved from the Instituto de Pesquisas Cito-Oncológicas of Fundação Faculdade Federal de Ciências Médicas de Porto Alegre - Santa Casa de Misericórdia and from the Unity of Pathology of Hospital Nossa Senhora da Conceição. The respective paraffin-embedded block with primary tumor f for each case was taken from the pathological archives for analysis. The inclusion criteria were age between 30 and 90 years, presence of invasive breast carcinoma, absence of signs of metastasis at diagnosis, had been submitted to surgical treatment, and radiotherapy, chemotherapy or hormone therapy when indicated. The exclusion criteria were no invasive carcinoma, inflammatory carcinoma, or the presence of carcinoma in the margins of the surgical resection. Tumor blocks from the PT and its LR were fixed in formalin and embedded in paraffin. All paraffin blocks were reviewed by a pathologist (CGZ) to confirm breast carcinoma according to the standard WHO Criteria [22].

Immunohistochemistry

All immunohistochemistry (IHC) assays were performed in a reference laboratory at the same time to reduce bias. The 3-µm-thick tissue sections were put on organosilane slides. Five slides for each block were prepared: one for hematoxylin-eosin and four for IHC. The slides were incubated at 60°C for 20 min for paraffin removal. After that they were immersed for 10 min in xylol followed by 5 sequential absolute alcohol immersions. For antigen retrieval, the slides were boiled in sodium citrate, pH 6, for 10 min in microwave. After reaching room temperature, the slides were rinsed for 2×5 min in PBS (pH 7.2–7.8) and incubated in hydrogen peroxide at 3% for 10 min. Non-specific sites were blocked with horse normal serum diluted in PBS according to the manufacturer's instructions (Dako A/S, Glostrup, Denmark). After non-specific blocking, the slides were rinsed in distilled water, followed by a 2×5 min PBS rinse. Primary antibodies were incubated according to the dilutions p53 (Dako D07) 1: 400, E-cadherin (Santa Cruz G10) 1: 400, estrogen receptor (DAKO 1D5) 1:2000, and C-erbB2 (DAKO polyclonal) 1:4000. After 12 hours of overnight incubation in a humid chamber and a 2×5 min PBS rinse, the slides were incubated with biotinylated rabbit-antimouse immunoglobulin (Ig) and biotinylated horseradish peroxidase streptavidin complex for detection of the primary antibody, according to the manufacture's instructions (System LSAB2, Dako). The slides were counter-stained with hematoxylin, mounted, and analyzed under an optical microscope.

All slides were submitted to rigorous standard methods and had positive and negative external controls, and estrogen receptor also had an internal positive control. Three different investigators (JLP, CGZ, and LM) evaluated all slides for immunostaining in a blind fashion. In case of disagreement, the slides were reviewed and a consensus view achieved. Positive expression for each tumor marker was considered as follows: Estrogen Receptor (ER) and p53 – staining of more than 10% of the nuclei in high-power field (HPF); E-cadherin and c-erbB2 – more than 10%

Table 1. Characteristics of the patients.

Characteristics	
Age (years) mean (range)	50.7 (33–86)
Type of Surgery	
Tumorectomy	30 (53%)
Radical modified mastectomy	27 (47%)
Tumor	
T1	15 (26%)
T2	30 (53%)
T3	12 (21%)
Axillary Status	
Positive	28 (53%)
Negative	25 (47%)
Histological type	
Ductal Invasive Carcinoma (DIC)	42 (64%)
DIC with Extensive Intraductal Component	11 (19%)
Lobular Carcinoma	4 (7%)
Clinical Stage	
I	11 (21%)
II	36 (68%)
III	6 (11%)
Disease-free Survival (months) mean (range)	32.5 (5–113)

complete staining of the cell membranes in HPF. Tumors were considered c-erbB2 positive only if there was a clear brown cell membrane staining. The remaining tumors, including those that showed a granular cytoplasmic staining, were considered to be c-erbB2 negative. The same criterion was applied to E-cadherin. These values were based on previous studies [23–25].

Ethical issues and statistical analysis

This study was submitted to and approved by the Ethics Board of Irmandade Santa Casa de Misericórdia of Porto Alegre.

The McNemar test was based on matching the cases and it was used to compare the dichotomic values (positive/negative) before and after treatment. The kappa index was used as a complementary method of reliability for the correlation of the markers expressed on the PT and its LR. The kappa coefficient (?) measures pair-wise agreement among a set of category judgments, correcting for expected chance agreement. Values of kappa range from –1, for total disagreement, through 0, representing the agreement expected by chance, to +1 for perfect correlation. A kappa value of <0.20 indicates weak correlation, 0.21 to 0.40 fair, 0.41 to 0.60 moderate, 0.61 to 0.80 good, and 0.81 to 1 excellent correlation [26]. A significant difference from zero in the value of kappa indicates similar samples, and likewise in the opposite case. EpiInfo-6 software (Center for Disease Control, Atlanta, USA) was used for data analysis.

Table 2. Tumor markers and immunohistochemistry expression in Primary Tumor (PT) and Local Recurrence (LR), pairing the samples. Values are numbers (percentages).

Status	PT (n=57)	LR (n=57)	*P**
Estrogen Receptor			
Positive	30 (53)	33 (58)	0.70
Negative	27 (47)	24 (42)	
p53			
Positive	17 (30)	13 (23)	0.52
Negative	40 (70)	44 (77)	
cerbB2			
Positive	29 (51)	25 (44)	0.57
Negative	28 (49)	32 (56)	
E-cadherin			
Positive	24 (42)	31 (54)	0.26
Negative	33 (58)	26 (46)	

* MacNemar test.

Results

This study is a retrospective analysis of 57 cases of histologically confirmed local recurrent breast cancer diagnosed between 1995 and 2000. The paraffin-embedded blocks of the respective PTs were retrieved from the pathological archives dated from 1990 to 2000. The immunohistochemical expression of p53, estrogen receptor, c-erbB2, and E-cadherin were analyzed in all paraffin-embedded blocks in the same run. Interobserver and interassay variability were very low (kappa: 0.85). Clinical, laboratory, and pathological findings are summarized in Table 1. The mean time of disease-free survival was 32.5±24 months (mean ±SD). Of the 57 cases, 30 patients (52.6%) changed the expression of at least one tumor marker.

When the pair-wise analysis was performed, no difference was observed (McNemar p>0.05, Table 2). Nevertheless, a different scenario was observed with the kappa index. Table 3 shows the immunohistochemical expressions of tumor markers in the PTs and in their LRs. Of the 27 negative ER expressions in the PT, 12 (44%) became positive in the LR, while of the 30 positive ER expressions in the PT, 9 (30%) became negative (kappa=0.25; p=0.051). p53, c-erbB2, and E-cadherin did not changed their expressions when the kappa index was used (p53=0.73; c-erbB2=0.86; E-cadherin=0.55; all p<0.001).

Discussion

In theory, the LR should have the same profile of tumor markers as the PT, since the LR is, by definition, the return of the

Table 3. Expression of p53, cerbB2, E-cadherin, and Estrogen Receptor (ER) in the primary tumor (PT) and in its respective local recurrence (LR). Values are numbers of cases (percentages).

Tumor Marker	A	B	C	D	kappa	*P*
P53	12 (22)	39 (68)	5 (9)	1 (1.7)	0.73	<0.001*
cerbB2	25 (44)	28 (49)	4 (7)	0	0.86	<0.001*
E-cadherin	21 (37)	23 (40)	3 (5)	10 (18)	0.55	<0.001*
ER	21 (37)	15 (26)	9 (30)	12 (44)	0.25	0.051**

A: Both PT and LR are positives, no change in tumor marker expressions; B: Both PT and LR are negatives, no change in tumor marker expressions; C: PT is positive and the LR became negative; D: PT is negative and the LR became positive;
* Concordance between the observations;
** Discordance between the observations.

same primitive tumor. The present study was conducted to confirm such a hypothesis. All patients were submitted to adequate treatment for breast cancer (i.e. surgery with safety margins free from neoplasia, being submitted to chemotherapy, radiotherapy, and hormone therapy when indicated). Rigorous immunohistochemical procedures were followed to avoid artifacts. The expressions of prognostic and predictive factors (ER and c-erbB2) and prognostic factors (p53 and E-cadherin) were analyzed in the PTs of breast cancer and in their LRs. Although small changes occurred in all tumor markers, the only instance in which 100% concordance between PT and LR was observed was when c-erbB2 was negative. As seen in Table 2, no difference was observed in all tumor markers when the McNemar test was used ($p>0.05$). This is because no statistical difference was observed among the tumors that changed their expression from positive to negative and vice versa. This statistical method does not consider the individual cases that remained unchanged. Conversely, 30 of the 57 patients (52.6%) changed the expression of the prognostic/predictive factor in some way, from positive to negative or vice versa. Such variation has a clinical significance. To confirm it statistically, we decided to use the kappa index, which considers these variations. The accuracy shown by the kappa index revealed a different scenario.

C-erbB2

C-erbB2 belongs to the family of epidermal growth factors (EGF) [18]. It is the main ontogeny that is activated in breast cancer and it is found in the mechanisms of tumor promotion, resistance of disease to therapy, and vigilance of immunity in breast cancer. The c-erbB2 gene is located on the 17q21 chromosome and its protein is expressed at low levels in the epithelial and myoepithelial tissue of normal breast cells. It is over-expressed in comedos, ductal carcinoma *in situ*, and in low levels in papillary and cribriform tumors *in situ* [18]. This protein is over-expressed in about 19% of breast tumors, thus reflecting a poor prognosis [27]. Haffty et al. compared the over-expression of c-erbB2 in patients with breast cancer with local recurrence with controls without recurrence. He described that a higher expression of c-erbB2 is more frequent in breast cancer with LR than c-erbB2 negative ones (56% vs. 18%) in a 10-year follow-up [28]. This finding suggests that c-erbB2 expression is an important marker for LR.

Our data showed a 50.8% of incidence of c-erbB2 in the PT, similar to the 43.6% found by Bijker et al. [13], but higher than that found by Borg et al. [27]. Although different stages of breast cancer were analyzed, and a different criterion for positiveness for c-erbB2 was used, our kappa indexes were very similar to those found by Bijker et al. [13].

p53

p53 is a tumor-suppressing gene located on chromosome 17p13.1 and is the most common single marker for genetic alterations in human tumors. Over 50% of human tumors show mutations in this gene. Loss of homozygosity of gene p53 is seen in virtually all cancers, particularly in lung, colon, and breast cancers. This suggests that p53 serves as a guardian against the formation of cancer, preventing the propagation of cell genetic damage [29]. We verified the mutant expression of this protein in our study. The incidence of p53 was positive in 30% (17/57) of the PT and 23% (13/57) of the LR, as shown in Table 2. Our findings are in accordance with the data described in the literature, i.e. 20 and 40% for p53 in breast cancer [17].

E-cadherin

A loss or change in the substrate of cell adhesion and alteration in cytoskeleton organization play important roles in the loss of differentiation. Moreover, these contribute to the formation of metastases: cell locomotion, proteolysis, survival, and proliferation in distant sites [20]. The mechanisms involved in breast cancer recurrence are still unknown. It is known that breast cancers at the same clinical stage show diverse clinical progression in different patients, independently of the therapeutic approach. It has been a challenge to identify the factors that will indicate the best therapeutic approach and follow-up according to its probable biological behavior [19]. E-cadherin expression was positive in 42% (24/57) of the PTs and in 54% (31/57) of the LRs when considered in the whole group (Table 2). These results are similar to those found by Yoshida et al. [30].

Estrogen receptor

By interacting with the ER, estrogen plays a central role in regulating the proliferation and differentiation of normal breast epithelium. During the past 20 years many studies have measured ER expression in breast cancer using biochemical ligand-binding assays. These studies showed that approximately 60 to 70% express ER. Even a weak expres-

sion has a favorable prognostic factor [31,32] . Depending of the estrogen assay, a significant difference can be found in the overall survival rate of women with mastectomy. Immunohistochemical assay of estrogen receptor alpha (irERalpha) seems to be a better prognosticator for the 5-year follow-up than estrogen receptor in the cytosol [33]. As seen in Table 2, the ER significantly changed either to positive or to negative expression in the local recurrence of the breast cancer. (kappa=0.25; p>0.05). These data are similar to the results found by Crawford et al, who used cytosol assays [34], and by Li et al. [12], but different from those found by Bijker [13]. The possible reasons for this discordance could be due to the different criteria of positiveness for ER and due to the sample analyzed. We analyzed invasive ductal carcinoma, while Bijker et al. included only ductal *in situ* carcinoma. We chose to consider an expression positive when >10% of nuclei were stained, because it seems to correlate well with enzyme immunoassay [23]. Conversely, Bijker et al. considered any staining as positive. This criteria has a low specificity (34%) [9].

Another possibility could be related to receptor heterogeneity in different areas of the tumor [14]. It is important to note that the assessment of staining of ER is a difficult area in terms of concordance of the results [35]. Unlike the cytosol assay, where a numerical result is produced, evaluation of staining is subjective. So far there is no agreement to the best way to assess the staining, nor what the cut-off should be [9]. Hawkins states that no single mode of expression was entirely satisfactory, and the probability of a good "out-come" (prognosis or response to endocrine therapy) increased with increasing activity (either fmol ER sites/mg protein or percentage of cells staining for ER). Thus the use of a single "cut-off" should be avoided and activity quantified, or stratified into categories [36].

Despite these differences, it is expected that some changes in ER expression happen. However, it should be noted that in a significant number of cases a major change in ER expression from the PT to the LR has occurred (Table 3). These findings lead us to believe that LR may mutate their receptors due to different types of cancer treatment, such as radio or chemotherapy [4,34] , or to the heterogeneity of the tumor [14].

Prophylactic hormone treatment (tamoxifen and similar drugs) is considered mainly for ER-positive tumors, since it leads to an increase in overall survival and disease-free time. In those tumors that have been reliably shown to be ER-negative, adjuvant tamoxifen remains a matter for research. However, some years of adjuvant tamoxifen treatment substantially improves the 10-year survival of women with ER-positive tumors and of women with tumors of unknown ER status; the proportional reductions in breast cancer recurrence and mortality appear to be largely unaffected by other patient characteristics or treatments. This new expression of ER would explain the reduction of breast cancer recurrence in tumors with unknown ER status or with unreliable assessment of ER status. For the purpose of a patient's treatment, it is not so relevant to define if it is an occurrence of a new neoplasm, or a loss of differentiation features, referred to as 'dedifferentiation', or the heterogeneity of the tumor. Our data shows that the local recurrence has a change in it markers. Although our sample has a moderate size, a change of more than 50% in tumor marker expressions seems to be a clinically relevant issue. Therefore, the tumor markers should be reassessed, especially those prognostic factors with a predictive value, here represented by ER and c-erbB2.

CONCLUSIONS

No significant change was observed in the expressions of cerbB2, E-cadherin, and p53 in PT and LR. Nevertheless, a significant change in the expression of estrogen receptor in the PT and its LR was observed in this group of patients with breast cancer. This variation suggests the need for revaluation of the estrogen receptor, independent of its result in the primary tumor.

REFERENCES:

1. Brasil.Ministério da Saúde.Instituto Nacional de Câncer – INCA. Estimativas da incidência e mortalidade por câncer no Brasil. Rio de Janeiro: Instituto Nacional de Câncer – INCA, 2003. *http: // www.inca.gov.br/conteudo_view.asp?id=336* accessed on 10/10/2004
2. Jones DA, Cho JJ, Salamon E, Stefano GB: Risk factors for breast cancer and the prognosis of African American women: estrogen's role. Med Sci Monit, 2003; 9(6): RA111-RA119
3. Hahnel R, Twaddle E: The relationship between estrogen receptors in primary and secondary breast carcinomas and in sequential primary breast carcinomas. Breast Cancer Res Treat, 1985; 5(2): 155–63
4. Mobbs BG, Fish EB, Pritchard KI et al: Estrogen and progesterone receptor content of primary and secondary breast carcinoma: influence of time and treatment. Eur J Cancer Clin Oncol, 1987; 23(6): 819–26
5. Holdaway IM, Bowditch JV: Variation in receptor status between primary and metastatic breast cancer. Cancer, 1983; 52(3): 479–85
6. Brunn RB, Kamby C: Immunohistochemical detection of estrogen receptors in paraffin sections from primary and metastatic breast cancer. Pathol Res Pract, 1989; 185(6): 856–59
7. Kamby C, Rasmussen BB, Kristensen B: Oestrogen receptor status of primary breast carcinomas and their metastases. Relation to pattern of spread and survival after recurrence. Br J Cancer, 1989; 60(2): 252–57
8. Allred DC, Harvey JM, Berardo M, Clark GM: Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Mod Pathol, 1998; 11(2): 155–68
9. Barnes DM, Millis RR, Beex LV et al: Increased use of immunohistochemistry for oestrogen receptor measurement in mammary carcinoma: the need for quality assurance. Eur J Cancer, 1998; 34(11): 1677–82
10. Nedergaard L, Haerslev T, Jacobsen GK: Immunohistochemical study of estrogen receptors in primary breast carcinomas and their lymph node metastases including comparison of two monoclonal antibodies. APMIS, 1995; 103(1): 20–24
11. Horigushi J, Iino Y, Takei H et al: Immunohistochemical study on primary and recurrent tumors in patients with local recurrence in the conserved breast. Oncol Rep, 2000; 7(2): 295–98
12. Li BD, Byskosh A, Molteni A, Duda RB: Estrogen and progesterone receptor concordance between primary and recurrent breast cancer. J Surg Oncol, 1994; 57(2): 71–77
13. Bijker N, Peterse JL, Duchateau L et al: Histological type and marker expression of the primary tumour compared with its local recurrence after breast-conserving therapy for ductal carcinoma in situ. Br J Cancer, 2001; 84(4): 539–44
14. Davis BW, Zava DT, Locher GW et al: Receptor heterogeneity of human breast cancer as measured by multiple intratumoral assays of estrogen and progesterone receptor. Eur J Cancer Clin Oncol, 1984; 20(3): 375–82
15. Bundred NJ: Prognostic and predictive factors in breast cancer. Cancer Treat Rev, 2001; 27(3): 137–42
16. Underwood JC: Oestrogen receptors in human breast cancer: review of histopathological correlations and critique of histochemical methods. Diagn Histopathol, 1983; 6(1): 1–22
17. Clahsen PC, van de Velde CJ, Duval C et al: p53 protein accumulation and response to adjuvant chemotherapy in premenopausal women with node-negative early breast cancer. J Clin Oncol, 1998; 16(2): 470–79

18. Molina R, Jo J, Filella X et al: C-erbB-2 oncoprotein in the sera and tissue of patients with breast cancer. Utility in prognosis. Anticancer Res, 1996; 16(4B): 2295–300
19. Cosentino D, Valli MC: Clinical application of integrated treatments in breast cancer. Tumori, 1998; 84(2): 223–28
20. Sommers CL: The role of cadherin-mediated adhesion in breast cancer. J Mammary Gland Biol Neoplasia, 1996; 1(2): 219–29
21. Donegan WL: Tumor-related prognostic factors for breast cancer. CA Cancer J Clin, 1997; 47(1): 28–51
22. World Health Organization. Pathology and Genetics of Tumours of the Breast and Female Genital Organs. 1 ed. Oxford: Oxford University Press, 2003
23. Ferrero-Pous M, Trassard M, Le Doussal V et al: Comparison of enzyme immunoassay and immunohistochemical measurements of estrogen and progesterone receptors in breast cancer patients. Appl Immunohistochem Mol Morphol, 2001; 9(3): 267–75
24. Ellis PE, Fong LFWT, Rolfe KJ et al: The role of p53 and Ki67 in Paget's disease of vulva and the breast. Gynecol Oncol, 2002; 86: 150–56
25. Jacobs TW, Gown AM, Yaziji H et al: Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. J Clin Oncol, 1999; 17(7): 1983–87
26. Jekel JF, Elmore JG, Katz DL: Understanding and Reducing Errors in Clinical Medicine. In: Editor Jekel JF, Elmore JG, Katz DL, eds. Epidemiology, Biostatistics and Preventive Medicine. Philadelphia: WB Saunders, 1996; 85–97
27. Borg A, Tandon AK, Sigurdsson H et al: HER-2/neu amplification predicts poor survival in node-positive breast cancer. Cancer Res, 1990; 50(14): 4332–37
28. Haffty BG, Brown F, Carter D, Flynn S: Evaluation of HER-2 neu oncoprotein expression as a prognostic indicator of local recurrence in conservatively treated breast cancer: a case-control study. Int J Radiat Oncol Biol Phys, 1996; 35(4): 751–57
29. Mirza AN, Mirza NQ, Vlastos G, Singletary SE: Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. Ann Surg, 2002; 235(1): 10–26
30. Yoshida R, Kimura N, Harada Y, Ohuchi N: The loss of E-cadherin, alpha- and beta-catenin expression is associated with metastasis and poor prognosis in invasive breast cancer. Int J Oncol, 2001; 18(3): 513–20
31. Elek J, Park KH, Narayanan R: Microarray-based expression profiling in prostate tumors. In Vivo, 2000; 14(1): 173–82
32. Allred DC, Bustamante MA, Daniel CO et al: Immunocytochemical analysis of estrogen receptors in human breast carcinomas. Evaluation of 130 cases and review of the literature regarding concordance with biochemical assay and clinical relevance. Arch Surg, 1990; 125(1): 107–13
33. Chrapusta SJ, Giermek J, Pienkowski T: Long-term survival in primary breast cancer: correlation with estrogen and progesterone receptor assay results and adjuvant tamoxifen therapy. Med Sci Monit, 2004; 10(10): CR577–CR586
34. Crawford DJ, Cowan S, Fitch R et al: Stability of oestrogen receptor status in sequential biopsies from patients with breast cancer. Br J Cancer, 1987; 56(2): 137–40
35. Wishart GC, Gaston M, Poultsidis AA, Purushotham AD: Hormone receptor status in primary breast cancer–time for a consensus? Eur J Cancer, 2002; 38(9): 1201–3
36. Hawkins RA: How best to express oestrogen receptor activity. Eur J Cancer, 2000; 36(Suppl.4): S21–S23

Index Copernicus

Global Scientific Information Systems for Scientists by Scientists

TM
INDEX COPERNICUS INTERNATIONAL

www.IndexCopernicus.com

- EVALUATION & BENCHMARKING
- PROFILED INFORMATION
- NETWORKING & COOPERATION
- VIRTUAL RESEARCH GROUPS
- GRANTS
- PATENTS
- CLINICAL TRIALS
- JOBS
- STRATEGIC & FINANCIAL DECISIONS

Index Copernicus integrates

IC Scientists

Effective search tool for collaborators worldwide. Provides easy global networking for scientists. C.V.'s and dossiers on selected scientists available. Increase your professional visibility.

IC Virtual Research Groups [VRC]

Web-based complete research environment which enables researchers to work on one project from distant locations. VRG provides:

- customizable and individually self-tailored electronic research protocols and data capture tools,
- statistical analysis and report creation tools,
- profiled information on literature, publications, grants and patents related to the research project,
- administration tools.

IC Journal Master List

Scientific literature database, including abstracts, full text, and journal ranking. Instructions for authors available from selected journals.

IC Patents

Provides information on patent registration process, patent offices and other legal issues. Provides links to companies that may want to license or purchase a patent.

IC Grant Awareness

Need grant assistance? Step-by-step information on how to apply for a grant. Provides a list of grant institutions and their requirements.

IC Conferences

Effective search tool for worldwide medical conferences and local meetings.

IC Lab & Clinical Trial Register

Provides list of on-going laboratory or clinical trials, including research summaries and calls for co-investigators.