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The Correlation between HER-2/neu Overexpression and Prognostic and Predictive Factor in Primary Breast Cancer

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Abstract

Background: **An over-expression of HER-2/neu protein has been reported to be associated with a poor clinical outcome in breast cancer. However, its prognostic value is important, especially in patients with node status, and in relation to the estrogen receptor (ER) and progesterone receptor (PR) status.**

Objectives: **To determine the correlation between HER-2/neu expression and several known, well-accepted prognostic indicators of invasive breast carcinoma.**

Materials and Methods: **This study analyzed data from 251 women with primary invasive breast cancer at Rajavithi Hospital (tertiary care hospital of Ministry of Public Health), Bangkok, Thailand. The pathological detection of lymph node metastasis and the detection of HER-2/neu protein, estrogen and progesterone receptor expression were performed using immunochemistry.**

Results: **Twenty-five percent of patients (64/251) showed HER-2/neu over-expression. HER-2/neu-positive tumors were not correlated with age and number of positive nodes (p value = 0.544 and 0.272, respectively) but there was an association between HER-2/neu-positive tumors and estrogen-negative tumors and progesterone-negative tumors (p value = 0.031 and 0.022, respectively).**

Conclusion: **There was good correlation between HER-2/neu expression and several known, well-accepted prognostic indicators of invasive breast carcinoma such as nodal status, estrogen receptor status and progesterone receptor status.**

Key words: **Breast Cancer, HER-2/neu/c-erb B2, Estrogen Receptor, Progesterone Receptor, Immunohistochemistry (IHC)**

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Primary breast cancer is the second most frequent cancer of Thai women. Today, primary breast cancer is considered a systemic disease from the beginning of the cancer, which is the micrometastasis. Therefore, breast cancer therapies include locoregional and systemic controls. The systemic controls comprise chemotherapy and hormonal therapy.

HER-2/*neu* (also known as c-erbB2) proto-oncogene is a normal cellular gene located on chromosome 17q21. HER-2/neu protooncogene encodes 185 kDa protein (p185 protein) which is present as the transmembrane glycoprotein growth factor receptor (i.e. HER-2) on the cell membrane of a breast cancer cell.[1] HER-2 is highly homologous with, but distinct from, the epidermal growth factor receptor (EGFR). When a specific ligand binds to the extracellular domain of the receptor, it will transmit a signal to the nucleus, resulting in gene activation which proliferates cancer cells.[2-5]

Recently a breakthrough new drug has been discovered that will compete to bind to HER-2 and will destroy the cancer cell by ADCC (Antibody Dependent Cellular Cytotoxicity) method. The drug is Recombinant Human-murine monoclonal antibody (Trastuzumab, Herceptin®; Genentech, Inc, South San Francisco, CA). Determining the HER-2 status of primary breast cancer is a prerequisite for the use of Trastuzumab. Initial clinical trials have indicated that this therapy has proven to be effective in prolonging the survival of patients with advanced, metastatic breast carcinoma.[6-9] Trastuzumab has been licensed for the treatment of metastatic breast cancer by the United States Food and Drug Administration (FDA) in 1998.

HER-2/*neu* is over-expressed or amplified in 15%-35% of breast cancers (Table 1).[10-12] Many studies published subsequently have demonstrated that women whose tumors express HER-2/*neu* at high levels have a relatively poor prognosis, shorter relapse time, and lower survival rate than HER-2/*neu*-negative. HER-2/neu is also associated with indicators of poor prognosis, such as loss of hormonal receptor expression (such as ER/PR negativity), high histological grade, high proliferative index, and an increased number of metastatic lymph nodes.[6,13-16] HER-2/*neu* testing has become of paramount importance as it represents a key therapeutic, prognostic and predictive parameter.

In fact, HER-2/*neu*-positive patients appear to be resistant to the CMF regimen and comparatively more sensitive to anthracyclins such as Trastuzumab.[17-20] Over-expression of HER-2/neu may be also associated with a less favorable response to endocrine therapy.[18,21-23] In cell culture experiments, transfection and over-expression of HER-2 in vitro promotes Tamoxifen resistance in ER-positive, estrogen dependent, human breast cancer cells.[14]

HER-2/*neu* testing is currently performed utilizing two main methods. Gene amplification is usually determined by fluorescence in situ hybridization (FISH), whereas protein over-expression is determined by immunohistochemistry (IHC). Three clinical assays have been approved by the Food and Drug Administration (FDA) for determination of HER-2/*neu* status

Table 1 Summary on incidence of HER-2/*neu* / c-erb B2 gene amplification or protein overexpression in primary breast cancer

Authors	HER-2/neu / c-erb B2 overexpression	Method
Bern EM, et al, 1995[21]	24% (62/259)	FISH
Chen Y, et al, 1995[30]	35% (35/101)	FISH
Valeron PF, et al, 1996[31]	15% (63/415)	FISH
Looi LM, et al, 1997[32]	38% (43/112)	IHC
Jacobs TW, et al, 1999[24]	26% (26/100)	FISH
	23% (23/100)	IHC
Jacobs TW, et al, 2000[29]	24% (24/100)	IHC
el-A Helal, et al, 2002[33]	40% (84/210)	IHC
Tsutsui, et al, 2002[13]	17% (120/698)	IHC
S Selvarajan, et al, 2004[28]	34% (14/41)	IHC
DiGiovanna MP, et al, 2005[34]	38% (306/807)	IHC

FISH = fluorescence in situ hybridization; IHC = immunohistochemistry

of breast cancer: HercepTest (DakoCytomation, Glostrup, Denmark), Pathway (Ventana Medical System Inc, Tucson, AZ), and PathVysion (Abbott Vysis, Abbott Park, IL). The first 2 are IHC assays and PathVysion is a FISH assay.

The comparison between FISH and IHC assays demonstrates a considerably high degree of concordance (over 90%). When dealing with cases scored as 3+ (according to the FDA licensed scoring method), such a concordance approaches 100%. This combination is not necessary at low (0-1+) or high (3+) level of IHC assays, because the correlation with gene amplification status is acceptably high. In consideration of greater technical simplicity as well as of cost-effectiveness, IHC represents the ideal screening method for HER-2/neu testing. The frequent 2+level of IHC assays requires complementary FISH test to verify gene amplification. FISH analysis remains valid for HER-2/*neu* evaluation in those cases in which IHC fails to provide unequivocal data.[17,24,25]

Materials and Methods

This study analyzed data derived from the medical records of patients from the Department of Surgery and the Department of Pathology at Rajavithi Hospital, Bangkok, Thailand. The study considered all women who were consecutively diagnosed with primary, invasive breast cancer from 1 January 2003 to 28 February 2005.

Subjects included patients who were pathologically diagnosed by specimens of incisional biopsy, excisional biopsy, breast conserved surgery (BCS), simple mastectomy, modified radical mastectomy (MRM). 251 women were eligible for analysis.

The following variables were analyzed in all patients: age at surgical intervention (<50 and ≥50 years), HER-2/*neu* protein and hormonal receptor status (positive and negative). Only 200 patients were analyzed for lymph node metastasis (positive and negative) and number of lymph node metastasis (≤3 and >3 nodes).

Immunohistochemical study of HER-2/neu protein[26]

For this study, paraffin-embedded blocks of primary breast cancer tissue were successfully retrieved in 251 cases for the IHC detection of HER-2/*neu* protein. After de-paraffinized process and antigen retrieval by microwave pre-treatment boiling in citrate buffer (0.01 M, pH 6), samples were stained with the DAKO A0485 polyclonal antibody (DakoCytomation, Glostrup, Denmark) at a dilution of 1:2,000 with incubation period of 60 min at room temperature.

Immunohistochemical study of hormonal receptor[26]

Paraffin-embedded blocks of primary breast cancer tissues were successfully retrieved in 251 cases for the IHC detection of hormonal receptors, such as estrogen receptor (ER) and progesterone receptor (PR).

One of the procedures is given, as follows. Formalin-fixed, paraffin-embedded sections are cut at 3 micron and placed on glass-slides that have been coated by 3-aminopropyltriethoxysilance. Sections are incubated at 60° Celsius for 1 hour. Slides are deparaffinized in three changes of xylene, for 5 min each, followed by stepwise dehydration. Epitope retrieval method is by microwave. Ten miliMolar citrate buffer is used. After blocking endogenous peroxidase activity and non-specific background, the primary antibodies (both ER and PR using 200 microlitres) are incubated at room temperature for 60 min. Visualized reagent is used for incubation for another 30 min. Finally, the slides go for DAB, with 10 min incubation and rinsed with distilled water, followed by hematoxylin counterstain.

The primary antibody was studied with the 1D5-monoclonal antibody of ER (DAKO M7047, Glostrup, Denmark) with microwave pre-treatment of tissue and

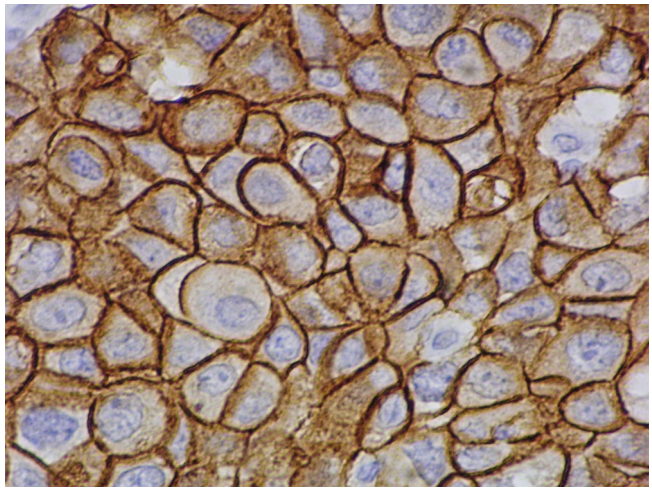

Fig. 1 HER-2/*neu*/c-erb B2 positive (3+)

PR88-monoclonal antibody of PR (BioGenex Ab No. M328, San Ramon CA) with microwave pre-treatment.

Interpretation criteria for HER-2/neu protein[26]

Immunostained slides were evaluated by pathologists:

Positive test is 3+ (Figure 1) which means over 10% tumor cells with complete and strong intensity membrane staining

Equivocally positive test is 2+ which means over 10% tumor cells with complete and mild or moderate intensity membrane staining

Negative test is 1+ and 0 (Figure 2, 3) which means incomplete membrane staining or no membrane staining or <10% tumor cells with complete membrane staining

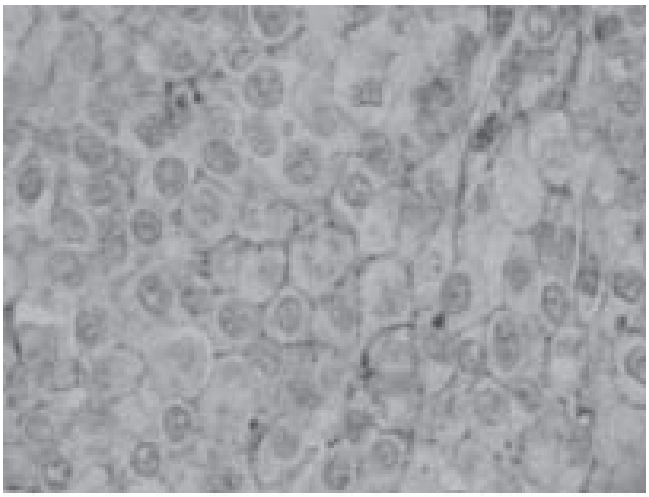

Fig. 2 HER-2/*neu*/c-erb B2 negative (1+)

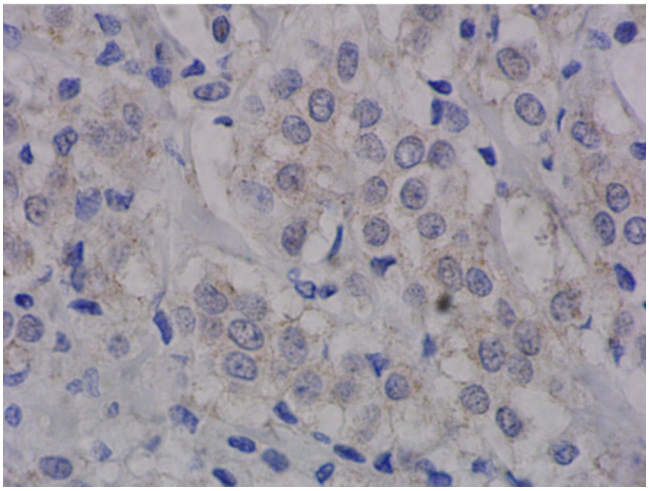

Fig. 3 HER-2/*neu*/c-erb B2 negative (0)

Interpretation criteria for hormonal receptor[26]

Immunostained slides were evaluated by pathologists:

Positive test: nuclear staining over 10% of tumor cells in whole section

Negative test: nuclear staining less than 10% of tumor cells in whole section

Statistical Analysis

The associations of the groups of breast cancer were calculated using the chi-square (χ^2) test. Fisher' s exact test was assessed when the chi-square (χ^2) test was not valid. A p value <0.05 was defined as significant.

Results

Thirty nine percent (99/251) and 28 percent (71/251) of the tumors had positive estrogen and progesterone receptors respectively. Twenty five percent (64/251) of the tumors showed HER-2/neu protein over-expression (Table 2).

Table 3 shows the association between HER-2/*neu* protein over-expression and clinicopathological or biological factors from the patients with primary breast cancer.

The factor of age: the percentage of HER-2/*neu*-positive tumors in "≥50 year old" group was slightly higher than in the "<50 year old" group and did not differ significantly (27.06 % and 23.72 %, p value = 0.544).

The factor of nodal status: the percentage of

Table 2 Characteristics of the patients

Sample Total (n = 251)	Number of patients
Age (range 24-84 years)	
- <50 year olds	118 (47.01 %)
- ≥50 year olds	133 (52.98 %)
Intervention for specimen	
- Biopsy	51 (20.32 %)
- BCS / MRM	200 (79.68 %)
Positivity of	
- Nodal status (n = 200)	82 (41.00 %)
- Estrogen Receptor	99 (39.44 %)
- Progesterone Receptor	71 (28.28 %)
- HER-2/*neu*/c-erb B2	64 (25.49 %)

BCS = breast conserving surgery : MRM = modified radical mastectomy

Table 3 Relationship between HER-2/*neu*/c-erb B2 expression and clinopathological or biological factor; A p value <0.05 was defined as significant

Factor	HER-2/*neu*/c-erb B2				
	Positive	Negative	Total	%Positive	p-value
Age					0.544
- <50 year olds	28	90	118	23.72	NS
- ≥50 year olds	36	97	133	27.06	
total (n)			251		
Node status					0.035
- positive	25	57	82	30.48	Significant
- negative	21	97	118	17.79	
total (n)			200		
Node positive					0.272
- 1-3 node	14	30	44	31.81	NS
- ≥4 nodes	8	30	38	21.05	
total (n)			82		
Estrogen receptor					0.031
- positive	18	81	99	18.18	Significant
- negative	46	106	152	30.26	
total (n)			251		
Progesterone receptor					0.022
- positive	11	60	71	15.49	Significant
- negative	53	127	180	29.44	
total (n)			251		

NS = Not significant

HER-2/neu-positive tumors in the positive-node group was significantly higher than for the negative-node group (30.48 % and 17.79 %, p value = 0.035). The positive-node group status was stratified by sub-grouping of the number of positive nodes. In the "1-3 nodes" subgroup, the percentage of HER-2/*neu*-positive tumors was inversely greater than in the ">3 nodes" subgroup (31.81% and 21.05%, p value =0.272).

The factor of estrogen receptor status: the percentage of HER-2/*neu*-positive tumors in the negative-estrogen receptor group was significantly higher than in the positive-estrogen receptor group (30.26% and 18.18%, p value = 0.031).

The factor of progesterone receptor status: the percentage of HER-2/*neu*-positive tumors in the negative-progesterone receptor group was also significantly higher than in the positive-progesterone receptor group (29.44 % and 15.49 %, p value = 0.022).

HER-2/*neu*-positive tumors were not correlated with age and the number of positive nodes, but they were significantly associated with metastatic lymph node status, estrogen-negative tumors and progesterone-negative tumors.

Discussion

IHC has been advocated as the primary test for identifying Trastuzumab candidates because it is readily available and easily performed in most clinical pathology labs. It is also relatively inexpensive.

In this study, the DAKO A0485 polyclonal antibody was used as IHC staining, the test has become an in-house validated assay since it is not used as approved by the FDA. But there are several studies which compare the results of the FISH and/or HercepTest with DAKO A0485. There was fully good concordance between both methods (FISH versus DAKO A0485 or HercepTest versus DAKO A0485), when the results were analyzed as binary variables (positive versus negative results) in more than 95 % of paraffin-embedded breast cancer specimens.[27,28] Excellent inter-laboratory agreement for HER-2/*neu* IHC was attained using the same primary antibody to HER-2/*neu*, even without standardization of assay method or scoring criteria.[29]

The FISH procedure required more technologist time and more interpretation time per case for the

pathologist than IHC. Reagent costs were substantially higher for FISH than for IHC and reagent of DAKO A0485 was much less expensive than HercepTest.[24,25]

However, standardization of these IHC staining remains an important objective to optimize inter-laboratory agreement. The potential clinical importance of HER-2/*neu* status in patient management, inter-laboratory variability in HER-2/*neu* IHC results in a matter of legitimate concern.

There was inverse association between HER-2/*neu* over-expression and, estrogen receptor and progesterone receptor expression significantly (p value = 0.031 and 0.022 respectively) (Table 2). HER-2/*neu* over-expression was also significantly associated with lymph node metastasis (p value = 0.035). Conversely, the age and the number of positive nodes were not significantly associated with HER-2/*neu* expression.

There was a good correlation between HER-2/*neu* over-expression and several known, well-accepted indicators of poor prognosis in invasive breast carcinoma such as lymph node metastasis, estrogen receptor non-expression and progesterone receptor non-expression tumors. So it may be regarded as another indicator of poor prognostic breast cancers when HER-2/*neu* is over-expressed. Furthermore, it would be important to study larger sample sizes of patients to obtain more conclusive results.

In addition to its prognostic value, evaluation of HER-2/*neu* status is necessary for the selection of patients who will benefit from treatment by Trastuzumab. HER-2/*neu* over-expression in ER-positive tumors may also be a marker of Tamoxifen resistance and may also aid prediction of response to hormonal therapy and chemotherapy.

Conclusion

There was good correlation between HER-2/*neu* expression and several known, well-accepted prognostic indicators of invasive breast carcinoma such as nodal status, estrogen receptor status and progesterone receptor status.

REFERENCES

1. Fukushige S, Matsubara K, Yoshida M, et al. Localization of a novel v-erbB-related gene, c-erbB-2, on human chromosome 17 and its amplification in a gastric cancer cell line. Mol Cell Biol 1986; 6: 955-8.
2. Coussens L, Yang-Feng TL, Liao YC, et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. Science 1985; 230(4730): 1132-9.
3. Schechter AL, Stern DF, Vaidyanathan L, et al. The neu oncogene: an erb-B-related gene encoding a 185,000-Mr tumour antigen. Nature 1984; 312(5994): 513-6.
4. Yamamoto T, Ikawa S, Akiyama T, et al. Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. Nature 1986; 319(6050): 230-4.
5. Pegram M, Slamon D. Biological rationale for HER2/neu (c-erbB2) as a target for monoclonal antibody therapy. Semin Oncol 2000; 27 (Suppl 9): 13-9.
6. Wang SC, Zhang L, Hortobagyi GN, Hung MC. Targeting HER2: recent developments and future directions for breast cancer patients. Semin Oncol 2001; 28 (Suppl 18): 21-9.
7. Cobleigh MA, Vogel CL, Tripathy D, et al. Efficacy and safety of Herceptin (humanized anti-HER2 antibody) as a single agent in 222 women with HER2 overexpression who relapsed following chemotherapy for metastatic breast cancer. Proc Am Soc Clin Oncol 1998; 17: 97A.
8. Slamon D, Leyland-Jones B, Shak S, et al. Addition of Herceptin (humanized anti-HER2 antibody) to first line chemotherapy for HER2 overexpressing metastatic breast cancer (HER+/MBC) markedly increases anticancer activity: A randomized, multinational controlled phase III trial. Proc Am Soc Clin Oncol 1998; 17: 98a.
9. Pegram MD, Lipton A, Hayes DF, et al. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/*neu* monoclonal antibody plus cisplatin in patients with HER2/*neu*-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 1998; 16: 2659-71.
10. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/*neu* proto-oncogene in human breast and ovarian cancer. Science 1989; 244(4905): 707-12
11. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177-82.
12. Revillion F, Bonneterre J, Peyrat JP. ERBB2 oncogene in human breast cancer and its clinical significance. Eur J Cancer 1998; 34: 791-808.
13. Tsutsui S, Ohno S, Murakami S, Hachitanda Y, Oda S. Prognostic value of c-erbB2 expression in breast cancer. J Surg Oncol 2002; 79: 216-23.
14. Hanna W, Kahn HJ, Trudeau M. Evaluation of HER-2/*neu* (erbB-2) status in breast cancer: from bench to bedside. Mod Pathol 1999; 12: 827-34.
15. Quenel N, Wafflart J, Bonichon F, et al. The prognostic value of c-erbB2 in primary breast carcinomas: a study on 942 cases. Breast Cancer Res Treat 1995; 35: 283-91.
16. Press MF, Bernstein L, Thomas PA, et al. HER-2/*neu* gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol 1997; 15: 2894-904.

17. Orvieto E, Dei Tos AP. Measurement of HER-2/*neu* in breast cancer: which methodologic approach? Pathologica 2001; 93: 183-8
18. Tetu B, Brisson J, Plante V, et al. p53 and c-erbB-2 as markers of resistance to adjuvant chemotherapy in breast cancer. Mod Pathol 1998; 11: 823-30.
19. Stal O, Sullivan S, Wingren S, et al. c-erbB-2 expression and benefit from adjuvant chemotherapy and radiotherapy of breast cancer. Eur J Cancer 1995; 31A: 2185-90.
20. Bitran JD, Samuels B, Trujillo Y, et al. Her2/*neu* overexpression is associated with treatment failure in women with high-risk stage II and stage IIIA breast cancer (>10 involved lymph nodes) treated with high-dose chemotherapy and autologous hematopoietic progenitor cell support following standard-dose adjuvant chemotherapy. Clin Cancer Res 1996; 2: 1509-13.
21. Berns EM, Foekens JA, van Staveren IL, et al. Oncogene amplification and prognosis in breast cancer: relationship with systemic treatment. Gene 1995; 159: 11-8.
22. Carlomagno C, Perrone F, Gallo C, et al. c-erb B2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph node metastases. J Clin Oncol 1996; 14: 2702-8.
23. Sjogren S, Inganas M, Lindgren A, et al. Prognostic and predictive value of c-erbB-2 overexpression in primary breast cancer, alone and in combination with other prognostic markers. J Clin Oncol 1998; 16: 462-9.
24. Jacobs TW, Gown AM, Yaziji H, Barnes MJ, Schnitt SJ. Comparison of fluorescence in situ hybridization and immunohistochemistry for the evaluation of HER-2/*neu* in breast cancer. J Clin Oncol 1999; 17: 1974-82.
25. Yaziji H, Goldstein LC, Barry TS, et al. HER-2 testing in breast cancer using parallel tissue-based methods. JAMA 2004; 291: 1972-7.
26. Sampatanukul P, Wannakrairot P, Thanakit V, et al. Immunohistochemical detections of ER, PR and HER2 status of invasive mammary carcinoma: a synopsis on current staining protocol, interpretation criteria and quality control measures by the breast pathology group. J R C Pathol Thai 2003; 2: 112-7.
27. Thomson TA, Hayes MM, Spinelli JJ, et al. HER-2/neu in breast cancer: interobserver variability and performance of immunohistochemistry with 4 antibodies compared with fluorescent in situ hybridization. Mod Pathol 2001; 14: 1079-86.
28. S Selvarajan, BH Bay, MJ Chng, PH Tan. The HercepTest and Routine C-erbB2 Immunohistochemistry in breast cancer: any difference? Ann Acad Med Singapore 2004; 33: 473-6
29. Jacobs TW, Gown AM, Yaziji H, Barnes MJ, Schnitt SJ. HER-2/*neu* protein expression in breast cancer evaluated by immunohistochemistry. A study of interlaboratory agreement. Am J Clin Pathol 2000; 113: 251-8.
30. Chen Y, Dong J, Lu Y, McGee JO. Quantitative detection of amplification of proto-oncogenes in breast cancer. Chin Med J (Engl) 1995; 108: 849-54.
31. Valeron PF, Chirino R, Fernandez L, et al. Validation of a differential PCR and an ELISA procedure in studying HER-2/*neu* status in breast cancer. Int J Cancer 1996; 65: 129-33.
32. Looi LM, Cheah PL, Yap SF. Correlation between histological grade and c-erbB2 oncoprotein overexpression in infiltrating ductal carcinoma of breast. Malays J Pathol 1997; 19: 35-9.
33. el-A Helal T, Khalifa A, Kamel AS. Immunohistochemical expression of p53 and c-erbB2 proteins in breast cancer in Egypt. Anticancer Res 2000; 20(3B): 2145-50.
34. DiGiovanna MP, Stern DF, Edgerton SM, et al. Relationship of epidermal growth factor expression to ErbB-2 signaling activity and prognosis in breast cancer patients. J Clin Oncol 2005; 23: 1152-60.