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ORIGINAL ARTICLE

Serum HER2 and CA 15-3 in breast cancer patients

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Summary

Purpose: *The HER2 oncogene encodes a transmembrane tyrosine kinase receptor. This molecule could be a new marker for prognosis and response to therapy in patients with advanced breast cancer. However, the extracellular domain of c-erbB-2 (HER2) transmembrane receptor undergoes proteolytic cleavage from the full-length protein by metalloproteases, and is shed into the blood as a circulating antigen. To determine the clinical utility of this oncoprotein, the soluble form of HER2 was assayed in the serum of breast cancer patients.*

Patients and methods: *Serum levels of breast carcinoma antigens CA 15-3 and HER2 were determined in 60 patients, 40 with localized (group A) and 20 with metastatic (group B) breast carcinoma. CA 15-3 measurements served as "gold standard" to which HER2 diagnostic and/or prognostic value was compared. Sera from 10 healthy women served as controls.*

Results: *Serum levels of the tested tumor markers HER2 and CA 15-3 were significantly higher in cancer patients compared to controls. CA 15-3 correlated with bulky initial tumor, whereas HER2 showed no differences between healthy individuals and group A patients. The serum levels of the tested markers in group B patients were significantly higher ($p < 0.001$) than the serum levels of patients in group A. Striking increase in serum levels of HER2 was found in 52.7% and CA 15-3 in 52.9% of patients with metastatic cancer. A combination of markers was more sensitive than using one marker alone. In this regard, 90% of the patients with metastasis had at least one of the markers increased.*

Conclusion: *The results of this study suggest that the HER2 oncoprotein may be potentially useful in detecting recurrence of breast cancer. However, to improve sensitivity and specificity in the diagnosis and monitoring of breast cancer, the use of multiple tumor markers should be employed.*

Key words: breast cancer, CA 15-3, HER2, serum levels

Introduction

In contrast to tissue tumor markers, serum tumor markers are reproducible and can be measured serially to reflect the dynamic changes in tumor burden. Their measurements offer an effective and objective way in monitoring remission and progression in patients with metastatic breast cancer. It was shown that a combination of markers is more sensitive than using a single marker [1]. In our study, two tumor markers were investigated: HER2 and CA 15-3.

The proto-oncogene HER2 (from Human Epidermal growth factor Receptor) is localized on chromosome 17q and encodes a transmembrane tyrosine kinase growth factor receptor. HER2 is a component of a 4-member family of closely related growth factor receptors, including HER1 (or EGFR, derived from Epidermal Growth Factor Receptor), HER2, HER3 and HER4 [2,3]. The full-length protein (HER2) undergoes proteolytic cleavage by metalloproteases, and its extracellular domain is shed into the blood as a circulating antigen. In addition to its association with

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disease outcome in gastrointestinal, pulmonary, genitourinary and other neoplasms, amplification of HER2 gene, or overexpression of the HER2 protein, has been identified in 10-34% of breast cancers [4]. The specific clinical benefit of serum HER2 measurement was clarified by numerous investigations worldwide. In most of the retrospective studies that included significant number of patients, serum HER2 levels showed prognostic significance with respect to disease-free survival and overall survival [5]. Circulating HER2 levels could successfully prove the presence and predict progression of HER2-positive breast cancers. In 22 published studies with 4,088 patients, 16 (73%) of them involving 3,458 (85%) of the patients, a significant correlation of serum HER2 levels with disease recurrence, metastasis or shortened survival was reported [6-13].

CA 15-3 (MUC-1, breast cancer mucin) is a cell membrane glycoprotein. CA 15-3 serum levels are elevated in 54-80% of patients with metastatic breast cancer. It may be increased in other malignant (lung, ovarian, endometrial, gastrointestinal and bladder carcinoma) or benign (chronic hepatitis, liver cirrhosis) conditions [14,15]. Elevated levels of CA 15-3 may indicate the first recurrence of breast cancer. In this regard, more than 75% of first recurrences (all sites included) are associated with a significant rise in CA 15-3 levels. Furthermore, CA 15-3 is a reliable marker for monitoring the clinical course of the disease and it seems to be of potential interest in monitoring disease during therapy, especially together with HER2 serum level estimation.

Patients and methods

Patients

The study enrolled 70 females, divided into two groups:

1. Experimental group – 60 patients with histologically confirmed breast carcinoma. This group consisted of two subgroups: group A (patients with localized tumor) and group B (patients with metastatic breast cancer).

2. Control group – 10 healthy women. Group A patients were included in the study before breast surgical treatment, and group B patients were included in the study after the final decision about the type of conservative treatment. In this way, any possible therapy effects (operative, chemo or hormonal therapy), which could influence the results, were excluded.

In addition, patients with inflammation (leukocytosis, high sedimentation rate and fibrinogen) were excluded.

Methods

HER2 and CA 15-3 serum levels were determined by using commercially available ELISA kits (Human sp185^{HER-2} ELISA, Bender MedSystems, Vienna, Austria and Breast Cancer Antigen CA 15-3 Enzyme Immunoassay Test Kit, DRG International, Inc. USA). Normal serum values were < 13.2 ng/ml for HER2 and < 35 U/ml for CA 15-3.

Statistical analysis

The data obtained were evaluated using the SPSS commercial program package (version 10.0, SPSS Inc., Chicago, IL). The normal data distribution was evaluated by the Kolmogorov-Smirnov test, Student t-test, and then retested by the c^2- test. Student t-test was used for parameters with normal data distribution, Mann-Whitney's U-test and Kruskal-Wallis's test compared two or more groups of non-parametric data. p – values less than 0.05 were accepted as significant.

Results

Patients in group A had almost identical HER2 serum levels as those in the control group (group A: 7.7 ng/ml; control group: 6.5 ng/ml; Figure 1). Increased HER2 serum values were detected in 52.6% of group B patients, with almost 5-fold average value compared with the control group (average HER2 serum values in the control and group B were 6.5 ng/ml and 30.6 ng/ml, respectively). Where the data were not homogeneous (CV=88.5%), the Kruskal-Wallis's test was applied, and showed statistically significant difference between the control and metastatic (B) groups (c^2=6.015, df=2, p=0.049). In addition, Mann-Whitney's test was used and showed also significant differences between control and group B (p=0.046; Figure 1).

Although patients with localized carcinoma (group A) had higher average CA 15-3 serum levels compared with the controls (localized: 30.5 U/ml; control: 22.5 U/ml), these values were, however, within normal range (0-36.0 U/ml, Figure 2). In patients with distant metastatic disease (group B) CA 15-3 serum levels were extremely increased (91.8 U/ml), in comparison with controls and group A patients (Kruskal-Wallis: c^2=18.398, df=2, p=0.000; Mann-Whitney: p <0.01).

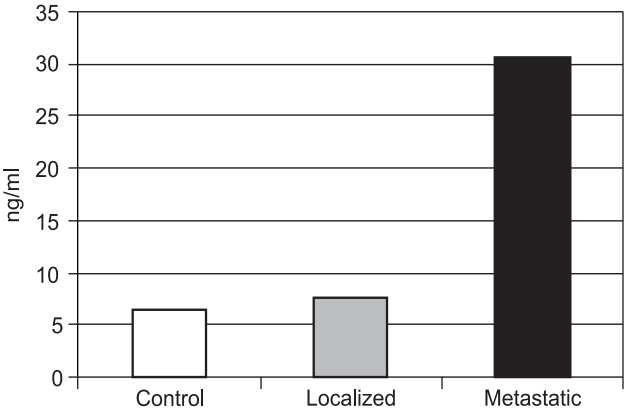

Figure 1. HER2 serum levels. Patients with breast cancer show increased HER2 serum levels. These values are highest in the group of patients with metastatic disease. Kruskal-Wallis: χ^2=6.015, df=2, p=0.049; Mann-Whitney: p=0.046.

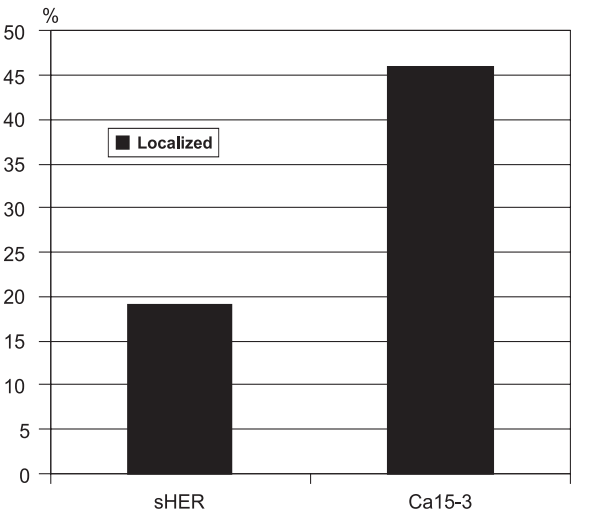

Figure 3. Deviation from control values in group A patients. Only CA 15-3 serum values show significant changes in comparison to control values. Results are shown as deviation from control values.

Figures 3 and 4 show HER2 and CA 15-3 serum values in breast cancer patients and the data are presented as percent of deviation from control values. As discussed before, HER2 and CA 15-3 could not establish the presence of localized breast carcinoma, because HER2 and CA 15-3 serum levels were almost identical in the control and group A patients. Interestingly enough, even 90% of the patients with distant metastatic disease had at least one tumor marker level increased (Figure 5).

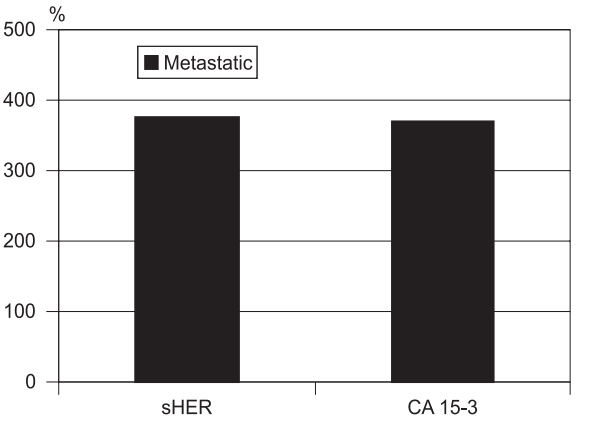

Figure 4. Deviation from control values in group B patients. Serum values of both tested tumor markers deviate significantly from control values. Results are shown as deviation from control values.

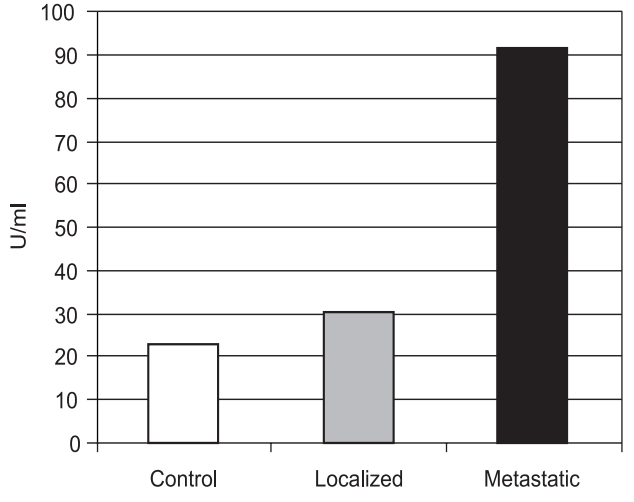

Figure 2. CA 15-3 serum levels. Patients with breast cancer show increased CA 15-3 serum levels. These values are highest in the group of patients with metastatic disease. Kruskal-Wallis: χ^2=18.398, df=2, p=0.000; Mann-Whitney: p<0.01.

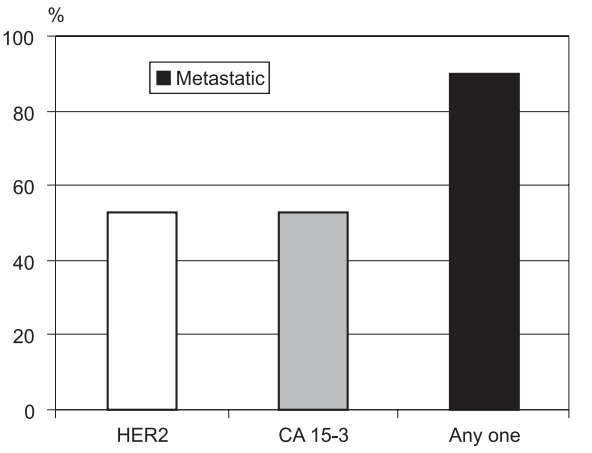

Figure 5. Specificity of HER2 and CA 15-3. Both HER2 and CA 15-3 values are similar in metastatic breast cancer patients group (52.7% and 52.9%, respectively). Nearly 90% of metastatic breast cancer patients had at least one tumor marker serum level increased.

Discussion

Tumors originate from normal tissues and malignant cells express cell surface protein molecules which can be found also, to a lower extent, on normal cells. These molecules, specific for malignant cells, were designated as tumor-associated antigens, or tumor markers. Detection and continuous monitoring of changes in tumor marker serum levels can offer data related to the estimation of risk for carcinoma appearance, diagnosis, prognosis and response to therapy [16-18]. Monitoring of the disease course, after diagnosis and first-line therapeutic procedures, reflects the clinical importance of tumor markers [19]. No single tumor marker has demonstrated a possibility of independent use for routine screening in early diagnosis [20]. Even CA 15-3, for which serum levels and tumor size and/or presence of distant metastases correlation was demonstrated, can not be independently used in screening and breast carcinoma diagnostic procedures because of its low sensitivity [21,22]. CA 15-3 and other tumor markers are mostly used for early detection of distant metastatic foci. In more than 75% of the cases, CA 15-3 serum level elevation indicates the onset of early breast carcinoma metastases [1]. Serum level elevation can precede by more than 6 months to clinical and radiological metastatic signs [22, 23]. However, the significance of continual increase of CA 15-3 serum levels, in the absence of signs of recurrent disease, is not entirely clear and does not provide enough information on the basis of which, under given circumstances, specific therapy could be defined. This tumor marker can be used in the evaluation of the therapeutic procedures, in addition to its diagnostic and prognostic use [22]. Numerous studies have shown that CA 15-3 serum levels can indicate the success of the applied therapy in more than 80% of the cases [22].

Our results were in accordance to those shown in similar studies. Although patients with localized breast carcinoma showed higher CA 15-3 serum concentrations than those of the control group, those values were still in the "normal" value range, according to detection kits manufacturers. Furthermore, the average values of CA 15-3 for this patient group exceeded the average CA 15-3 concentration of the control group by nearly 50%. This finding clearly shows that CA 15-3 has undoubted diagnostic importance. On the other hand, values observed in patients with distant metastases were extremely higher compared with the control group, as well as the localized tumor group. Our data confirmed the clinical importance of this marker, not just for the early detection of recurrent disease, but even in routine examination if breast cancer is suspected. However, the combination of different tumor markers can enhance both specificity and sensitivity. Because of this, CA 15-3 and HER2 detection were combined in our study.

According to numerous studies [24,25], in 20-30% of breast cancer cases HER2 amplification and overexpression was observed (detected by immunohistochemistry) and HER2 could be an independent prognostic factor. In different neoplasms, as well as in breast carcinoma, overexpression of HER2 correlates with aggressive forms of the disease and with bad prognosis. Besides that, overexpression often was associated with other important breast cancer prognostic factors: high grade and extensive ductal carcinoma *in situ* [26-28], DNA aneuploidy, high index of proliferation, p53 mutations and changes in other biomarkers of breast carcinoma metastatic potential [29-36].

HER2 oncoprotein belongs to the EGFR receptor family (transmembrane tyrosine kinase receptor). The extracellular domain of this molecule undergoes enzymatic cleavage, and can be detected in the blood as circulating tumor marker [29, 37]. Several studies showed high serum levels of HER2 in 18.5% (median) of patients with primary breast carcinoma (range 7-38%), as well as in patients with distant metastases (median 43%) [38]. According to these studies, HER2 serum level elevation was an indicator of bad prognosis and inadequate response to therapy.

Our results were, mostly, in accordance with data of other relevant studies: notable differences in HER2 serum concentration between patients with localized tumor and controls did not occur; HER2 serum level in the group with distant metastases was significantly higher. Based on the results of our study we can conclude that HER2 serum level cannot contribute in primary breast carcinoma diagnosis, but it can offer useful data for disease monitoring.

Moreover, besides the lower sensitivity in the group with localized carcinoma, CA 15-3 established its clinical value since those patients had 50% higher values than controls (Figure 2). The impressive finding is that both tested tumor markers clearly implied the presence of distant metastatic foci (values in metastatic group were almost 5 times higher than those of the control group, Figure 2). Nevertheless, the distribution of the measured values was not homogeneous; only 23.7% of group A patients had higher HER2 serum values. This finding suggests, according to the data discussed above, that HER2 and CA 15-3 cannot be independent diagnostic tumor markers and the average values did not describe the real

state, but represented a reflection of the sensitivity of the applied tests.

CA 15-3, its low sensitivity excepted, proved its diagnostic value, considering that average values of CA 15-3 in the localized tumor group exceeded those of the control group by nearly 50%. However, HER2 serum level distribution was nonhomogeneous; only 23.7% of the patients with localized tumor had higher values. This finding suggests, according to the data discussed above, that HER2 and CA 15-3 cannot be independent diagnostic tumor markers.

The development of distant metastases basically reflects the breakdown of host defense mechanisms accompanied with loss of homeostatic balance. Thus, it is not surprising that CA 15-3 and HER2 indicate the presence of distant metastatic foci. In fact, we observed elevated serum values of HER2 and CA 15-3 in 52.7% and 52.9% patients with metastases, respectively. It is interesting that 90% of the patients with distant metastases had at least one of these tumor markers increased.

We conclude that serum estimations of CA 15-3 in the diagnosis, as well as in the monitoring of breast carcinoma patients, are more than useful. It is clear that HER2 serum level is not adequate for the diagnosis of primary breast carcinoma, but it can provide relevant data for disease monitoring and can be used for the detection of distant metastatic foci.

HER2 can be used for the detection of distant metastatic foci. Combination of different tumor markers can enhance their overall sensitivity. The cclinical importance of HER2 serum estimation demands additional research.

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References

1. Cheung KL, Robertson JFR. Objective measurement of remission and progression in metastatic breast cancer by the use of serum tumour markers. Minerva Chir 2003; 58: 297-303.
2. Plowman GD, Whitney GS, Neubauer GD et al. Molecular cloning and expression of an additional epidermal growth factor receptor-related gene. Proc Natl Acad Sci USA 1990; 87: 4905-4909.
3. Plowman GD, Green JM, Culouscou JM et al. Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. Proc Natl Acad Sci USA 1993; 366: 473-475.
4. Ross S, Flecher JA. The HER-2/neu oncogene in breast cancer: prognostic factor, predictive factor, and target for therapy. Stem Cells 1998; 16: 413-428.
5. Lüftner D, Lüke C, Possinger K. Serum HER-2/neu in the management of breast cancer patients. Clin Biochem 2003; 36: 233-240.
6. Fehm T, Maimonis P, Katalinic A et al. The prognostic significance of c-erbB-2 serum protein in metastatic breast cancer. Oncology 1998; 55: 33-38.
7. Mehta RR, McDermott JH, Hieken TJ et al. Plasma c-erbB-2 levels in breast cancer patients: prognostic significance in predicting response to chemotherapy. J Clin Oncol 1998; 16: 2409-2416.
8. Colomer R, Montero S, Lluch A et al. Circulating HER2 extracellular domain and resistance to chemotherapy in advanced breast cancer. Clin Cancer Res 2000; 6: 2356-2362.
9. Bewick M, Conlon M, Gerard S et al. HER-2 expression is a prognostic factor in patients with metastatic breast cancer treated with a combination of high-dose cyclophosphamide, mitoxantrone, paclitaxel and autologous blood stem cell support. Bone Marrow Transplant 2001; 27: 847-853.
10. Harris LN, Liotcheva V, Broadwater G et al. Comparison of methods of measuring HER-2 in metastatic breast cancer patients treated with high-dose chemotherapy. J Clin Oncol 2001; 19: 1698-1706.
11. Ali SM, Leitzel K, Chinchilli VM et al. Relationship of serum HER-2/neu and serum CA 15-3 in patients with metastatic breast cancer. Clin Chem 2002; 48: 1314-1320.
12. Classen S, Kopp R, Possinger K et al. Clinical relevance of soluble c-erbB-2 for patients with metastatic breast cancer predicting the response to second-line hormone or chemotherapy. Tumour Biol 2002; 23: 70-75.
13. Lipton A, Ali SM, Leitzel K et al. Elevated serum Her-2/neu level predicts decreased response to hormone therapy in metastatic breast cancer. J Clin Oncol 2002; 20: 1467-1472.
14. Molina R, Zanón G, Filella X et al. Use of serial carcinoembryonic antigen and CA 15-3 assays in detecting relapses in breast cancer. Breast Cancer Res Treat 1995; 36: 41-48.
15. van Dalen A. New markers for breast carcinoma-associated mucin antigen in comparison with CA 15-3. Anticancer Res 1996; 16: 2339-2343.
16. Stearns V, Yamauchi H, Hayes DF. Circulating tumour markers in breast cancer: accepted utilities and novel prospects. Breast Cancer Res Treat 1998; 52: 239-259.
17. Hayes DF. Tumor markers for breast cancer. Ann Oncol 1993; 4: 807-819.
18. Hayes DF. Serum (circulating) tumor markers for breast cancer. Recent Results Cancer Res 1996; 140: 1-13.
19. Lamerz R, Stieber P, Fateh-Moghadam A. Serum marker combinations in human breast cancer. In Vivo 1993; 7: 607-613.
20. Duffy MJ. Biochemical markers in breast cancer: which ones are clinically useful? Clin Biochem 2001; 34: 347-352.
21. Antoine E, Kayitalire L, Spielmann A. CA 15-3 and breast cancer. M Rev Med Interne 1994; 15: 650-662.
22. Crombach G. Considerations in rational use of tumor markers in breast carcinoma. Schweiz Rundsch Med Prax 1998; 87: 589-594.

23. Pujol H. Importance of early detection of metastases? Impact on survival and quality of life. Nucl Med Biol 1994; 21: 303-315.
24. Ross JS, Gray GS. Targeted therapy for cancer: the HER-2/neu and Herceptin story. Clin Leadersh Menag Rev 2003; 17: 333-340.
25. Ross JS, Flatcher JA, Linette GP et al. The Her-2/neu gene and protein in breast cancer biomarker and target of therapy. Oncologist 2003; 8: 307-325.
26. Bose S, Lesser ML, Norton L et al. Immunophenotype of intraductal carcinoma. Arch Pathol Lab Med 1996; 120: 81-85.
27. Moreno A, Lloveras B, Figueras A et al. Ductal carcinoma in situ of the breast: Correlation between histologic classification and biologic markers. Mod Pathol 1997; 10: 1088-1092.
28. Mack L, Kerkzelit N, Doig G et al. Relationship of a new histological categorization of ductal carcinoma in situ of the breast with size and the immunohistochemical expression of p53, c-erbB-2, bcl2 and ki-67. Hum Pathol 1997; 28: 974-979.
29. Navolanic PM, Steelman LS, McCubrey JA et al. EGFR family signaling and its association with breast cancer development and resistance to chemotherapy. Int J Oncol 2003; 22: 237-252.
30. Stark A, Hulka BS, Joens S et al. HER-2/neu amplification in benign breast disease and the risk of subsequent breast cancer. J Clin Oncol 2000; 18: 267-274.
31. Hayes DF, Thor AD. c-erbB-2 in breast cancer: development of a clinically useful marker. Semin Oncol 2002; 29: 231-245.
32. Masood S, Bui MM. Prognostic and predictive value of HER2/neu oncogene in breast cancer. Microsc Res Tech 2002; 59: 102-108.
33. Eccles SA. The role of c-erbB-2/HER2/neu in breast cancer progression and metastasis. J Mammary Gland Biol Neoplasia 2001; 6: 393-406.
34. Piccart M, Lohrisch C, Di Leo A et al. The predictive value of HER2 in breast cancer. Oncology 2001; 61 (Suppl 2): 73-82.
35. Yarden Y. Biology of HER2 and its importance in breast cancer. Oncology 2001; 61 (Suppl 2): 1-13.
36. Zemzoum I, Kates RE, Ross JS et al. Invasion factors uPA/PAI-1 and HER2 status provide independent and complementary information on patient outcome in node negative breast cancer. J Clin Oncol 2003; 21: 1022-1028.
37. Pavlovic S, Baskic D, Ristic P, Arsenijevic N. HER2. Overexpression on malignant cells. Medicus 2004; 5: 22-26.
38. Carney WP, Neumann R, Lipton A et al. Potential clinical utility of serum HER-2/neu oncoprotein concentrations in patient with breast cancer. Clin Chem 2003; 49: 1579-1598.