

Utility of Serum HER-2/ neu in Prediction of Tissue HER-2 /neu Status of Primary Breast Cancer

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Please cite this article as:
Mokhtari M, Khosravi MH. Utility of serum HER-2/ neu in prediction of tissue HER-2 /neu status of primary breast cancer. Middle East J Cancer. 2021;12(4):483-90. doi: 10.30476/mejc.2021.84845.1234.

Abstract

Background: The use of biomarkers has become increasingly important in the diagnosis and treatment of several malignancies, including breast cancer. Among the established biomarkers, receptor tyrosine-protein kinase erbB-2 (HER-2/ neu) has been found to be of a predictive and prognostic role in breast cancer patients.

Method: 94 patients with primary breast cancer were enrolled in our prospective study between 2016 and 2017. 5 cc clot blood samples were taken for serum HER-2/neu testing at the time of diagnosis of breast cancer. The patients' demographic data, tumor characteristics, including tumor size, tumor grade, presence of lymph node involvement, and stage, hormone receptor, and tissue HER-2/neu status of the subjects were recorded following tumor removal.

Results: All of the patients were female with the age range of 27 to 80 years (the mean of age was 49.66. 36 patients (38.3%) had positive tissue HER-2 results and 58 (61.7%) had negative results. In those with high level serum HER-2 ECD, 28(77.7%) had positive tissue HER-2 and 8(22.2%) had negative results. Moreover, in the patients with low level serum HER-2 ECD, 8(13.7%) had positive tissue HER-2 and 50(86.2%) had negative results with the sensitivity of 77.7% and specificity of 86.2%. HER2 ECD levels were highly concordant with tissue HER2 status, with a *P* value of less than 0.001, which was considered to be statistically significant. Among different clinicopathologic variables, serum and tissue HER-2/nu were significantly correlated with only tumor grade.

Conclusion: A significantly increased release of HER2 ECD levels may accurately predict tumor HER2 status as detected with immunohistochemistry and/or in situ hybridizations studies.

Keywords: Breast neoplasms, HER 2 proto-oncogene protein, In situ hybridization

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Introduction

Breast cancer is the most prevalent type of cancer and the second most common cause of cancer death in

female population, annually affecting about 2.3 million women worldwide.¹

It has been stated that the pathogenesis of some human

carcinomas, including breast cancer, are related to mutations of the human epidermal growth factor receptor (HER family of receptors). This glycoprotein mediates proliferation and differentiation in both normal and cancer cells. This family comprises four main members with the same structure (extracellular ligand binding site, transmembrane part, and an intracellular domain with tyrosine kinase catalytic activity).²

HER2 overexpression has been postulated as an independent predictor of poor prognosis in invasive breast cancer.³

Even though HER2 gene encodes for the full-length membrane-spanning receptor p185 HER2, approximately 30% of HER2+ tumors express other types of receptor fragments sized between 90 and 115 kDa, which are known as p95 HER2 carboxy-terminal fragments (CTFs) or truncated HER2 protein. The HER-2/neu ECD, known as “serum HER2/neu”, is a glycoprotein of 97 to 115 kD. The ECD undergoes proteolytic cleavage from the full-length protein by metalloproteases and released into the blood as a circulating antigen.⁴

Once HER-2/neu ECD is cleaved, the truncated intracellular tyrosine kinase retains its signaling ability. Thus, serum HER-2/neu levels may reflect the activation state associated with the shedding process.⁴

Accurate determination of HER2 status of tumor is mandatory for HER2-targeted therapy.

Both HER-2/neu gene amplification and protein overexpression could be evaluated in tumor tissue.⁴ In situ hybridization is considered as the gold standard method for gene amplification status determination. Over the recent two decades, several studies have evaluated the utility of measuring serum HER2 in breast cancer by different immunoassays, yet the results are controversial. Shed HER2 ECD has been detected in various percentages of primary and metastatic breast cancer patients, which is higher in metastatic patients (20 to 40%) compared with early breast cancer patients (up to 20%).⁵

Estimation of ECD of HER2 receptor shed in blood is simple, easily performed, and cost-effective. The present study aimed to assess the

correlation between serum HER2 ECD levels and tissue HER2 status on top of the relationship between serum HER2 ECD levels and other clinicopathologic features at the time of breast cancer diagnosis.

Material and Method

This was a cross-sectional study conducted in Faghihi Hospital affiliated to Shiraz University of Medical Sciences, Shiraz, Iran. The Medical Ethics Committee of Shiraz University of Medical Sciences approved the study (Ethics code: IR.sums.med.rec.1396.s373).

After taking the informed consent, the samples were collected from 94 patients with breast cancer between 2016 and 2017. All the patients were subjected to a complete work-up, including imaging modalities like mammography, chest radiography, ultrasonography, and computed tomography (CT), particularly for detecting metastases. The patients who were already on treatment and metastatic breast cancer (MBC) were excluded. Serum samples were collected at the time of cancer diagnosis prior to the treatment; 5 ml of peripheral blood was collected in a sterile test tube without anticoagulants and centrifuged at 1000 g for 10 min at room temperature. It was then stored at -20 °C. Serum HER2 ECD was measured with a 2-site chemiluminescence sandwich immunoassay (Siemens ADVIA Centaur CP System) which employs two monoclonal antibodies against two distinct epitopes of serum HER2. The test was carried out according to the manufacturer's protocol.

The participants were considered to have positive serum HER-2 results when the value was over 15 ng/ml.

Following tumor excision, the tissue was placed in neutral buffered formalin and sent to pathology department for routine permanent sectioning and staining. The demographic data, including age and sex, were recorded. In addition, clinicopathologic data were retracted from pathology reports, including tumor type, tumor size, tumor grade, status of lymph node involvement, tumor stage, hormone receptor, HER-2/neu status, and molecular subtype. The status of estrogen receptor (ER), progesterone

receptor (PR), and HER2 were assessed on tumor samples applying standard immunochemistry (IHC) methods. In IHC equivocal cases (2+ results), chromogenic in situ hybridization (CISH) method was utilized with Novocastra-Leica 2C SPEC ERBB2/CEN 17 Probe Kit. Statistical analysis was done with SPSS version 23. Group comparisons of categorical variables was analyzed using the Pearson's chi-square test.

Results

This study was performed in pathology department, Shiraz University, which includes 94 cases of newly diagnosed breast cancer.

All of them were female with the age range of 27 to 80 years (mean of age was 49.66). 84 patients (89.4%) had invasive ductal carcinoma, not otherwise specified (NOS), 7 (7.4%) had invasive carcinoma with medullary differentiation, 2 (2.1%) had invasive lobular carcinoma, and 1 (1.1%) had mucinous carcinoma.

Regarding tumor grading, 27 patients (28.7%) had breast cancer with grade I, 41 (43.6%) and 26 (27.6%) patients had grade II and grade III, respectively. Regarding TNM stage, 26 (27.7%)

subjects were in stage IA, 1 (1.1%) was in stage IB, 31 (33%) were in stage IIA, 13 (13.8%) were in stage IIB, 13 (13.8%) were in stage IIIA, and 10 (10.6%) were in stage IIIC. The tumor sizes ranged between 0.5 to 11 cm with the mean of 2.59 cm; 43 patients (45.7%) had a tumor size of ≤ 2 cm, which was between 2 to 5 cm and > 5 cm respectively for 46 patients (48.9%) and 5 patients (5.3%).

In 90 cases with submitted lymph nodes, 45 (50%) had positive nodes and 45 (50%) had negative nodes. The number of involved lymph nodes ranged from 1 to 24. Among the lymph node positive patients, 31 (68.9%) showed lymph nodes with macro metastasis (size involvement ≥ 2 mm).

Regarding the hormone receptor status, 69 patients (73.4%) were positive for ER and PR and 25 (26.6%) were negative for ER and PR. Concerning molecular subtypes (6), 48 participants (51.1%) had luminal A, 21 (22.3%) had luminal B, 15 (16%) had HER-2 enriched, and 10 (10.6%) had basal-like tumor.

Regarding HER-2 IHC results, 20 patients (21.3%) showed no staining (considered as

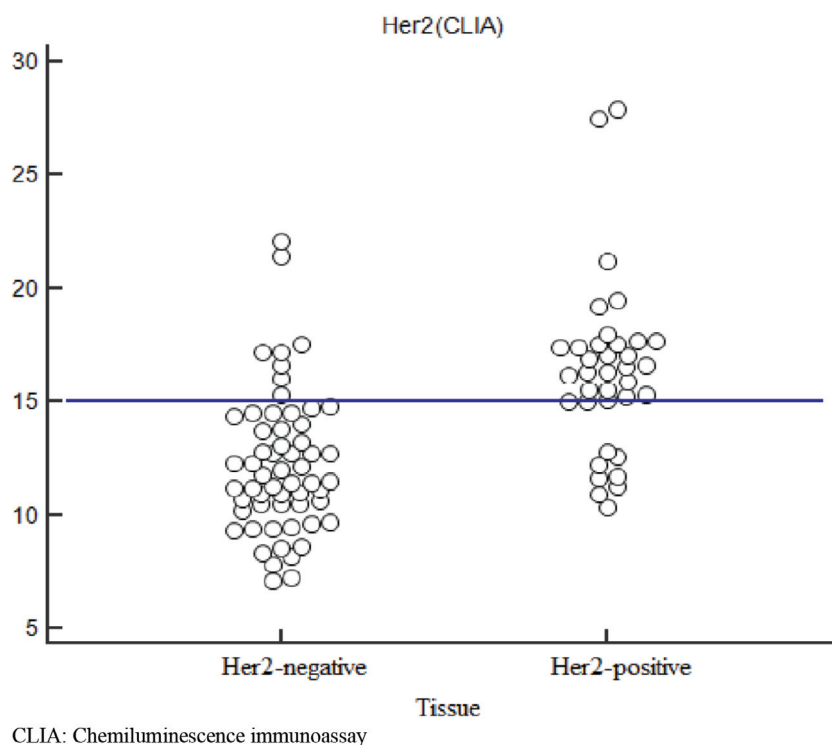


Figure 1. This figure shows the association between serum and tissue HER-2 at the cut-off value of 15 ng/ml.

negative), 17 (18.1%) had IHC +1 (considered as negative), 30 (31.9%) had IHC +2 (as equivocal), and 27 (28.7%) had IHC +3 (considered as positive). CISH study was performed on 30 subjects with +2 IHC results, which revealed positive results in 9 patients (30%) and 21 patients (70%) had negative results. Therefore, based on the results of IHC and CISH studies, 36 patients (38.3%) had positive tissue HER-2 results and 58 (61.7%) had negative results.

Serum HER-2 results

Serum HER-2 ECD ranged between 7.1 to 27.9 ng/ml (the mean of HER2 ECD was 13.8 ng/ml). With a cut-off value of 15 ng/ml, 36 out of the 94 patients (38.3%) had HER2 ECD levels ≥ 15 ng/ml (high levels) and 58 (61.7%) had negative HER2 ECD levels. In the patients with high level serum HER-2 ECD, 28(77.7%) had positive tissue HER-2 and 8(22.2%) had negative results. In the subjects with low level serum HER-2 ECD, 8(13.7%) had positive tissue HER-2 and 50(86.2%) had negative results. HER2 ECD levels

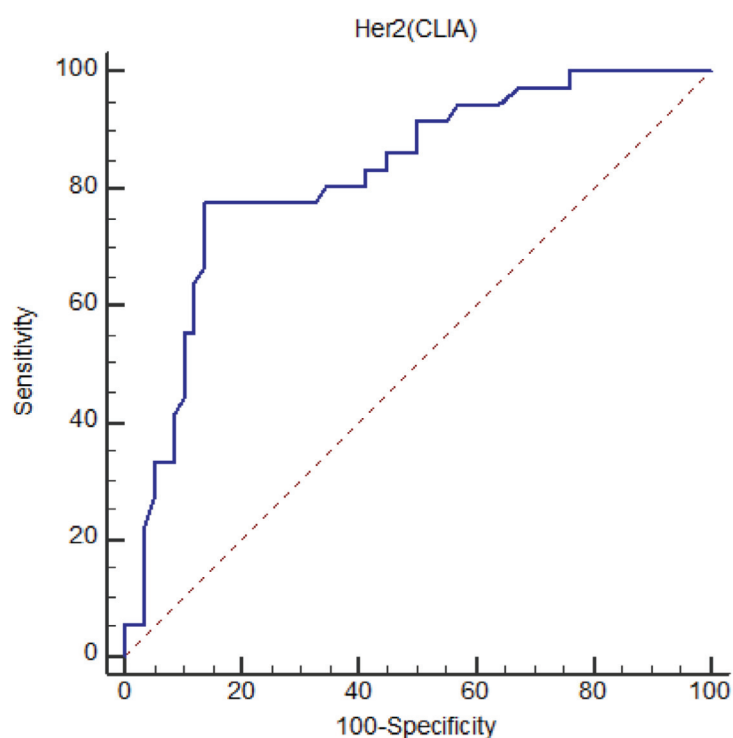
were highly concordant with tissue HER2 status with a P value of less than 0.001, which was considered statistically significant (Figure 1).

We also performed a ROC analysis based on the assumption that IHC 3+ and/or IHC 2+ and CISH positive tumors represent the positive cases and all the other scores show the negative cases. The cut-off, which was measured in our lab, was 14.8 ng/ml. As recommended by the US Food and Drug Administration, 15 ng/ml is the most frequently used cut-off for HER2 ECD elevation. We validated this cut-off value in our laboratory based on the ROC curve (Figure 2).

Using a cut-off value of 15 ng/ml, the HER2 status was defined with IHC and CISH, which was quite specific (86.2%) with a sensitivity of 77.7%.

Association of tissue and serum HER-2

In 36 cases with IHC result of +3 and/or CISH positivity, 28 had HER-2 ECD levels of ≥ 15 ng/ml and 8 had HER-2 ECD levels of < 15 ng/ml. In 21 patients with +2 IHC results and negative CISH results, 1 had HER-2 ECD levels of ≥ 15



CLIA: Chemiluminescence immunoassay

Figure 2. This figure shows a ROC curve for finding the serum HER-2/neu cut-off value.

ng/ml and the remaining 20 cases had serum level of <15 ng/ml. In 20 cases with IHC result of 0, 4 had HER-2 ECD levels of ≥ 15 ng/ml and 16 had HER-2 ECD levels of <15 ng/ml. In 17 subjects with IHC result +1, 3 had HER-2 ECD levels of ≥ 15 ng/ml and 14 had HER-2 ECD levels of <15 ng/ml ($P < 0.001$).

Relationship between serum HER-2 ECD levels with clinicopathological variables

In the patients with HER-2 ECD levels of ≥ 15 ng/ml, 4 had grade I, 17 had grade II, and 15 had grade III tumor. In positive tissue HER-2 results, 3 subjects had grade I, 20 had grade II, and 13 had grade III. In negative tissue HER-2 patients, 24 had grade I, 21 had grade II, and 13 had grade III with a P -value of 0.005 and 0.006 for HER-2 ECD and tissue results, respectively, which was considered to be statistically significant.

In those with HER-2 ECD levels of ≥ 15 ng/ml, 11 had stage IA, 7 had stage IIA, 4 had stage IIB, 7 had stage IIIA, and 7 had stage IIIC tumors. In positive tissue HER-2 results, 9 patients had stage IA, 11 had stage IIA, 5 had stage IIB, 5 had stage IIIA, and 6 had stage IIIC tumors. In negative tissue HER-2 results, 17 cases had stage IA, 1 had stage IB, 20 had stage IIA, 8 had stage IIB, 8 had stage IIIA, and 4 had stage IIIC tumor with a P -value of 0.01 and 0.20 respectively for HER-2 ECD and tissue results, which was considered to be statistically not significant.

In the participants with HER-2 ECD levels of ≥ 15 ng/ml, we observed 16 patients with tumor size of ≤ 2 cm, 18 with tumor size between 2 to 5 cm, and 2 with tumor size of > 5 cm. In those with HER-2 ECD levels of ≤ 2 cm, 28 were found to have tumor size between 2 to 5 cm and 3 had tumor size of > 5 cm with a P value of 0.10, which was considered statistically not significant. In positive tissue HER-2 results, 15 patients had tumor size of ≤ 2 cm, 19 had tumor size between 2 to 5 cm, and 2 had tumor size of > 5 cm. In negative tissue HER-2 results, 28 subjects had tumor size of ≤ 2 cm, 27 had tumor size between 2 to 5 cm, and 3 had tumor size of > 5 cm with a P -value of 0.57, which was considered statistically not significant.

In the patients with HER-2 ECD levels of

≥ 15 ng/ml and in positive tissue HER-2 results, there were 19 with positive lymph nodes and 14 with negative lymph nodes involvement. In those with HER-2 ECD levels of <15 ng/ml, there were 26 with positive nodes and 31 with negative nodes involvement with a P value of 2.97 and 0.08, respectively, which was considered to be statistically insignificant. In the cases with HER-2 ECD levels of ≥ 15 ng/ml, 18 with positive nodes showed macro metastasis whereas in those with HER-2 ECD levels of <15 ng/ml, 13 showed macro metastasis. ($P = 0.41$). In positive tissue HER-2 results, 17 subjects showed lymph node with macro metastasis ($P = 0.12$).

Discussion

In our cross-sectional study, we investigated the role of serum HER2 ECD in prediction of HER2 status in tissue and also the relationship between serum HER2 ECD levels and other clinicopathologic features (tumor size, lymph node status, stage, and grade). We observed that elevated serum HER2/neu levels were accompanied with increased tissue expression of HER2/neu receptors and the association was highly significant ($P = 0.0001$). Certain previous studies documented the correlation of the serum HER2 ECD concentration with tissue HER2 status with IHC and/or FISH or CISH.^{7,8,9} In contrast, some authors pointed out that there is no correlation between HER2 in serum and in tissue.^{10,11,12}

It is indeed difficult to compare all these studies due to the heterogeneity of the study populations, the small sample sizes, the use of different assays, and the use of different cut-off values.¹³

Over the last 15 years, the number of methods available for HER2 testing has increased and the accuracy and reproducibility of these assays has improved. In this study, we utilized an automated chemiluminescent immunoassay (CLIA) method for detection of HER-2 ECD.

Estimation of ECD of the HER2 receptor shed in blood by CLIA is simple, easily performed, and cost-effective. The CLIA results are quantitative, which allows the formation of biologic subgroups within the subsets of patients

who were identified as being HER-2/neu positive via semi-quantitative tissue testing. The method is non-invasive and allows repetition of testing and monitoring of breast cancer patients undergoing all the forms of systemic therapy. While determination of tissue HER-2 is a one-time event, serum HER-2 could be measured any time during the follow-up. Thus, owing to its simple and inexpensive methodology, CLIA could be useful in assessing the response to therapy, estimating the tumor burden in the body, detecting occult metastases, and selecting cases for trastuzumab therapy.¹⁴

The sensitivity and specificity of HER2 ECD in our study were 77.7 and 86.2, respectively. We had eight false positive results of HER2 ECD. This may be explained by tumor heterogeneity which leads to false tissue results and also methodological problems. Although in theory, serum HER2 levels cannot increase without the existence of a HER2- positive tumor, Molina et al. found abnormal HER2 ECD levels in almost 40% of the patients with liver cirrhosis and in more than 25% of the patients with primary liver cancer. However, no significant differences were found between the serum HER2 ECD concentrations in liver cirrhosis or primary liver cancer, suggesting that the possible cause is the catabolism of this antigen in the liver.¹⁵ None of our patients had liver cirrhosis or any other malignancies; therefore, this explanation is not accurate in our patients. Interference by heterophilic antibodies can be a problem in immunoassays, which causes false high values and possible misdiagnosis. We reported eight false negative results. This may be caused by low levels of serum HER-2 below the detection limit of our CLIA kit or the selected cut-off value of HER-2 ECD. We used the cut-off of 15 ng/ml, according to the kit, to categorize the results into positive or negative categories. Furthermore, we determined our cut-off based on our data, which resulted in 14.8 ng/ml. Nonetheless, there is still no general consensus about a discriminant threshold for clinical use of HER2 ECD. As recommended by the US Food and Drug Administration, 15 ng/ml is the most frequently

used cut-off for HER2 ECD elevation. Since tumor marker values depend on the assay used, a general cut-off value for all the methods cannot be applied. Tumor markers higher than the reference range (which corresponds to the 95th percentile of the healthy individuals investigated) have been and are still interpreted as 'positive', whereas the values within the reference range have been regarded as 'normal'.

Therefore, it is only possible to choose a relatively high cut-off value level for reaching the maximum reliability. It has been shown that the increase in HER2 gene copy numbers could be induced with chemotherapy. The assessment of HER2 status with HER2 ISH assays can be a challenging task for pathologists with those patients who have been treated with chemotherapy.¹⁶ It is not the issue in our study since the cases were selected before chemotherapy.

We found that the level of HER-2 is significantly correlated with tumor grade, but the level is not correlated significantly with tumor stage, lymph node involvement, and tumor size. We excluded the breast cancer with distant metastasis and we analyzed the HER-2 serum level in stage I-III. It has been reported that the serum HER2 ECD concentration correlates with advanced or late-stage diseases.¹⁷ In breast cancer, the sensitivity of the serum HER-2/neu assay increases with the stage of disease, from 0% for stage I up to 40 to 50% for stage IV of the disease. The data presented by Kong et al. showed that the presence of liver metastases reduces the specificity. This fact may suggest again that HER2 ECD concentrations are influenced by liver dysfunction.¹⁸ It has been postulated that HER2 overexpression is an independent predictor of shorter overall survival and disease-free survival in invasive primary breast cancer.¹⁹ Isola et al.²⁰ demonstrated the utility of serum HER2 for monitoring the tumor progression of patients with HER2-positive breast carcinoma. Kandl et al.²¹ reported a correlation between elevated serum HER2 and poor prognosis of advanced breast carcinoma. In addition, both Willsher et al.²² and Rocca et al.²³ showed the prognostic importance of serum HER2 in early breast carcinoma patients.

Molina et al.²⁴ demonstrated that high pre-operation serum HER2 was related to the poor prognosis of node-positive and node-negative breast carcinoma. Moreover, Ludovini et al.²⁵ indicated a shorter disease-free survival in patients with elevated serum HER2 ECD levels.²⁰⁻²⁵ The elevated serum HER2 level was observed in patients with high histological grades and ER negativity, suggesting that these patients had a higher risk of metastasis. Based on a systematic review of the literature, Carney et al.²⁶ reported that the prevalence of elevated serum HER2 ECD levels was approximately 18% (range 0%-38%) in primary breast cancer, yet increased up to 43% in metastatic breast cancer.

Conclusion

Thus, determination of HER-2 ECD could be an adjunct to tissue HER-2 in diagnosis of patients who benefit from anti-HER-2 therapy. Serum testing for HER-2 may be helpful in the cases in which ISH study results are equivocal for finding HER-2 positive patients. In conclusion, a significantly increased release of HER2 ECD levels may accurately predict tumor HER2 status as detected with IHC and/or ISH studies. Despite the use of HER2 IHC and HER2 ISH assays for the determination of patient selections for HER2 therapy, there are no real gold standards for HER2 status assessments in breast cancer today. Serum HER2 assay cannot replace IHC/FISH/CISH testing, but may complement the tissue assay in monitoring therapy of breast cancer patients by providing information that is lacking in IHC and FISH testing, such as early diagnosis of recurrence and metastases. Additionally, HER-2 ECD CLIA may be performed once tissue is not available or is unsuitable for IHC and ISH studies. It is a well-known fact that HER2 genetic heterogeneity is a factor in discrepant HER2 status assessments between HER2 IHC and HER2 ISH testing methods. In this situation, serum HER-2 testing may be useful for determination of patients' HER-2 status.

Conflict of Interest

None declared.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021. doi: 10.3322/caac.21660.
2. Broughton MN, Westgaard A, Paus E, Øijordsbakken M, Henanger KJ, Naume B, et al. Specific antibodies and sensitive immunoassays for the human epidermal growth factor receptors (HER2, HER3, and HER4). *Tumour Biol*. 2017;39(6):1010428317707436. doi: 10.1177/1010428317707436.
3. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235(4785):177-82. doi: 10.1126/science.3798106.
4. Nitta H, Kelly BD, Allred C, Jewell S, Banks P, Dennis E, et al. The assessment of HER2 status in breast cancer: the past, the present, and the future. *Pathol Int*. 2016;66(6):313-24. doi: 10.1111/pin.12407.
5. Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers: Overexpression and therapeutic implications. *Mol Biol Int*. 2014;2014:852748. doi: 10.1155/2014/852748.
6. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ, et al. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol*. 2011;22(8):1736-47. doi: 10.1093/annonc/mdr304.
7. Pallud C, Guinebretiere JM, Guepratte S, Hacene K, Neumann R. Tissue expression and serum levels of the oncoprotein HER-2/neu in 157 primary breast tumours. *Anticancer Res*. 2005;25(2):1433-40.
8. Ludovini V, Gori S, Colozza M, Pistola L. Evaluation of serum HER2 extracellular domain in early breast cancer patients: correlation with clinicopathological parameters and survival. *Ann Oncol*. 2008;19(5):883-90.
9. Kong SY, Nam BH, Lee KS, Kwon Y, Lee ES, Seong MW, et al. Predicting tissue HER2 status using serum HER2 levels in patients with metastatic breast cancer. *Clin Chem*. 2006;52(8):1510-5.
10. Willsher PC, Beaver J, Pinder S, Bell JA, Ellis IO. Prognostic significance of serum c-erbB-2 protein in breast cancer patients. *Breast Cancer Res Treat*. 1996;40(3):251-5.
11. Kong SY, Kang JH, Kwon Y, Kang HS, Chung KW. Serum HER-2 concentration in patients with primary breast cancer. *J Clin Pathol*. 2006;59(4):373-6.

12. Dittadi R, Zancan M, Perasole A, Gion M. Evaluation of HER-2/neu in serum and tissue of primary and metastatic breast cancer patients using an automated enzyme immunoassay. *Int J Biol Markers*. 2001;16(4):255-61.
13. Kandl H, Seymour L, Bezwoda WR. Soluble c-erbB-2 fragment in serum correlates with disease stage and predicts for shortened survival in patients with early-stage and advanced breast cancer. *Br J Cancer*. 1994;70(4):739-42.
14. Lüftner D, Lüke C, Possinger K. Serum HER-2/neu in the management of breast cancer patients. *Clin Biochem*. 2003;36(4):233-40.
15. Molina R, Jo J, Filella X, Bruix J. Serum levels of C-erbB-2 (HER-2/neu) in patients with malignant and non-malignant diseases. *Tumour Biol*. 1997;18(3):188-96.
16. Valent A, Penault-Llorca F, Cayre A, Kroemer G. Change in HER2 (ERBB2) gene status after taxane-based chemotherapy for breast cancer: polyploidization can lead to diagnostic pitfalls with potential impact for clinical management. *Cancer Genet*. 2013;206(2):37-41.
17. Broughton MN, Westgaard A, Paus E, Øijordsbakken M, Henanger KJ. Specific antibodies and sensitive immunoassays for the human epidermal growth factor receptors (HER2, HER3, and HER4). *Tumour Biol*. 2017;39(6):521-36.
18. Kong SY, Nam BH, Lee KS, Kwon Y, Lee ES. Predicting tissue HER2 status using serum HER2 levels in patients with metastatic breast cancer. *Clin Chem*. 2006;52(8):1510-5.
19. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987;235(4785):177-82.
20. Isola JJ, Holli K, Oksa H, Teramoto Y, Kallioniemi OP. Elevated erbB-2 oncoprotein levels in preoperative and follow-up serum samples define an aggressive disease course in patients with breast cancer. *Cancer*. 1994;73(3):652-8.
21. Kandl H, Seymour L, Bezwoda WR. Soluble c-erbB-2 fragment in serum correlates with disease stage and predicts for shortened survival in patients with early-stage and advanced breast cancer. *Br J Cancer*. 1994;70(4):739-42.
22. Willsher PC, Beaver J, Pinder S, Bell JA. Prognostic significance of serum cerbB-2 protein in breast cancer patients. *Breast Cancer Res Treat*. 1996;40(3):251-5.
23. Rocca A, Canello G, Bagnardi V, Sandri MT, Torrisi R. Perioperative serum VEGF and extracellular domains of EGFR and HER2 in early breast cancer. *Anticancer Res*. 2009;29(12):5111-9.
24. Molina R, Jo J, Filella X, Zanon G, Pahisa J. C-erbB-2 oncoprotein in the sera and tissue of patients with breast cancer. Utility in prognosis. *Anticancer Res*. 1996;16(4):2295-300.
25. Ludovini V, Gori S, Colozza M, Pistola L, Rulli E. Evaluation of serum HER2 extracellular domain in early breast cancer patients: correlation with clinicopathological parameters and survival. *Ann Oncol*. 2008;19(5):883-90.
26. Carney WP, Neumann R, Lipton A, Leitzel K, Ali S, Price CP. Monitoring the circulating levels of the HER2/neu oncoprotein in breast cancer. *Clin Breast Cancer*. 2004;5(2):105-16.