PREDICTIVE CIRCULATING MARKERS FOR ANTHRACYCLINE CHEMOTHERAPY IN NON-METASTATIC BREAST CANCER

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Abstract

Anthracyclines are used in breast cancer both in early and advanced stages and their recommendation together with taxanes, either concurrently or sequentially, is debatable and individualized by phenotype. Circulating biomarkers have already been introduced in clinical practice for metastatic disease monitoring. We questioned whether it might be a role for these markers in neoadjuvant and adjuvant settings too and a general review was conducted.

CK18 and CTC were found predictive for anthracycline related response in preoperative setting. Soluble E-cadherin is promising, a retrospective analysis showing a direct correlation with clinical response. CEA, CA 15-3 and HER2 ECD are not of interest for their predictive role.

Key words: breast cancer, circulating biomarkers, anthracyclines.

INTRODUCTION

Breast cancer is a heterogeneous disease with various clinical, morphological and molecular characteristics (1).

Anthracyclines represent one of the most effective classes of anti-cancer agents in breast cancer treatment both in early and advanced stages. Their therapeutic effect, compared with taxanes, another class of chemotherapeutic drugs, normally recommended in this localization, whether simultaneously or sequentially, is controversial and differentiated by biomolecular immunophenotype (2). As we previously shown, gynecological malignancies could have a fulminant evolution, therefore a complex treatment could be required (3).

Biomolecular markers for chemotherapy response assessment and disease monitoring are required for an adequate treatment. Topoisomerase 2 alpha (TOP2A) tumor tissue expression is one of the few biomarkers showing a predictive value to

anthracycline treatment response in human epidermal growth factor receptor type 2 (HER2) overexpressing breast cancers (4).

A growing field is represented by circulating biomarkers which offer information about tumor features and the advantage of a rapid, low-cost and easily accessible monitoring of tumor response. Some of them have already been introduced in clinical practice for metastatic disease monitoring (5). It is questionable whether there could be a role for such markers in the neoadjuvant and also in the adjuvant setting.

In neoadjuvant situation, the correlation between clinical and pathological responses and markers level variation during chemotherapy is easier to validate.

Considering the long term toxicity associated with anthracyclines, identification of predictive circulating biomarkers for their effectiveness is highly important (6).

A general review was conducted to identify potential predictive circulating factors to perioperative anthracyclines' efficacy in breast cancer population.

MATERIALS AND METHODS

We searched on PubMed and Medline databases using the searching terms: circulating predictive biomarkers, breast cancer, anthracyclines, neoadjuvant chemotherapy, pathologic response and relapse free survival. We selected articles published between 2004 and 2016 considering the discrepancy in laboratory methods by the time. The outcomes of interest were pathological response and disease-free survival. We focused on neoadjuvant situation given the promptness of evaluation through tumor shrinkage and pathological response and a better correlation with tumor markers. The risk of confusion with the markers' prognostic value and the lack of correlation

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with clinical response are the limits of the evaluation in the adjuvant setting.

Protein markers

Carbohydrate antigen 15-3 (CA 15-3) is one of the routinely used biomolecular markers in breast cancer follow-up, detecting the extracellular domain of mucin 1 transmembrane glycoprotein (MUC-1) (7). Mucins have an important role in the protection, lubrication and repair of epithelial mammary ducts (8). Abnormal mucin production is found in chronic inflammation and in malignization. More than 90% of breast cancers express MUC-1 (9, 10). High circulating CA 15-3 levels are found in the breast, ovarian, pancreatic, gastric and lung carcinoma (11).

Carcinoembryonic antigen (CEA), widely distributed in malignant and embryonic gastrointestinal tissues, is a high weight membrane glycoprotein involved in intercellular adhesion and acts as an epithelial phenotype protector (12, 13). Its serum concentration is usually very low in healthy adults, but it increases in breast, gastrointestinal and lung cancer cases (14, 15). By losing polarity and adhesiveness, the tumor cell undergoes epithelial-mesenchymal transition, a key process in metastasis (16). High circulating levels are found in approximately 25% of primary breast cancer and in up to 80% of metastatic breast cancer patients (17).

Guidelines are not recommending CA 15-3 and CEA measurement for screening, diagnostic and follow-up after primary treatment because of low organ specificity. Even in metastatic setting, the recommendations are to use these markers along with radiological and clinical assessment (18-20). CA 15-3 and CEA serum levels increase has been found to anticipate by several months the radiological relapse and a hypothesis might be the release of markers in the blood stream from occult metastatic sites and micro metastasis (21).

A prospective analysis run in 51 locally advanced breast cancer patients, exposed to neoadjuvant anthracyclines revealed a direct correlation between the levels of CEA and CA 15-3 ($R=0.43$; $p<0.001$) before treatment initiation, but their kinetics during the first two cycles of anthracyclines was found non-significantly correlated with pathological response ($p=0.491$ and $p=0.981$, respectively). The comparison in CEA and CA 15-3 serum marker concentration between the two different therapy response groups (patients with partial and complete response on the one hand and those with no change of the disease on the

other hand) was performed by Wilcoxon test (22).

Cytokeratins (CKs) comprise a heterogeneous group of polypeptides that form intermediate filaments of cytoskeleton. The main role of CKs is to ensure cell protection and integrity, but also to regulate different intracellular functions such as polarization and protein translation control (23). Considering that tumor cells display a specific cytokeratin profile, mostly being used in adenocarcinoma-carcinoma differentiation, they too have been proposed as predictive markers (24). Some circulating cytokeratins have demonstrated clinical importance for anthracycline response monitoring right from the early phases of induction. The dynamics of serum cytokeratin 18 fragments (CK 18) has been retrospectively evaluated during neoadjuvant therapy in 43 patients with localized breast carcinoma. Significant increase in markers' median value has been recorded 24 hours after anthracycline-based chemotherapy completion compared to initial levels in responders compared to patients with no clinical response ($p<0.0001$). Compared to taxanes, anthracyclines induced a significantly accelerated increase in median levels of total CK18 levels at 24 and 72 hours after chemotherapy compared to initial concentration $p<0.00001$ and $p=0.0023$, respectively (25).

Chemotherapy drugs including anthracyclines lead to cell death mainly by inducing apoptosis. A specific neoepitope, caspase cleaved, derived from CK18 fragmentation (M30 antigen) and detected by M30 monoclonal antibody may be a useful biomarker for chemotherapy response. Significantly higher levels of caspase-cleaved CK18 fragments were observed at 24 and 48 hours compared to baseline ($p<0.001$, $p=0.003$, respectively) in 42 patients with localized breast carcinoma exposed to neoadjuvant anthracyclines and this increase was statistically significant in responders and insignificant in non-responders ($p<0.001$ and $p=0.004$ at 24 and 48 hours vs. baseline, respectively) (26).

In addition to CKs, other markers of cellular apoptosis or necrosis, such as nucleosomes, nuclear complexes composed of DNA and histones, seem promising regarding their predictive value to anthracyclines' efficacy. Nucleosome blood stream concentration measured with the ELISA technique by two antibodies directed against DNA and histones, in 51 locally advanced breast cancer patients, seems to have been correlated with response to neoadjuvant anthracyclines. A significant difference in the initial level was observed in patients with partial or complete pathologic remission compared to those with stable

disease (median value 53.7 ng/mL, range 28.8-170 vs. 92.4 ng/mL, range 28.8-475, respectively, $p=0.038$). The drop-down in serum nucleosome value at the start of the second cycle of epirubicine and cyclophosphamide chemotherapy was more significant in stable disease group compared to responders, who already had decreased initial values ($p=0.004$). CK 18 fragment level kinetics during anthracycline chemotherapy was insignificant for chemotherapy response ($p=0.225$) (27).

Circulating membrane markers

Soluble E-cadherin (sE-cadherin) is an 80 kDa fragment, measurable in human bloodstream derived from the proteolytic cleavage of the cell surface E-cadherin, because of its continuous regeneration (28). E-cadherin is a member of a large family of adhesion transmembrane proteins and is involved in cell growth and apoptosis regulation. The loss of this protein facilitates invasion and metastasis (29, 30).

High circulating levels of E-cadherin were found in more than half of individuals with various types of epithelial carcinomas (31). In breast cancer, high soluble E-cadherin levels predict a worse outcome and were found significantly correlated with TNM stage, tumor grade and nodal status. A high sensitivity and specificity (79.1% vs. 89.3%, respectively) for breast cancer compared to control (ROC curve (AUC) of 0.935 (95% CI 0.900-0.969) at a cut-off value of 1239.3 ng/mL) was observed (32). It was presumed that soluble E-cadherin levels before and after neoadjuvant chemotherapy and marker levels changes during treatment correlated with clinical and pathological response.

In a retrospective analysis, the predictive value of sE-cadherin was investigated in one hundred and six locally advanced breast cancer patients that have undergone neoadjuvant anthracycline sequential containing chemotherapy. Significant differences in median pre-therapeutic sE-cadherin level were found in patients with pathologic complete response (pCR) ($n=10$) compared to low-responders ($n=98$) (3440.8 ng/mL, range 2241.3-4545.4 vs. 4363.5 ng/mL, range 1779.4-8677.5, $p=0.002$). Marker's level variations during neoadjuvant chemotherapy were significantly correlated with treatment response and important decreases were reported in patients with pCR ($p=0.043$). In the subgroup exposed to anthracyclines ($n=55$), sE-cadherin and pCR were marginally correlated (OR=0.99, 95% CI (0.99-1.00), $p=0.063$) (33).

Furthermore, another retrospective analysis in

133 females diagnosed with invasive breast carcinoma exposed to preoperative anthracyclines demonstrated a positive correlation between the median sE-cadherin levels after chemotherapy and clinical response ($p=0.006$) (34).

The extracellular domain of human epidermal growth factor 2 receptor (HER2 ECD) is expressed in about 38% of primary breast cancer and between 20 and 80% in metastatic disease (35). Human epidermal growth factor 2 receptor (HER2) undergoes a proteolytic cleavage of its extracellular domain mediated by membrane matrix metalloproteinases and thus being detectable by immuno-enzymatic assays (36). The circulating HER2 ECD is assessed for clinical relevance as a surrogate for tissue HER2 status (37). High HER2 ECD serum levels were described in approximately 40% of the patients with HER2 overexpressing breast tumors. Androgen receptor expression does not correlates with HER2 positive tumors (38). HER2 ECD level's variation during neoadjuvant anthracycline chemotherapy was found correlated with tumor response.

Two populations of locally advanced breast cancer patients were analyzed for their serum HER-2/neu levels kinetics and pathologic response according to neoadjuvant type of chemotherapy in a ($n=108$) retrospective study. The first group ($n=55$) underwent concomitant anthracyclines and taxanes chemotherapy and the second one ($n=53$) was exposed to anthracyclines solely. A significant correlation was found between histopathologic response and both pre-chemotherapy serum Her-2 levels and type of chemotherapy (all $p<0.01$). Median pretherapeutic HER2 ECD concentration in patients with complete histopathological response was significantly higher compared to patients with partial or no therapy response (10.95 vs. 9.1 ng/mL, $p=0.041$; 95% CI (0.036-0.046)) but was found non-significantly correlated with the type of chemotherapy (39).

Circulating tumor cells (CTCs)

We assist to a rapidly growing field of circulating tumor cells in cancer diagnosis and therapy follow-up. Released in the bloodstream right from the early phases of tumorigenesis, they are detected via novel technologies based on their biological and physical characteristics (40).

However, the phenotype of CTCs is thought to be a consequence of an epithelial mesenchymal transition characterized by a decreased expression of epithelial markers, increased plasticity and capacity for

invasion and migration (41).

Immunohistochemistry allows CTC detection using monoclonal antibodies against cytoskeletal and membrane antigens that are specific for epithelial cells. CellSearch® assay was recently approved by the Food and Drug Administration (FDA) for treatment follow-up in metastatic breast cancer and allows counting the circulating cancer cells by means of an immunofluorescence reaction, separating them with magnetic layers (42). Regarding the predictive value of CTCs during neoadjuvant treatment, serial assays of a prospective analysis found a significant correlation with tumor burden ($p=0.001$) in 58 locally advanced breast cancer patients receiving anthracycline-taxanes chemotherapy. A less than 10-fold decrease in the CTC count during anthracycline-based chemotherapy was associated with worse distant metastasis free survival compared to patients with more than 10-fold reduction ($p<0.0001$, HR=11.91). (43)

Another prospective analysis in 55 stage II/III invasive breast carcinoma patients undergoing sequential doxorubicin and paclitaxel or paclitaxel followed by doxorubicin also assessed the CTC kinetics during neoadjuvant chemotherapy. No significant correlation was found in either baseline median CTC levels or after four cycles of chemotherapy and clinical response in any of the two treatment sequences ($p=0.89$ and $p=0.36$, respectively) (44).

Experimental biomarkers

Cell free deoxyribonucleic acids (cfDNAs) are small fragments of deoxyribonucleic acid (DNA) circulating freely in the plasma or serum in both cancer and healthy individuals alike. Necrotic, apoptotic cells or circulating tumor cells are presumed to be the sources for plasma cell free DNA (45).

After being found in the serum of incipient breast cancer patients, it is currently emerging as a diagnostic, prognostic and therapy monitoring biomarker. There are two approaches for DNA analysis: quantification of cfDNA and study of tumor specific DNA abnormalities as mutations and methylation using polymerase chain reactions and next generation sequencing methods (46, 47).

Non-coding cfDNA, repetitive sequence quantification, may differentiate between apoptotic, the main source of cfDNA in healthy individuals, and necrotic cell death, an important feature in differentiating circulating free cell DNA derived from tumors *versus* healthy humans (48).

ALU non-coding sequences (named by

the action of *Arthrobacter luteus* (ALU) restriction endonuclease on DNA) vary in dimension, unlike equally truncated cfDNA in healthy individuals and are quantifiable by real-time polymerase chain reaction amplification (49).

Levels of repetitive ALU 115 and ALU 247 obtained before and after the completion of neoadjuvant anthracycline-based chemotherapy and their level variation during therapy were evaluated in a small group ($n=65$) of locally-advanced breast cancer patients. A significant decrease in ALU 115 serum level ($p=0.033$) and a borderline in ALU 247 level during chemotherapy was noted in patients with complete pathological response ($p=0.071$) (50).

Among classical, easily accessible and feasible in daily practice protein biomarkers, CEA and CA 15-3 are not correlated with anthracycline efficacy in perioperative situation. Soluble E-cadherin is promising, a retrospective analysis showing a direct correlation with clinical response.

Of cytokeratins, analyzed prospectively, CK18 was found predictive for anthracycline related clinical response in preoperative setting, right from the early phases of neoadjuvant anthracycline-based chemotherapy.

The so-called “liquid biopsy” comprising CTC and novel cell free DNA offers promising for their predictive purpose. Disease monitoring by CTC was approved in metastatic breast cancer, but it also proves helpful in the neoadjuvant setting. Free cell DNA measurement seems to be predictive for anthracycline response in small trials. HER2 ECD remains useful only in prognostic assessment.

In conclusion, using biomarkers in predicting tumor treatment response is challenging. Further prospective studies are needed to confirm the predictive role for these biomarkers.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

References

1. Li CI, Uribe DJ, Daling JR. Clinical characteristics of different histologic types of breast cancer. *Br J Cancer*. 2005;93(9):1046-1052.
2. Rocca A, Bravaccini S, Scarpi E, Mangia A, Petroni S, Puccetti M, Medri L, Serra L, Ricci M, Cerasoli S, Biglia N, Maltoni R, Giunchi DC, Gianni L, Tienghi A, Brandi M, Faedi M, Sismondi P, Paradiso A, Silvestrini R, Amadori D. Benefit from anthracyclines in relation to biological profiles in early breast cancer. *Breast Cancer Res Treat*. 2014;144(2):307-318.

3. Anghel RM, Trifanescu OG, Mitrica RI, Curea FG, Botnariuc I, Herhelegiu CG, Orlov CM, Ilie SM. Good Prognosis Went Badly: Fulminant Evolution of a 29-Year-Old Patient with Verrucous Carcinoma of the Cervix. *J Adolesc Young Adult Oncol* 2017;doi: 10.1089/jayao.2017.0009.
4. Scandinavian Breast Group Trial 9401, Tanner M, Isola J, Wiklund T, Erikstein B, Kellokumpu-Lehtinen P, Malmström P, Wilking N, Nilsson J, Bergh J. Topoisomerase II α gene amplification predicts favorable treatment response to tailored and dose-escalated anthracycline-based adjuvant chemotherapy in HER-2/neu-amplified breast cancer: Scandinavian Breast Group Trial 9401. *J Clin Oncol*. 2006;24(16):2428-2436.
5. Di Gioia D, Heinemann V, Nagel D, Untch M, Kahlert S, Bauerfeind I, Koehne T, Stieber P. Kinetics of CEA and CA15-3 correlate with treatment response in patients undergoing chemotherapy for metastatic breast cancer (MBC). *Tumour Biol*. 2011;32(4):777-785.
6. Ghigo A, Li M, Hirsch E. New signal transduction paradigms in anthracycline-induced cardiotoxicity. *Biochim Biophys Acta*. 2016;1863(7 Pt B):1916-1925.
7. Kallioniemi OP, Oksa H, Aaran RK, Hietanen T, Lehtinen M, Koivula T. Serum CA 15-3 assay in the diagnosis and follow-up of breast cancer. *Br J Cancer*. 1988;58(2):213-235.
8. Hollingsworth MA, Swanson BJ. Mucins in cancer: protection and control of the cell surface. *Nat Rev Cancer*. 2004;4(1):45-60.
9. Ehsanullah M, Filipe MI, Gazzard B. Mucin secretion in inflammatory bowel disease: correlation with disease activity and dysplasia. *Gut*. 1982;23(6):485-489.
10. Rakha EA, Boyce RW, Abd El-Rehim D, Kurien T, Green AR, Paish EC, Robertson JF, Ellis IO. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Mod Pathol*. 2005;18(10):1295-1304.
11. Gang Y, Adachi I, Ohkura H, Yamamoto H, Mizuguchi Y, Abe K. CA 15-3 is present as a novel tumor marker in the sera of patients with breast cancer and other malignancies. 1985;12(12):2379-2386.
12. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. *J Exp Med*. 1965;122(3):467-481.
13. Oikawa S, Inuzuka C, Kuroki M, Matsuoka Y, Kosaki G, Nakazato H. Cell adhesion activity of non-specific cross-reacting antigen (NCA) and carcinoembryonic antigen (CEA) expressed on CHO cell surface: homophilic and heterophilic adhesion. *Biochem Biophys Res Commun*. 1989;164(1):39-45.
14. Polat E, Duman U, Duman M, Derya Peker K, Akyuz C, Fatih Yasar N, Uzun O, Akbulut S, Birol Bostanci E, Yol S. Preoperative serum tumor marker levels in gastric cancer. *Pak J Med Sci*. 2014;30(1):145-149.
15. Shao Y, Sun X, He Y, Liu C, Liu H. Elevated Levels of Serum Tumor Markers CEA and CA15-3 Are Prognostic Parameters for Different Molecular Subtypes of Breast Cancer. *PLoS One*. 2015;10(7):e0133830.
16. Wegwitz F, Lenfert E, Gerstel D, von Ehrenstein L, Einhoff J, Schmidt G, Logsdon M, Brandner J, Tiegs G, Beauchemin N, Wagener C, Deppert W, Horst AK. CEACAM1 controls the EMT switch in murine mammary carcinoma *in vitro* and *in vivo*. *Oncotarget*. 2016.
17. Steward AM, Nixon D, Zamcheck N, Aisenberg A. Carcinoembryonic antigen in breast cancer patients: serum levels and disease progress. *Cancer*. 1974;33(5):1246-1252.
18. Stieber P, Nagel D, Blankenburg I, Heinemann V, Untch M, Bauerfeind I, Di Gioia D. Diagnostic efficacy of CA 15-3 and CEA in the early detection of metastatic breast cancer-A retrospective analysis of kinetics on 743 breast cancer patients. *Clin Chim Acta*. 2015;448:228-231.
19. Harris L, Fritsche H, Mennel R, Norton L, Ravdin P, Taube S, Somerfield MR, Hayes DF, Bast RC Jr; American Society of Clinical Oncology. 2007 update of recommendations for the use of tumor markers in breast cancer. *J Clin Oncol*. 2007;25(33):5287-5312.
20. Senkus E, Kyriakides S, Ohno S, Penault-Llorca F, Poortmans P, Rutgers E, Zackrisson S, Cardoso F; ESMO Guidelines Committee. Primary breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26 Suppl 5:v8-30.
21. Kallioniemi OP, Oksa H, Aaran RK, Hietanen T, Lehtinen M, Koivula T. Serum CA 15-3 assay in the diagnosis and follow-up of breast cancer. *Br J Cancer*. 1988;58(2):213-215.
22. Stoetzer OJ, Fersching DM, Salat C, Steinkohl O, Gabka CJ, Hamann U, Braun M, Feller AM, Heinemann V, Siegele B, Nagel D, Holdenrieder S. Circulating immunogenic cell death biomarkers HMGB1 and RAGE in breast cancer patients during neoadjuvant chemotherapy. *Tumour Biol*. 2013;34(1):81-90.
23. Pastuszak M, Groszewski K, Pastuszak M, Dyrła P, Wojtuś S, Gil J. Cytokeratins in gastroenterology. Systematic review. *Prz Gastroenterol*. 2015;10(2):61-70.
24. Chu P, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol*. 2000;13(9):962-972.
25. Olofsson MH, Ueno T, Pan Y, Xu R, Cai F, van der Kuip H, Muerdter TE, Sonnenberg M, Aulitzky WE, Schwarz S, Andersson E, Shoshan MC, Havelka AM, Toi M, Linder S. Cytokeratin-18 is a useful serum biomarker for early determination of response of breast carcinomas to chemotherapy. *Clin Cancer Res*. 2007;13(11):3198-3206.
26. Demiray M, Ulukaya EE, Arslan M, Gokgoz S, Saraydaroglu O, Ercan I, Evrensel T, Manavoglu O. Response to neoadjuvant chemotherapy in breast cancer could be predictable by measuring a novel serum apoptosis product, caspase-cleaved cytokeratin 18: a prospective pilot study. *Cancer Invest*. 2006;24(7):669-676.
27. Stoetzer OJ, Fersching DM, Salat C, Steinkohl O, Gabka CJ, Hamann U, Braun M, Feller AM, Heinemann V, Siegele B, Nagel D, Holdenrieder S. Prediction of response to neoadjuvant chemotherapy in breast cancer patients by circulating apoptotic biomarkers nucleosomes, DNase, cytokeratin-18 fragments and survivin. *Cancer Lett*. 2013;336(1):140-148.
28. Steinhilber U, Weiske J, Badock V, Tauber R, Bommert K, Huber O. Cleavage and shedding of E-cadherin after induction of apoptosis. *J Biol Chem*. 2001;276(7):4972-80. Epub 2000 Nov 13.
29. Lu M, Marsters S, Ye X, Luis E, Gonzalez L, Ashkenazi A. E-cadherin couples death receptors to the cytoskeleton to regulate apoptosis. *Mol Cell*. 2014;54(6):987-98.
30. Yoshida R, Kimura N, Harada Y, Ohuchi N. The loss of E-cadherin, α - and β -catenin expression is associated with metastasis and poor prognosis in invasive breast cancer. *Int J Oncol*. 2001;18(3):513-520.
31. Katayama M, Hirai S, Kamihagi K, Nakagawa K, Yasumoto M, Kato I. Soluble E-cadherin fragments increased in circulation of cancer patients. *Br J Cancer*. 1994;69(3):580-585.
32. Liang Z, Sun XY, Xu LC, Fu RZ. Abnormal expression of serum soluble E-cadherin is correlated with clinicopathological features and prognosis of breast cancer. *Med Sci Monit*. 2014 ;20:277-282.
33. Hofmann G, Balic M, Dandachi N, Resel M, Schippinger W, Regitnig P, Samonigg H, Bauernhofer T. The predictive value of serum soluble E-cadherin levels in breast cancer patients undergoing preoperative systemic chemotherapy. *Clin Biochem*. 2013;46(15):1585-1589.
34. Hofmann G, Dandachi N, Balic M, Schippinger W, Wernecke K-D, Samonigg H, Bauernhofer T. Quantitative measurement of soluble E-cadherin in sera of breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy and its predictive and prognostic value. *J Clin Oncol*. 2005; 23(16): 768-768.
35. Carney WP, Neumann R, Lipton A, Leitzel K, Ali S, Price CP. Potential clinical utility of serum HER-2/neu oncoprotein concentrations in patients with breast cancer. *Clin Chem*. 2003;49(10):1579-1598.
36. Codony-Servat J, Albanell J, Lopez-Talavera JC, Arribas J,

- Baselga J. Cleavage of the HER2 ectodomain is a pervanadate-activable process that is inhibited by the tissue inhibitor of metalloproteases-1 in breast cancer cells. *Cancer Res.* 1999;59(6):1196-1201.
37. Kong SY, Nam BH, Lee KS, Kwon Y, Lee ES, Seong MW, Lee DH, Ro J. Predicting tissue HER2 status using serum HER2 levels in patients with metastatic breast cancer. *Clin Chem.* 2006;52(8):1510-1515.
38. Raica M, Muresan A, Cimpean A, Suciu C. Clinical Significance of Androgen Receptor Expression in Breast Cancer. *Acta Endocrinologica -Buc* 2009; 5(4):459-469.
39. Schippinger W, Dandachi N, Regitnig P, Hofmann G, Balic M, Neumann R, Samonigg H, Bauernhofer T. The predictive value of EGFR and HER-2/neu in tumor tissue and serum for response to anthracycline-based neoadjuvant chemotherapy of breast cancer. *Am J Clin Pathol.* 2007;128(4):630-637.
40. Sheng Y, Wang T, Li H, Zhang Z, Chen J, He C, Li Y, Lv Y, Zhang J, Xu C, Wang Z, Huang C, Wang L. Comparison of analytic performances of Cellsearch and iFISH approach in detecting circulating tumor cells. *Oncotarget.* 2017;8(5):8801-8806.
41. Barrière G, Riouallon A, Renaudie J, Tartary M, Rigaud M. Mesenchymal and stemness circulating tumor cells in early breast cancer diagnosis. *BMC Cancer.* 2012;12:114.
42. Balic M, Dandachi N, Hofmann G, Samonigg H, Loibner H, Obwallner A, van der Kooi A, Tibbe AG, Doyle GV, Terstappen LW, Bauernhofer T. Comparison of two methods for enumerating circulating tumor cells in carcinoma patients. *Cytometry B Clin Cytom.* 2005;68(1):25-30.
43. Camara O, Rengsberger M, Egbe A, Koch A, Gajda M, Hammer U, Jörke C, Rabenstein C, Untch M, Pachmann K. The relevance of circulating epithelial tumor cells (CETC) for therapy monitoring during neoadjuvant (primary systemic) chemotherapy in breast cancer. *Ann Oncol.* 2007;18(9):1484-1492.
44. Boutrus RR, Abi Raad RF, Kuter I, Ancukiewicz M, Roberts L, Solomon N, Ngo T, Borick H, Ryan P, Moy B, Gadd M, Chien J, Younger J, Smith B, Taghian AG, Harris L. Circulating tumor cells as predictors of response and failure in breast cancer patients treated with preoperative chemotherapy. *Int J Biol Markers.* 2013;28(1):17-23.
45. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, Knippers R. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. *Cancer Res.* 2001;61(4):1659-1665.
46. Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, Dunning MJ, Gale D, Forsheew T, Mahler-Araujo B, Rajan S, Humphray S, Becq J, Halsall D, Wallis M, Bentley D, Caldas C, Rosenfeld N. Analysis of circulating tumor DNA to monitor metastatic breast cancer. *N Engl J Med.* 2013;368(13):1199-1209.
47. Iqbal S, Vishnubhatla S, Raina V, Sharma S, Gogia A, Deo SS, Mathur S, Shukla NK. Circulating cell-free DNA and its integrity as a prognostic marker for breast cancer. *Springerplus.* 2015;4:265.
48. Avraham A, Uhlmann R, Shperber A, Birnbaum M, Sandbank J, Sella A, Sukumar S, Evron E. Serum DNA methylation for monitoring response to neoadjuvant chemotherapy in breast cancer patients. *Int J Cancer.* 2012;131(7):E1166-72.
49. Umetani N, Giuliano AE, Hiramatsu SH, Amersi F, Nakagawa T, Martino S, Hoon DS. Prediction of breast tumor progression by integrity of free circulating DNA in serum. *J Clin Oncol.* 2006;24(26):4270-4276.
50. Stötzer OJ, Lehner J, Fersching-Gierlich D, Nagel D, Holdenrieder S. Diagnostic relevance of plasma DNA and DNA integrity for breast cancer. *Tumour Biol.* 2014;35(2):1183-1191.