Pilot Study on the Utility of Circulating HER2/Neu Levels in the Serum of Breast Cancer Patients

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Abstract. *Background/Aim:* Accurate and timely assessment of the human epidermal growth factor receptor 2 (HER2/neu) overexpression is pivotal for the identification of breast cancer (BC) patients that could benefit from HER2-targeted therapy. Currently approved tissue-based HER2 assays (tHER2) are limited to testing HER2 status on tumor samples obtained at a few points in time during the course of the disease. Herein, we assessed serum HER2 (sHER2) status longitudinally in 81 serial samples prospectively collected from 43 consenting patients pre- and post-therapy to revisit the idea of serum testing in the follow-up of BC patients. *Patients and Methods:* The cohort included 11 patients with early BC (EBC), 17 with locally advanced BC (LABC), and 15 with metastatic BC (MBC). sHER2 concentrations were measured using a quantitative ELISA-based technique, using 15 ng/ml as the cut-off for positivity. *Results:* At baseline, sHER2 was negative in all EBC patients while positive in 1 LABC and 5 MBC patients. Sixteen BC patients (10 LABC, 1 EBC, and 5 MBC) were tHER2 positive. sHER2 and tHER2 results were discordant in 14 patients. Among the 16 tHER2 positive patients, 9 LABC, 1 EBC and 2 MBC patients were sHER2 negative. Conversely, 2 MBC patients were sHER2 positive, despite being tHER2 negative. A rise or drop of sHER2 by >20% correlated with

disease progression or pathological response to therapy, respectively. *Conclusion:* The study demonstrated the technical validity and feasibility of the sHER2 assay. Findings suggest that post initial tissue diagnosis (tHER2), sHER2 assay may supplement subsequent tissue tests to monitor disease status and response to therapy. Further studies to assess the role of HER2 targeted therapies in sHER-positive/tHER2-negative cases upon disease progression are warranted.

Gene amplification and overexpression of human epidermal growth factor receptor 2 (HER2/neu), detected in ~15-20% of breast cancer (BC), is a significant prognostic and predictive marker of the disease (1, 2). HER2 overexpression has been associated with disease aggressiveness, poor prognosis, and survival benefit from HER2-targeted therapy (3-5). The standard diagnostic modalities to determine tissue HER2 (tHER2) status, including immunohistochemistry (IHC) and *in situ* hybridization (ISH), have become a mandatory test in all BC patients at the time of diagnosis (6-8). However, these tissue-based assays are affected by tumor discrepancy, testing accuracy, reproducibility and subjectivity in result interpretation (9-11). Although rare, heterogeneity in tHER2 expression between primary tumor and metastases requires repeated biopsy to determine tHER2 status during disease progression (12, 13). Acquisition of tumor tissue is invasive and difficult to perform for certain disease sites. This underscores the unmet clinical need for an alternative method for accurate, real-time monitoring of HER2 status in a non-invasive manner to augment tissue-based information and guide BC therapeutic decision making in the neoadjuvant, adjuvant, and metastatic disease settings.

BC patients would greatly benefit from a blood test that could provide a real time assessment of circulating biomarkers, including circulating tumor cells, exosomes, cell-free tumor nucleic acid and proteins shed into the blood. Such

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Table I. Clinicopathological characteristics of the study cohort.

	Early breast cancer (EBC), n (%)	Locally aggressive breast cancer (LABC), n (%)	Metastatic breast cancer (MBC), n (%)
Total	11	17	15
Histologic subtype			
Invasive ductal carcinoma (IDC)	8 (18.6%)	15 (34.9%)	10 (23.3%)
Invasive lobular carcinoma (ILC) carcinoma (ILC)	1 (2.3%)	2 (4.7%)	2 (4.7%)
IDC/ILC	1 (2.3%)	0	1 (2.3%)
Mucinous carcinoma	1 (2.3%)	0	0
Invasive carcinoma, NOS	0	0	2 (4.7%)
Tumor grade			
I	3 (7.0%)	2 (4.7%)	1 (2.3%)
II	6 (14.0%)	11 (25.6%)	4 (9.3%)
III	2 (4.7%)	3 (7.0%)	6 (14.0%)
Unknown	0	1 (2.3%)	4 (9.3%)
Lymphovascular invasion (LVI)			
Negative	9 (20.9%)	14 (32.6%)	8 (18.6%)
Positive	2 (4.7%)	3 (7.0%)	4 (9.3%)
Unknown	0	0	3 (7.0%)
Axillary lymph node involvement			
Negative	9 (20.9%)	5 (11.6%)	2 (4.7%)
Positive	2 (4.7%)	11 (25.6%)	12 (27.9%)
Unknown	0	1 (2.3%)	1 (2.3%)
tHER2			
Negative	10 (23.3%)	7 (16.3%)	10 (23.3%)
Positive	1 (2.3%)	10 (23.3%)	5 (11.6%)
sHER2			
Negative	13 (30.2%)	16 (37.2%)	11 (25.6%)
Positive	0	1 (2.3%)	4 (9.3%)
Follow-up			
Mean follow-up time (months)*	7 (range=1-16)	9 (range=4-17)	10 (range=3-27)
Complete remission	9 (20.9%)	16 (37.2%)	0
Stable/partial remission	2 (4.7%)	1 (2.3%)	3 (7.0%)
Progression	0	0	10 (23.3%)
Recurrence	0	0	0
Death	0	0	2 (4.7%)

*Follow-up time was defined from the date of the first blood sample drawn for the study to date of last follow up or date of disease progression or death of disease.

collected before and after neoadjuvant chemotherapy. The third group was metastatic BC (MBC) patients from whom 2 blood samples were collected 6 months apart regardless of systemic therapy cycles. Clinicopathological characteristics were retrieved from electronic medical records (Table I). Both tHER2 positive and negative patients were included in the study. Informed consent was obtained from all individual participants included in the study. All procedures performed involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was reviewed and approved by Sunnybrook Health Sciences Centre research ethics board.

Serum specimen collection and processing. Blood samples (6 ml) were taken in plain vacutainer serum tubes under sterile conditions and were allowed to clot for 0.5-1 h. Samples were then centrifuged at 1500 g for 15 min. Serum was aliquoted in amber aliquot tubes and stored at -80°C.

Enzyme-linked immunosorbent assay (ELISA). The HER2/neu ELISA IVD kit (Nuclea Diagnostic Laboratories LLC, MA, USA) was used for the quantitative estimation of soluble p105 (molecular weight 105 kDa) ECD of the HER2 protein in serum. All procedures were performed according to manufacturer's recommendations. Standard curves were generated using 6 standards of recombinant human HER2 ECD with known concentrations of 0.0, 2.5, 7.5, 15.0, 25.0 and 35.0 ng/ml. The serum specimens were diluted to 1:50 with the sample diluent provided in the kit. A dilution of 1:100 and/or 1:200 was needed only if the absorbance values exceeded the range of the standard curve. For monitoring quality of the assay, three different concentrations of HER-2/neu ELISA controls were also included on every microtiter plate. The standards, specimens and controls (100 µl per well) were added to the wells of microtiter plate in duplicate and incubated for 3 h at 37°C. The plate was then washed six times with 300 µl of plate wash buffer (1x) using automated plate washer equipment (TECAN Columbus Pro, TECAN U.S. Inc, Morrisville, NC, USA). After washing, the

detector antibody (100 µl per well) was added and incubated for 1 h at 37°C, then washed 6 times with 300 µl of plate wash buffer.

The conjugate concentrate was diluted 1:50 with conjugate diluent, added to the plate (100 µl per well) and incubated for 30 min at room temperature, then washed 6 times with 300 µl of plate wash buffer. Working substrate was added to each well (100 µl per well) and incubated in the dark for 45 min at room temperature. The reaction was interrupted by adding 100 µl of stop solution. The absorbance of each well was determined at 492 nm by a microtiter plate reader (TECAN Sunrise, TECAN U.S. Inc).

Data analysis. Controls and standard curves were inspected to confirm test performance prior to data analysis. Mean absorbance values from duplicate wells for each of the 6 different standards were used to generate a standard curve, which was used to determine HER2/neu protein concentration (y) for each sample by inserting the specimen's absorbance value (x). Samples with values >15 ng/ml were considered positive as per manufacturer's instructions.

Statistical analysis. Pearson χ^2 test was performed to assess correlation among sHER2, tHER2, clinicopathological variables and response to treatment. Fisher exact method was used when spreadsheet cell counts were <5. All statistical analyses were two-sided and conducted using IBM SPSS Statistics version 24 (IBM Corporation, New York, NY, USA). All *p*-values <0.05 were considered statistically significant. Data are presented as mean±standard deviation (SD).

Results

Patient characteristics. Forty-three BC patients were prospectively enrolled to the pilot study (Figure 1); 11 EBC; 17 LABC; and 15 MBC. Clinicopathological characteristics by groups are outlined in Table I. Among the 15 MBC patients, the metastatic sites included bone (13/15), liver (10/15), lung (7/15), and brain (4/15); Six out of fifteen patients (6/15) had metastases at other sites. The majority of patients (13/15) had at least two metastatic sites.

Evaluation of tissue and serum HER2 in EBC. Among the 11 EBC patients, 1 was tHER2 positive (Table II). sHER2 was negative in all EBC patients at baseline and at 6-month follow up. The tHER2 positive patient was sHER2 negative and had complete disease remission on-follow up. Mean pre- and post-therapy sHER2 levels were 10.1±1.9 ng/ml and 9.0±2.0 ng/ml, respectively (Table II).

Tissue and serum HER2 status in LABC. Within the context of LABC, 10/17 patients were tHER2 positive (Table II). Mean pre- and post- therapy sHER2 levels were 11.4±5.9 ng/ml, and 8.5±2.0 ng/ml, respectively. One tHER2-positive LABC patient was also sHER2-positive pre-therapy. This patient was found to have inflammatory BC with nodal involvement. Post-therapy, sHER2 level showed an 82.0% drop with a value below the cut-off, which correlated with complete pathologic response to therapy (Table III). The 9 remaining tHER2+/sHER2- LABC

Table II. Concordance between tissue and serum HER2 status.

HER2 status		EBC (N=11)	LABC (N=17)	MBC (N=15)	Total (N=43)
Tissue	Serum				
-	-	10	7	8	25
	+	0	0	2	2
+	-	1	9	2	12
	+	0	1	3	4

EBC, Early breast cancer; LABC, locally aggressive breast cancer; MBC, metastatic breast cancer.

patients experienced complete pathologic response to neoadjuvant systemic therapy.

Prevalence of tissue and serum HER2 in MBC. Of the 15 MBC patients in the study, 5 were tHER2 positive (Table II). Mean sHER2 levels in the baseline and second samples were 17.6±14.7 ng/ml and 22.4±24.1 ng/ml, respectively. sHER2 positivity was detected in the baseline sample of 4 MBC patients (Table III). Among these, 3 were positive for both tHER2 and sHER2 while 1 was tHER2 negative/sHER2 positive. The latter patient had diffuse liver metastasis with impaired liver function and progressed on several lines of chemotherapy. Furthermore, the 2 tHER2 positive/sHER2 negative MBC patients showed disease progression (Table I & II).

In second samples, sHER2 positivity was detected in 4 MBC patients. Of these, 1 patient had a 54.1% rise in sHER2 levels from a negative baseline sample to a positive second sample (Table III). The remaining 3 MBC patients were positive for sHER2 in both samples and also demonstrated a rise (from 1.2% to 65.5%) in sHER2 levels from the baseline sample. All MBC patients positive for sHER2 in the second serum sample showed disease progression on numerous lines of chemotherapy, one of whom died of disease 26 months post diagnosis (mean follow-up time 10 months, range 3-27 months). Of note, 1 MBC patient who was initially positive for sHER2 and became negative post-chemotherapy plus targeted HER2 therapy demonstrated a 64.9% decrease in sHER2 levels, which correlated with partial disease remission (Table III).

Discussion

The implementation of sHER2 testing in routine management of patients is hampered by lack of consistent findings regarding its concordance with tHER2 testing and lack of data from randomized clinical trials regarding its ability to predict response to HER2-targeted therapies in patients with negative tHER2. This is attributed to lack of standardisation in sHER2 assessment, different assay techniques, study designs and use of different cut-offs for sHER2 positivity. In

Table III. Characteristics of patients positive for serum HER2.

	HER2 status		Tissue	Disease	Histologic presentation	Grade subtype	LVI	Nodal involvement	ER status	PR status	Outcome
	Serum sample 1 (ng/ml)	Serum sample 2 (ng/ml)									
1	41.4	68.5	+	MBC	IDC	III	–	–	+	–	Died of disease
2	10.9	16.8	–	MBC	IDC	III	–	+	+	–	Disease progression
3	16.6	16.8	–	MBC	IDC/ILC	II	–	+	+	+	Disease progression
4	57.6	78.4	+	MBC	Invasive carcinoma		–	+	–	–	Disease progression
5	33.9	11.9	+	MBC	IDC	III	–	+	+	+	Partial response/Stable disease
6	32.7	5.9	+	LABC	IDC	II	–	+	+	–	Complete pathological response

MBC, Metastatic breast cancer; LABC, locally aggressive breast cancer; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor

this setting, in the current study we used a standardized sHER2 ELISA kit and a cut-off of 15 ng/ml, which was recommended by the manufacturer and used in several other studies (22, 24, 29). This cut-off was established in a study by Wang *et al.* (30), where the prognostic value of sHER2 ECD, at a cut off of 15 ng/ml, was demonstrated using univariate ($HR=1.664$, $p<0.0001$) and multivariate analysis ($HR=1.547$, $p=0.011$). Kaplan-Meier survival curves revealed that patients with sHER2 >15 ng/ml had shorter progression-free survival ($p<0.0001$).

A greater proportion of sHER2 positivity was identified in the metastatic setting (5/15 of MBC patients, *vs.* 0/11 of EBC and 1/17 LABC patients), irrespective of tHER2 status at diagnosis. This finding is consistent with results from prior studies. Molina *et al.* (31) have showed that sHER2 concentrations in patients with advanced disease were related to the site of recurrence, with significantly higher values in metastatic patients compared to those with loco-regional recurrence. Similarly, they stated that over 40% of patients with MBC demonstrated elevated sHER2 levels, whereas only 14.3% of patients with stage I or II disease demonstrated elevated sHER2 levels (29, 32).

sHER2 positivity also correlated with burden of disease. Of the 5 tHER2 positive MBC patients, 2 were sHER2 negative; 1 of whom was newly diagnosed with a small liver metastasis, and 1 with stable bone and liver metastases on chemotherapy. Furthermore, sHER2 negative status was seen in 9/10 tHER2 positive LABC patients and 1/1 tHER2 positive EBC patient, which emphasizes that patients with low tumor burden are less likely to be sHER2 positive.

Although the prognostic value of the initial sHER2 could not be evaluated in the current study, we did find that a larger subset of sHER2 positive patients showed disease progression (4/6) compared to sHER2 negative patients (8/37). In particular, 1 tHER2 positive patient showed a very high

baseline sHER2 concentration (57.6 ng/ml) and even higher subsequent sHER2 level (78.4 ng/ml). sHER2 results in this patient were synchronous with disease progression on up to five lines of chemotherapy. This is consistent with studies that have shown associations between raised concentrations of sHER2 and poor prognosis (29, 30, 32, 33). Others have suggested that the prognostic value of sHER2 is limited to advanced stage BC patients only (27, 28, 34).

The findings also demonstrate that changes in sHER2 level may correlate with treatment response. All 4 patients with consistently positive sHER2 levels post-treatment had poor response to treatment with disease progression, whereas the 2 patients with negative sHER2 seroconversion post-treatment had partial or complete pathological response to HER2-targeted therapy. These findings are similar to two large prospective studies, in which patients who did not achieve $\geq 20\%$ decline in sHER2 levels had less response to Trastuzumab-based therapy (31, 35). Interestingly, two other large studies demonstrated that tHER2-positive patients with $\geq 20\%$ decrease in sHER2 achieved therapeutic success, even if therapy was not HER2-targeted (29, 36, 37). Our study adds further evidence to support the published cut-off of 20% to define significant decrease in sHER2, as the two patients in our study with partial or complete response to therapy had a 64.9% and an 82% drop in sHER2, respectively.

Previous studies showed that baseline sHER2 positivity at diagnosis lacked predictive value for response to HER2-targeted therapy. This was demonstrated in a study of 296 LABC and MBC patients treated with Trastuzumab-based therapies, of which 205 (69%) had sHER2 >15 ng/ml (26). No significant associations were found between baseline sHER2 and tumour response, as 48% of patients with <15 ng/ml baseline sHER2 responded to the treatment regimen *vs.* 52% of those with >15 ng/ml baseline sHER2. Similar result was seen in patients with Trastuzumab monotherapy (30).

Figure 2. Proposed algorithm to HER2 testing utilizing both serum and tissue HER2/neu assays in breast cancer patients.

Some studies have proposed to use sHER2 concentration as an alternative to histological determination of tHER2 status (26, 29, 38). Our data did not support this notion as 14 of 43 patients in the present study had discordant tHER2 and sHER2 status. Similarly, several other groups have failed to reliably correlate tHER2 status to sHER2 levels (26, 39, 40). The discordance can be indicative of different biological subgroups of breast tumors with various levels of HER2 cleavage and shedding irrespective of tHER2 status. Alternatively, it could be attributed to several other factors. First, heterogeneity in primary and metastatic BC could result in small areas of HER2 positive cancer that is not assessed on tissue analysis, but sheds detectable sHER2 into the circulation. Second, extensive liver metastasis may impede its function and ability to degrade normally shed sHER2 (41, 42). Indeed, one patient in our study with tHER2 negative/sHER2 positive profile had extensive liver metastasis. Hence, sHER2 and tHER2 are not interchangeable and should be used as complementary markers.

In conclusion, the results of this proof-of-concept study support the potential use of sHER2 as a tool complementary to tHER2 for monitoring BC progression, particularly in the

advanced and metastatic settings. A suggested algorithm is shown in Figure 2. Although sHER2 positivity was associated with response to therapy, a predictive value could not be concluded from this study, due to small sample size and data requirement beyond the main objective of the study. Due to lack of correlation between sHER2 and tHER2 results, it is recommended to use clinical parameters and tissue HER2 testing according to the national guidelines when making decisions about initiation, continuation, and discontinuation of HER2-targeted treatments.

Conflicts of Interest

The Authors declare that they have no competing interests.

Authors' Contributions

SM: Sample collection, literature search and design of the work; YA: performed experiments and interpretation of data; ES, FL, CPH, SNM: identification and assessment of patient cohort; MT: research concept and access to patients; EOM: drafting the work; AS: revising manuscript critically for intellectual content; WMH: conception of the work.

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Data Availability Statement

The datasets generated and/or analyzed for the current study are not publicly available, because of the proprietary information contained within, but are available from the corresponding Author on reasonable request.

References

- Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A and McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235: 177-182, 1987. PMID: 3798106. DOI: 10.1126/science.3798106
- Owens MA, Horten BC and Da Silva MM: HER2 Amplification ratios by fluorescence *in situ* hybridization and correlation with immunohistochemistry in a cohort of 6556 breast cancer tissues. *Clin Breast Cancer* 5: 63-69, 2004. PMID: 15140287. DOI: 10.3816/CBC.2004.n.011
- Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T, Eiermann W, Wolter J, Pegram M, Baselga J and Norton L: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 344: 783-792, 2001. PMID: 11248153. DOI: 10.1056/NEJM200103153441101
- Tafe LJ and Tsongalis GJ: The human epidermal growth factor receptor 2 (HER2). *Clin Chem Lab Med* 50: 23-30, 2011. PMID: 21919545. DOI: 10.1515/ccm.2011.707
- Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn H-J, Albain KS, André F, Bergh J, Bonnefoi H, Bretel-Morales D, Burstein H, Cardoso F, Castiglione-Gertsch M, Coates AS, Colleoni M, Costa A, Curigliano G, Davidson NE, Di Leo A, Ejlertsen B, Forbes JF, Gelber RD, Gnant M, Goldhirsch A, Goodwin P, Goss PE, Harris JR, Hayes DF, Hudis CA, Ingle JN, Jassem J, Jiang Z, Karlsson P, Loibl S, Morrow M, Namer M, Kent Osborne C, Partridge AH, Penault-Llorca F, Perou CM, Piccart-Gebhart MJ, Pritchard KI, Rutgers EJT, Sedlmayer F, Semiglazov V, Shao Z-M, Smith I, Thürlimann B, Toi M, Tutt A, Untch M, Viale G, Watanabe T, Wilcken N, Winer EP and Wood WC: Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. *Ann Oncol* 24: 2206-2223, 2013. PMID: 23917950. DOI: 10.1093/annonc/mdt303
- Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, Allred DC, Bartlett JMS, Bilous M, Fitzgibbons P, Hanna W, Jenkins RB, Mangu PB, Paik S, Perez EA, Press MF, Spears PA, Vance GH, Viale G and Hayes DF: Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol* 31: 3997-4013, 2013. PMID: 24101045. DOI: 10.1200/JCO.2013.50.9984
- Hanna WM, Barnes PJ, Chang MC, Gilks CB, Magliocco AM, Rees H, Quenneville L, Robertson SJ, SenGupta SK and Nofech-Mozes S: Human epidermal growth factor receptor 2 testing in primary breast cancer in the era of standardized testing: A Canadian prospective study. *J Clin Oncol* 32: 3967-3973, 2014. PMID: 25385731. DOI: 10.1200/JCO.2014.55.6092
- Hanna WM, Slodkowska E, Lu F-I, Nafisi H and Nofech-Mozes S: Comparative analysis of human epidermal growth factor receptor 2 testing in breast cancer according to 2007 and 2013 American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations. *J Clin Oncol* 35: 3039-3045, 2017. PMID: 28445098. DOI: 10.1200/JCO.2016.70.5319
- Perez EA, Suman VJ, Davidson NE, Martino S, Kaufman PA, Lingle WL, Flynn PJ, Ingle JN, Visscher D and Jenkins RB: HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 Intergroup Adjuvant Trial. *J Clin Oncol* 24: 3032-3038, 2006. PMID: 16809727. DOI: 10.1200/JCO.2005.03.4744
- Sauter G, Lee J, Bartlett JMS, Slamon DJ and Press MF: Guidelines for human epidermal growth factor receptor 2 testing: Biologic and methodologic considerations. *J Clin Oncol* 27: 1323-1333, 2009. PMID: 19204209. DOI: 10.1200/JCO.2007.14.8197
- Sapino A, Goia M, Recupero D and Marchiò C: Current Challenges for HER2 Testing in Diagnostic Pathology: State of the Art and Controversial Issues. *Front Oncol* 3, 2013. PMID: 23734345. DOI: 10.3389/fonc.2013.00129
- Niikura N, Liu J, Hayashi N, Mittendorf EA, Gong Y, Palla SL, Tokuda Y, Gonzalez-Angulo AM, Hortobagyi GN and Ueno NT: Loss of human epidermal growth factor receptor 2 (HER2) expression in metastatic sites of HER2-overexpressing primary breast tumors. *J Clin Oncol* 30: 593-599, 2012. PMID: 22124109. DOI: 10.1200/JCO.2010.33.8889
- Wilkink U, Karlsson E, Skoog L, Hatschek T, Lidbrink E, Elmberger G, Johansson H, Lindström L and Bergh J: HER2 status in a population-derived breast cancer cohort: discordances during tumor progression. *Breast Cancer Res Treat* 125: 553-561, 2011. PMID: 20628810. DOI: 10.1007/s10549-010-1029-2
- Wan JCM, Massie C, Garcia-Corbacho J, Mouliere F, Brenton JD, Caldas C, Pacey S, Baird R and Rosenfeld N: Liquid biopsies come of age: towards implementation of circulating tumour DNA. *Nat Rev Cancer* 17: 223-238, 2017. PMID: 28233803. DOI: 10.1038/nrc.2017.7
- Scripcariu V, Scripcariu D-V, Filip B, Gavrilescu M-M, Muşină A-M and Volovăţ C: "Liquid biopsy" – Is it a feasible option in colorectal cancer? *Chirurgia* 114: 162, 2019. PMID: 31060647. DOI: 10.21614/chirurgia.114.2.162
- Mulhngwi P, Valdes R, Fernandez-Botran R, Burton E, Williams B and Linder MW: Cell-free DNA diagnostics: Current and emerging applications in oncology. *Pharmacogenomics* 20: 357-380, 2019. PMID: 30983510. DOI: 10.2217/pgs-2018-0174
- Sias PE, Kotts CE, Vetterlein D, Shepard M and Wong WLT: ELISA for quantitation of the extracellular domain of p185HER2 in biological fluids. *J Immunol Methods* 132: 73-80, 1990. PMID: 1975263. DOI: 10.1016/0022-1759(90)90400-P
- Cook GB, Neaman IE, Goldblatt JL, Cambetas DR, Hussain M, Lüftner D, Yeung KK, Chan DW, Schwartz MK and Allard WJ: Clinical utility of serum HER-2/neu testing on the Bayer Immuno 1 automated system in breast cancer. *Anticancer Res* 21: 1465-1470, 2001. PMID: 11396233

- 19 Carney WP: Potential clinical utility of serum HER-2/neu oncoprotein concentrations in patients with breast cancer. *Clin Chem* 49: 1579-1598, 2003. PMID: 14500583. DOI: 10.1373/49.10.1579
- 20 Codony-Servat J, Albanell J, Lopez-Talavera JC, Arribas J and 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* 59: 1196-1201, 1999. PMID: 10096547.
- 21 McDermott E, Dully K, O'Higgins N, Fennelly J and Duffy MJ: Enzyme-linked immunosorbent assay of c-erbB-2 oncoprotein in breast cancer. *Clin Chem* 38: 1471-147, 1992. PMID: 1353709.
- 22 Lam L, McAndrew N, Yee M, Fu T, Tchou JC and Zhang H: Challenges in the clinical utility of the serum test for HER2 ECD. *Biochim Biophys Acta (BBA) - Reviews on Cancer* 1826: 199-208, 2012. PMID: 22521738. DOI: 10.1016/j.bbcan.2012.03.012
- 23 Leary AF, Hanna WM, van de Vijver MJ, Penault-Llorca F, Rüschhoff J, Osamura RY, Bilous M and Dowsett M: Value and limitations of measuring HER-2 extracellular domain in the serum of breast cancer patients. *J Clin Oncol* 27: 1694-1705, 2009. PMID: 19255333. DOI: 10.1200/JCO.2008.17.3989
- 24 Tsé C, Gauchez A-S, Jacot W and Lamy P-J: HER2 shedding and serum HER2 extracellular domain: Biology and clinical utility in breast cancer. *Cancer Treat Rev* 38: 133-142, 2012. PMID: 21549508. DOI: 10.1016/j.ctrv.2011.03.008
- 25 Ardavanis A, Kountourakis P, Kyriakou F, Malliou S, Mantzaris I, Garoufali A, Yiotis I, Scorilas A, Baziotis N and Rigatos G: Trastuzumab plus paclitaxel or docetaxel in HER-2-negative/HER-2 ECD-positive anthracycline- and taxane-refractory advanced breast cancer. *Oncologist* 13: 361-369, 2008. PMID: 18448549. DOI: 10.1634/theoncologist.2007-0207
- 26 Lennon S, Barton C, Banken L, Gianni L, Marty M, Baselga J and Leyland-Jones B: Utility of serum HER2 extracellular domain assessment in clinical decision making: Pooled analysis of four trials of trastuzumab in metastatic breast cancer. *J Clin Oncol* 27: 1685-1693, 2009. PMID: 19255335. DOI: 10.1200/JCO.2008.16.8351
- 27 Ali SM, Carney WP, Esteva FJ, Fornier M, Harris L, Köstler WJ, Lotz J-P, Luftner D, Pichon M-F and Lipton A: Serum HER-2/neu and relative resistance to trastuzumab-based therapy in patients with metastatic breast cancer. *Cancer* 113: 1294-1301, 2008. PMID: 18661530. DOI: 10.1002/cncr.23689
- 28 Reix N, Malina C, Chenard M-P, Bellocq J-P, Delpous S, Molière S, Sevrin A, Neuberger K, Tomasetto C and Mathelin C: A prospective study to assess the clinical utility of serum HER2 extracellular domain in breast cancer with HER2 overexpression. *Breast Cancer Res Treat* 160: 249-259, 2016. PMID: 27709352. DOI: 10.1007/s10549-016-4000-z
- 29 Colomer R, Montero S, Lluch A, Ojeda B, Barnadas A, Casado A, Massutí B, Cortés-Funes H and Lloveras B: Circulating HER2 extracellular domain and resistance to chemotherapy in advanced breast cancer. *Clin Cancer Res: an official journal of the American Association for Cancer Research* 6: 2356-2362, 2000. PMID: 10873087.
- 30 Wang T, Zhou J, Zhang S, Bian L, Hu H, Xu C, Hao X, Liu B, Ye Q, Liu Y and Jiang Z: Meaningful interpretation of serum HER2 ECD levels requires clear patient clinical background, and serves several functions in the efficient management of breast cancer patients. *Clin Chim Acta* 458: 23-29, 2016. PMID: 27109901. DOI: 10.1016/j.cca.2016.04.025
- 31 Molina R, Jo J, Filella X, Zanon G, Pahisa J, Muñoz M, Farrus B, Latre ML, Gimenez N, Hage M, Estape J and Ballesta AM: C-erbB-2 oncoprotein in the sera and tissue of patients with breast cancer. Utility in prognosis. *Anticancer Res* 16: 2295-2300, 1996. PMID: 8694559.
- 32 Krainer M, Brodowicz T, Zeillinger R, Witschke C, Scholten C, Seifert M, Kubista E and Zielinski CC: Tissue expression and serum levels of HER-2/neu in patients with breast cancer. *Oncology* 54: 475-481, 1997. PMID: 9394844. DOI: 10.1159/000227606
- 33 Leyland-Jones B and Smith BR: Serum HER2 testing in patients with HER2-positive breast cancer: the death knell tolls. *Lancet Oncol* 12: 286-295, 2011. PMID: 21376291. DOI: 10.1016/S1470-2045(10)70297-7
- 34 Lipton A, Leitzel K, Ali SM, Carney W, Platek G, Steplewski K, Westlund R, Gagnon R, Martin A-M and Maltzman J: Human epidermal growth factor receptor 2 (HER2) extracellular domain levels are associated with progression-free survival in patients with HER2-positive metastatic breast cancer receiving lapatinib monotherapy: Serum HER2 Predicts Lapatinib Response. *Cancer* 117: 5013-5020, 2011. PMID: 21456017. DOI: 10.1002/cncr.26101
- 35 Krainer M, Brodowicz T, Zeillinger R, Witschke C, Scholten C, Seifert M, Kubista E and Zielinski CC: Tissue expression and serum levels of HER-2/neu in patients with breast cancer. *Oncology* 54: 475-481, 1997. PMID: 9394844. DOI: 10.1159/000227606
- 36 Kong S-Y: Serum HER-2 concentration in patients with primary breast cancer. *J Clin Pathol* 59: 373-376, 2006. PMID: 16461567. DOI: 10.1136/jcp.2005.029603
- 37 Finn RS, Gagnon R, Di Leo A, Press MF, Arbushites M and Koehler M: Prognostic and predictive value of HER2 extracellular domain in metastatic breast cancer treated with lapatinib and paclitaxel in a randomized phase III study. *J Clin Oncol* 27: 5552-5558, 2009. PMID: 19858400. DOI: 10.1200/JCO.2008.21.1763
- 38 Christianson TA, Doherty JK, Lin YJ, Ramsey JEE, Holmes R, Keenan EJ and Clinton GM: NH2-terminally truncated HER-2/neu protein: Relationship with shedding of the extracellular domain and with prognostic factors in breast cancer. *Cancer Res* 58: 5123-5129, 1998. PMID: 9823322.
- 39 Ross JS and Fletcher JA: The HER-2/neu oncogene in breast cancer: Prognostic factor, predictive factor, and target for therapy. *Stem Cells* 16: 413-428, 1998. PMID: 9831867. DOI: 10.1002/stem.160413
- 40 Vargas-Roig LM, Gago FE, Tello O, Martin de Civetta MT and Ciocca DR: c-erbB-2 (HER-2/neu) protein and drug resistance in breast cancer patients treated with induction chemotherapy. *Int J Cancer* 84: 129-134, 1999. PMID: 10096243. DOI: 10.1002/(SICI)1097-0215(19990420)84:2<129::AID-IJC6>3.0.CO;2-4
- 41 Molina R, Jo J, Filella X, Bruix J, Castells A, Hague M and Ballesta AM: Serum levels of C-erbB-2 (HER-2/neu) in patients with malignant and non-malignant diseases. *Tumour Biol* 18: 188-196, 1997. PMID: 9143415. DOI: 10.1159/000218029
- 42 Ha J-H, Seong M-K, Kim E-K, Lee JK, Seol H, Lee JY, Byeon J, Sohn Y-J, Koh JS, Park I-C, Noh WC and Kim H-A: Serial serum HER2 measurements for the detection of breast cancer recurrence in HER2-positive patients. *J Breast Cancer* 17: 33, 2014. PMID: 24744795. DOI: 10.4048/jbc.2014.17.1.33.

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