Monitoring serum HER2 levels in the neoadjuvant "Geparquattro" trial - a decrease predicts pathological complete remission

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Keywords

Breast cancer, neoadjuvant therapy, HER2, serum

Abbreviations

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Abstract

Background: In the context of neoadjuvant treatment for breast cancer patients, different targeted therapy approaches are currently evaluated in clinical trials. Serum markers could help to monitor and optimize treatment strategies.

Methods: We investigated the HER2 serum levels (sHER2) in 175 breast cancer patients participating in the GeparQuattro trial. This study incorporated neoadjuvant chemotherapy (NT) approaches and additional trastuzumab treatment for all patients with HER2-positive tumors. sHER2 levels were measured by ELISA before initiation of NT and after NT (pre-surgery).

Results: Median pre-chemotherapy sHER2 levels were higher in patients with positive HER2 status than in patients with negative HER2 status (14.9 ng/ml versus 7.7 ng/ml, $p<0.001$). ROC-curve analysis revealed that a sHER2 cut-off level of 10 ng/ml has a sensitivity of 72%, a specificity of 85%, a positive predictive value of 85% and a negative predictive value of 73% in discriminating between positive and negative HER2 status. Median pre-chemotherapy sHER2 was significantly higher in patients with pCR compared to patients with no pCR (14.9 ng/ml versus 8.7 ng/ml, $p=0.001$). In 87 HER2 positive patients, we found a significant positive association between pathological complete remission (pCR) and decrease of sHER2 levels ($p=0.02$), which was also significant in multivariate analysis (OR=3.2, 95% CI 1.13 – 9.55, $p=0.029$). In 73 HER2 negative patients, we observed no association between change of sHER2 levels and pCR ($p>0.05$).

Conclusion: sHER2 values in breast cancer patients before NT are associated with positive HER2 status of the primary tumor. Results of this study demonstrate pre-chemotherapy sHER-2 levels as well as a decrease of serum levels to be a significant predictor of response to NT for breast cancer in HER2 positive patients. Thus, monitoring sHER2 levels in the presence of anti-HER2 treatment might be a adjunct to the clinical evaluation during NT.

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Introduction

Neoadjuvant treatment (NT) strategies allow the assessment of therapeutic efficacy of chemotherapy and novel targeted approaches in breast cancer patients without long follow-up periods which are required in the adjuvant setting. The German Breast Group has conducted successful clinical trials in the neoadjuvant setting over the last years [1, 2]. The study Geparquattro is a phase III trial program that incorporated different NT approaches (epirubicin/cyclophosphamide prior to randomization to either docetaxel alone, docetaxel in combination with capecitabine or docetaxel followed by capecitabine) and additionally trastuzumab into current neoadjuvant chemotherapy regimens for primary breast cancer (JOC, in press, Figure1). This offered an opportunity for translational research projects examining biomarkers that should allow improving the knowledge of mechanisms underlying modern treatment strategies.

Human epidermal growth factor receptor 2 (HER2) is a growth factor receptor with a molecular weight of 185 kD. HER2 is overexpressed in 15–20% of primary breast cancers. Breast tumors that exhibit HER2 protein overexpression or gene amplification are more aggressive and more likely to recur [3-5]. The HER2 protein is a 185-kDA transmembrane tyrosine kinase with 3 defined domains: the intracellular tyrosine kinase portion, a short transmembrane portion, and the extracellular domain (ECD). This 105-kDa ECD can be cleaved from the surface by metalloproteases and detected in the peripheral blood (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, 59:1196-1201). It was reported that trastuzumab inhibits HER2 extracellular domain cleavage and that the remaining cleaved HER2 receptor is constitutively activated (Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J: Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. Cancer Res 2001, 61:4744-4749; Hudelist G, Kostler WJ, Attems J, Czerwenka K, Muller R, Manavi M, Steger GG, Kubista E, Zielinski CC, Singer CF: Her-2/neu-triggered intracellular tyrosine kinase activation: in vivo relevance of ligand-independent activation mechanisms and impact upon the efficacy of trastuzumab-based treatment. Br J Cancer 2003, 89(6):983-991.), suggesting that the presence of sHER2 also reflects a biologic process leading to more aggressive tumor behavior (Molina MA, Saez R, Ramsey EE, Garcia-Barchino MJ, Rojo F, Evans AJ, Albanell J, Keenan EJ, Lluch A, Garcia-Conde J et al: NH(2)-terminal truncated HER-2 protein but not full-length receptor is associated

with nodal metastasis in human breast cancer. ClinCancer Res 2002, 8:347-353.). Elevated levels of HER2 ECD are observed in patients with primary breast cancer [6] or metastatic breast cancer (MBC) [7, 8].

Trastuzumab, a monoclonal antibody directed against HER2, has become a standard treatment in HER2 positive patients with breast cancer and has also increased remission rates after neoadjuvant therapy [9]. A number of new treatment approaches directed against HER2 are currently examined in clinical trials, but the optimal use of these compounds is still unclear.

Also, the increasing use of neoadjuvant chemotherapy in patients with primary breast cancer requires the identification of predictive markers of pathologic response which is a surrogate marker of survival [10, 11].

The role of sHER2 in the neoadjuvant setting with use of HER2-targeted therapies is unclear. Therefore, the purpose of this study was to determine sHER2 levels in patients with non-metastatic breast cancer before NT and after NT prior to surgery. We evaluated the potential utility of sHER2 levels for predicting pCR to treatment, in particular in patients treated with trastuzumab-based regimens and compare results of a HER2 positive cohort with a HER2 negative cohort.

Material and Methods

The clinical study Geparquattro

Patients with either large operable or locally advanced tumors; tumors with negative hormone receptor status; or receptor positive tumors but clinically node-positive disease were recruited to receive preoperatively 4 cycles of epirubicin/cyclophosphamide (EC) (90 mg/m^2 / 600 mg/m^2) and to be then randomized to either 4 cycles of docetaxel (T) (100 mg/m^2) or 4 cycles of T + capecitabine (X) (75 mg/m^2 / 1800 mg/m^2) (TX) or 4 cycles of T (75 mg/m^2) followed by 4 cycles of X (1800mg/m^2) (T→X). Patients with HER2 positive tumors received trastuzumab (6 mg/kg i.v. every 3 weeks) concomitantly to cytotoxic treatment, starting with a loading dose of 8 mg/kg i.v. on day 1 of the first EC-cycle. Primary objectives were to assess the effect of X and to assess the effect of therapy duration (Figure 1). Primary endpoint of the study was pathological complete remission (pCR). Secondary endpoint was breast conserving therapy (BCT). Trastuzumab was given to patients with HER2 positive tumors. HER2 positivity was defined as IHC 3+ or fluorescence in-situ hybridization (FISH) positive of the primary tumor. The standardized immunohistochemistry assay HercepTest® by DakoCytomation was mandatory and all IHC 2+ cases had to be centrally analyzed by FISH assay in one of five German reference centers.

Inclusion criteria for the translational subprotocol and ethical considerations

Full blood samples before and after chemotherapy were collected for patients eligible for the Geparquattro study in the participating centers. All patients gave informed consent to provide a prespecified amount of extra blood before entering the Geparquattro study with the informed consent form. Participation on the clinical trial was still possible if a patient did not agree to provide extra blood samples. Patients were not informed about the laboratory results due to their experimental character. The clinical treatment study as well as the translational research project described here was approved by the central ethics committee at the University of Frankfurt as well as in all ethics committees of the participating centers.

Interventions

Serum samples were collected in the study centers and send to the German Breast Group. Correct labeling was checked and 175 serum samples (90 of HER2 positive and 85 of HER2 negative patients) were forwarded to the Department of Gynecology, University Medical Center Hamburg-Eppendorf. Medical records were stored in a central database at the GBG and the

patient identifiers were kept confidential. After serum determination results were assigned to the medical records and double-checked. Detailed patient characteristics are listed in Table 1.

ELISA

sHER2 was quantified by a commercially available enzyme-linked immunosorbant assay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) as previously described (Mueller et al. 2004). The results were expressed in nanogramme per millilitre. Each sample, standard and control was assayed in duplicate. Inter-assay and intra-assay coefficients of variation were less than 10%.

Statistical Analysis

The statistical analysis was performed using SPSS 15.0 software (SPSS, Chicago, IL, USA). HER2 levels were plotted against clinicopathological parameters by analysis of variance (ANCOVA). The following groups were compared: Tumour size less than 5 cm (pT1+2) versus more than 5 cm (pT3+4), G1/G2 versus G3; node-positive versus node-negative tumours; estrogen receptor positive versus negative, progesterone receptor positive versus negative; premenopausal versus postmenopausal, age <40 years versus ≥40 years and HER2 positive versus HER2 negative tumours. Pathological complete remission (pCR) was defined as no microscopic evidence of residual viable tumor cells in all resected specimens of the breast. The correlation between pCR and baseline variables was tested by the T-test in the univariate analysis and by a binary logistic regression in the multivariate analysis. A two tailed p-value less than 0.05 was considered as statistically significant.

Results

175 patients were included in this analysis (Table 1).

There was no between HER2 positive and the 85 HER2 negative patients regarding median age, menopausal status , nodal status, grading and rate of breast conserving therapy. In the HER2 negative cohort, more patients were estrogen receptor positive (p=0.02), progesterone receptor positive (p=0.01) and less patients had a pCR (p<0.01). The comparison between HER2 positive and negative patients regarding clinicopathological parameters is listed in Table 2.

Correlation between sHER2 levels and HER2 status

Pre-chemotherapy sHER2 levels in were determined in 167 patients. 89 patients had HER2 positive and 78 patients HER2 negative primary tumors. Median pre-chemotherapy sHER2 levels were higher in patients with positive HER2 status than in patients with negative HER2 status (14.9 ng/ml versus 7.7 ng/ml, p<0.001, figure 1). Post-chemotherapy sHER2 levels (n=164) were also associated with HER2 status (median 14.5 ng/ml in HER2 positive patients versus 8.2 ng/ml in HER2 negative patients, p<0.001).

A currently used cut off in the metastatic setting is 15ng/ml (Lipton A, Ali SM, Leitzel K, Demers L, Chinchilli V, Engle L, Harvey HA, Brady C, Nalin CM, Dugan M, Carney W, Allard J: Elevated serum her-2/neu level predicts decreased response to hormone therapy in metastatic breast cancer. J Clin Oncol 20: 1467-1472, 2002; Esteva FJ, Valero V, Booser D, Guerra LT, Murray JL, Pusztai L, Cristofanilli M, Arun B, Esmaeli B, Fritsche HA, Sneige N, Smith TL, Hortobagyi GN: Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. J Clin Oncol 20: 1800-8, 2002). Pre-chemotherapy sHER2 levels above 15 ng/ml were associated with a positive HER2 status in 44 of 89 patients (49%). sHER2 levels below 15 ng/ml were associated with a negative HER2 status in 77 of 78 patients (99%). Thus, the specificity of the common sHER2 cut-off value of 15 ng/ml is high, with a low sensitivity in non-metastatic patients. ROC-curve analysis revealed that a sHER2 cut-off level of 10 ng/ml has a higher sensitivity of 72%, a specificity of 85%, a positive predictive value of 85% and a negative predictive value of 73% in discriminating between positive and negative HER2 status of primary tumors (figure 2).

Change of sHER2 levels during chemotherapy

In HER2 positive patients, we determined a significant decline of sHER2 levels (> 20%) during therapy in 49% of patients (n=43). In HER2 negative patients, we found a significant decline only in 14% of the patients (n=12). This difference between both groups was statistically significant (p=0.039).

Correlation between sHER2 levels and clinicopathological parameters

In the HER2 positive cohort, we observed a positive correlation between nodal status and pre-chemotherapy sHER2 levels (p=0.042). We observed no other correlations between clinicopathological parameters including age, menopausal status, tumor size, grading, histological type, estrogen and progesterone receptor status and pre-chemotherapy sHER2 levels or post-chemotherapy sHER2 levels.

In the HER2 negative cohort, we observed a positive correlation between pre-chemotherapy sHER2 levels and menopausal status (p=0.019) and a positive correlation between post-chemotherapy sHER2 levels and age (p=0.017) and estrogen receptor status (p=0.012).

We found no other statistically significant correlations between pre or post-chemotherapy sHER2 levels and the above mentioned parameters.

pCR in relation to sHER2 levels and clinicopathological parameters

In HER2 positive patients, we found an association between pCR and negative hormone receptor status ($p<0.001$ for ER and p=0.002 for PR), prechemotherapy sHER2 levels above 15 ng/ml (p=0.045) and a decline of sHER2 levels (>20%) during NT (p=0.02) in univariate analysis (table 3), which was also significant in multivariate analysis (OR=3.29, 95% CI 1.001 – 10.89, p=0.049).

In the HER2 positive patient cohort, patients with pCR had higher sHER2 levels before chemotherapy than patients with no pCR (median 17.7 ng/ml versus 13.1 ng/ml, p=0.13). After the end of chemotherapy, sHER2 levels did not differ in patients with pCR or no pCR (median 15.2 ng/ml versus 13.6 ng/ml, p=n.s.). HER2 positive patients with pCR had a median decrease of sHER2 levels of 26% in comparison to a median decrease of 10% in HER2 positive patients without pCR (p=0.023).

In conclusion, HER2 positive patients with sHER2 levels above 15 ng/ml before therapy or patients with a minimum decline of sHER2 levels of 20% during therapy had a 60% higher probability of achieving a pCR than patients with sHER2 levels below 15 ng/ml or patients with a decline of less than 20% during therapy.

In HER2 negative patients, only negative progesterone receptor status correlated with pCR (p=0.001), but no such differences were observed for patients with or without pCR with regard to sHER2 levels before and after chemotherapy.

Breast Conserving Therapy in relation to sHER2 levels and clinicopathological parameters

In HER2 positive patients, Breast Conserving Therapy was associated with smaller tumor size ($p<0.001$) and decline of sHER2 levels ($p=0.035$) in univariate analysis and only with smaller tumor size in multivariate analysis (OR=0.17, 95%-CI 0.06 – 0.48, $p=0.001$).

In HER2 negative patients, only smaller tumor size correlated with Breast Conserving Therapy in univarariate ($p<0.001$) and multivariate analysis (OR=0.4, 95%-CI 0.18-0.71, $p=0.003$), but no such correlation was observed for sHER2 levels or decline of serum levels.

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Discussion

To add:
1. differences between groups with HER2 positive and negative primary tumors concearning pCR and ER status. This is representative of whole study cohort since positive HER2 status of primary tumor is associated with ER/PR negative status and pCR rate is higher in patients receiving trastuzumab treatment.
2. Sample size of our study in relation to whole study cohort. Our group is representative of whole cohort (data not shown).

Pathologic complete response to induction chemotherapy is considered an important prognostic factor for patients with primary breast cancer undergoing neoadjuvant chemotherapy. To our knowledge, the present study is the largest one to evaluate sHER2 levels in the context of NT and the only one in prospective trial with trastuzumab treatment. Furthermore, a HER2 negative cohort was selected as comparison group.

HER2 is a prominent therapeutic target in breast cancer and trastuzumab, a monoclonal antibody against this epidermal growth factor receptor, prolongs survival in the adjuvant and metastatic setting [12, 13]. The expression of HER2 in primary tumors is essential for trastuzumab treatment decisions of breast cancer patients [12]. However, the optimal use of trastuzumab and the various other drugs targeting HER2 that are currently in different phases of development are not clear yet. The neoadjuvant setting offers the opportunity to optimize treatment strategies in non-metastatic breast cancer patients. The effect of different therapies can be evaluated as the tumor is removed after treatment and tissue can be examined. In this context, different targeted therapy approaches are currently evaluated in clinical trials. The role of sHER2 in the adjuvant setting is still not well defined [14].

The sHER2 ELISA system used in our study was approved by the US Food and Drug Administration in the year 2000. The currently approved cut-off for an elevated sHER2 is greater than 15 ng/ml and, circulating trastuzumab has been shown not to interfere with the assay [15] because the antibodies used recognize different and non-overlapping epitopes on the extracellular domain (ECD) from those recognized by trastuzumab. Most published studies found no correlation between serum and tissue HER2 status among women with newly diagnosed breast cancer. Koestler et al. found the HER2 ELISA to have a sensitivity of 28% in 39 HER2 positive patients (FISH), Mazouni et al. reported a sensitivity of 56% in 16 HER2 positive patients (IHC) with a cut-off of 15 ng/ml (REFERENZEN). This is in line with our finding that the cut-off of 15 ng/ml has a low sensitivity, but high specificity in predicting the HER2 status of the primary tumour. This fact suggests that some women with HER2–negative tumors secrete detectable amounts of sHER2. However, we found no association between tumor size and sHER2 levels which was described as a parameter that might increase sHER2 levels by others [16, 17]. We

found a positive association between nodal status and sHER2 in HER2 positive patients and between age and sHER2 in HER2 negative patients. In our patient cohort, ROC-curve analysis showed that a cut-off level of 10 ng/ml had a sensitivity of 72% and a specificity of 85% in predicting the HER2 status of the primary tumor. In contrast, Quaranta et al. could not find a correlation between serum and tissue HER2 levels in an unselected patient group of 108 patients using the same cut-off as we did (10ng/ml) [18].

In metastatic breast cancer, no clear relationship was found between baseline sHER2 levels and tumor response to trastuzumab based treatment in a recently published metaanalysis [19], while other groups have suggested a role of sHER2 determination (Ali SM, Carney WP, Esteva FJ, Fornier M, Harris L, Kostler WJ, Lotz JP, Luftner D, Pichon MF, Lipton A: Serum HER-2/neu and relative resistance to trastuzumab-based therapy in patients with metastatic breast cancer. Cancer 2008, 113(6):1294-1301; 1. Fornier MN, Seidman AD, Schwartz MK, Ghani F, Thiel R, Norton L, Hudis C: Serum HER2 extracellular domain in metastatic breast cancer patients treated with weekly trastuzumab and paclitaxel: association with HER2 status by immunohistochemistry and fluorescence in situ hybridization and with response rate. Ann Oncol 2005, 16(2):234-239) so that no definitive conclusions can be drawn (Ali SM, Leitzel K, Lipton A, Carney WP, Kostler WJ: Value of serum human epidermal growth factor receptor 2 (HER2)/neu testing for early prediction of response to HER2/neu-directed therapies is still an open one and deserves further study in large prospective trials. J Clin Oncol 2009, 27(36):273). There is limited information regarding sHER2 to predict benefit from trastuzumab in primary breast cancer. None of the recent adjuvant trastuzumab trials have reported an analysis of sHER2 [20]. However, the rationale of determining sHER2 with no primary tumor present is weak. In our randomized NT-trial, we could show in a relatively large number of patients that elevated sHER2 levels above 15 ng/ml as well as a decrease of sHER2 levels of more than 20% from the beginning of NT to the end of NT was associated with pCR in HER2 positive patients. This effect observed in univariate and multivariate analysis. Two smaller published reports investigated the correlation between pretreatment sHER2 and pathologic complete response during neoadjuvant chemotherapy plus trastuzumab. Koestler et al. evaluated sHER2 levels in a trastuzumab-based neoadjuvant setting in 16 patients. In this small group of patients, they could show that a decrease of sHER2 levels was associated with response to therapy [21]. In 39 patients with NT-treatment, 29 patients receiving a trastuzumab combination, Mazouni et al. could only find a role of decreasing sHER2 levels in predicting therapy response from week 3 to week 6 after therapy initiation[22].

In our patient group, sHER2 levels were higher in HER2 positive patients than in HER2 negative patients before initiation of chemotherapy and also after finalization of chemotherapy. The fact that sHER2 levels were not associated with tumor size supports the hypothesis that secretion of HER2 is dependent on biologic mechanisms like active shedding. One potential drawback of our study is the fact that we have no sHER2 determination after surgery since the ethics approval did not allow blood sampling at this time point. Therefore, we are not able to determine if sHER2 after chemotherapy was secreted only by the primary tumor. Forty-nine % of HER2 positive patients and only 14% of HER2 negative patients had a decline of sHER2 levels of more than 20% during chemotherapy. It was described that shedding of the HER2 extracellular domain is inhibited by trastuzumab (Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J: Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. Cancer Res 2001, 61:4744-4749). In the HER2 positive patient cohort, median prechemotherapy sHER2 levels were significantly higher in patients with pCR than in patients without pCR and the median decrease of sHER2 levels during therapy was also higher in patients with pCR. However, we were not able to find a specific cut-off level that was able to discriminate between patients with and without pCR. This is in concordance with findings in the metastatic setting, where the baseline level of sHER2 alone was not a predictor of response to treatment [23] [7]. In contrast to our findings, in the series reported by Mazouni et al in the neoadjuvant setting, mean sHER2 baseline values were not different between the pCR group and the group with residual disease [22].

In the current report, we can summarize that patients who did not have a significant decline (>20%) in sHERs levels had decreased benefit from trastuzumab-based therapy. Possible mechanisms of trastuzumab resistance include altered receptor antibody interaction, PTEN loss and enhanced Akt signaling, p27 loss, signaling through other receptors [24].

Monitoring changes in sHER2 levels after a specific time-period after trastuzumab treatment to predict clinical response might be valuable for identifying a patient population that might benefit from additional treatment regimens with other HER2 targeted therapies. Prospective clinical trials evaluating the use of other HER-2-directed therapies (e.g., receptor antibodies, sheddase inhibitors, signal transduction inhibitors, heat shock protein inhibitors, proteasome inhibitors, anti-angiogenic agents and immune-stimulatory therapies) with and without continued trastuzumab therapy that are currently in development to exploit the pathway following the onset of resistance. Since the truncated receptor might be not ideally treated with

trastuzumab but rather with agents targeting the intracellular region of the receptor like the tyrosine kinase inhibitor lapatinib (Scaltriti M, Rojo F, Ocana A, Anido J, Guzman M, Cortes J, Di Cosimo S, Matias-Guiu X, Ramon y Cajal S, Arribas J et al: Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. J Natl Cancer Inst 2007, 99(8):628-638), the determination of sHER2 levels might be useful in this context. Serum samples can be easily obtained and repeated determinations can be performed. Based on these considerations, we will investigate sHER2 levels in the currently ongoing neoadjuvant GeparQuinto trial where HER2 positive patients receive either trastuzumab or lapatinib in addition to chemotherapy. Therefore, our findings support the basis for further studies which may lead to an improvement of current treatment strategies.

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Table 1: Patients` characteristics before and after neoadjuvant therapy (NT)

Characteristics	Before NT	After NT
	No. of patients (percent)	
Tumour size		
Tis/T0		57 (33)
T 1	6 (3)	65 (37)
T 2	126 (72)	36 (21)
T 3	22 (13)	5 (3)
T 4	21 (12)	1 (0.6)
Nuclear grading		
(pCR)		39 (22)
G 1	8 (5)	5 (3)
G 2	102 (58)	73 (42)
G 3	58 (33)	32 (18)
Grading unknown	7 (4)	26 (15)
Estrogen receptor status		
(pCR)		31 (18)
Positive	78 (45)	66 (38)
Negative	97 (55)	40 (23)
Unknown		38 (22)
Progesterone receptor status		
(pCR)		32 (18)
Positive	86(49)	56 (32)
Negative	89 (51)	50 (29)
Unknown		37 (21)
Axillary lymph node status		
Positive	99 (57)	51 (29)
Negative	76 (43)	117 (67)
Unknown		7(4)

Table 2: Comparison between the HER2 positive and negative patient cohort.

	HER2 positive	HER2 negative	p-value
Age [median]	48 yrs.	47 yrs.	n.s.
Premenopausal status	54/90 (60%)	53/85 (62%)	n.s.
nodal status (positive)	51/90 (57%)	48/85 (57%)	n.s.
High grading (G3)	32/90 (36%)	26/85 (31%)	n.s.
ER positive	42/90 (47%)	55/85 (65%)	**p=0.02**
PR positive	37/90 (41%)	52/85 (61%)	**p=0.01**
pCR	44/90 (49%)	12/85 (14%)	**p<0.01**
BCT	60/90 (67%)	54/85 (64%)	n.s.

Table 3: Correlation of clinicopathological parameters with pathological complete remission (t-test)

Baseline characteristics	t-value	p-value
T3/4 vs. T1/2	0.39	n.s.
G3 vs. G1/2	1.5	n.s.
cN1 vs. cN0	1.25	
ER neg vs. ER pos*	4.99	**p<0.001**
PR neg vs. PR pos	3.17	**p=0.002**
sHER2 > 15 mg/ml vs. sHER2<15 ng/ml	2.03	**p=0.045**
sHER2 decrease > 20% vs. sHER2 decrease <= 20%*	2.36	**p=0.02**

* also significant in multivariate analysis

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Figure Legends

Figure 1: sHER2 levels in HER2 positive and HER2 negative patients. sHER2 levels are significantly higher in HER2 positive patients than in HER2 negative patients ($p<0.001$).

Figure 2: Receiver Operating Characteristic (ROC) curve for different sHER2 cut-off points corresponding to the HER2 status of the primary tumour. A sHER2 cut-off of 10ng/ml has a sensitivity of 72% and a specificity of 85% in discriminating between HER2 positive and HER2 negative tumours.