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To cite this version:

A. Noske, S. Loibl, S. Darb-Esfahani, M. Roller, R. Kronenwett, et al.. Comparison of different approaches for assessment of HER2 expression on protein and mRNA level: prediction of chemotherapy response in the neoadjuvant GeparTrio trial (NCT00544765). Breast Cancer Research and Treatment, 2010, 126 (1), pp.109-117. 10.1007/s10549-010-1316-y . hal-00604408

HAL Id: hal-00604408

https://hal.science/hal-00604408

Submitted on 29 Jun 2011

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Comparison of different approaches for assessment of HER2 expression on protein and mRNA level - prediction of chemotherapy response in the neoadjuvant GeparTrio trial (NCT00544765)

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Running Title: Different HER2 assessment approaches

Abstract

Introduction: Human epidermal growth factor receptor 2 (HER2) testing is an essential part of pathological assessment in breast cancer patients, as HER2 provides not only prognostic but also predictive information on response to targeted therapy. So far, HER2 test accuracy of immunohistochemistry/ in-situ-hybridization techniques is still under debate, and more reliable and robust technologies are needed. To address this issue and to evaluate the predictive value of HER2 on chemotherapy, we investigated a cohort of 278 patients from the GeparTrio trial, a prospective neoadjuvant anthracycline/taxane-based multicenter study. In the GeparTrio trial, patients were not treated with any anti-HER2 therapy, as this was not standard therapy at this time.

Methods: The HER2 status was analyzed by three different approaches: local and central evaluation using immunohistochemistry combined with in-situ-hybridization as well as evaluation of HER2 mRNA expression using kinetic RT-PCR from formalin-fixed, paraffin-embedded (FFPE) tissue samples using a predefined cutoff.

Results: HER2 overexpression/amplification was observed in 37.3% (91/244) and 17.9% (41/229) of the informative samples in the local and central evaluations, respectively. Positive HER2 mRNA levels were found in 19.8% (55/278). We observed a highly significant correlation between central HER2 expression and HER2 status measured by kinetic RT-PCR ($r=0.856$, $p<0.0001$) and an overall agreement of 95.6% (κ statistic, 0.862, CI 0.77-0.94). Further, central HER2 as well as HER2 mRNA expression were predictors for a pathologically complete response after neoadjuvant anthracycline/taxane-based primary chemotherapy in a univariate binary logistic regression analysis (OR 3.29, $p=0.002$; OR 2.65, $p=0.004$). The predictive value could be confirmed for the central HER2 status by multivariate analysis (OR 3.046, $p=0.027$). The locally assessed HER2 status was not predictive of response to chemotherapy.

Conclusions: Our results suggest that standardized methods are preferable for evaluation of HER2 status. The kinetic RT-PCR from FFPE tissue might be an additional approach for assessment of this important prognostic and predictive parameter but has to be confirmed by other studies.

Key words: HER2, kinetic RT-PCR, breast cancer, neoadjuvant therapy, pathological complete response

Introduction

Human epidermal growth factor receptor 2 (HER2) testing is the prototype of a predictive biomarker for therapeutic decisions in breast cancer. There are several studies that have compared different HER2 testing methods to find out the most accurate and precise technique [1], [2], [3], [4]. In these studies, a high correlation between HER2 protein level evaluated by immunohistochemistry and gene amplification assessed by fluorescence or silverenhanced in-situ-hybridization (FISH/SISH) was demonstrated. Therefore the current state-of-the-art approach is immunohistochemistry combined with additional testing of equivocal cases by in-situ-hybridization techniques.

However, several reports have pointed out considerable difficulties in the assessment of HER2 by immunohistochemistry, with discordance rates of up to 20% [5]. This is mainly related to the fact that the immunohistochemical assessment is only semi-quantitative.

It has been suggested that HER2 expression is a continuous parameter that could be measured on mRNA level by RT-PCR in formalin-fixed, paraffin-embedded (FFPE) tissue [6]. On the basis of this observation, we have developed a quantitative method for determination of HER2 mRNA expression in FFPE tissue [7,8]. The aim of this study was to compare different methods for HER2 testing and to identify the predictive value of HER2 expression for a pathological complete response to neoadjuvant anthracycline/taxane-based chemotherapy. To address this issue, we evaluated a cohort of 278 pretherapeutic FFPE core biopsies of patients from the GeparTrio neoadjuvant trial. The patients had not been treated with any anti-HER2 therapy regardless of individual HER2 status, since this was not standard treatment for HER2-positive tumors at the time the study was conducted. HER2 status was investigated by kinetic RT-PCR on 278 available RNA samples isolated from FFPE tissue to determine mRNA expression. Further, the samples were analyzed for HER2 expression by local pathological institutes of the participating centers as well as by central evaluation using immunohistochemistry and in-situ-hybridization.

Patients and methods

Clinical study background

The GeparTrio study enrolled women with operable as well as locally advanced breast carcinomas and was organized by the German Breast Group between 2002 and 2005 [9-11]. After two cycles with TAC (docetaxel 75 mg/m^2, doxorubicin 50 mg/m^2, cyclophosphamide 500 mg/m^2), patients were randomized depending on their clinical response. Patients with an early response were randomized to proceed with either 4 or 6 additional cycles of TAC, whereas non-responding patients were randomized either to continue with 4 cycles of TAC or 4 cycles of NX (vinorelbine 25 mg/m^2 plus capecitabine 1000 mg/m^2).

Breast cancer diagnosis was made by core needle biopsy and the pathological examination was performed by local pathologists of the participating sites. The histological type was defined according to the WHO classification. Estrogen receptor (ER), progesterone receptor (PgR), and HER2 status were determined locally at each participating center by immunohistochemistry on tissue sections of the true/core-cut-biopsies obtained at the time of primary diagnosis before treatment. In case of moderate Her2 overexpression (Score 2+) by immunohistochemistry, confirmatory FISH testing was required. All pathology reports were centrally reviewed at the German Breast Group headquarters, Neu-Isenburg, Germany. This study includes essential elements of the REMARK criteria [12,13].

Inclusion criteria for evaluation of HER2 expression

For this study, only patients of those arms of the GeparTrio trial that had received 6 cycles of TAC were included, to ensure a homogenous therapy. All samples were taken at the time of initial diagnosis and were collected prospectively at the tumor bank of the German Breast Group at the Institute of Pathology, Charité Hospital, Germany. The study was approved by the institutional review boards of the centers participating in the clinical trial as well as the Hospital Charité. All patients gave written informed consent on participating in the trials and biomaterial collection.

Inclusion criteria for this study were treatment with 6 cycles of TAC, available FFPE pretherapeutic core biopsies, and available RNA from the tissue biopsies containing more than 30% tumor cells. Of the total of 1025 patients that had received 6 cycles of

TAC, mRNA samples from 278 patients were included. Data on clinical parameters were taken from the clinical trials database.

Pathological complete remission (pCR) was defined as a histopathological complete remission of all invasive tumor cells from both breast and axillary tissue removed at surgery (ypT0, ypTis, ypN0).

Assessment of HER2 status using different approaches

A total of three approaches were used for determination of HER2 status. First, HER2 expression was analyzed by local pathological institutes of the participating centers by immunohistochemistry and/or fluorescence in-situ-hybridization (FISH).

In addition, we performed a central assessment of HER2 expression by immunohistochemical staining in combination with silverenhanced in-situ-hybridization (SISH), according to the ASCO/CAP guidelines for HER2 testing in breast carcinomas and as described recently [14,15]. For this evaluation, tissue microarrays were constructed from needle core biopsies as described recently [15].

For immunohistochemical determination of HER2 were incubated with the rabbit polyclonal antibody against human HER2 (HercepTest antibody, Dako, 1:500) using the Discovery XT autostainer (Ventana, Tuscon, AZ, USA). Stained slides were digitized by a slide scanner (MIRAX SCAN, Zeiss Jena, Germany). The central review was done by two independent pathologists using custom-made software for whole slide imaging of virtual slides (VMscope, Berlin, Germany). SISH analyses were carried out in cases with equivocal Her2 expression. This technique was performed on a Benchmark XT autostainer (Ventana) using the INFORM HER2 probe (Ventana) according to the manufacturer's instructions. Internal and external quality controls were performed to assure test accuracy.

RNA isolation and kinetic RT-PCR

As a third method, HER2 mRNA expression was measured by kinetic RT-PCR in the pretherapeutic core biopsies. RNA was isolated from formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens employing a Siemens Healthcare Diagnostics proprietary experimental method based on magnetic beads (not commercially available) as previously described [8]. All tumor samples included in the study contained at least 30% tumor tissue, as evaluated by hematoxylin-eosin staining. RPL37A, a house keeping gene and HER2 mRNA expression were

assessed by kinetic one-step RT-PCR on ABI7900 systems using primers and probes as described previously [8]. Forty amplification cycles were applied and the threshold (Ct) values of genes were determined by ABI PRISM software. Relative HER2 mRNA expression levels were calculated as ΔCt values ($\Delta Ct=20-[Ct_{Her2}-Ct_{RPL37A}]$). For mRNA-based classification of HER2 status, a ΔCt cutoff point of 18 was used. The cutoff value was pre-defined on the basis of the bi-modal distribution of HER2 expression in a previous project [16].

Statistics

For the statistical calculations, the software package SPSSv17.0 was used. Correlation analyses were performed by using binary logistic regression analysis, and as indicated by Fisher's exact test or chi-square test for trends. Correlations among the HER2 assessment methods were evaluated using the Spearman's correlation coefficient method and Mann-Whitney test. Agreement between the immunohistochemistry/ in-situ-hybridization methods and kRT-PCR uncorrected for chance was calculated as the number of samples which agree divided by the total number of samples. Agreement corrected for chance was determined by kappa statistics estimating Cohen's kappa coefficient. In general, $\kappa > 0.91$ is considered to represent almost perfect concordance. To identify parameters independently associated with pCR, a multivariate logistic regression was performed. In this analysis, all parameters that had been described as predictive in the primary clinical study cohort were included. Generally, p-values of <0.05 were considered significant.

Results

Characteristics of the study cohort

A cohort of 278 patients from the GeparTrio trial was selected (Table 1). Patient age ranged from 23.5 to 78.7 years (mean 50.8). The distribution of clinicopathological parameters and pCR rate was comparable to the full study population that received 6 cycles of TAC.

Table 1 Characteristics of the study cohort and results of the HER2 determination with three approaches

Parameter	Study cohort No (%)	GeparTrio 6xTAC No (%)
cases	278	1025
Pathological complete response		
pCR	56 (20.1)	199 (19.4)
no pCR	222 (79.9)	826 (80.6)
Tumor grade		
G1-2	165 (59.4)	573 (55.9)
G3	81 (29.1)	399 (38.9)
missing	32 (11.5)	53 (5.2)
Tumor type		
invasive ductal/others	241 (86.7)	884 (86.2)
invasive lobular	37 (13.3)	141 (13.8)
ER/ PgR status		
ER-/ PgR-	63 (22.7)	346 (33.8)
ER+ and/ or PgR+	181 (65.1)	647 (63.1)
missing	34 (12.2)	34 (3.1)
Palpable tumor size		
≤4 cm	122 (43.9)	546 (53.3)
>4 cm	152 (54.7)	464 (45.3)
unknown	4 (1.4)	15 (1.4)
Nodal status		
cN0	133 (47.8)	441 (43.0)
cN+	130 (46.8)	542 (52.9)
missing	15 (5.4)	42 (4.1)
Age		
≤40 yrs	49 (17.6)	197 (19.2)
> 40 yrs	82.4 (82.4)	828 (80.8)
central HER2 status		
negative	188 (67.6)	602 (58.7)
positive	41 (14.7)	264 (25.8)

missing	49 (17.6)	159 (15.5)
local HER2 status		
negative	153 (55.0)	551 (53.8)
positive	91 (32.7)	315 (30.7)
missing	34 (12.2)	159 (15.5)
HER2 mRNA level		
negative	223 (80.2)	n.a
positive	55 (19.8)	n.a

Evaluation of HER2 status by local and central IHC/ISH and quantitative RT-PCR

Results on the three different measurements of HER2 are summarized in Table 1 and Figure 1. Each analysis method was performed blinded to the results of the other methods. Data on local HER2 analysis were available in 244 cases and revealed HER2 positivity in 37.3% (91 out of 244). However, in our central analysis, the rate of HER2-positive cases was considerably lower, with 17.9% (41 out of 229 interpretable samples). Assessment of HER2 mRNA by kinetic RT-PCR was carried out on 278 breast cancer samples. Normalized HER2 mRNA expression (ΔCT) ranged from 12.6 to 21.8, with a median of 16.3 ΔCT. We used a pre-defined cutoff value of a ΔCT of 18 that was based on independent studies and found a positive HER2 mRNA expression in 19.8% of tumors.

We observed a significant association between all HER2 assessment methods using the Mann-Whitney test ($p<0.0001$) and Spearman's rho correlation. The correlation coefficient between HER2 mRNA and central HER2 expression was particularly high (0.867, $p<0.0001$). In contrast, the correlation between HER2 mRNA and local HER2 (0.449, $p<0.001$) as well as central and local HER2 (0.438, $p<0.0001$) was significant as well, but the correlation coefficients were much lower.

The concordance (Table 2) between central HER2 and HER2 mRNA status was 95.6% (219 out of 229 cases), while the results of local HER2 and HER2 mRNA testing were concordant in only 182 of 244 cases (74%). Local HER2 assessment found 52 additional positive cases that were negative by kRT-PCR. In these discrepant cases, the mean relative RNA expression was 16.6 indicating relatively high levels of HER2 mRNA.

Table 2 Concordance analysis between the different HER2 testing methods

	mRNA vs. central	mRNA vs. local	central vs. local
kappa (CI 95%)	0.862 (0.778-0.946)	0.401 (0.272-0.530)	0.339 (0.257-0.541)
overall agreement	95.6%	74.6%	74.50%
positive agreement	97.6%	42.9%	34.1%
negative agreement	95.2%	93.5%	72.4%

As shown in Table 3, there was no relationship between HER2 status and clinico-pathological features like tumor size, nodal stage, histological grade, and subtype or patient age.

Table 3 Expression of HER2: relationship of different methods as well as correlation of mRNA HER2 status with outcome and clinical parameters

Characteristic	n	HER2 mRNA negative	HER2 mRNA positive	p-value
cases	278	223 (80.2%)	55 (19.8%)	
Central HER2				<0.0005*
negative	188	179 (95.2%)	9 (4.8%)	
positive	41	1 (2.4%)	40 (97.6%)	
Local HER2				<0.0005*
negative	153	143 (93.5%)	10 (6.5%)	
positive	91	52 (57.1%)	39 (42.9%)	
Pathological response				0.005*
pCR	56	37 (66.1%)	19 (33.9%)	
no pCR	222	186 (83.8%)	36 (16.2%)	
Tumor grade				ns
G1-2	165	134 (81.25)	31 (18.8%)	
G3	81	62 (76.5%)	19 (23.5%)	
Tumor type				0.074*
invasive ductal/other	241	189 (78.4%)	52 (21.6%)	
invasive lobular	37	34 (91.9%)	3 (8.1%)	
ER/PgR Status				ns
ER+ and/or PgR+	181	148 (81.8%)	33 (18.2%)	
ER-/PR-	63	48 (76.2%)	15 (23.8%)	
Palpable tumor size				ns
≤4 cm	122	98 (80.3%)	24 (19.7%)	
>4 cm	152	123 (80.9%)	29 (19.1%)	
Nodal status				ns
cN0	133	107 (80.5%)	26 (19.5%)	
cN+	130	104 (80.0%)	26 (20.0%)	
Age				ns
≤40 yrs	49	36 (16.1%)	13 (23.6%)	
>40 yrs	229	187 (83.9%)	42 (76.4%)	

*chi-square test for trends

Predictive effect of central HER2 and HER2 mRNA on pCR

In univariate logistic regression, we evaluated the HER2 status assessed by different methods as well as other established prognostic factors like patient age, histological grading, clinical tumor size, clinical nodal status, histological subtype, and hormone receptor status. We found that age, grading, tumor size as well as hormone receptor expression were significant predictive factors for pCR as known for invasive breast cancer. The histological subtype and clinical nodal status were not significant predictive factors in this cohort. Further, we observed significant results for central HER2 as well as HER2 mRNA status in predicting a pathological complete response in univariate binary logistic regression analyses. In contrast, local HER2 assessment did not have any predictive information (Table 4).

The predictive effect of central HER2 expression was confirmed in a multivariate binary logistic regression model using all clinicopathological parameters that were significant on univariate analysis (Table 5). However, the local HER2 status (OR 1.5 CI 0.67-3.35, p=0.32) as well as the HER2 mRNA status (OR 1.56 CI 0.66-3.68, p=0.311) was not significant in multivariate analysis. .

Table 4 Univariate logistic regression analysis: factors predicting a pCR

Characteristic	n	pCR (%)	Univariate analysis Odds ratio (95% CI)	p-value
Central HER2	229			0.002
negative	188	28 (14.9%)	1.00	
positive	41	15 (36.6%)	3.297 (1.555-6.991)	
HER2 mRNA	278			0.004
negative	223	37 (16.6%)	1.00	
positive	55	19 (34.5%)	2.653 (1.374-5.125)	
Local HER2	244			0.678
negative	153	27 (17.6%)	1.00	
positive	91	18 (19.8%)	1.151 (0.593-2.232)	
Hormone receptor status	244			<0.0001
ER+ and/ or PgR+	181	23 (12.7%)	1.00	
ER-/PgR -	63	25 (39.7%)	4.519 (2.318-8.813)	
Age	278			0.006
>40 yrs	229	39 (17.0%)	1.00	
≤40 yrs	49	17 (34.7%)	2.588 (1.309-5.117)	
Tumor type	278			0.138
invasive lobular	37	4 (10.8%)	1.00	
invasive ductal /other	241	52 (21.6%)	2.270 (0.769-6.698)	
Tumor grade	246			0.003
G1-2	165	21 (12.7%)	1.00	
G3	81	23 (28.4%)	2.719 (1.398-5.290)	
Palpable tumor size	274			0.024
>4 cm	152	22 (14.5%)	1.00	
≤4 cm	122	31 (25.4%)	2.013 (1.095-3.699)	
Nodal status	263			0.833
cN+	130	26 (20.0%)	1.00	
cN0	133	28 (21.1%)	1.067 (0.586-1.941)	

Table 5 Multivariate logistic regression analysis: factors predicting a pCR

Characteristic	Odds Ratio (95% CI)	p-value
Central HER2 (positive vs. negative)	3.04 (1.13-8.16)	0.027
Hormone receptor status (ER-/PgR – vs. ER+ and/ or PgR+)	3.99 (1.58-10.06)	0.003
Age (<40 vs. >40 yrs)	1.27 (0.44-3.60)	ns
Tumor grade (G3 vs. G1-2)	2.86 (1.12-7.29)	0.028
Palpable tumor size (< 4 cm vs. > 4 cm)	2.71 (1.09-6.72)	0.031

Discussion

Our study shows a significant correlation of HER2 levels by conventional immunohistochemistry and in-situ-hybridization methods as well as kinetic RT-PCR. We found that central HER2 status and HER2 mRNA expression are predictors for a pathological complete response after neoadjuvant TAC treatment in a univariate binary logistic regression. Moreover, central HER2 status is an independent predictor for pCR in a multivariate binary logistic regression.

This study demonstrates that evaluation of HER2 mRNA levels is feasible in routine formalin-fixed, paraffin-embedded tissue from clinical multicenter studies, which is in line with other studies [16,17]. Since the interpretation of HER2 immunostaining and in-situ-hybridization may be influenced by laboratory and observer variability, the kRT-PCR could improve the assessment of HER2 status as an additional molecular test. This approach enables a relatively fast, reproducible quantification of HER2 expression and reduces inter-observer variability. Previous studies revealed an overall concordance from 82 to 93% between different HER2 assessment methods of protein, DNA, and mRNA levels [18,19].

Pathological complete response (pCR) following neoadjuvant chemotherapy (NCT) has been shown to be predictive of longer recurrence-free and overall survival [20,21]. However, only 15 to 20% of the patients achieve a pathological complete response. In the last few years, several studies have shown a higher chemosensitivity in certain tumor subtypes. Tumors with high-grade, non-lobular invasive histology, and negative lymph node status correlate with a good response to NCT [22],[23]. Additionally, several reports suggest a significance of the hormone receptor and HER2 status in assessment of chemotherapy response. Thus, negative hormone receptors are one of the strongest predictive markers [24], and triple-negative breast carcinomas have higher pCR rates than non-triple-negative tumors [25], [26].

Our findings are in line with other studies demonstrating a predictive value for HER2 expression in achieving a good response to neoadjuvant chemotherapy [27,28]. Until now, the reports concerning the predictive value of HER2 were inconsistent due to heterogeneous study populations and different systemic treatment regimens.

Regarding the molecular mechanisms, it is not completely clear why HER2-expressing breast carcinomas show a better response to NCT. Thus, it was

suggested that HER2-positive tumors are more sensitive to anthracycline chemotherapy due to the frequent coamplification of HER2 and topoisomerase II [29]. Generally, basal-like and HER2+/HR- breast carcinomas have shown a higher sensitivity to neoadjuvant anthracycline-based therapy than the luminal subtype [23,30-32]. However, the therapy response was higher in the so called luminal B (HER2+/HR+) than in the luminal A subtype (HER2-/HR+) [31,32]. In the study of Guarneri and colleagues, the pCR was higher in HER2+/HR+ than in HER2-/HR+ carcinomas (15.3% vs. 6%). This was also observed in a cohort of the GeparDuo trial on anthracycline/taxane-based neoadjuvant chemotherapy showing higher pCR rates in HER2+/HR+ than in HER2-/HR+ breast carcinomas [15] as well as in the whole analysis of GeparTrio [26].

HER2 overexpression is associated with a higher rate of pCR to sequential paclitaxel/FAC preoperative chemotherapy regardless of ER status in a cohort of 534 patients. Further, HER2 was a particularly strong predictor of pCR in ER-positive carcinomas (pCR rate 19% vs. 6%) [33]. The authors report also on an association of higher TOP2A mRNA, lower MAP-tau expression, and HER2 positivity in a subset of ER-positive breast carcinomas suggesting an increased sensitivity for neoadjuvant chemotherapy.

We are aware of the limitations our study: It is a retrospective analysis of pretherapeutic core biopsies in the neoadjuvant setting, which results in restrictions in the number of available samples as well as in comparably small amounts of tissue used for TMA construction resulting in false-negative results, and missing data from local histology reports. Further, only cases which were informative for kinetic RT-PCR were included in this investigation which may cause a potential selection bias. However, we evaluated a study cohort of a large prospective clinical trial with similar well-documented clinical data and pre-defined cutoffs for HER2 mRNA. It is not completely clear why central HER2 status, but not HER2 mRNA status is an independent predictor in multivariate analysis, considering the fact that both factors are highly correlated and both are significant in univariate analysis. This result should be interpreted with caution and validated in additional cohorts.

Our study shows that the local HER2 status was not predictive for pCR in a univariate logistic regression analysis. The rate of HER2-positive breast carcinomas by local analysis (37.3%) was considerably higher as compared with the central HER2 status (17.9%) and HER2 mRNA (19.8%) expression. This is not unexpected because

preanalytic and analytic process steps in HER2 testing as well as the inter-observer variability in evaluation of HER2 immunostaining may influence the testing accuracy. Furthermore, at the time the GeparTrio trial was conducted, the experience with HER2 testing in pathology laboratories was much lower than today. As reviewed by Gown, HER2 studies performed at local and central laboratories showed significant levels of discordance [5]. However, the application of ASCO/CAP guidelines as well as proficiency tests have improved the accuracy of HER2 determination in the last few years [34].

Conclusions

As the HER2 status plays a critical role in the treatment of breast cancer patients, a standardized HER2 assessment with implementation of the ASCO/CAP guidelines is essential for future clinical trials. Quality assurance standards in assessing hormone receptors and HER2 status are also needed in routine clinical practice. Furthermore, due to the false-positive rate in the determination of HER2 by immunohistochemistry and time-consuming ISH methods, reliable and accurate assessment approaches are essential. In addition to the established methods, the kinetic RT-PCR might be a diagnostic alternative in HER2 testing. Further studies are needed to approve the advantages of this quantitative approach.

Figure legends

Figure 1 Relationship between HER2 assessment methods
A: Distribution of HER2 expression assessed by local and central evaluation as well as by kinetic RT-PCR. **B and C:** Association of the different assessment methods presented in a scatter diagram.

Abbreviations

CI: Confidence interval, ER: estrogen receptor, FISH: fluorescence in-situ-hybridization, FFPE: formalin-fixed, paraffin-embedded, HER2: human epidermal growth factor receptor 2, HR: hormone receptor, NCT: neoadjuvant chemotherapy, NX: Vinorelbine, Capecitabine, pCR: pathological complete response, OR: Odds ratio, PgR: progesterone receptor, SISH: silverenhanced in-situ-hybridization, TAC Docetaxel, Doxorubicin, and Cyclophosphamide.

Acknowledgements

We would like to thank Mrs. Petra Wachs, Mrs. Gabriela Altmann and Mrs. Claudia Roth for her excellent technical assistance.

Author contributions

AN, SL, SDE, MR, BMM, JS, RK, CT, RW, CD, GM conceived study design, data collection, data analysis and interpretation. IB, GH, GH, STG, HUU carried out data collection. All authors were involved in writing the paper and had final approval.

Competing interests

The authors declare that they have no competing interests. Ralf Kronenwett, Christian von Törne and Ralph Wirtz are employees of Siemens Healthcare Diagnostics, Cologne, Germany.

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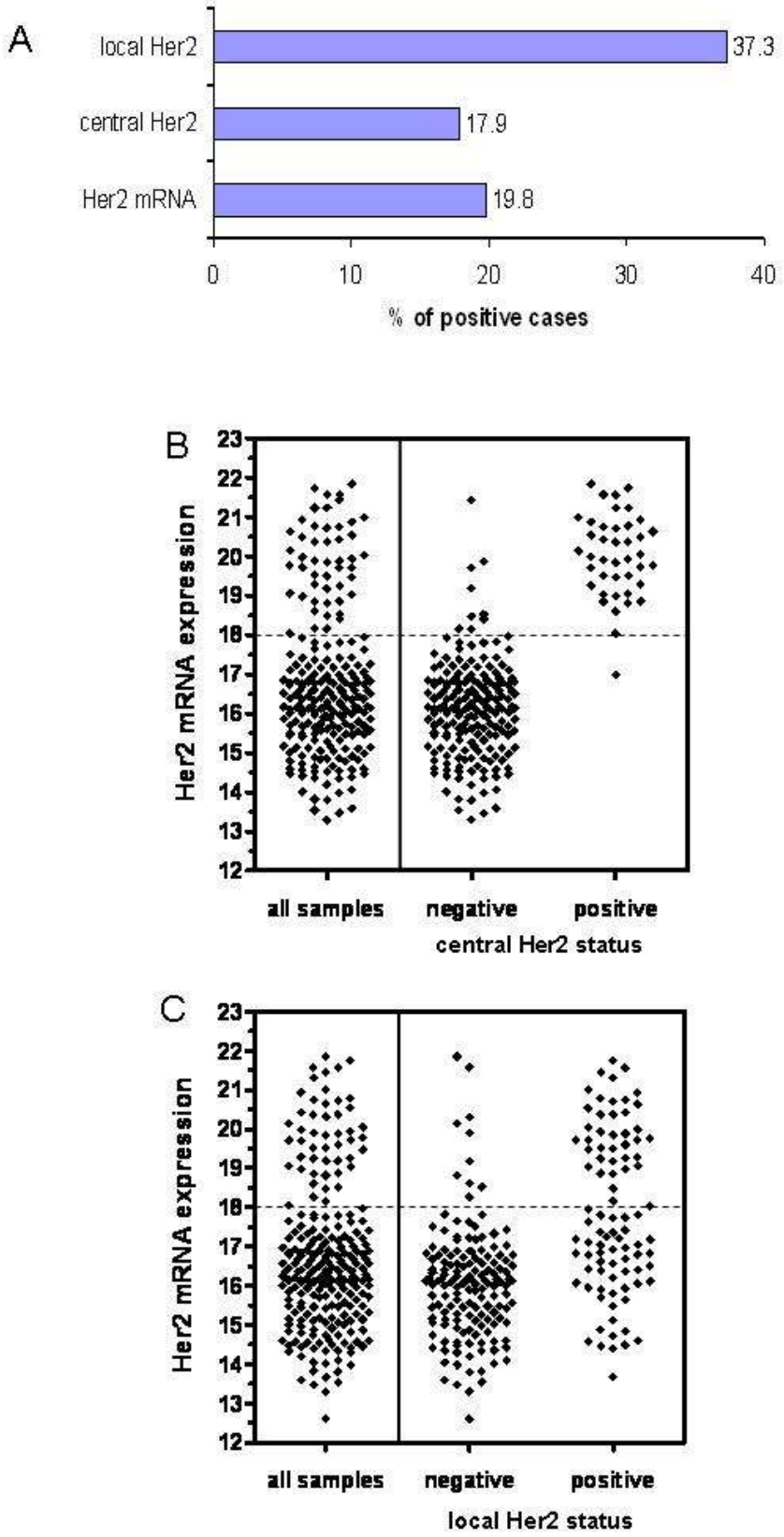
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