ANTICANCER RESEARCH *37*: 3117-3128 (2017)
doi:10.21873/anticanres.11669

Clinical Relevance of Serum HER2 and Circulating Tumor Cell Detection in Metastatic Breast Cancer Patients

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Abstract. *Background/Aim: Presence of circulating tumor cells (CTCs) is associated with impaired survival in metastatic breast cancer (MBC). This study was designed to evaluate whether assessment of serum HER2 (sHER2) levels provide additional prognostic information in MBC. Materials and Methods: Two hundred and fifty-three MBC patients were enrolled in this multicentre trial. CTCs were detected before the start of first- or later-line treatment using the CellSearch system. sHER2 was determined using ELISA. Results: ≥5 CTCs were detected in 122 of 245 evaluable patients (49.8%). One hundred and nineteen of 251 patients (47%) had sHER2 levels above 15 ng/ml. Median overall survival (OS) was 16.3 months in patients with elevated sHER2; median OS in patients with non-elevated sHER2 has not been reached (p=0.001). Patients with ≥5 CTCs were more likely to present with elevated sHER2 (61% vs. 33% in those with <5 CTC; p<0.001). In patients with HER2-negative tumors, elevated sHER2 was associated with shorter OS and PFS; in HER2-positive patients with OS only. Including sHER2, CTC status and established prognostic factors into a multivariate analysis, only the presence of CTCs and higher-line of therapy remained independent predictors of OS. Conclusion: Elevated levels of sHER2 are associated with worse survival, irrespective of the HER2 status of the tumor. However, sHER2 does not provide additional prognostic information in patients with known CTC status.*

Hematogenous spread of single cancer cells that broke free from the primary tumor is an essential step in the metastatic cascade (1). These cells enter the blood circulation and may persist in their secondary homing sites where they interact through complex mechanisms with the microenvironment. As putative precursors of metastatic growth, circulating tumor cells (CTCs) can be detected in most solid tumors of epithelial origin, but no entity has been studied in this context as extensively as breast cancer (BC). Numerous studies have confirmed the presence of CTCs as an independent prognostic factor in metastatic BC, with an established cut-off of five or more tumor cells per 7.5 ml of peripheral blood (2, 3). Since metastatic cancer is a dynamic and heterogeneous disease, its features, particularly with regard to predictive markers, may change over time. Evaluation of CTCs and their expression profiles may therefore improve our understanding of the current status of the disease and serve as a 'liquid biopsy'.

This study was conducted on behalf of the DETECT Study Group.

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Key Words: Breast cancer, circulating tumor cell, serum HER2, prognosis, survival, biomarker.

One of the most important prognostic and predictive factors in BC is the overexpression of the human epidermal growth factor receptor 2 (HER2). The current guidelines define a primary tumor as HER2-positive if more than 10% of the cells show a strong staining by immunohistochemistry or if a specific cut-off is reached on *in situ* hybridisation. While this definition has been confirmed in multiple trials, one has to bear in mind that tumors as well as different metastatic sites are heterogeneous in nature, and those considered HER2-negative by these criteria may consist of HER2-positive cells as well. HER2 receptor seems to provide these cells with higher metastatic potential and thus with a survival advantage; indeed, the HER2 status of tumor cells encountered in blood or bone marrow differs from primary tumor in a significant proportion of patients (4-6).

The HER2 protein contains three domains, of which the extracellular one can be released into the blood after being cleaved from the cell surface by metalloproteases. The possibility of measuring levels of circulating HER2 in the serum has gained considerable interest in the past two decades. While this strategy has not been implemented into clinical routine, several studies have shown that elevated serum HER2 (sHER2) levels are associated with impaired survival and response to treatment (7-11). Furthermore, since patients with HER2-negative primary tumors frequently present with elevated sHER2 levels, sHER2 testing might potentially identify a subgroup that might derive benefit from anti-HER2 directed therapies (12, 13).

The aim of the present study was to evaluate sHER2 levels and the presence of CTCs in patients with metastatic breast cancer and assess the association of these markers with clinical outcome.

Materials and Methods

A total of 253 patients with metastatic breast cancer from nine German Breast Cancer Centres were enrolled in this prospective, multicentre, open-label, non-randomized study. All patients were diagnosed with metastatic epithelial breast carcinoma. Further inclusion criteria were: age 18 years and older, and first diagnosis of metastatic disease or disease progression before start of a new treatment line. Patients with a second primary malignancy (except *in situ* carcinoma of the cervix or adequately treated cutaneous basal cell carcinoma) were excluded. Blood samples were collected before start of a new line of therapy chosen according to national and institutional standards. Response to therapy was evaluated by computed tomography every 12 weeks. Informed consent was obtained from all individual participants included in the study.

Detection of circulating tumor cells. CTCs were detected using the CellSearch™ system (Veridex LLC, NJ, USA). Briefly, 7.5 ml peripheral blood was collected into CellSave Tubes and processed according to manufacturer's instructions. The assay consisted of an immunomagnetic enrichment step employing immunomagnetic beads coated with anti-epithelial cell adhesion molecule (EpCAM) antibody, followed by staining with several antibodies. A circulating tumor cell is defined as a CD45-negative cytokeratin-positive cell with a DAPI-stained nucleus. In the current study, CTC-positive patients were defined as those with at least five tumor cells per 7.5 ml blood.

Characterization of HER2 status of CTCs. HER2 expression of CTCs was characterized within the CellSearch assay by addition of a fluorescein isothiocyanate-labeled anti-HER2 antibody (Veridex LLC, NJ, USA), as described previously (6). To evaluate the intensity of HER2 immunostaining, approximately 500 breast cancer cells from cell lines with known HER2 status (MCF-7: no HER2 gene amplification, MDA-MB-453: 2- to 3-fold HER2 gene amplification, SK-BR-3 and BT474: 5- to 7-fold HER2 gene amplification) were spiked into 7.5 ml blood from healthy donors and were processed under identical conditions with the CellSearch assay. The intensity of the HER2-specific immunofluorescence was categorized into negative (0), weak (1+), moderate (2+), and strong (3+). CTCs were considered HER2-positive if at least one CTC had strong HER2 staining (3+) based on the cut-off level described previously (14).

Detection of serum HER2. sHER2 was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) (Martell Diagnostic Laboratories, Roseville, MN, USA; formerly Wilex Inc, Cambridge, MA, USA), as described previously (11). This test is based on the quantitative measurement of the extracellular domain of the HER2 protein. Briefly, the assay uses one mouse monoclonal antibody to capture the extracellular domain and another one to detect and quantify it, and has been cleared by the Food & Drug Administration (FDA) with the recommended cut-off of 15 ng/ml (15-17).

Statistical analysis. Chi-squared test and Fisher's exact test were used to evaluate the relationship between CTC and sHER2 detection and clinicopathological factors. For the survival analysis, we considered in separate analyses the following primary end points: 1) death and 2) progression. Survival intervals were measured from the time of blood sampling to the time of death or of the first clinical, histological or radiographic diagnosis of progression. We constructed Kaplan–Meier curves and used the log-rank test to assess the univariate significance of the parameters. Cox regression analysis was used for multivariate analysis. All reported *p*-values are two-sided. Statistical analysis was performed by SPSS, version 18 (SPSS Inc., Chicago, IL, USA). The analysis was performed according to the REporting recommendations for tumor MARKer prognostic studies (REMARK) criteria on reporting of biomarkers (18). The primary question was the prognostic impact of sHER2 and CTC in the entire patient cohort.

Ethical approval. All procedures performed in this study were in accordance with the ethical standard of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local ethical committees of participation institutions.

Informed consent. Informed consent was obtained from all individual participants included in this study.

Table I. *Patients' characteristics (significant values are shown in bold).*

	Serum HER			CTC status		
	Total	sHER2 elevated n (%)	*p*-Value	Total	≥5 CTCs/7.5 ml n (%)	*p*-Value
Overall	251	119 (47%)		245	122 (50%)	
ER status			0.616			0.265
Negative	76	38 (50%)		74	33 (45%)	
Positive	174	81 (47%)		170	89 (52%)	
PR status			0.252			0.514
Negative	102	53 (52%)		99	47 (48%)	
Positive	148	66 (45%)		145	75 (52%)	
HER2 status			**<0.001**[4]			0.055[4]
Negative[1]	143	55 (38%)		138	76 (55%)	
Positive[2]	75	49 (65%)		75	31 (41%)	
Unknown[3]	33	15 (45%)		32	15 (47%)	
Metastatic site			**0.030**			**0.007**
Visceral	99	49 (49%)		96	39 (41%)	
Bone	34	9 (26%)		35	14 (40%)	
Both	118	61 (52%)		114	69 (61%)	
Extent of metastatic disease			**0.004**			**0.035**
One site	84	29 (34%)		84	34 (41%)	
Multiple sites	167	90 (54%)		161	88 (55%)	
Therapeutic setting			0.092			0.482
1st-line	96	38 (40%)		94	48 (51%)	
2nd-line	66	31 (47%)		64	28 (44%)	
3rd-line or more	88	49 (56%)		86	46 (545)	
Grading			0.592			0.679
G1	6	2 (33%)		6	2 (33%)	
G2	128	60 (37%)		123	63 (51%)	
G3	103	53 (51%)		102	50 (49%)	
CTCs[5]			**<0.001**			
<5 CTCs/7.5 ml	122	40 (33%)				
≥5 CTCs/7.5 ml	121	74 (61%)				
HER2 status of CTCs			**0.035**			
Positive	56	35 (62%)				
Negative	123	56 (45%)				

[1]IHC score: 0 /+1 or FISH negative; [2]ICH score: +3 or FISH positive; [3]not determined or ICH score +2 and FISH not performed; [4]Analysis performed only in patients with known HER2 status; [5]in 8 patients CTC status could not be determined.

Results

Patients' characteristics and CTC detection. Two hundred and fifty-three patients diagnosed with metastatic breast cancer were included into the analysis. Clinical-pathological data are summarized in Table I. Median age of all patients was 60 years. 39% of patients were included into the study before start of first-line therapy. HER2 was overexpressed by the primary tumor and/or metastasis in 35% of patients. Five or more CTCs per 7.5 ml of peripheral blood were detected in 122 of 245 evaluable patients (49.8%). As reported previously, elevated CTC counts were significantly associated with the extent of metastatic disease (*i.e.* more than one metastatic site [p=0.030] and both visceral and bone involvement [p=0.007]) but not with other classical clinical-pathological factors (19). The HER2 status of CTCs was positive in 57 out of 180 patients (32%) in whom at least one CTC was detected. 26% of patients with HER2-negative primary tumor and/or metastasis had HER2-positive CTCs, compared to 45% of HER2-positive patients (p=0.022). HER2 status of CTCs was not associated with other clinical-pathological factors. The distribution of patients is summarized in a REMARK diagram (Figure 1).

Serum HER2 detection. 119 of 251 (47%) patients had sHER2 levels above the cut-off level of 15 ng/ml. Patients with HER2-positive primary tumor and/or metastasis were significantly more likely to present with elevated sHER2 levels. 65% of tissue HER2-positive patients had elevated sHER2 levels in comparison with 38% of tissue HER2-negative patients

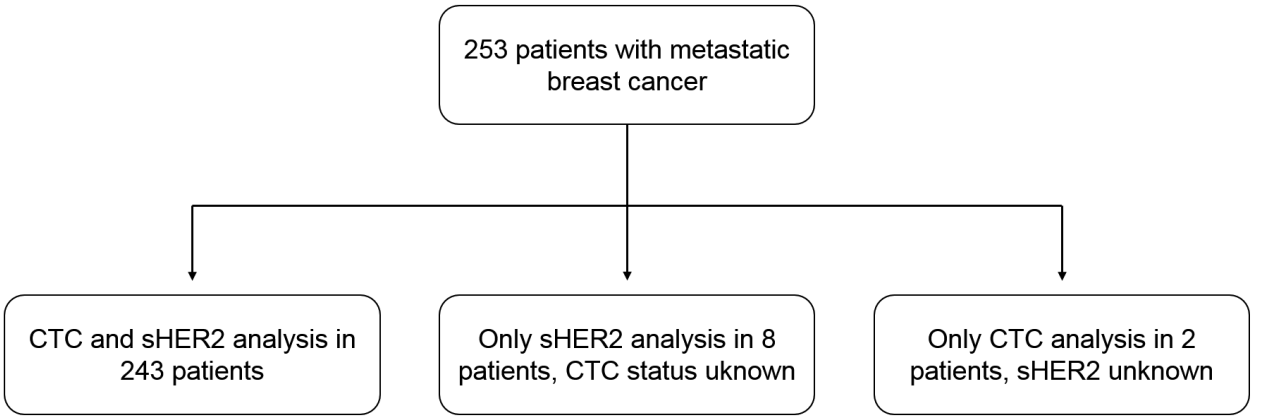

Figure 1. *Patient distribution diagram according to the REMARK criteria.*

(p<0.001). Multiple metastatic sites (p=0.004), visceral and bone involvement (p=0.030) and positive HER2 status of CTCs (p=0.035) were associated with higher sHER2 levels as well. Neither hormone receptor status nor line of therapy correlated with sHER2 levels (Table I). CTC-positive patients were more likely to present with elevated levels of sHER2: in patients with ≥5 CTCs, serum levels were above the cut-off for sHER2 in 61% *vs*. 33% in those with less than 5 CTCs per 7.5 ml of peripheral blood (p<0.001).

Survival analysis. During a median follow up of 19 months, 85 patients deceased and 183 were diagnosed with progressive disease. Median progression-free survival (PFS) was 7.2 months (95% CI=4.9-9.5 months) for patients with elevated sHER2 *versus* 8.7 months (7.0-10.5 months) with non-elevated levels (p=0.06). Median overall survival (OS) was 16.3 months (95% CI=10.0-22.6 months) in patients with elevated sHER2 levels; median OS in patients with non-elevated sHER2 has not been reached (p=0.001) (Figure 2a). Presence of ≥5 CTCs per 7.5 ml blood was significantly associated with shorter PFS (5.7 [95% CI=4.5-7.0] *vs*. 10.3 [8.4-12.2] months, p=0.001) and OS (median 12.6 [95% CI=7.8-17.3] months *vs*. not reached, p<0.001) (Figure 2b). Patients with elevated sHER2 and ≥5 CTCs had the shortest PFS (5.6 [95%-CI=3.3-7.8] months) and OS (median 12.3 [95% CI=5.3-19.3] months), compared to those with non-elevated sHER2 and <5 CTCs (PFS: 11.4 [95% CI=8.7-12.2] months; median OS not reached) (p=0.04 for PFS and p<0.001 for OS) (Figure 2c). In a multivariate analysis, presence of ≥5 CTCs, higher grading and higher line of therapy remained independent predictors of shorter OS (Table II). Negative ER status, higher line of therapy and elevated CTC counts were independent predictors of shorter PFS in the multivariate analysis.

Table II. *Multivariate analysis of overall survival.*

	p-Value	Hazard Ratio	95%-Confidence Interval
sHER2			
Elevated *vs*. non elevated	0.063	1.702	0.97-2.98
Therapy line			
>1st line *vs*. 1st line	<0.001	3.105	1.65-5.84
Menopausal status			
Post- *vs*. Premenopausal	0.446	0.807	0.47-1.40
ER status			
Positive *vs*. Negative	0.068	0.488	0.23-1.05
PR status			
Positive *vs*. Negative	0.549	1.240	0.61-2.51
HER2 status			
Positive *vs*. Negative	0.101	0.614	0.34-1.10
Number of metastatic sites			
Multiple *vs*. Single site	0.202	2.556	0.60-10.82
CTC counts			
≥5 *vs*. <5 CTCs/7.5 ml blood	<0.001	4.096	2.23-7.54

No significant association was found between the HER2 status of CTCs and overall (p=0.492) or progression-free survival (p=0.692).

Survival analysis in subgroups stratified by the HER2 status of the tumor. Separate survival analyses were conducted in tissue HER2-negative and tissue HER2-positive patients. In patients with HER2-negative tumors (Figure 3a), sHER2 was significantly associated with OS (p=0.001) and PFS (p=0.021). In this subgroup, the CTC status was a strong predictor of OS and PFS as well.

Table III. *Univariate survival analysis in the (a) HER2-positive and (b) HER2-negative subgroup.*

(a)

		sHER2 elevated		sHER2 non-elevated
Overall survival (months)	Mean	15.0 [12.3-17.6]		21.1 [19.0-23.4]
	Median	Not reached		Not reached
	p-value		0.013	
Progression-free survival (months)	Mean	8.3 [6.4-10.2]		10.6 [7.9-13.4]
	Median	7.5 [4.9-10.2]		9.6 [7.1-12.1]
	p-value		0.313	

(b)

		sHER2 elevated		sHER2 non-elevated
Overall survival (months)	Mean	12.3 [10.1-14.6]		18.2 [16.3-20.0]
	Median	10.6 [5.0-16.2]		Not reached
	p-value		0.001	
Progression-free survival (months)	Mean	7.5 [6.0-8.9]		10.2 [8.6-11.9]
	Median	5.8 [3.8-7.9]		8.9 [6.1-11.8]
	p-value		0.021	

In patients with HER2-positive tumors, elevated sHER2 predicted shorter OS (p=0.013) but not PFS (p=0.313) (Figure 3b). The presence of ≥5 CTCs correlated with shorter OS and PFS in these patients.

Discussion

In the current study, we aimed at evaluating the correlation between sHER2 levels and another promising biomarker, CTCs, and their impact on survival in a multicentre cohort of metastatic breast cancer patients.

The current body of evidence supporting CTCs as a strong independent prognostic factor is much larger than that regarding sHER2. The clinical relevance of CTC detection in early and advanced BC has been confirmed in two pooled analyses including 1944 non-metastatic and 3,173 metastatic patients (20, 21). Various molecular and immunocytological assays have been developed for CTC diagnostics, most of them based on the detection of epithelial markers, such as cytokeratins and EpCAM. Among those, the FDA approved CellSearch® system is the most widely used detection tool and has been employed in a number of clinical trials, such as the Southwest Oncology Group (SWOG) 0500, CirCe01 (Circulating Tumor Cells to Guide Chemotherapy for Metastatic Breast Cancer), STIC-CTC (Medico-economic Interest of Taking Into Account Circulating Tumor Cells to Determine the Kind of First Line Treatment for Metastatic, Hormone-receptors Positive, Breast Cancers), DETECT III (A Multicenter, Phase III Study to Compare Standard Therapy +/- Lapatinib in HER2-ve MBC-Patients With HER2+ve CTCs) and DETECT IV (A Study in Patients With HER2-negative Metastatic Breast Cancer and Persisting HER2-negative Circulating Tumor Cells) (22). While the majority of studies conducted in the adjuvant setting considers patients CTC-positive if at least one tumor cell is detected, a cut-off of ≥5 tumor cells per 7.5 ml blood is currently being used as gold standard to identify patients with unfavorable prognosis in the metastatic situation (23).

Serum HER2 detection. Several approaches to measure the extracellular domain of HER2 in the serum have been introduced, most of them based on the ELISA method (24-27). While a direct comparison of the results reported by early studies in the 1990s has been difficult due to the lack of standardization and the variety of cut-offs employed, most recent trials used the FDA-cleared ELISA test with the standardized cut-off of 15 ng/ml to distinguish between non-elevated and elevated sHER2 levels, determined as part of the data submitted to the FDA (26, 28, 29) (Tables III and IV). Patients with HER2-overexpressing tumors are more likely to have elevated sHER2 than those with sHER2-negative disease: in a meta-analysis including 1902 patients, median sHER2 levels before start of treatment were significantly higher in patients with HER2-positive tumors (25.1 ng/ml *vs*. 10.1 ng/ml, respectively) (13). This is in accordance with the present study: we found elevated sHER2 levels in 65% of patients with HER2-positive tumors compared to 38% of those with HER2-negative disease.

The majority of studies on sHER2 in breast cancer have focussed on one of the following aspects: (A) the prognostic relevance of sHER2, (B) the feasibility of sHER2 for therapy monitoring and (C) therapy selection based on sHER2.

Prognostic relevance of sHER2 in breast cancer. The largest dataset on the prognostic significance of sHER2 in metastatic setting is the meta-analysis of three prospective randomized lapatinib-based trials (EGF30008, EGF30001 and EGF100151) (13). In these trials patients received standard chemo- (paclitaxel or capecitabine) or endocrine therapy +/- lapatinib. Patients with elevated sHER2 levels benefited most from lapatinib; this effect was independent of the HER2 status of the tumor. In the control group, which did not receive HER2-targeted therapy, high baseline sHER2 was an independent predictor of shorter PFS and OS. Several large trials in metastatic setting showed a significant association between higher sHER2 and worse clinical outcome while others found no prognostic significance (Table III). In non-metastatic BC, numerous trials demonstrated that elevated sHER2 levels at time of diagnosis predict poor clinical outcome (Table IV).

Furthermore, Fehm *et al*. analyzed blood samples from 152 initially non-metastatic BC and showed that sHER2 levels may change during the clinical course of disease (30). A significant proportion of sHER2-negative patients at time of diagnosis of early breast cancer turned sHER2-positive when diagnosed with metastatic disease. Interestingly, only few of the initially sHER2-positive patients became sHER2-negative. In this study sHER2 status at time of diagnosis of metastatic disease was associated with poor clinical outcome as well.

In our study, patients with elevated sHER2 levels had shorter overall survival, but sHER2 was no longer an independent prognostic factor when included into a multivariate regression analysis besides – among other factors – the CTC status. To our knowledge, this is the largest study examining both biomarkers in metastatic BC. Fehm *et al*. analyzed blood samples from 77 metastatic BC patients with negative or unknown HER2 status and determined sHER2 as well as the HER2 status of CTCs (31). 23 patients were HER2-positive using at least one method; the concordance between both techniques was 71%. Another smaller study determined sHER2 and CTC status in 68 mostly HER2-negative metastatic BC patients (32). sHER2 did not affect clinical outcome but was associated with CTC number and the HER2 status of CTCs. In our study, we were able to confirm that CTC-positive patients were more likely to have elevated sHER2 levels (61% *vs*. 33% in CTC-negative patients, $p<0.001$).

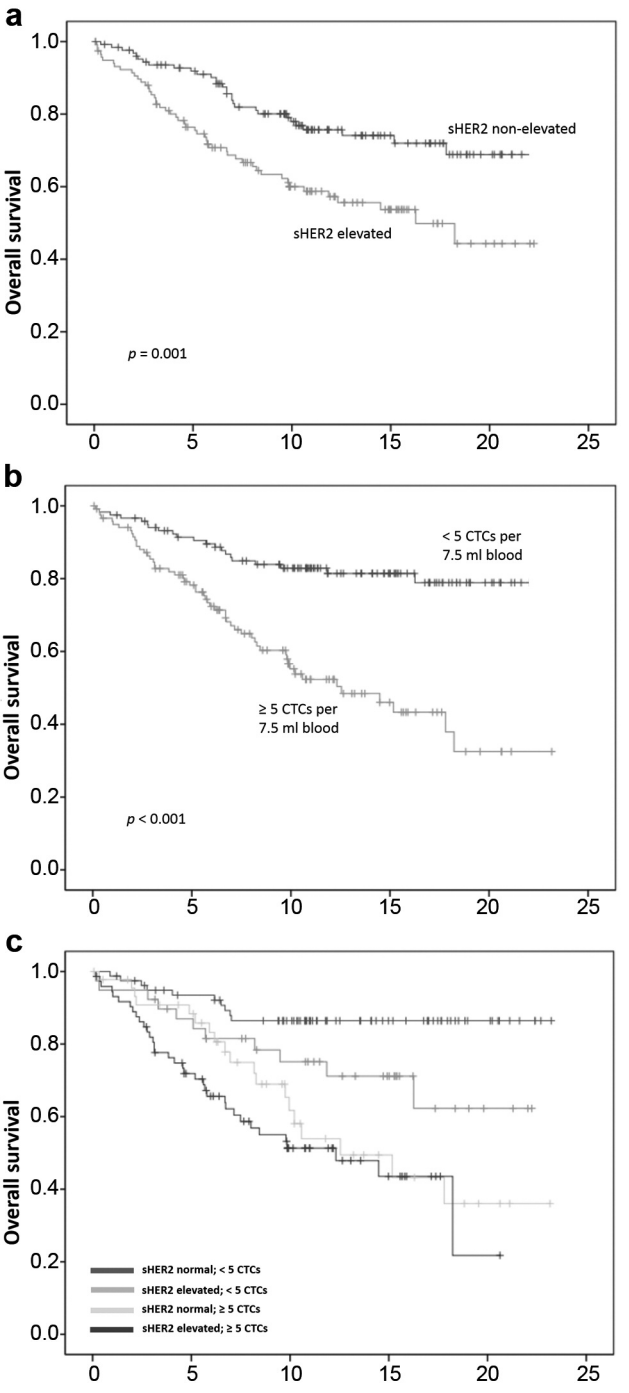

Figure 2. *Correlation between overall survival [months] and a) serum HER2 levels; b) CTC counts; c) sHER2 and CTCs.*

Serum HER2 and therapy monitoring. The possibility of assessing the current status of the disease is regarded as one of the most promising potentials of blood-based biomarkers. A simple and non-invasive blood test represents an attractive tool allowing real-time insight into the features and activity of the disease ("liquid biopsy"). With respect to CTCs, it has already been shown that CTC dynamics in metastatic BC predict response to therapy sooner than conventional imaging-based evaluation and that clinical outcome is poorest in patients with persistently high CTC levels (33, 34). However, the optimal management of such patients has not yet been clarified (34).

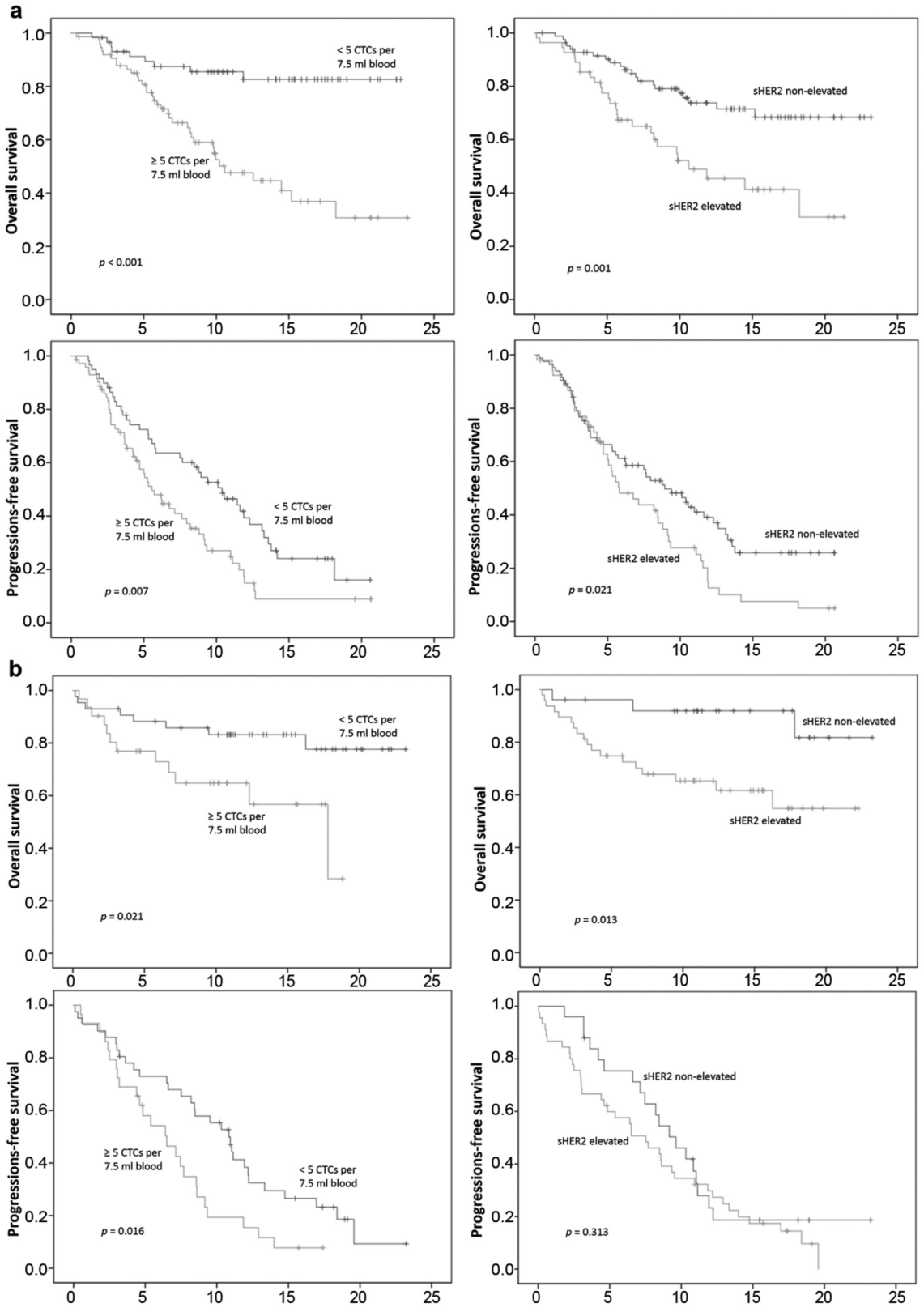

Figure 3. *Kaplan–Meier overall and progression-free survival analysis in a) HER2 negative and b) HER2 positive patients according to sHER2 levels and CTC status [months].*

Table IV. *Current evidence regarding serum HER2 in metastatic breast cancer.*

Study	Setting	Patients, n	Method	Follow up (months)	Prognostic significance
Our study	Metastatic HER2-pos and neg	251	ELISA[1]	19	Univariate: OS (irrespective of the HER2 status of the tumor); PFS in HER2-negative patients Multivariate: No
Lee *et al*. (13) Meta-analysis of EGF30008, EGF30001 and EGF100151	Metastatic HER2-pos and neg	1,902	ELISA[1]	n.a.	PFS[3], OS[3] (in patients not treated with HER2-targeted therapy) Elevated sHER2 associated with higher PFS benefit from lapatinib
EGF30001 (35)	Metastatic HER2-neg or unknown	579	ELISA[5]	n.a.	Baseline sHER2: none Changes in sHER2 levels: PFS 4
Lipton *et al*. (41)	Metastatic HER2-pos and neg	562	ELISA[1]	n.a.	PFS[3]
CALGB 8662 (24)	Metastatic HER2-pos and neg	242	Sandwich enzyme immunoassay	n.a.	Univariate: OS Multivariate: None
Lipton *et al*. (42)	Metastatic/Advanced[2] HER2-pos and neg	240	ELISA[1]	n.a.	OS[3]
Fehm *et al*. (30)	Metastatic HER2-pos and neg	152	ELISA[1]	35	OS[3]
EGF20009 (43)	Metastatic HER2-pos	138	ELISA[1]	n.a.	Baseline sHER2: none Decline in sHER2 levels: PFS[3]
Esteva *et al*. (37)	Metastatic HER2-pos	103	ELISA[1]	8	Baseline sHER2: none Decline in sHER2 levels: PFS[4]
Aurilio *et al*. (32)	Metastatic HER2-pos and neg	68	ELISA[1]	n.a.	None
Fornier *et al*. (38)	Metastatic HER2-pos and neg	55	ELISA[1]	n.a.	Baseline sHER2: none Decline in sHER2 levels: response to therapy[4]
Burstein *et al*. (44)	Metastatic HER2-pos	54	ELISA[1]	n.a.	Decline in sHER2 levels: PFS[4]
Hayashi *et al*. (27)	Metastatic ER2-pos and neg	52	ELISA[6]	22	None
Kong *et al*. (45)	Metastatic HER2-pos	50	ELISA[1]	n.a.	Baseline sHER2: none Decline in sHER2 levels: response to therapy[4]
Reix *et al*. (9)	Metastatic HER2-pos	48	ELISA[1]	68	PFS[3], OS[3]

[1]Cut-off 15 ng/ml; [2]measured at time of progression in patients with initially non-elevated sHER2; [3]multivariate analysis; [4]univariate analysis; [5]cut-off 16 ng/ml; [6]two methods with different cut-offs.

Numerous trials aimed at determining the usefulness of sHER2 for therapy monitoring in both metastatic and early BC setting (Tables III and IV). In the EGF30001 trial, 579 patients with newly diagnosed metastatic BC were randomized to receive paclitaxel with lapatinib or placebo (35). Blood samples were collected before start of first-line therapy and every 9-12 weeks thereafter. An additional analysis of sHER2 dynamics was performed in 391 patients with HER2-negative tumors. In patients with initially elevated sHER2 levels, conversion to non-elevated sHER2 during treatment predicted better PFS compared to those with persistently high sHER2. Conversely, patients whose sHER2 levels rose in the course of therapy had a worse PFS that those whose sHER2 levels remained low. Changes in sHER2 levels in patients with HER2-positive tumors were analyzed in the EGF20009 trial (36). sHER2 was determined before start of first-line therapy with lapatinib and every four weeks thereafter in 138 metastatic BC patients. A decrease in sHER2 levels of at least 20% predicted better PFS; conversely, an increase of ≥ 20% was associated with worse PFS. Similar results were demonstrated in smaller studies as well (37, 38).

The feasibility of sHER2 analysis or therapy monitoring in non-metastatic BC has been evaluated in a number of trials (Table V). Reix *et al*. examined changes of sHER2 during neoadjuvant chemotherapy in 106 BC patients and observed a significant decrease in sHER2 levels only in patients who achieved complete remission (9). This is in line with two German neoadjuvant trials: GeparQuattro and

Table V. *Current evidence regarding serum HER2 in non-metastatic breast cancer.*

Study	Setting	Patients, n	Method	Follow up (months)	Prognostic significance	Association with pCR*
NCCTG N9831 (8)	Adjuvant Recurrent	2,318 124	ELISA[1]	56	DFS[4] OS[4]	-
Lee *et al*. (46)	Adjuvant	436	ELISA[1]	50	DFS[3]	-
Reix *et al*. (9)	(Neo)Adjuvant	286	ELISA 1	68	DFS[4], OS[3]	Decline in sHER2 levels observed only in pCR patients
Ludovini *et al*. (47)	Adjuvant	256	ELISA[1]	38	DFS[3]	-
Fehm *et al*. (50)	Adjuvant	211	ELISA[5]	n.a.	DFS[3]	-
Witzel *et al*. (10)	Neoadjuvant	210	ELISA[1]			Decline in sHER2 levels significantly associated with pCR in patients treated with lapatinib
Ryu *et al*. (39)	Adjuvant	200	ELISA[1]	38	Baseline sHER2: DFS4, OS[4] Decline in sHER2: OS[4]	-
Witzel *et al*. (12)	Neoadjuvant	175	ELISA[1]			Decline in sHER2 levels and initially elevated sHER2 significantly associated with pCR in patients receiving HER2-targeted therapy
Tchou *et al*. (48)	Adjuvant	118	MBB-ELISA[2]	21	DFS[3]	-
Thureau *et al*. (49)	Adjuvant	65	ELISA[1]	53	DFS[3], OS[3]	-

*In case of neoadjuvant study; [1]cut-off 15 ng/ml; [2]cut-off 7 ng/ml; [3]multivariate analysis; [4]univariate analysis; [5]cut-off 120 fmol/ml.

GeparQuinto. Witzel *et al*. analyzed the data from the translational project of the GeparQuinto trial and reported that a significant decrease of sHER2 levels predicted complete remission in a univariate analysis (10). In the multivariate analysis, the association of sHER2 decrease and pCR was significant only in patients receiving lapatinib but not trastuzumab. In contrast, the GeparQuattro trial confirmed sHER2 decline as an independent predictor in trastuzumab-treated patients (12). sHER2 dynamics have been explored in the adjuvant setting as well: Ryu *et al*. collected blood samples before and after adjuvant chemotherapy in 200 patients with non-metastatic BC patients (39). An increase in sHER2 levels during treatment predicted shorter DFS in a univariate analysis.

Therapy selection based on serum HER2. Treatment decisions are based on the histological characteristics of tumor tissue without considering features of minimal residual disease, although the latter is the aim of systemic therapy as well. With regard to HER2, Riethdorf *et al*. reported that in one-fifth of patients with HER2-negative BC HER2-positive CTCs may be detected (14). However, these patients are not eligible for anti-HER2 targeted treatment. In our study, elevated sHER2 levels were detected in 38% of patients with HER2-negative tumors. In this patient group, sHER2 was significantly associated with OS and PFS. It remains unclear, whether these patients might benefit from anti-HER2 directed therapy. Interestingly, in the meta-analysis by Lee *et al*., patients with high sHER2 levels benefited from lapatinib therapy regardless of the HER2 status of the tumor (13). It has also been shown that HER2-targeted agents can eliminate HER2-positive CTCs in metastatic BC irrespectively of the HER2 status of the primary tumor (40). The question whether the choice of systemic treatment might be driven by blood-based biomarkers, is currently being clarified in the DETECT trials (2).

Conclusion

The present study is the first large analysis of serum HER2 and CTCs in metastatic breast cancer patients. Our results confirm the previously reported association between elevated sHER2 levels and poor clinical outcome both in HER2-negative and HER2-positive patients. However, determining sHER2 status does not provide additional prognostic information when CTC status is available.

Trial Registration

Current Controlled Trials ISRCTN59722891 (DETECT).

Conflicts of Interest

The Authors declare that they have no conflicts of interest.

Acknowledgements

This work was supported by a research grant from Roche Pharma AG, Germany. Furthermore, this study was partly supported by Adnagen AG (Langenhagen, Germany). ELISA kits were provided at no cost by Oncogene Science, a former part of Siemens Medical Solutions Diagnostics and now part of Wilex.

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Received May 1, 2017
Revised May 11, 2017
Accepted May 15, 2017