Moderate HER2 expression as a prognostic factor in hormone receptor positive breast cancer

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Abstract

Overexpression and/or amplification of human epidermal growth factor receptor 2 (HER2) is associated with poor prognosis in breast cancer and predicts response to anti-HER2 therapy in breast cancer. The prognostic relevance of moderate expression of HER2 is unclear. Data of 9872 patients with primary nonmetastatic breast cancer from the cancer registries of Magdeburg and Halle, Germany, were analyzed retrospectively. A total of 5907 patients with complete data sets including follow-up were eligible for analysis. HER2 status was determined as recommended by international guidelines. Of 5907 patients investigated, 5023 (68.4%) had HER2 0 and 1+ expression and 884 (12.0%) had HER2 (2+)/HER2– expression. Patients with hormone receptor positive (HR+) and HER2 (2+) tumors had a shorter median disease-free survival (DFS; $P < 0.0001$) and breast cancer specific survival (BCSS; $P = 0.019$) than HR+ patients with HER2 (0/1+) tumors. Among patients with HR– breast cancer there was no significant difference between HER2 (2+) and HER2 (0/1+) tumors. In multivariate analysis after adjustment for other prognostic factors, HER2 (2+) status remained an unfavorable prognostic factor for DFS (hazard ratio (HR) = 1.217, 95% CI = 1.052–1.408; $P = 0.008$) but not for BCSS (HR = 1.045, 95% CI = 0.926–1.178; $P = 0.474$). The HER2 (2+) status is an unfavorable prognostic factor for survival of patients with HR+ breast cancer. The impact of anti-HER2 therapy in this group of patients should be evaluated.

Key Words

- HER2
- HER2 2+
- HER2 score
- breast cancer

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Introduction

The human epidermal growth factor receptor 2 (HER2) is a cell surface transmembrane receptor. HER2 amplification occurs in 20–30% of invasive breast cancers (Akiyama *et al.* 1986, Slamon *et al.* 1987, 1989, Gasparini *et al.* 1994) and is associated with poor prognosis (Slamon *et al.* 1987, Ross & Fletcher 1998, Berry *et al.* 2000, Gennari *et al.* 2008). The HER2 status is not only a prognostic but also a predictive factor for response to the anti-HER2 therapy,

e.g., trastuzumab (Slamon *et al.* 2011). Based on standardized criteria, HER2 expression has been divided on the basis of immunohistochemical (IHC) results into the following groups: 0, 1+, 2+, and 3+ (Jacobs *et al.* 1999). It is believed that HER2 tumors scored 0 and 1+ do not respond to trastuzumab therapy. On the contrary, a HER2 (3+) status implies strong expression of the receptor and is highly predictive for a response to this drug

(Ross & Fletcher 1998, Wolff *et al.* 2007). The moderate group of HER2 (2+) cancers requires an additional gene amplification assay (e.g., fluorescent *in situ* hybridization (FISH) or silver *in situ* hybridization (SISH) respectively) to identify patients who overexpress HER2 and benefit from anti-HER2 therapy (Ross & Fletcher 1998). Interestingly, in retrospective analysis with small patient cohorts, the moderate (2+) and low (1+) HER2 expression has been shown to be a negative prognostic survival factor for breast cancer patients (Gilcrease *et al.* 2009, Rossi *et al.* 2012). Patients with negative HER2 (0) expression demonstrated significantly longer median survival compared to patients with low HER2 (1+) and moderate HER2 (2+) expression respectively. Thus, the prognostic value of moderate HER2 (2+) expression requires reevaluation. In this study we retrospectively analyzed 5907 patients with breast cancer. The HER2 status was compared with clinical and pathological characteristics. The clinical outcome of patients with moderate HER2 (2+) expression was compared with that of HER2 negative (0/1+) patients.

Patients and methods

Patients

We investigated cases of female breast cancer included in the regional cancer registry of Saxony-Anhalt, federal state of Germany. The tumor registry contains information on diagnosis, age, tumor stage, receptor status, tumor grading,

lymph node status, date of diagnosis, date of disease recurrence, date of death, and the treatment regimens used. In our cohort study we analyzed women with primary non-metastatic breast cancer diagnosed between 2003 and 2013 in nine hospitals in Saxony-Anhalt, federal state of Germany: University Hospital Magdeburg, University Hospital Halle, Harzklinikum Dorothea Christiane Erxleben, Johanniter Clinic Genthin-Stendal, HELIOS Clinic Burg, and the AMEOS Clinics in Aschersleben, Halberstadt, Haldensleben, and Schönebeck.

The primary outcome of this study was disease-free survival (DFS) of patients with HER2 (2+) breast cancer compared to patients with HER2 (0/1+) breast cancer. Secondary endpoints were DFS of patients with hormone receptor positive (HR+)/HER2 (2+) breast cancer compared to patients with HR+/HER2 (0/1+) breast cancer; DFS of patients with HR–/HER2 (2+) breast cancer compared to patients with HR–/HER2 (0/1+) breast cancer; breast cancer specific survival (BCSS) of patients with HER2 (2+) breast cancer compared to patients with HER2 (0/1+) breast cancer; BCSS of patients with HR+/HER2 (2+) breast cancer compared to patients with HR+/HER2 (0/1+) breast cancer; and BCSS of patients with HR–/HER2 (2+) compared to patients with HR–/HER2 (0/1+) breast cancer.

We identified 9872 female patients with primary non-metastatic invasive breast cancer. In 2526 cases the HER2 status was unknown and these patients were excluded from this study (Fig. 1). Another 1329 patients with HER2

Figure 1

Study cohort. HER2, human epidermal growth factor receptor 2.

(3+) and 110 patients with HER2 (2+)/HER2+ expression were also excluded. A total of 5907 eligible cases were included in the study. The follow-up period ended with the patient's death or with the latest available information

in the tumor registry. This study was approved by the Research and Ethical Committee of Otto-von-Guericke University, Magdeburg, Germany. Patients gave written informed consent for data transfer to the tumor registry.

Table 1 Patient and tumor characteristics

Characteristics	No. of patients	HER2 status		P value
		Negative, n (%)	Moderate, n (%)	
Total	5907	5023	884	
Age (range)		63 (24–97)	63 (25–101)	0.103
Menopausal status				
Premenopausal	1175	994 (19.9)	181 (20.6)	0.648
Postmenopausal	4695	3997 (80.1)	698 (79.4)	
Tumor size (cm)				
≤2	4741	4048 (86.6)	693 (83.4)	0.017
>2	627	627 (13.4)	138 (16.6)	
Missing	539	348	191	
Lymph node status				
Negative	3820	3283 (68.0)	537 (62.9)	0.004
Positive	1863	1546 (32.0)	317 (37.1)	
Missing	224	194	30	
Histotype				
Ductal	4592	3883 (77.4)	709 (80.3)	0.019
Lobular	790	675 (13.5)	115 (13.0)	
Mixed	355	322 (6.4)	33 (3.7)	
Other	164	138 (2.8)	26 (2.9)	
Missing	6	6	0	
Grade				
1	1083	964 (19.5)	119 (13.8)	0.0001
2	3205	2730 (55.4)	475 (55.1)	
3	1505	1237 (25.1)	268 (31.1)	
Missing	114	92	22	
Ki-67				
≤14	544	452 (43.7)	92 (23.2)	0.0001
>14	887	583 (56.3)	304 (76.8)	
Missing	4476	3988	488	
ER status				
Negative	958	852 (17.8)	106 (12.5)	0.0001
Positive	4684	3938 (82.2)	746 (87.5)	
Missing	265	233	32	
PR status				
Negative	1403	1215 (25.4)	188 (22.1)	0.039
Positive	4234	3570 (74.6)	664 (77.9)	
Missing	270	238	32	
Operative therapy				
No	2	2 (0.01)	0	0.0001
Breast conserving	3934	3396 (69.7)	538 (62.8)	
Mastectomy	1791	1472 (30.2)	319 (37.2)	
Missing	180	153	27	
Radiation				
No	1687	1391 (29.8)	296 (34.7)	0.005
Yes	3833	3277 (70.2)	556 (65.3)	
Missing	387	355	32	
Chemotherapy				
No	3107	2661 (55.6)	446 (51.4)	0.024
Yes	2549	2099 (44.4)	422 (48.6)	
Missing	251	239	16	
Hormonal therapy				
No	2248	1888 (40.0)	360 (41.6)	0.386
Yes	3433	2837 (60.0)	506 (58.4)	
Missing	226	298	18	

HER2 status

The HER2 status was assessed using the HercepTest (Hamburg, Germany) according to the manufacturer's instructions. The IHC staining of HER2 is based on the staining intensity and distribution (Jacobs *et al.* 1999). The HER2 status is grouped as follows: 0, no staining; 1+, faint/barely perceptible membrane staining detected in >10% of the tumor cells; 2+, weak to moderate membrane staining >10% of the tumor cells; and 3+, strong, circumferential membrane staining in >30% of tumor cells. Very recently new updated guidelines for HER2 determination have been published (Wolff *et al.* 2013). FISH or SISH testing is routinely used to assess the *HER2* gene amplification in cases with moderate HER2 (2+) expression. Patients with positive HER2 expression defined as HER2 (3+) or HER2 (2+) by IHC and positive *HER2* amplification by FISH or SISH (HER2 (2+)/HER2+) ($n=1439$, 19.6%) were excluded (Fig. 1). The remaining 5907 patients were divided into two main groups (Fig. 1): HER2 negative ($n=5023$, 68.4%) and HER2 moderate defined as HER2 (2+) by IHC without gene amplification ($n=884$, 12.0%). The HER2 negative group consisted of 2374 (47.3%) HER2 (0), 1393 (27.7%) HER2 (1+), and 1256 (25.0%) HER2 negative cases with no exact scoring available (0 or 1+). The HER2 moderate group consisted of 884 (12.0%) cases with moderate expression according to IHC (HER2 (2+)) and with *HER2*– by FISH or SISH (HER2 (2+)/HER2–).

Statistical analysis

The statistical calculations were performed using SPSS version 21.0 (SPSS). The association between HER2 status and clinical and pathological variables was assessed using the χ^2 test or Fisher's exact test. The DFS and BCSS probability distribution was studied by the Kaplan–Meier method. The equality of survival curves was tested by the log rank test. DFS was defined as the time between primary diagnosis and disease recurrence of all types as well as breast cancer specific death. BCSS was defined as the time between primary diagnosis and breast cancer specific death. Patients who died of other causes or patients lacking follow-up data were censored. Univariate Cox proportional hazards regression analysis was used to identify significant prognostic factors and then the prognostic significance was evaluated using multivariate analysis. The statistical analyses were two sided and P values of <0.05 were considered statistically significant.

Figure 2

DFS probability according to HER2 status. DFS in the whole cohort (A), HR+ (B), and HR– (C) breast cancer is demonstrated. The log rank test was used to calculate the P value. HER2, human epidermal growth factor receptor 2; DFS, disease-free survival.

Results

Study design

Between January 2003 and December 2013, 9872 women with primary non-metastatic invasive breast cancer underwent medical treatment in nine hospitals. Patients with unknown HER2 status ($n=2526$) and patients with positive HER2 expression defined as HER2 (3+) or HER2 (2+) by IHC and positive *HER2*+ amplification by FISH or SISH (HER2 (2+)/HER2+) ($n=1439$) had to be excluded (Fig. 1). The remaining 5907 patients with a median age of 63 years (range 19–101 years) were eligible for analysis. Patients were divided into two groups based on their HER2 expression and *HER2* gene amplification (Fig. 1). Table 1 summarizes patient and tumor characteristics according to the HER2 IHC-score. Clinical data including surgery, chemotherapy, and hormonal therapy were also analyzed with regard to the HER2 score.

HER2 status and patient and tumor characteristics

Patient and tumor characteristics showed significant differences between the two groups. Moderate HER2 (2+) expression was associated with larger tumor size ($P<0.0001$), positive lymph node status ($P=0.017$), invasive ductal histologic type ($P=0.019$), higher tumor grade ($P<0.0001$), increased expression of Ki-67, lymph node metastases ($P=0.004$) and positive estrogen receptor (ER) ($P<0.0001$) and progesterone receptor (PR) status ($P=0.039$). Patients with moderate HER2 (2+) expression were more likely to receive chemotherapy ($P=0.024$) but significantly less likely to receive breast conserving surgery ($P<0.0001$) followed by radiation ($P=0.005$) in comparison to the patients with HER2 (0/1+) negative expression.

HER2 status and survival

HER2 (2+) moderate expression appeared to be a worse prognosticator for DFS in comparison to the HER2 (0/1+) negative cases in view of the first 10 years following diagnosis, without statistical significance (Fig. 2A, $P=0.072$). Interestingly, analysis of DFS according to HR status showed that HER2 (2+) moderate expression is an unfavorable prognostic factor in HR+ cases (Fig. 2B, $P<0.0001$) but not in HR– cases (Fig. 2C, $P=0.470$). HER2 (2+) moderate status was not associated with BCSS in the whole cohort (Fig. 3A, $P=0.659$). Patients with HR+ and moderate HER2 (2+) expression had been found to be associated with shorter median BCSS than HR+ and HER2

Figure 3 BCSS probability according to HER2 status. BCSS in the whole cohort (A), HR+ (B), and HR– (C) breast cancer is demonstrated. The log rank test was used to calculate the P value. HER2, human epidermal growth factor receptor 2; BCSS, breast cancer specific survival.

Table 2 Uni- and multivariate analysis of disease-free survival in hormone receptor positive patients

Parameter	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
HER2						
Negative vs moderate	1.272	1.112–1.454	0.0001	1.216	1.056–1.400	0.007
Age (years)						
≤45 vs >45	0.500	0.365–0.685	0.0001	0.493	0.280–0.870	0.015
Menopausal status						
Pre vs post	0.875	0.720–1.065	0.183	1.281	0.883–1.859	0.192
Tumor size (cm)						
≤2 vs >2	3.420	2.838–4.123	0.0001	1.709	1.244–2.347	0.001
Lymph node status						
Negative vs positive	2.799	2.357–3.325	0.0001	1.738	1.291–2.340	0.0001
Histologic grade						
1 vs 2 and 3	2.504	1.806–3.470	0.0001	2.707	1.662–4.409	0.0001
Operative therapy						
Breast conserving vs mastectomy	2.079	1.753–2.465	0.0001	1.332	1.030–1.805	0.039
Radiation						
No vs yes	0.964	0.809–1.150	0.688	1.010	0.735–1.389	0.950
Chemotherapy						
No vs yes	1.580	1.331–1.875	0.0001	1.078	0.819–1.419	0.790
Hormonal therapy						
No vs yes	0.762	0.615–0.946	0.014	0.877	0.646–1.191	0.401

(0/1+) negative patients (Fig. 3B, $P=0.018$). In HR– patients, HER2-status had no influence on BCSS (Fig. 3C, $P=0.544$).

Univariate analysis revealed that HER2 (2+) moderate status was a prognostic factor for poor DFS (hazard ratio (HR)=1.272, 95% CI=1.112–1.454; $P<0.0001$; Table 2) and BCSS (HR=1.139, 95% CI=1.022–1.270; $P=0.019$; Table 3) in HR+ breast cancer patients. In HR– breast

cancer there was no significant difference (Table 4). After adjustment for patients' age, menopausal status, tumor size, lymph node status, tumor grade, operative therapy, radiation, chemotherapy, and hormone therapy, multivariate analysis rendered HER2 (2+) as an independent, unfavorable prognostic factor for DFS (HR=1.217, 95% CI=1.052–1.408; $P=0.008$; Table 2) but not for BCSS in HR+ breast cancer patients (HR=1.045, 95%

Table 3 Uni- and multivariate analysis of breast cancer specific survival in hormone receptor positive patients

Parameter	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
HER2						
Negative vs moderate	1.139	1.022–1.270	0.019	1.070	0.952–1.201	0.256
Age (years)						
≤45 vs >45	2.376	1.508–3.745	0.0001	0.632	0.309–1.292	0.209
Menopausal status						
Pre vs post	3.230	2.557–4.079	0.0001	2.755	1.766–4.299	0.0001
Tumor size (cm)						
≤2 vs >2	2.999	2.581–3.485	0.0001	2.319	1.807–2.977	0.001
Lymph node status						
Negative vs positive	1.848	1.613–2.118	0.0001	1.438	1.143–1.808	0.002
Histologic grade						
1 vs 2 and 3	1.588	1.282–1.967	0.0001	1.814	1.329–2.475	0.0001
Operative therapy						
Breast conserving vs mastectomy	2.143	1.865–2.462	0.0001	1.225	0.962–1.560	0.100
Radiation						
No vs yes	0.531	0.465–0.606	0.0001	0.794	0.626–1.008	0.058
Chemotherapy						
No vs yes	0.468	0.407–0.537	0.0001	0.527	0.424–0.656	0.0001
Hormonal therapy						
No vs yes	0.294	0.257–0.338	0.0001	0.353	0.289–0.431	0.0001

Table 4 Univariate analysis of disease-free survival and breast cancer specific survival in hormone receptor negative patients

Parameter	DFS			BCSS		
	Hazard ratio	95% CI	P value	Hazard ratio	95% CI	P value
HER2						
Negative vs moderate	0.913	0.714–1.166	0.464	0.874	0.688–1.110	0.270
Age (years)						
≤45 vs >45	0.484	0.595–1.208	0.361	1.165	0.789–1.720	0.443
Menopausal status						
Pre vs post	0.852	0.664–1.093	0.207	1.340	1.022–1.757	0.034
Tumor size (cm)						
≤2 vs >2	2.423	1.829–3.209	0.0001	3.308	2.552–4.287	0.0001
Lymph node status						
Negative vs positive	2.615	2.075–3.297	0.0001	2.377	1.883–3.002	0.0001
Histologic grade						
1 vs 2 and 3	1.847	0.592–5.765	0.291	1.073	0.443–2.599	0.876
Operative therapy						
Breast conserving vs mastectomy	1.928	1.529–2.430	0.0001	2.436	1.919–3.093	0.0001
Radiation						
No vs yes	0.697	0.545–0.892	0.0001	0.474	0.376–0.598	0.0001
Chemotherapy						
No vs yes	1.010	0.737–1.385	0.948	0.424	0.330–0.546	0.0001
Hormonal therapy						
No vs yes	1.401	0.984–1.995	0.061	1.011	0.687–1.488	0.956

CI=0.926–1.178; $P=0.474$). Age <45 years old, increased tumor size, positive lymph node invasion, high histological grade, and mastectomy were additional unfavorable prognostic factors for DFS (Table 2). Menopausal status, increased tumor size, positive lymph node status, higher histological grade, and the administration of radiotherapy, chemotherapy, and hormonal therapy were associated with worse BCSS (Table 3).

Discussion

This is the largest study of its kind investigating the impact of HER2 (2+) moderate expression on breast cancer survival. Our data provide evidence that tumors with moderate HER2 (2+) expression with no gene amplification differ from cases with negative or low HER2 expression (0/1+). As expected HER2 positivity (3+) was associated with a poor clinical outcome and aggressive tumor behavior, which is consistent with recently published data (Templeton et al. 2014). In our study, HR+ breast cancer patients with tumors displaying moderate HER2 (2+) expression had a poorer DFS but not BCSS compared with low (1+) or negative (0) HER2 expression, confirming previous findings from smaller cohort studies (Gilcrease et al. 2009, Iorfida et al. 2012). In other studies, HER2 (2+) was a prognostic factor only in lymph node positive breast cancer (Menard et al. 2008). However, the impact of moderate HER2 (2+) expression on DFS by multivariate analysis after adjustment for other

prognostic factors has been not shown yet (Menard et al. 2008). HER2 (2+) moderate status is defined as ~500 000 detectable HER2 receptors on the cell surface (Ross & Fletcher 1998) required for the activation of underlying intracellular HER2 pathways to drive tumor growth and invasiveness. This hypothesis is supported by the finding that even low levels of HER2 (1+) expression involving 100 000 cell surface receptors (Ross & Fletcher 1998) could be associated with a poor outcome (Camp et al. 2003, Gilcrease et al. 2009, Iorfida et al. 2012). According to our data, HER2 (1+) tumors seem to have characteristics between HER2 (0) and HER2 (2+) cancers suggesting a possible linear correlation between HER2 expression and tumor behavior. Such results have been observed for prostate cancer granted that HER2 overexpression is a rare phenomenon (Minner et al. 2010). Tumors with low or moderate expression of HER2 (2+) might be more sensitive to different growth factors stimulating breast cancer cells because HER2 signaling is a key factor in breast cancer proliferation (Ross & Fletcher 1998). Interestingly, in our survey moderate expression of HER2 (2+) receptor was identified as an unfavorable prognostic factor in patients with HR+ tumors but not in those with HR– tumors. As already demonstrated, HER2 expression is inversely related to the expression of HR (ER and PR) and is associated with endocrine resistance (Ross & Fletcher 1998, Massarweh et al. 2008). One might speculate that low/moderate expression of HER2 indicates some activity of the HER2 pathway and thus an existing influence on

growth and proliferation via cross-talk with ER signaling. Notably, in our study HER2 expression was associated with increased proliferative features of the tumors: increased Ki-67 expression and reduced grade of proliferation.

HER2 overexpression is routinely used to predict response to anti-HER2 therapy and at present only patients with an IHC score 3+ and gene amplification should receive anti-HER2 therapy (Goldhirsch et al. 2013). It has been demonstrated that tumors with moderate HER2 (2+) expression without gene amplification do not respond to trastuzumab therapy (Slamon et al. 2011, Goldhirsch et al. 2013). However, recently published data suggest that a small number of patients with lower levels of HER2 (1+) may benefit from anti-HER2 treatment (Paik et al. 2008, Perez et al. 2010).

The association of low HER2 (1+) expression with patient survival has been investigated in a limited number of small sample-sized retrospective studies. Menard et al. (2008) conducted a retrospective study and were able to show that the HER2 (2+) status is associated with increased recurrence rates in patients with positive lymph-nodes. Notably, their cohort study identified 24.1% patients as HER2 (2+), whereas our cohort study identified only 12% patients as HER2 (2+). This difference might be due to the fact that Menard et al. made no distinction between HER2 (2+)/HER2+ and HER2 (2+)/HER2– tumors. Another explanation is the use of different IHC test systems. In other smaller studies with 1150 (Rossi et al. 2012) and 91 (Gilcrease et al. 2009) patients, HER2 (2+) and HER2 (1+) statuses, have also been reported to count as negative prognostic factor for DFS compared to HER2 (0) status.

Our results provide evidence that moderate expression of HER2 (2+) is biologically different from low and negative HER2 (0/1+) expression. We believe that the prognostic significance of moderate HER2 (2+) expression needs to be reevaluated as a predictor for anti-HER2 therapy. Notably, intratumoral heterogeneity of the HER2 gene is a characteristic property of tumors with low and moderate HER2 expression and is associated with decreased DFS (Barrett et al. 2007, Seol et al. 2012). Therefore, HER2 overexpression without gene amplification should be further evaluated, and the determination of the HER2 status and in particular the predictive value of HER2 (2+) expression should be reconsidered.

The main limitation of the present study is its retrospective character. However, this is a multicenter study with the largest cohort of HER2 (2+) cases to date. Our results suggest that HER2 (2+) tumors have a specific biological behavior associated with an unfavorable clinical

outcome. The hypothesis that HER2 (2+) tumors might benefit from an anti-HER2 therapy is going to be addressed in a prospective clinical trial (NSABP-47 trial).

Declaration of interest

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