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Serum HER2 supports HER2-testing in tissue at the time of primary diagnosis of breast cancer

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ABSTRACT

Aim: HER2 in tissue is of high prognostic value. Soluble HER2, the extracellular domain (ECD), has been suggested to be a helpful biomarker. We investigated whether there is a relationship between HER2 ECD and HER2 in tissue and whether this relationship could be used for diagnostic purposes.
Methods: HER2 ECD was measured in healthy individuals (N = 283, 184 females, 99 males), in patients with history of breast cancer (BC) with no evidence of disease (N = 249) as well as in BC patients before any treatment (N = 565). HER2 in tissue was determined by immunohistochemistry and HER2 ECD was analyzed by immunoassay.
Results: HER2 ECD levels were higher in healthy men than in healthy women (medians 12.9 ng/mL vs. 9.9 ng/mL, $p < 0.001$). We observed an age dependency in women that means the older the women the higher the HER2 ECD level. In treated BC patients there was only a weak difference between younger and older women. For patients without distant metastases as well as patients with metastatic disease we observed a correlation of HER2 in serum and tissue. The median concentrations of HER2 ECD were 11.7 ng/mL (13.2 ng/mL) for the HER2-negative (HER2-positive) patients in the non-metastatic-group ($p < 0.001$) and 11.9 ng/mL (16.0 ng/mL) in the metastatic-group ($p = 0.01$). Using a cut-off of 30 ng/mL the HER2 in tissue was always positive, corresponding to a specificity of 99.8% and a sensitivity of 10.3%.
Conclusions: There is a strong correlation between HER2 ECD and HER2 in tissue. HER2 ECD supports the HER2 testing in tissue and may reveal false-negative tissue findings.

1. Introduction

The HER2/neu gene (c-erbB2) expresses a 185 kDa transmembrane glycoprotein (HER2/neu) which belongs to the ErbB family of receptor tyrosine kinases. The HER2/neu protein consists of three domains: a 105 kDa extracellular domain (ECD), a transmembrane lipophilic segment and an intracellular domain with tyrosine kinase activity. HER2 ECD can be released by proteolytic cleavage from HER2 receptor and can be detected in serum [1,2].

Approximately 25–30% of breast cancers have gene amplification and/or overexpression of HER2 which results in a clinically more aggressive tumor type [3]. Increased HER2 ECD levels could be found in 18% of women with primary breast cancer and in 46% of patients with metastatic disease [4,5]. Elevated levels of HER2 ECD are also measured in patients with other malignancies like ovarian carcinomas, lung cancers and prostate cancers [6].

The approval of the recombinant humanized monoclonal antibody trastuzumab and the small molecule tyrosine kinase inhibitor lapatinib for the treatment of women with HER2-amplified breast cancer has dramatically improved the clinical outcome of HER2-positive breast cancer patients. It was the most relevant improvement in breast cancer treatment in the last years [7,8]. Recent data suggest that elevated levels of HER2 ECD may be associated with a lower probability of response to hormonal, chemotherapeutic and trastuzumab treatment in patients with metastatic breast cancer [9–12].

The aim of our retrospective analysis was to assess the correlation between serum HER2 ECD levels and tissue HER2 status and also the relationship between serum HER2 ECD levels and other clinicopathologic features (like age, menopausal status, tumor size, lymph node involvement, UICC stage, grading, and hormonal receptor status).

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2. Patients and methods

2.1. Patients

Between 1998 and 2007, we analyzed the sera of 565 patients treated for breast cancer at the University Hospital Klinikum Großhadern, Munich. Inclusion criteria for this retrospective analysis were known HER2 status in tissue and known serum level of HER2 ECD prior to any treatment. Median age at the time of primary diagnosis of breast cancer was 58 years (range 25–93 years). Tumor size using the UICC classification was mostly T1 in 274 (48.5%) and T2 in 182 (32.2%) patients. Approximately 55.0% of the patients had no positive lymph nodes, 25.8% had one to three positive nodes, and 7.1% had four or more positive nodes. The major pathologic type was invasive ductal carcinoma (89.3%). IHC assays showed overexpression of HER2/neu in tumors from 116 patients (20.5%) and hormone receptors in 448 (79.3%) patients including 418 (74.0%) positive for estrogen receptor (ER) and 363 (64.3%) positive for progesterone receptor (PR). Thirty-four patients (6%) presented with distant metastases at primary diagnosis (M1). Detailed information about patient characteristics is shown in Table 1.

All patients underwent operation (breast conserving surgery followed by irradiation or mastectomy ± irradiation) and systemic adjuvant therapy depending on clinicopathologic features.

As control groups, we investigated the sera of two hundred and eighty-three asymptomatic healthy individuals (184 females and 99 males) who gave blood voluntarily for scientific purpose between 2006 and 2007. Due to the younger age of the healthy women (median 37 years) in comparison to breast cancer patients (median 58 years) we also analyzed blood from 249 patients with a history of breast cancer. These patients participated in a prospective follow-up study in the Institute of Clinical Chemistry of the University Hospital Klinikum Großhadern, Munich. Approval of the institutional review board and written patient consent was obtained for this subgroup of patients. All patients entered into the study after completing adjuvant therapy including chemo- and radiotherapy. Adjuvant treatment was finished before August 2008 and all patients showed no evidence of disease for at least 18 months at the time of blood sample drawing. Median age at time of blood sample was 53.6 years (range 32.6–72.6 years).

Table 1
Clinical characteristics of 565 patients.

	All (N = 565)	M0 (N = 531)	M1 (N = 34)
Age, median (range), years	58 (25–93)	58 (25–89)	57 (36–93)
Tumor size, n (%)			
Tis	8 (1.4)	8 (1.5)	0 (0)
T1	274 (48.5)	269 (50.7)	5 (14.7)
T2	182 (32.2)	173 (32.6)	9 (26.5)
T3	27 (4.8)	23 (4.3)	4 (11.8)
T4	16 (2.8)	8 (1.5)	8 (23.5)
Unknown	58 (10.3)	50 (9.4)	8 (23.5)
Axillary lymph nodes, n (%)			
N0	311 (55.0)	309 (58.2)	2 (5.9)
N1	146 (25.8)	129 (24.3)	17 (50)
N2	30 (5.3)	26 (4.9)	4 (11.8)
N3	10 (1.8)	9 (1.7)	1 (2.9)
Unknown	68 (12.0)	58 (10.9)	10 (29.4)
Grading, n (%)			
G1	60 (10.6)	60 (11.3)	0 (0)
G2	307 (54.3)	294 (55.4)	13 (38.2)
G3	184 (32.6)	168 (31.6)	16 (47.1)
G4	2 (0.4)	0 (0)	2 (5.9)
Unknown	12 (2.1)	9 (1.7)	3 (8.8)
Hormone receptors, n (%)			
ER/PR positive	448 (79.3)	424 (79.8)	24 (70.6)
Both negative	117 (20.7)	107 (20.2)	10 (29.4)
HER2/neu-status, n (%)			
0	247 (43.7)	231 (43.5)	16 (47.1)
1+	140 (24.8)	137 (25.8)	3 (8.8)
2+	68 (12.0)	64 (12.1)	4 (11.8)
3+	110 (19.5)	99 (18.6)	11 (32.4)
HER2/neu-status, n (%)			
Negative (0/1+ or 2+/FISH−)	409 (72.4)	388 (73.1)	21 (61.8)
Positive (3+ or 2+/FISH+)	116 (20.5)	104 (19.6)	12 (35.3)
HER2+/FISH unknown	40 (7.1)	39 (7.3)	1 (2.9)
Menopausal status, n (%)			
Premenopausal	141 (25.0)	134 (25.2)	7 (20.6)
Postmenopausal	398 (70.4)	373 (70.2)	25 (73.5)
Unknown	26 (4.6)	24 (4.6)	2 (5.9)

2.2. Serum HER2 ECD assays

Serum samples were collected at the time of cancer diagnosis before treatment and stored at −80 °C. Serum HER2 ECD was measured by a 2-site chemiluminescence sandwich immunoassay (ADVIA Centaur System, Bayer HealthCare LLC, Diagnostics Division, Tarrytown, NY, USA). Measurements were performed strictly according to the manufacturer's instructions and quality control was ensured. The serum assays were performed completely blinded to the clinical outcomes.

2.3. Tissue HER2 determined by IHC and FISH

Tissue samples were obtained during operations or as biopsies and were formalin-fixed and embedded in paraffin by standard methods. Status of estrogen receptor (ER), progesterone receptor (PR) and HER2 were assessed on tumor samples by standard immunochemistry (IHC) methods.

The DAKO scoring system for HER2/neu was defined as negative for scores of 0 as well as 1+ and positive for tumors with a score of 3+. Fluorescence in situ hybridization (FISH) was applied to tumors in the case of a score of HER2 2+ to ensure HER2 gene amplification. DAKO-Score 2+ and gene amplification in FISH analysis were also regarded as HER2/neu-positive tumors. All tissue assays were performed completely blinded to the clinical outcomes in the Institute of Pathology, Klinikum Großhadern, University Hospital, Munich.

2.4. Statistical analyses

Data were analyzed using the statistical package SAS (V 9.2, SAS Inc., Cary, NC). $p < 0.05$ was considered to indicate statistical significance. Data are represented graphically as single values and medians.

Association of positive HER2 status in tissue with other tumor characteristics and clinical features was evaluated by means of chi-square test.

To compare HER2 ECD levels between subgroups, the Wilcoxon rank-sum test in the case of two subgroups and the Jonckheere–Terpstra test in the case of more than two ordered subgroups were used, respectively. Simultaneous influence of tumor characteristics on HER2 ECD levels was tested by multivariate analysis of variance using SAS procedure GLM. HER2 ECD levels were transformed into ranks and all effects, significant in univariate analysis, were included.

Sensitivity and specificity of HER2 ECD to predict a positive HER2-status in tissue were graphically represented by means of a receiver operating characteristic (ROC) curve, and the area under the curve (AUC) was calculated. HER2 status in tissue also was the target variable in logistic regression analysis, and the best model including HER2 ECD, estrogen receptor (ER) status, and grading (G) was visualized by a ROC curve, too.

3. Results

3.1. Serum HER2 ECD levels in healthy individuals, patients with no evidence of disease (NED) and untreated breast cancer patients

In the healthy group there was a significant difference between men and women. Male healthy individuals had higher HER2 ECD levels than