

## Clinical significance and prognostic value of serum sHER-2/neu levels in patients with solid tumors

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Received: 18 January 2008 / Accepted: 22 September 2008 / Published online: 15 October 2008  
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**Abstract** The purpose of this study was to determine HER-2/neu in the serum of patients with solid tumors and to investigate its potential usefulness in predicting the clinical course of the disease. At the same time, we compared the ability of serum HER-2/neu, CA15.3, CA12-5, CA19-9, carcino embryonic antigen (CEA), and  $\alpha$ -feto-protein (AFP) in breast, colorectal, and lung cancer patients. Forty, thirty-six, and twenty-three patients with lung, colon and breast cancer were included in this study, respectively. Serum levels of HER-2/neu, CA15.3, CA12-5, CA19-9, CEA, and AFP were measured. Her-2 neu levels were significantly higher in the breast cancer groups than colorectal and lung cancer and controls groups ( $P < 0.01$ ). There is no significant difference when compared with others groups ( $P > 0.05$ ). There was a positive correlation between the HER-2/neu and CA15-3 values in breast cancer groups. We found 0.75(0.59–0.90) for Her-2/neu from the area under

the curve (AUC).  $P$ -value for breast cancer is 0.003, and we discovered that 9 ng/ml was the best intersection point. In this situation, we calculated that sensitivity was 65.2%, specificity was 100%, positive predictive value was 100%, negative predictive value 75.8%, and accuracy was 83.4%. These findings indicate that serum HER2/neu levels are clinically valuable in monitoring metastatic breast cancer and non-small cell lung cancer patients. Prognosis of breast cancer provides an additional value over the commonly used CA15-3 test. Measurements of levels of serum HER-2/neu provide prognostic and predictive information to the clinician and can especially be used for monitoring metastatic breast cancer patients. Further clinical validation is needed to confirm these findings.

**Keywords** Solid tumor · Cancer · HER-2/neu · Prognostic factor · Tumor marker

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### Introduction

The HER-2 gene encodes for p185, a 185-kDa transmembrane tyrosine kinase growth factor receptor belonging to the epidermal growth factor receptor family. The specific ligand for p185 is unknown [1]. Both HER-2 gene amplification and overexpression have important biological implications, as they represent prognostic markers and predict breast tumor responsiveness to conventional therapies [2]. HER-2/neu (C-erbB-2) is a self-protein expressed in a variety of tissues of epithelial origin and it plays a fundamental role in cellular proliferation and differentiation during fetal development. In adults, the HER-2/neu gene presents as a single copy in normal cells; however, amplification of the gene and resultant protein overexpression are seen in various cancers including

breast, ovarian, uterine, colorectal, gastric, prostate, and adenocarcinoma of the lung [2–8]. Furthermore, the overexpression of HER-2/neu is implicated in the malignant transformation of breast cancer and is a biologically relevant protein in the pathogenesis of several other epithelial-based tumors, for example leading to the development of hormone resistance in prostate cancer [9].

The extracellular domain (ECD) of the Her-2/neu protein (Her-2/neu ECD) is cleaved and shed from the receptor and can be detected in serum as a protein of ~105 kDa. The shedding is regulated actively by a proteolytic process [10]. Since HER-2/neu bearing epithelial cells shed the ECD into the serum, serum HER-2/neu levels can be detected by enzyme-linked immunosorbent assays (ELISA). Elevated serum HER-2/neu levels in breast cancer patients following primary surgery as well as immediately after the completion of adjuvant chemotherapy provide prognostic information. These results suggest that those patients exhibiting elevated levels of serum HER-2/neu might benefit from specific anti-HER-2/neu-target treatment options early in the course of their disease, i.e., in the adjuvant setting [11].

In the present study, we determined the levels of ECD of HER-2 (sHER-2) in the serum of patients with solid tumor and investigated its potential usefulness in predicting the clinical course of the disease. We compared the ability of serum HER-2/neu, CA15.3, CA12-5, CA19-9, carcino embryonic antigen (CEA), and  $\alpha$ -feto-protein (AFP) in breast, colorectal, and lung cancer patients. At the same time, we evaluated to associate these levels with other prognostic factors like age, menopausal status, stage of disease, node, and hormone receptor status in women with primary breast cancer.

## Materials and methods

### The study population

This case–control study was conducted at the Department of Internal Medicine, Cerrahpasa Medical Faculty, University of Istanbul, Turkey. A total of 99 patients were admitted during the period. Twenty-five age- and sex-matched healthy women were enrolled into this study. Cases either study group or control, who had pathologies that could cause secondary lipid disorders, cardiovascular diseases, diabetes mellitus, renal failure, chronic infection and inflammation, alcohol abuse, and those who used antilipidemic and antioxidant drugs were excluded from the study. All patients with previously performed chemotherapy, radiotherapy, and surgery were also excluded from the study.

The remaining 40 patients with previously untreated, histopathologically verified newly diagnosed as of lung cancer (LC). Subjects' history and physical examinations were documented. The diagnosis of LC was based on histopathologic findings. Of 40 patients, 7 were with small cell LC, 33 patients were with non-small cell LC (NSCLC).

This study included 23 consecutive patients with primary breast cancer, who attended the Faculty of them, had distant metastases at the time of diagnosis. We evaluated clinicopathological features (histology, menopausal status, estrogen receptor (ER), and progesterone receptor (PR) status, number of axillary lymph nodes involved, grade, tumor size, and stage according to the American Joint Committee on Cancer staging system).

Thirty-six patients with newly diagnosed and histologically confirmed primary colorectal cancer were included in this prospective study. Tumor staging was performed according to the Dukes' and TNM classifications.

The protocol for sample collection was approved by the Istanbul University Cerrahpasa Medicine Faculty Ethics Committee. The study was performed in accordance with the Helsinki Declaration and informed consent was obtained from all patients and controls prior to their inclusion in the study.

### Serum preparation

Blood was drawn after 12–14 h of fasting in the morning. Serum were obtained after at least 30 min of clotting by centrifugation at 2,500g for 15 min. Serum were removed and used directly for measurements of biochemical parameters and tumor markers. Other serum were stored at  $-70^{\circ}\text{C}$  until assayed for determination of all parameters. All icteric or hemolytic blood samples were discarded. All parameters were analyzed in all samples together in a single batch, after we had finished our protocol (control and patient samples were analyzed in the same batch).

Tumor markers (CEA, CA125, CA19-9, CA15-3, AFP) were measured using an IMMULITE 2000 (DPC, Los Angeles, CA). CA15-3, CA125, and AFP were analyzed using the chemiluminescent immunometric assay. CA19-9 and CEA were measured by immunometric assay.

### Serum HER-2/neu levels

The levels of serum HER-2/neu were measured by ELISA. An anti-sp185<sup>HER-2</sup> human monoclonal coating antibody (Bender MedSystem, Vienna, Austria) was adsorbed on to micro wells. sp185<sup>HER-2</sup> present in the sample or standards bound to antibodies adsorbed to the micro wells; a horse

radish peroxidase (HRP)-conjugated monoclonal anti-sp185<sup>HER-2</sup> antibody was then added to the wells. Following incubation, unbound enzyme-conjugated anti-sp185HER-2 was removed by washing, and substrate tetramethylbenzidine (TMB) solution reactive with HRP was added to the wells. A colored product was formed in proportion to the amount of soluble p185HER-2 present in the sample. The reaction was terminated by addition of sulfuric acid, and absorbance was measured at 450 nm. The sHER-2 concentration was measured according to the manufacturer's instructions. The intra-assay coefficient of variation (CV) was less than 6% and the interassay CV was 9%. An investigator who was blinded to diagnosis and clinical details performed all assays.

### Statistical analysis

The statistical analysis was made with SPSS (Statistical Package for Social Sciences 10.0). All data were expressed as median and min–max excluding age. In the current study, we have performed one way analysis of variance (ANOVA), Kruskal–Wallis, and Mann–Whitney *U* tests for the various statistical comparisons. Our statistical consultant advice that sample size for the each patient group should be equal to or more than 20 to get an accurate estimates. Sample size for each patient group were not less than 20 in the current study. ANOVA for age, Kruskal–Wallis, and Mann–Whitney were used for others parameters. Correlations between changes in variables were tested using Pearson's correlation.  $P < 0.05$  was considered statistically significant. The receiver-operating characteristic (ROC) analysis was performed to determine the optimal cutoff value in terms of sensitivity and specificity for detecting HER-2 by ELISA in breast cancer.

### Results

Serum levels of tumor markers and HER-2/neu in studied groups were given in Table 1. Age was not different among the groups ( $P > 0.05$ ).

CA12-5 levels were significantly higher in the LC groups than in the breast cancer and control groups. CA12-5 levels were significantly higher in the colorectal cancer and control groups than in the breast cancer groups ( $P < 0.001$ ).

CEA levels were significantly higher in the lung and colorectal cancer groups than in the breast cancer and control groups ( $P < 0.01$ ). There is no significant difference when compared with others groups ( $P > 0.05$ ).

AFP levels were significantly higher in the colorectal cancer and control groups than in the breast and LC groups ( $P < 0.01$ ). There is no significant difference when compared with others groups ( $P > 0.05$ ).

Her-2 neu levels were significantly higher in the breast cancer groups than colorectal and LC and controls groups ( $P < 0.01$ ). There is no significant difference when compared with others groups ( $P > 0.05$ ).

In case of a lung and breast cancer, Her-2/neu levels were significantly higher in groups with metastase than in non-metastase groups ( $P < 0.05$ ). However, in case of a colorectal cancer, there is no significant difference in groups when compared with metastase or non-metastase groups ( $P > 0.05$ ) (Table 2).

There is no correlation between Her-2/neu levels and CA15-3, CA12-5, CA19-9, CEA, and AFP levels in the colorectal and LC groups ( $P > 0.05$ ). There was a positive correlation between the HER-2/neu and CA15-3 values in the breast cancer groups ( $r = 0.723$ ,  $P < 0.001$ ). There is no correlation between Her-2/neu levels and CA12-5, CA19-9, CEA, and AFP levels in the breast cancer groups ( $P > 0.05$ ). There is association between serum and tissue Her-2/neu levels in a range of tumors including carcinomas of the breast.

Our study showed that increased serum HER-2/neu levels were not significantly related with age, clinical stage of disease, node, and hormone receptor status.

The diagnostic performances of serum Her-2 neu levels were examined by ROC analysis, in the breast cancer groups ( $n = 23$ ), and the results are shown in Fig. 1. To distinguish the breast cancer group from the control group and to determine the best intersection point, we have done ROC analysis. From this analysis, we found 0.75(0.59–0.90) for Her-2 neu from the area under the curve (AUC). *P*-value for breast cancer is 0.003, and we discovered that 9 ng/ml was the best intersection point. In this situation, we calculated that sensitivity was 65.2%, specificity was 100%, positive predictive value was 100%, negative predictive value 75.8%, and accuracy was 83.4%.

### Discussion

The specific clinical benefits of serum HER-2/neu testing are being clarified by numerous investigations ongoing worldwide. Several studies indicate a role for forecasting of disease-free and overall survival (i.e., prognostication). Other trials, specifically in metastatic breast cancer populations, patient subsets with elevated baseline serum HER-2/neu levels vs. the subgroup with a normal serum HER-2/neu baseline concentration are compared for predicting response to treatment (i.e., a predictive role). Both HER-2/neu gene amplification and protein overexpression can be evaluated in tumor tissue. However, circulating levels of serum HER-2/neu can also be used to evaluate the HER-2/neu status. Since HER-2/neu bearing epithelial cells shed

**Table 1** Serum levels of tumor markers and HER-2/neu in studied groups

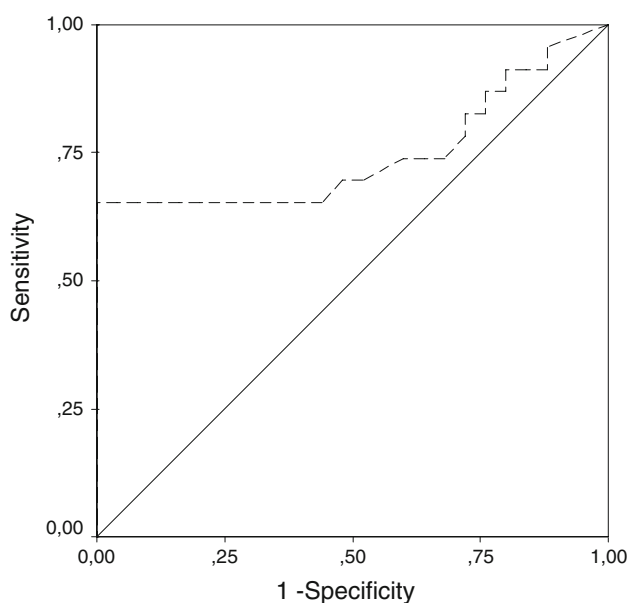
	Kontrol ( <i>n</i> :25)	Lung ( <i>n</i> :40)	Colon ( <i>n</i> :36)	Breast ( <i>n</i> :23)	<i>P</i>
Years	46.16 ± 5.44	59.94 ± 11.85	54.12 ± 11.97	53.86 ± 10.95	.102
	Median(min–max)	Median(min–max)	Median(min–max)	Median(min–max)	
CA15-3 (U/mL)	13.3(4–24)	32.0(7–470)	22.0(7–92)	23.0(8–142)	.198
CA12-5 (U/mL)	14.3(4–27)	20.5(2–140)	16.0(2–296)	6.0(2–18)	.001***
CA19-9 (U/mL)	5.26(2–14)	7.0(1–485)	13.0(1–1200)	5.0(1–83)	.075
CEA (ng/mL)	1.24(1–2)	5.0(1–1424)	3.0(1–2249)	1.0(0–12)	.002**
AFP (IU/mL)	3.35(1–6)	2.0(1–19)	3.0(1–39)	1.0(1–6)	.003**
Her-2/neu (ng/ml)	7.5(2–9)	5.0(2–63)	5.6(4–38)	11.4(2–21)	.002**

\*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$

**Table 2** Serum levels of HER-2/neu in groups with metastases

HER-2/neu	Metastasis(–) Median(min–max)	Metastasis(+) Median(min–max)	<i>P</i>
Lung (23M–, 15M+)	4.45(2–8)	6(2–63)	0.036*
Colon (12M–, 22M+)	5.4(4–38)	5.6(4–36)	0.968
Breast (16M–, 7M+)	9.95(2–17)	14.6(7–21)	0.013*

\*  $P < 0.05$

**Fig. 1** ROC plots for the diagnostic accuracy of serum Her-2/neu levels in breast cancer ( $n = 23$ )

the ECD into the serum, serum HER-2/neu levels can be detected by ELISA [11].

We and other investigators [12–15] have reported increased soluble HER-2/neu in the plasma or serum of patients with metastatic breast cancer. Many reports have shown that increased serum HER-2/neu is associated with a worse prognosis. Here, in addition, we determined that 9 ng/ml serum HER2 ECD showed 65.2% specificity and 100% sensitivity, positive predictive value 100%, negative predictive value 75.8%, and accuracy 83.4% in predicting

tissue HER2. We and Kong et al. [16] suggest that value can be used as the cutoff because we focused on a cutoff concentration at which the false-positive rate for tissue positivity was low. Determination of serum HER2 ECD concentration in metastatic breast cancer may provide a useful index of tissue HER2 status, especially in light of its relatively easy and inexpensive methodology and the simplicity of sample collection.

Antibodies similar to those used for HER-2/neu protein detection in breast tumor tissues have been applied to human serum samples to test whether circulating HER-2/neu receptor protein levels can predict the progression of breast cancer [17]. Serial serum HER-2/neu protein levels did not correlate with the clinical course in a group of patients with metastatic breast carcinoma treated with second-line hormonal therapy [18].

Serum HER-2/neu remains a significant independent predictive and prognostic factor in hormone receptor-positive metastatic breast cancer even when adjusted for tumor burden as measured by CA15-3. The combination of increased serum HER-2/neu and increased serum.

CA15-3 predicted a poorer prognosis compared with increased CA15-3 alone [15]. There is no correlation between Her-2/neu levels and CA12-5, CA19-9, CEA, and AFP levels in breast cancer groups except for CA15-3 values. In this study, the combination of increased serum HER-2/neu and serum CA15-3 predicted a strong prognosis in breast cancer. Mansour et al. [19] have found that the serum HER2 level was a better prognostic parameter than the tissue expression of HER2, suggesting that the shedding of the soluble fragments of HER2 into the serum may be a characteristic of the malignant cell. Although,

Goksel et al. [20] demonstrated that there was no significant difference in the levels of serum Her-2/neu between early-stage breast cancer patients and healthy controls. No statistically significant relationships were found between the levels of serum Her-2/neu and various characteristics of the patients and their disease, such as disease stage, age, menopausal status, and hormone receptor status.

The present study failed to find any correlation for serum Her-2/neu levels with any of the other variables measured in LC patients. Although, in case of a LC, Her-2/neu levels were significantly higher in groups with metastas than in non-metastatic groups. Heinmoller et al. [21] reported that circulating HER-2/neu protein is an independent prognostic factor in patients with advanced NSCLC. Its pretreatment measurement in clinical practice may be a useful addition to the assessment of other classic clinical and pathologic variables for a better prediction of survival outcome. Ardizzoni et al. [22] have shown that serum HER2 ECD levels >15 ng/ml were found in 31 of 511 samples (6%; median 19.6 ng/ml; mean 22.5 ng/ml). The serum HER2 ECD concentration in NSCLC patients ranged from 0 to 47.4 ng/ml. Patients with positive fluorescence in situ hybridization (FISH) result tend to have higher serum HER2 levels compared with immunohistochemistry (IHC)-positive patients. ELISA would have detected several patients without IHC/FISH-positive disease. FISH and IHC assess the level of HER2 gene amplification and HER2 protein overexpression, respectively, directly in tumor cells, whereas retaining the characteristic morphology of the tissue studied. In contrast, the ELISA evaluates the ECD of HER2, which is shed from tumor cells into the blood. If sufficiently predictive, ELISA would be an ideal technique for the evaluation of HER2 status, because it is rapid and economical, and the use of blood samples means that no tissue sample is necessary. Our study showed that increased serum HER-2/neu levels were not significantly related with age, clinical stage of disease, node, and hormone receptor status.

In our study, there was no correlation between serum HER-2/neu and CA15-3, CA12-5, CA19-9, CEA, and AFP levels in colorectal and LC groups which may reflect differences in the sensitivity of different tumor markers for the diagnosis of relapse, heterogeneity of tumor-associated antigen expression and variations in the kinetics of epitope release [23].

In our findings, serum Her-2/neu levels in patients with colon cancer were not significantly different from those of healthy controls. There was no correlation between circulating c-erbB-2 and CEA levels in stage-matched cases in colorectal cancer. Tsigris et al. [24] investigated that colorectal cancer patients had higher serum c-erbB-2 levels when compared with healthy controls, with a significant association between preoperative levels and both disease

stage and the presence of liver metastases. All of these areas will require prospective study along with its role as a novel tumor target in colorectal cancer employing anti-HER-2 specific therapies.

Retrospective studies have shown that HER2/neu gene amplification, measured using FISH, is the best predictive marker of response to trastuzumab-based therapy [25]. Nevertheless; because the manual and automated ELISA testing methods of serum HER2 are standardized and is not invasive, expensive, taking too long time, this method is preferable.

Measurements of levels of serum HER-2/neu provide prognostic and predictive information to the clinician and can especially be used for monitoring the metastatic breast and LC patients. Further clinical validation is needed to confirm these findings.

**Acknowledgements** This work was supported by Istanbul University Research Fund (Project No: 428/13092005).

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