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SERUM HER2 LEVELS ARE INCREASED IN PATIENTS WITH CHRONIC HEART FAILURE

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ABSTRACT

Background

The use of trastuzumab, an antibody directed against HER2, in patients with HER2 positive metastatic breast cancer, is related to an increased incidence of heart failure, suggesting that HER2 is required in the normal heart. The cleaved extracellular domain of HER2 can be measured in serum. We evaluated whether serum human epidermal growth factor receptor 2 (HER2) levels are increased in heart failure patients and related to disease severity or circulating apoptosis-related cytokines.

Patients and methods

Serum HER2 and plasma levels of tumor necrosis factor (TNF)-α, soluble (s) TNF-receptor (R) 1, and sTNF-R2, were determined by enzyme-linked immunosorbent assay (ELISA) in heart failure patients and compared to age and gender matched healthy controls.

Results

Serum HER2 was higher ($P = 0.013$) in 50 heart failure patients (32 male and 18 female; median age 57 (range 33-77) years), mean 12.1 ±SD 2.3 ng/mL, than in 15 controls, 10.4 ±2.6 ng/mL. Serum HER2 levels were highest among NYHA class III patients, followed by NYHA class II patients. Controls had the lowest serum HER2 levels ($P = 0.029$, Kruskal-Wallis test). Serum HER2 correlated inversely with LVEF ($P = 0.037$). No differences were observed in plasma TNFα levels, but sTNF-R1 ($P < 0.001$) and sTNF-R2 ($P = 0.015$) were higher in patients than in controls, and correlated positively with serum HER2 ($P = 0.027$ and $P = 0.036$, respectively).

Conclusions

The increased serum HER2 levels in heart failure patients could suggest that HER2 plays a role in the pathophysiology of heart failure. HER2 may be involved in cardiac apoptosis, based on the association with increased sTNF-R plasma levels.

INTRODUCTION

An increased incidence of heart failure is observed with the use of trastuzumab, a monoclonal antibody against the human epidermal growth factor receptor 2 (HER2), also known as erbB-2, in the treatment of HER2-positive metastatic breast cancer patients.[1] The pathophysiological mechanism of trastuzumab-related heart failure is still unclear. HER2 is indispensable for normal cardiac development in animals.[2] Additionally, mice with a cardiac-restricted *HER2* gene deletion developed severe dilated cardiomyopathy, after they were born without overt phenotypic abnormalities and at normal Mendelian frequencies.[3,4] This coincided with increased apoptosis in the left ventricles of these mice.[5] Based on these data, HER2 is considered to play a role in cardiac pathophysiology.

In heart failure due to dilated cardiomyopathy (DCM) or ischemic heart disease (IHD), apoptosis leading to progressive myocellular loss, plays a pathophysiologic role.[6,7] Circulating levels of tumor necrosis factor (TNF)-α and its soluble (s) receptors, TNF-R1 and TNF-R2, which are elevated in patients with chronic heart failure, are associated with heart failure severity,[8-10] and increased cardiomyocyte apoptosis in these patients.[11]

The extracellular domain of the transmembrane HER2 protein can be proteolytically cleaved from the cell membrane. After shedding into the circulation, this HER2 extracellular domain can be measured in serum with an enzyme-linked immunosorbent assay (ELISA). Patients with HER2 positive breast cancer have high serum HER2 levels, which correlate positively with the number of metastatic sites.[12]

To date, no knowledge is available regarding serum HER2 levels in patients with heart failure. It is hypothesized that HER2 is involved in compensatory mechanisms following cardiac stress. Anthracyclines for instance, can induce cardiac stress through the induction of free oxygen radicals.[13] The increased cardiac stress may lead to upregulation of cardiomyocyte-expressed HER2, rendering these cardiomyocytes more susceptible to trastuzumab. We hypothesize that HER2 is involved in chronic heart failure, reflected by an increase in serum HER2 concentrations in heart failure patients. The aim of the present study was to evaluate circulating HER2 concentrations in patients with chronic heart failure, caused by idiopathic DCM or IHD. In addition, we evaluated whether serum HER2 is associated with plasma levels of the apoptosis-related proteins TNF-α, sTNF-R1 and sTNF-R2.

PATIENTS AND METHODS

Subjects

Consecutive patients who visited the outpatient clinic of the Thorax Center of the University Medical Center Groningen, The Netherlands, for chronic heart failure were asked to participate in the study. Exclusion criteria were unstable cardiac

disease, such as decompensated heart failure or unstable angina pectoris, prior anthracycline-based chemotherapy, and serious uncontrolled concurrent disease, or concurrent malignancy.
A control group consisting of gender and age-matched healthy volunteers was recruited by an advertisement. None of the volunteers were admitted to the hospital, had acute or chronic illness, or reported symptoms related to the cardiovascular system. All subjects gave their written informed consent, in accordance with the approval of the local medical ethics committee.

Cardiac evaluation
Signs and symptoms of heart failure were classified according to the New York Heart Association (NYHA) functional class. Radionuclide angiography or cardiac ultrasound was performed to determine left ventricular ejection fraction (LVEF). Left ventricular end systolic and diastolic diameter (LVESD and LVEDD) were determined with echocardiography. Peak oxygen consumption was assessed using exercise testing with gas exchange analysis.

Blood sampling
For the determination of serum HER2, B-type natriuretic peptide (BNP), TNF-α, sTNF-R1 and sTNF-R2, peripheral blood samples were collected and transferred in 10 mL disposable tubes, tubes containing 2-natrium-ethylenediamine tetra-acetic acid (EDTA), heparin or no additive. After sampling, tubes were placed on ice immediately. Serum or plasma was separated within 30 min of collection by centrifugation at 4 °C, and stored at –80 °C until determination. HER2 extracellular domain concentrations were measured in serum, BNP and TNF-α in EDTA plasma. Heparin plasma was used for quantification of sTNF-R1 and sTNF-R2.

Serum HER2, apoptosis markers and neurohormones
BNP was assessed with an immunoassay (Abbott Laboratories, Abbott Park, IL, USA). Serum HER2 levels were measured using a sandwich enzyme assay (HER-2/ECD Assay, Oncogene Sciences, Cambridge, MA, USA). The assay has a cut-off value of 15 ng/mL, based on serum HER2 levels in breast cancer patients, which correspond with tumor HER2 expression.[14] Plasma TNF-α, sTNF-R1 and sTNF-R2 were determined using commercially available enzyme-linked immunosorbent assays (Quantikine, R&D systems, Minneapolis, MN, USA) following the manufacturer's instructions.

Statistics
Values are given in median (range). Quantitative variables were compared between two groups using a Mann-Whitney-U test for skewed distributed variables. For comparisons between more than two groups, a Kruskal-Wallis test was used. Correlations between variables were calculated using Pearson's correlation

coefficient test. All P-values were two-sided and $P < 0.05$ was considered statistically significant.

RESULTS

Clinical characteristics

Clinical characteristics are presented in table 1. The study population consisted of 50 patients with moderate to severe chronic heart failure. Medical treatment for heart failure consisted of angiotensin-converting enzyme inhibitors or angiotensin receptor-2 antagonists, β-blockers, diuretics, nitrates and/or digoxin. BNP values were higher in patients, than in controls ($P<0.01$), and higher in NYHA III than in NYHA II patients ($P < 0.05$) (Table 1). LVEF values correlated inversely with BNP values ($R = -0.385$, $P = 0.012$) and positively with peak oxygen consumption values ($R = 0.284$, $P = 0.046$). The control group consisted of 15 gender and age-matched healthy subjects (10 men and 5 women) with a median age of 56 (range 39-70) years.

Table 1. Clinical characteristics

	Controls (N=15)	Patients (N=50)	Patients according to NYHA classification	
			II (N=31)	III (N=19)
Age (years)	56 (39-70)	57 (33-77)	58 (33-77)	56 (34-68)
Male/Female	10/5	32/18	19/12	13/6
Cause of heart failure				
DCM	N/A	28	19	9
IHD	N/A	22	12	10
Diabetes mellitus	0	5	5	0
Hypertension	0	7	4	3
Smoking	3	6	3	3
LVEF	N/A	0.23 (0.10-0.54)	0.27 (0.12-0.54)	0.22 (0.10-0.40)†
Peak oxygen consumption (mL/kg min^{-1})	N/A	15.8 (4.2-19.8)	17.0 (8.7-19.8)	14.4 (4.2-18.5)†
BNP (pg/mL)	32 (9-273)	279 (5-1938)*	216 (12-1545)*	519 (5-1938)*†

Values are presented as median (range). * $P < 0.01$ compared to controls. † $P < 0.05$ compared to NYHA class II patients

Serum HER2 and plasma apoptosis markers

Serum HER2 levels were higher in heart failure patients, compared to controls ($P = 0.013$) (Table 2). Plasma sTNF-RI and sTNF-RII were also higher in patients, than in controls ($P < 0.001$ and $P = 0.015$, respectively).

Table 2. Serum HER2, plasma TNFα and sTNF-R levels

	Control group (N=15)	Heart failure patients(N=50)	P-value
HER2 (ng/mL)	10.4 ± 2.6	12.1 ± 2.3	0.013
TNF-α (pg/mL)	4.3 ± 7.0	3.6 ± 6.3	0.889
sTNF-R1 (ng/mL)	0.8 ± 0.3	1.4 ± 0.5	< 0.001
sTNF-R2 (ng/mL)	2.0 ± 0.5	2.6 ± 0.8	0.015

Values are presented as mean ± SD

When comparing NYHA class III to NYHA class II and controls, NYHA class III patients had the highest circulating levels of HER2 (P = 0.029), sTNF-R1 (P < 0.001) and sTNF-R2 (P = 0.010), followed by NYHA class II patients and controls (Kruskal-Wallis test) (Figure 1).

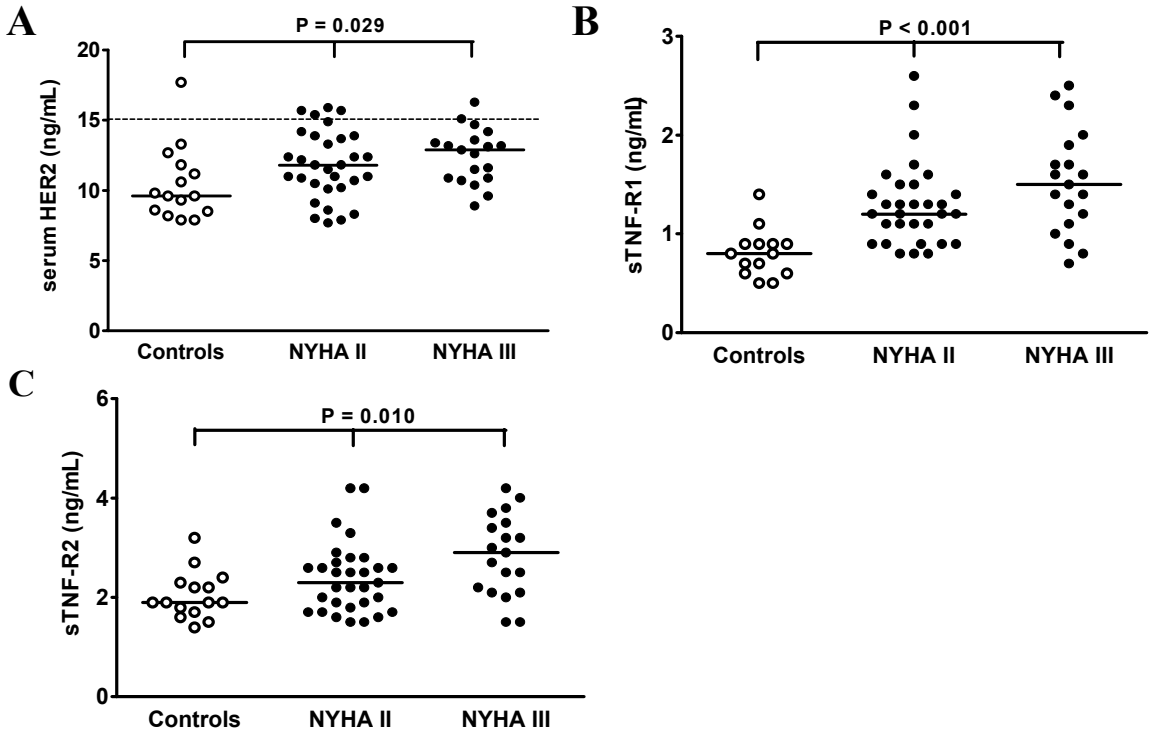

Figure 1. Dot plot of serum human epidermal growth factor receptor 2 (HER2). Dotted line represents the serum HER2 cut-off value (upper limit of normal) of 15 ng/L. (**A**) tumor necrosis factor receptor (sTNF-R) 1 (**B**) and sTNF-R2 (**C**) levels in NYHA class II vs. NYHA class III heart failure patients, compared to controls. Lines represent mean values.

Serum HER2 levels were not associated with the time since the first diagnosis of heart failure. No associations were observed between serum HER2 levels and gender, age or the presence of diabetes mellitus, hypertension or present smoking. TNFα and sTNF-R levels were also not associated with gender, age or any of the aforementioned comorbidities. Serum HER2 correlated inversely with LVEF (R = -0.296, P = 0.037) (Figure 2).

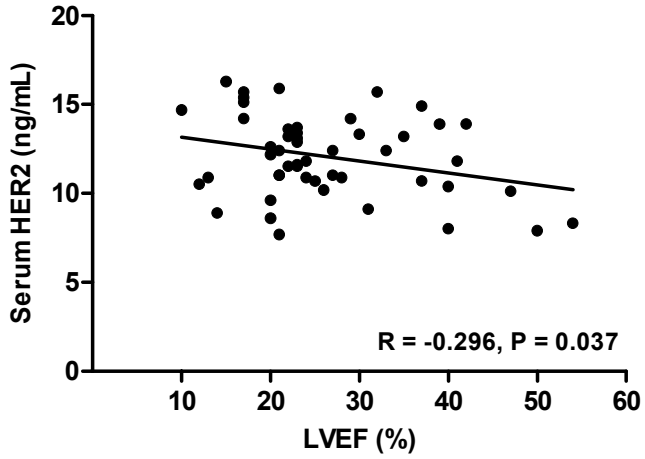

Figure 2. Dot plot of serum human epidermal growth factor receptor 2 (HER2) levels in relation to left ventricular ejection fraction (LVEF) values in heart failure patients. Line represents Pearson correlation coefficient.

HER2 did not correlate with peak oxygen consumption, plasma BNP values, nor with LVESD or LVEDD. No differences were observed regarding serum HER2, plasma TNF-α and sTNF-R levels between patients with DCM and IHD as the cause for heart failure. A weak positive correlation existed between serum HER2 and sTNF-R levels; sTNF-R1 ($R = 0.277$, $P = 0.027$), sTNF-R2 ($R = 0.260$, $P = 0.036$).

DISCUSSION

One of the major findings of the present study, is that serum HER2 levels are elevated in heart failure patients. Higher serum HER2 levels were associated with increasing NYHA class, and a weak negative correlation between serum HER2 and LVEF values was observed. Furthermore, serum HER2 levels correlated positively with plasma levels of the apoptosis-related proteins sTNF-R1 and sTNF-R2.

Although the differences in serum HER2 levels between patients and controls are small and not likely to be of clinical relevance, the elevated serum HER2 in heart failure patients suggests that HER2 plays a role in cardiac pathophysiology. This may be an important observation with regard to our understanding of the mechanism responsible for trastuzumab-related cardiotoxicity.

In rats with banding-induced aortic stenosis, compensatory myocardial hypertrophy precedes heart failure. Myocardial HER2 mRNA expression, which is present during compensatory hypertrophy in the animals with banding-induced aortic stenosis, is decreased during the transition from compensatory hypertrophy to heart failure.[15] In 36 patients with severe (NYHA class IV) heart failure due to ischemic or non-ischemic cardiomyopathy, myocardial HER2 mRNA levels were raised following left ventricular assist device implantation.[16] It can be imagined that the slightly higher serum HER2, as was observed in our study population, is indicative of increased shedding of HER2 from the cardiomyocyte membrane into the circulation. This may result, via decreased HER2 mRNA expression, in decreased HER2 cardiomyocyte membrane expression. To date however, no data are

available with regard to the relationship between HER2 mRNA and membrane expression in the heart.

Recently, HER2 expression was shown with immunohistochemistry in left ventricle biopsies from 6 of 60 patients with severe cardiac disease and heart failure symptoms. Only weak HER2 staining was observed in these positive biopsies, compared to HER2 overexpressing or *HER2* gene amplifying breast tumors.[17] This might explain why circulating HER2 levels in our heart failure patients are only slightly elevated in comparison to high serum HER2 values in HER2 positive breast cancer patients.[18]

The pathophysiological mechanism of the higher serum HER2 values in heart failure patients remains unclear. We observed an inverse association between serum HER2 levels and systolic left ventricular function as assessed with LVEF measurement. Serum HER2 was however not related to other markers of heart failure severity such as BNP, peak oxygen consumption nor with left ventricular diameters. Hypothetically, loss of cardiomyocyte-expressed HER2 may result in loss of functional myocytes leading to systolic functional impairment and heart failure. In addition, whether or not the myocardium is the origin of the increased HER2 serum levels remains unclear. It can also be imagined that the HER2 is produced by other organs, or as a result of systemic responses, such as passive congestion, ischemia or as an acute phase reactant. HER2 appears to be involved in the prevention of apoptosis.[19]

HER2 signal transduction is initiated through heterodimerization with one of the other three HER family members. For review regarding the role of the HER family in the heart see.[20] Neuregulins are a family of ligands that can activate the HER family of growth promoting receptors. Although no direct activating ligand has been discovered for HER2, neuregulin signaling appears to be crucial for cardiac development since targeted disruption of neuregulin signaling results in embryonic lethality due to incomplete cardiac and neurologic development.[21,22]

Inhibition of HER2 in isolated neonatal rat ventricular myocytes resulted in increased expression of the pro-apoptotic protein Bcl-xS and a decrease in the anti-apoptotic Bcl-xL.[23] It can be hypothesized that in heart failure, the protective trophic signal normally provided by HER2 is lost through increased shedding of cardiomyocyte membrane-bound HER2 into the circulation. Loss of cardiomyocyte-expressed HER2 may induce cardiac apoptosis leading to heart failure. The slight elevation in serum HER2 levels in our population of heart failure patients may be indicative of this loss of membrane-expressed HER2.

HER2 cleavage might be affected by apoptosis-related cytokines, which are elevated in patients with heart failure.[24] In the current study, plasma levels of both TNF receptors were higher than in controls. In addition, we observed a weak positive correlation between plasma levels of both TNF receptors, which may point in this direction. Further study is needed to determine whether the higher serum HER2 levels in heart failure patients are related to increased cardiomyocyte apoptosis or to augmented myocardial HER2 expression and shedding.

For evaluating the importance of HER2 in the heart, scintigraphy with radiolabeled trastuzumab may prove of value. This technique enables visualization of HER2 by trastuzumab tissue uptake, using gammacamera imaging.[25-27] Radiolabeled trastuzumab scintigraphy in patients with chronic heart failure may increase our understanding of the role played by HER2 in the heart.
A limitation of the current study is the relatively small sample size, which may have restricted the statistical significance of our findings. For instance, elevated plasma TNF-α levels have been described extensively in heart failure patients.[28] Although plasma TNF-α levels were slightly higher in heart failure patients than in controls in the current study, this did not reach statistical significance.

In conclusion, the increased serum HER2 levels observed in heart failure patients in our study suggest that HER2 might play a role in heart failure. Although serum HER2 did not correlate with TNFα, HER2 may be involved in cardiac apoptosis, based on the positive association between HER2 and the sTNF-R levels. However, the underlying pathophysiological mechanisms for the higher circulating levels of HER2 remain unclear and require further investigation.

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