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High-sensitivity cardiac troponin I detection for 2 types of drug-induced cardiotoxicity in patients with breast cancer

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Abstract

Background Breast cancer treatment with trastuzumab, a monoclonal antibody that targets human epidermal growth factor receptor type 2 (HER2), has largely been successful in improving the prognosis of HER2-positive disease. However, a critical issue associated with trastuzumab treatment is its cardiotoxic adverse effects, including cardiac insufficiency.

Methods We measured levels of cardiac troponin I, a marker of myocardial damage, with a highly sensitive method (hs-cTnI) using a fully automated chemiluminescent immunoassay system (ADVIA Centaur® XP) in breast cancer patients and examined the relationship between administration of trastuzumab and epirubicin and concentrations of hs-cTnI.

Results The coefficient of variation for within-run repeatability was 1.34–5.93 %, using plasma pools and

controls of 3 concentrations, and that for between-run reproducibility was 3.99–8.79 %, indicating high precision of the assay. In a dilutional linearity test with highly concentrated specimens, hs-cTnI values could be measured up to 50 ng/mL with linearity. No influence from coexisting substances was observed. The concentration of hs-cTnI was at or above the reference range (0.04 ng/mL) in 9 of 214 total breast cancer cases (4.2 %). The hs-cTnI concentration was at or above the reference range in 4 of 49 cases (8.2 %) that were administered trastuzumab, and in 5 of 165 cases (3.0 %) that were not. Trastuzumab did not cause elevation of hs-cTnI when not administered in combination with epirubicin.

Conclusions These results suggest that epirubicin and trastuzumab cause cardiotoxicity through different mechanisms. Epirubicin can cause myocardial necrosis, while trastuzumab can cause cardiomyopathy without myocardial necrosis.

Keywords Cardiotoxicity · High-sensitivity cardiac troponin I · Trastuzumab · Epirubicin

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Introduction

Human epidermal growth factor receptor type 2 (HER2), which is overexpressed in 20–25 % of breast cancer cases [1], is involved in cellular proliferation and differentiation, and HER2-positive breast cancer is reported to have a poor prognosis [2]. The humanized monoclonal antibody against HER2, trastuzumab, is a highly effective therapeutic drug for treatment of HER2-positive breast cancer [3]. However, an association between trastuzumab and impairment of the left ventricular ejection fraction (LVEF) leading to conventional heart failure has become evident, and it has also

been clarified that the class of conventional cytotoxic chemotherapeutic drugs, the anthracyclines [4], increases the risk of this cardiotoxic effect [2]. However, certain differences in the cardiotoxicities induced by these 2 drugs have been reported. Cardiotoxicity induced by an anthracycline depends on its total dose. In contrast, cardiotoxicity due to trastuzumab is unrelated to the total dose or administration span [5]. Thus, chemotherapy-related cardiac dysfunction could be classified into 2 types: anthracycline-induced type I cardiac dysfunction and trastuzumab-induced type II cardiac dysfunction [6]. Although the mechanisms of cardiotoxicity in both types are not well understood, both types of cardiotoxicity are serious complications, and early detection and care are needed.

Since cardiotoxicity is difficult to detect by echocardiography in its early stage [7, 8], blood tests, including detection of creatinine kinase (CK), CK MB fraction, myoglobin, heart-type fatty acid-binding protein, cardiac troponin T (cTnT), cardiac troponin I (cTnI), myosin light chain, brain natriuretic peptide (BNP), and N-terminal (NT)-pro BNP, have been used to screen patients for cardiac dysfunction. One of these blood tests involves detection of cardiac troponin, which is a protein complex consisting of troponin T, I, and C [9, 10]. Troponin suppresses actin-myosin muscle contraction in the myocardium, and the T and I subunits are specific for the myocardium [10, 11]. The troponin subunits leak into the blood when myocardial cells became necrotic, and their presence in the blood is indicative of myocardial damage [11]. Until several years ago, measurement of troponin tended to produce false negative results since it was difficult to make an accurate measurement at low concentrations because of the low sensitivity of the test [12, 13]. However, new and highly sensitive methods of measuring cTnT and cTnI have been developed, making it possible to not only diagnose myocardial infarction at a hyperacute stage, but also to detect latent cardiovascular disease, evaluate the effect of treatment, and detect drug-induced cardiotoxicity earlier than before. However, there have been few reports on the use of high-sensitive cardiac troponin (hs-cTnI) detection to diagnose early-stage cardiotoxicity caused by molecularly targeted therapeutic drugs, specifically trastuzumab.

First, we determined the precision of hs-cTnI measurements using the chemiluminescent immunoassay. Then, the effect of trastuzumab on the blood concentration of hs-cTnI was examined and compared to the effect of the anthracycline epirubicin on blood levels of hs-cTnI. Here, we show that trastuzumab did not cause an increase in hs-cTnI levels when it was consumed after epirubicin.

Materials and methods

Blood samples

Blood samples were obtained from 214 female breast cancer patients who were examined at the Department of Breast and Endocrine Surgery at Tokai University Hospital from February 2010 to June 2011 (median age 60.5 years; range 30–83 years). Patients were eligible if their blood urea nitrogen (BUN), serum creatinine (CRE), and CK levels were within the reference ranges (BUN 8.0–20.0 mg/dL, CRE 0.50–0.80 mg/dL, CK 30–140 U/L), and there were no abnormalities detected in an electrocardiogram or in LVEF by an echocardiography, since this could affect the level of hs-cTnI. Hs-cTnI was measured using the samples for a routine blood test, regardless of the presence of cardiac symptoms, since the aim of the measurements was early detection of cardiotoxicity. This study was approved by the Institutional Review Board for Clinical Research in Tokai University. A written informed consent was obtained from all the participants.

Usage of drugs

Four cycles of epirubicin plus cyclophosphamide therapy were administered to 101 breast cancer patients in the present study. Then, to prevent deterioration of cardiac function, 4 cycles of trastuzumab with docetaxel were administered to patients with HER2-positive breast cancer when LVEF, assessed by echocardiography, was within the range of reference values. Trastuzumab without docetaxel was further administered up to 1 year from the date of initial administration.

Measurements of hs-cTnI

To examine cardiotoxicity using an assay system for detecting hs-cTnI, the definition for an increased value of cardiac troponin was defined as a measurement exceeding the 99th percentile of a normal reference population, with optimal precision of the assay system to include a low coefficient of variation at the 99th percentile for each assay [14]. Hs-cTnI was measured with a chemiluminescent immunoassay, based on the 2-step sandwich method using the Chemilumi Centaur—Troponin I Ultra (Siemens Healthcare Diagnostics K.K., Tokyo, Japan). Briefly, acridinium ester-labeled anti-cTnI goat polyclonal antibodies and 2 types of biotin-labeled anti-cTnI mouse monoclonal antibodies were added to a blood specimen to form antigen–antibody complexes, and then streptavidin-bound magnetic latex particles were added.

Table 1 The precision results of hs-cTnI assay

	Within-run						Between-run		
	hs-cTnI			hs-cTnI			hs-cTnI		
	Pool plasma			Control			Control		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
<i>n</i>	10	10	10	10	10	10	15	15	15
Min (ng/mL)	0.065	0.782	10.836	0.046	0.811	4.769	0.059	0.928	4.752
Max (ng/mL)	0.071	0.824	11.623	0.057	0.875	5.021	0.084	1.119	5.745
Mean (ng/mL)	0.0680	0.8031	11.2449	0.0516	0.8397	4.8783	0.0688	1.0104	5.2279
SD (ng/mL)	0.0023	0.0120	0.2320	0.0031	0.0230	0.0652	0.0060	0.0403	0.2561
CV (%)	3.32	1.50	2.06	5.93	2.74	1.34	8.79	3.99	4.90

SD standard deviation, CV coefficient of variation

The resultant antigen–antibody–magnetic latex particle complexes were precipitated with a magnet. The reactions were performed and detected in the ADVIA Centaur® XP Immunoassay System (Siemens Healthcare Diagnostics K.K., Tokyo, Japan). The chemiluminescence generated by adding an oxidizer was measured with the detector, and the data were analyzed automatically.

Predetermination of precisions of the assay system

Prior to the study on hs-cTnI in breast cancer patients, the precision, dilutional linearity, and influence of coexisting substances in measurements of hs-cTnI were examined using blood samples randomly chosen from breast cancer patients. To evaluate the assay precision, the within-run repeatability (intra-assay variability) and day-to-day precision were examined and calculated. For within-run repeatability, 10 consecutive measurements were taken using plasma pools and controls of 3 concentrations. For between-run reproducibility, 15 days of measurements were done using controls of 3 concentrations. To examine dilutional linearity, plasma pools of 4 different concentrations were used, and serial dilutions were made independently. To examine the influence of coexisting substances, levels of hs-cTnI were measured in human pools combined with free bilirubin, conjugated bilirubin, hemoglobin, chyle, and rheumatoid factor (RF) using Interference Check A Plus and RF Plus (Sysmex Corporation, Kobe, Japan).

Statistics

The Mann–Whitney *U* test was used to detect significant differences between groups that did not show a normal distribution.

Fig. 1 Dilution linearity for the measurement of hs-cTnI

Results

Precision of the assay system

The coefficient of variation for within-run repeatability was 1.34–5.93 % using plasma pools and controls of 3 concentrations, and that for between-run reproducibility was 3.99–8.79 %, indicating high precision of the assay (Table 1). Next, the dilutional linearity was examined (Fig. 1). Values of hs-cTnI showed linearity in the range of 0–50 ng/mL. No effect on measurements of hs-cTnI was observed with up to 20.0 mg/dL of free bilirubin, 20.0 mg/dL of conjugated bilirubin, 500 mg/dL of hemolytic hemoglobin, 3,000 formazin turbidity units of chyle, and 550 IU/mL of RF (Fig. 2).

Fig. 2 The effect of interfering substances on the measurement of hs-cTnI

Hs-cTnI concentration in breast cancer patients

We then analyzed the distribution of hs-cTnI concentrations in breast cancer patients. Table 2 shows the patient characteristics. Nine of 214 breast cancer patients (4.2 %), whether or not receiving drug therapy, showed high levels of hs-cTnI above the reference value (0.04 ng/mL) (Fig. 3a). Then, the relationship between the use of therapeutic drugs and the levels of hs-cTnI was analyzed. Among 49 patients to whom trastuzumab was administered [trastuzumab (+) group], 4 patients (8.2 %) showed high levels of hs-cTnI. Among 165 patients to whom trastuzumab was not administered [trastuzumab (−) group], 5 patients (3.0 %) had high hs-cTnI levels. There was no significant difference between these 2 groups (Fig. 3b). Among 101 patients to whom epirubicin was administered [epirubicin (+) group], 9 patients (8.9 %) showed high hs-cTnI levels, while none of the 113 patients to whom epirubicin was not administered [epirubicin (−) group] had high levels of hs-cTnI, and this difference was significant (Fig. 3c). Since 29 patients received epirubicin followed by trastuzumab [epirubicin (+)/trastuzumab (+)], the possible

Table 2 Patient characteristics

	<i>n</i>	%
Age (years)		
Median (range)	60.5 (30–83)	
HER2 status		
Negative	165	77.1
Positive	49	22.9
Trastuzumab		
Not administered	165	77.1
Administered	49	22.9
Epirubicin		
Not administered	113	52.8
Administered	101	47.2
Trastuzumab(−), Epirubicin(+)	72	71.3
Trastuzumab(+), Epirubicin(+)	29	28.7

HER2 human epidermal growth factor receptor type 2

enhancement of the cardiotoxic effect of epirubicin was also analyzed. The results showed that hs-cTnI was high in 5 out of 72 epirubicin (+)/trastuzumab (−) patients, and in 4 out of 29 epirubicin (+)/trastuzumab (+) patients, and

Fig. 3 Distributions of serum hs-cTnI in patients with breast cancer. **a** Distribution of serum hs-cTnI in total breast cancer patients ($n = 214$). **b** Comparison of hs-cTnI levels between patients that were not administered trastuzumab ($n = 165$) and those that were administered trastuzumab ($n = 49$). **c** Comparison of hs-cTnI levels between patients that were not administered epirubicin ($n = 113$) and those that were administered epirubicin ($n = 101$). **d** Comparison of

hs-cTnI levels between patients that were administered epirubicin, but not trastuzumab [epirubicin (+)/trastuzumab (-); $n = 72$] and those that were administered both epirubicin and trastuzumab [epirubicin (+)/trastuzumab (+); $n = 29$]. The upper limit of the reference range was 0.04 ng/mL (**bold line**). Significant differences between the 2 compared groups were evaluated by the Mann–Whitney U test

Fig. 4 Relationship between total dose of epirubicin and serum hs-cTnI in all patients with breast cancer

this difference was not statistically significant (Fig. 3d). Then, the relationship between total doses of epirubicin and hs-cTnI levels was plotted (Fig. 4). Although there was not a strong correlation when the relationship was analyzed by linear regression, high levels of hs-cTnI were present only in the patients who received epirubicin doses of 400 mg/m² or higher.

Since prior radiation to the breast or chest wall may influence cardiac dysfunction, the effect of radiation on hs-cTnI levels was also examined. The results showed there was no significant association between radiation therapy and hs-cTnI levels (Fig. 5).

Fig. 5 Relationship between prior radiation to the breast or chest wall and serum hs-cTnI levels in all patients with breast cancer

Discussion

The chemiluminescent hs-cTnI immunoassay system in the present study had a good performance with regard to reproducibility and linearity. Cardiac troponin has been recommended as a diagnostic criterion for acute myocardial infarction as defined by the joint European Society of Cardiology/American College of Cardiology [15]. Later,

the European Society of Cardiology/American College of Cardiology/American Heart Association/World Heart Federation (ESC/ACC/AHA/WHF) defined an increased value for cardiac troponin as a measurement exceeding the 99th percentile of a normal reference population, and optimal precision was defined as the coefficient of variation $\leq 10\%$ at the 99th percentile value [14]. Because the present assay fulfilled this definition, it was considered highly sensitive, enabling earlier detection of myocardial injury [16].

In the present study, high hs-cTnI levels in both the trastuzumab and non-trastuzumab group were attributed to the use of anthracyclines, and resulted in the conclusion that administration of trastuzumab did not cause elevation in serum hs-cTnI. It has been reported that breast cancer patients who only take trastuzumab have a cardiotoxicity incidence rate of 2.0–7.0 % [8, 17–19]. When anthracyclines are used in combination with trastuzumab, damage to the myocardium occurs in 26.6 % of patients [20]. For this reason, echocardiography for examining LVEF is recommended every 3 months during trastuzumab administration to detect the occurrence of cardiotoxicity [21]. Furthermore, the reagent used in this study is useful as a risk indicator and in the early diagnosis of acute myocardial infarction [13]. It has also been reported that hs-cTnI measurements can be useful predictive factors for cardiotoxicity in HER2-positive patients who receive combination chemotherapy with trastuzumab and anthracyclines [22].

Although the number of patients in the present study was relatively low when compared to larger-scale studies, we would expect several patients to have trastuzumab-induced cardiotoxicity based on reported incidence rates. Thus, another interpretation should be considered, other than trastuzumab directly causing injury of myocardial cells. The mechanism by which trastuzumab causes cardiotoxicity still remains unclear; however, some researchers believe the cardiotoxicity is due to the participation of HER2 in the formation and growth of the myocardium [23]. When trastuzumab acts through HER2 receptor signals, it reduces the myocardium-protecting effect of HER2 [24]. Therefore, the trastuzumab-induced dysfunction may be due to cardiomyopathy. Microscopic studies of non-drug-induced classical cardiomyopathy usually do not show myocardial necrosis, but instead show reversible myocardial degeneration and fibrosis [25]. The joint ESC/ACC/AHA/WHF Task Force for the Redefinition of Myocardial Infarction [14] has emphasized the significance of troponin elevation, even in the absence of ischemic heart disease, including congestive heart failure. However, as shown in the present study, trastuzumab may not in fact have caused myocardial necrosis, though epirubicin definitely did.

Because we attempted to detect early cardiotoxicity due to treatment with trastuzumab and epirubicin, the levels of hs-cTnI were not measured in patients who had heart failure; therefore, we do not know if an advanced state of trastuzumab-induced cardiotoxicity also correlates with high levels of hs-cTnI. However, it is unlikely that detectable levels of hs-cTnI will be present in stages of early cardiotoxicity induced by trastuzumab alone.

In addition, we examined the relationship between the anthracycline epirubicin and hs-cTnI levels and found that the 9 patients in whom hs-cTnI concentration was at or above the reference range had taken total epirubicin doses of 476–816 mg/m². Generally, because of cardiotoxicity, 900 mg/m² is recommended as the upper limit for the lifetime total dose of epirubicin [26–28], although there are reports of cardiac insufficiency occurring at 178 and 540 mg/m² [27, 29]. The results of this study were also thought to have been influenced by the use of anthracyclines, which suggests the need for regular cardiac function tests, even when the total dose of anthracyclines is <900 mg/m².

There are other sensitive biomarkers, such as BNP and NT-proBNP, which may be used to detect cardiac failure before a decline of LVEF [30]. As shown in the present study, a structural myocardial protein, cardiac troponin, is released from damaged myocytes. On the other hand, the neurohormones BNP and NT-proBNP are released when hemodynamic stress increases, acting as a cardiac failure biomarker independent of hs-cTnI [31]. Furthermore, at times, their serum or plasma levels elevate concurrently, resulting in high mortality [30, 32]. Taken together, it may be useful to measure both of these markers.

In conclusion, these results suggest that epirubicin and trastuzumab cause cardiotoxicity through different mechanisms. Epirubicin can cause myocardial necrosis, while trastuzumab can cause cardiomyopathy without myocardial necrosis. Therefore, extreme caution should be exercised when administering trastuzumab and epirubicin, and further studies are warranted to improve detection of early cardiotoxicity in these breast cancer patients.

Conflict of interest None declared.

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