# Troponin-T and myoglobin plus echocardiographic evaluation for monitoring early cardiotoxicity of weekly epirubicin–paclitaxel in metastatic breast cancer patients

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**Increased serum level of troponin-T and myoglobin has been recently reported to be related to cumulative anthracycline exposure. Left ventricular ejection fraction seems accurate in monitoring systolic function according to the latest version of Toxicity Criteria by chemotherapeutics 3.0. From January 2002, 20 patients with untreated advanced breast cancer received epirubicin (25 mg/m$^2$/week) and paclitaxel (80 mg/m$^2$/week) for 24 weeks. Troponin-T, myoglobin and biochemical serum enzymes circulating levels were measured immediately before and 4 h after epirubicin administration every week. Patients underwent electrocardiography and echocardiography at weeks 0, 8, 16 and 24. The number of courses administered was 352 (median 18, range 4–24). Epirubicin median dose administered was 600 mg/m$^2$ and paclitaxel median dose administered was 1760 mg/m$^2$. Troponin-T never overcame the upper normal limit; one patient experienced troponin-T elevation without any clinical or instrumental sign of cardiac failure. Myoglobin never significantly increased with the exception of a patient who underwent several abdominal fluid drainages. Creatine kinase MB and C-reactive protein never moved outside the upper normal limit. No symptomatic cardiac event was recorded. In 55 performed echocardiograms at weeks 0, 8, 16 and 24, neither left ventricular ejection fraction nor early peak flow/atrial flow velocity registered any significant decrease. No troponin-T or myoglobin serum elevations and Left ventricular ejection fraction/early peak flow/atrial flow velocity changes were registered in our series of nonsymptomatic women during epirubicin/paclitaxel weekly chemotherapy in the absence of clinical cardiac toxicity. Longer follow-up is needed, however, to understand whether the troponin-T or myoglobin circulating level measurement is able to detect subclinical, early-stage doxorubicin-induced cardiotoxicity. *Anti-Cancer Drugs* 18:227–232 © 2007 Lippincott Williams & Wilkins.**

**Anti-Cancer Drugs** 2007, **18**:227–232

**Keywords: cardiotoxicity, epirubicin, myoglobin, paclitaxel, troponin, weekly**

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Previous presentation: Presented as poster at the 96th American Association of Cancer Research (AACR) Annual Meeting, 16–20 April 2005.

**Received** 9 August 2006 **Revised form accepted** 24 September 2006

## Introduction

As anthracyclines remain one of the most frequently used antineoplastic drugs, the early detection and/or prediction of anthracycline-induced cardiotoxicity is urgently needed. Serum levels of cardiac troponin-T (cTnT) are increasingly becoming recognized as potential biochemical markers of even subclinical myocardial injury. In a recent paper, Lipshultz *et al.* [1] affirmed that 'the serum level of cTnT is an accurate surrogate for acute myocardial injury in children, specifically that related to doxorubicin (DOX)'. The same authors previously showed that low-level elevations of cTnT induced by DOX were associated with histological evidence of myocardial injury [2], and that dexrazoxane-treated rats had less frequent elevations in cTnT and less severe cardiac injury on histological analysis, and were in better health than rats that did not receive dexrazoxane [3].

cTnT and myoglobin (M) have been described to predict the anthracycline-induced cardiac injury. In fact, increasing serum level of TnT has been recently reported to be related to cumulative anthracycline exposure. Left ventricular ejection fraction (LVEF) seems accurate in monitoring systolic function for cardiac damage measurement and its decrease has recently been included among the parameters of Toxicity Criteria by Chemotherapeutics 3.0. Early peak flow/atrial flow velocity (E/A) ratio is supposed to be useful to assay diastolic function to predict systolic failure [4].

Despite these findings, there is still only a little information about the exact mechanisms responsible for the cTnT release, especially with regard to the gradual development of myocardial injury, and the role of cTnT in diagnosing and monitoring cardiac damage remains

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controversial. Moreover, the lack of evaluation of diastolic heart function represents a negative point of design of the Lipshultz study.

Therefore, the question immediately arises as to whether diastolic heart function may have a nonnegligible power for the prediction of chronic/late cardiotoxicity of anthracyclines. From these perspectives, the identification of serum predictors of anthracycline-induced cardiac damage is certainly an emerging issue.

## Methods

### Patients

Patients affected by HER-2-negative advanced/metastatic breast cancer not previously treated, entered into a phase II study, in which the combination of weekly epirubicin (E, 25 mg/m$^2$/week) and paclitaxel (P, 80 mg/m$^2$/week), and granulocyte colony-simulating factor support on days 2 and 4 were delivered for 24 consecutive weeks to assess tolerability and activity. We previously demonstrated that a 2-day granulocyte colony-stimulating factor administration is able to reduce neutropenia and also maintain dose intensity in a sustained weekly schedule [5]. Patients younger than 18 or older than 75 years or having a history of cardiovascular disease, drug-uncontrolled systemic hypertension, LFEV < 50% or chronic renal failure were excluded. Patients had to sign a detailed informed consent considering all diagnostic and therapeutic procedures associated with the antineoplastic treatment of their underlying disease.

### Cardiac evaluation

The cardiac function was evaluated by complete physical examination, electrocardiography and echocardiocolor-doppler examination, which assessed both systolic and diastolic function. LVEF was determined by applying the Teichholz formula and calculated from the measured left ventricular end-diastolic diameter and left ventricular end-systolic diameter, with the mean of three measurements being used. Each study consisted of two-dimensional echocardiography (Eco) and Doppler evaluation. E/A ratio was measured to test diastolic function. Pulsed Doppler volume was calculated at mitral leaflets.

Baseline Eco was performed immediately before initiation of the first cycle of chemotherapy, and after 8, 16 and the last (24th) weekly administration of chemotherapy. After completion of treatment, patients were followed up, performing the examination every 3 months, in the absence of cardiac damage. Patients experiencing a decrease in LFEV greater than 20% underwent a MUGA scan and discontinued chemotherapy.

Results of the follow-up Ecos were included in the study only if the patient did not receive any further antineoplastic therapy after monitoring of cTnT was completed, because of potential impacts on cardiac function. Patients with the most severely impaired cardiac function had the most Ecos and, thus, including all Ecos in the analysis could have biased the results. Therefore, we analyzed the latest Eco obtained from each patient in each period: before, during and after E/P chemotherapy.

### Biochemical analysis

We measured cTnT, M, alanine transaminase, aspartate transaminase, lactic dehydrogenase, creatine phosphokinase, creatine kinase MB and C-reactive protein circulating levels immediately before and 4 h after chemotherapy administration every week. Serum collected at clinical sites was immediately frozen and stored at −70°C until it was assayed for cTnT at a central core laboratory with the use of the Elecsys Troponin T STAT Immunoassay (Roche Diagnostics, Indianapolis, Indiana, USA). Values were considered normal between 0.01 and 0.10 ng/ml. Each week, pre-chemotherapy and post-chemotherapy determination of all parameters were collected.

The protocol included the collection of serum at standardized times: cTnT was measured immediately whenever serum was obtained for routine laboratory tests, starting before initiation of chemotherapy until discharge of the patient. The same schedule of measurements was followed if patients were monitored for more than one treatment cycle. Lyophilized human control serum in two concentration ranges (PreciControl Cardiac; Roche Diagnostics) served as positive control.

### Statistical analysis

Age, sex, baseline LVEF and concomitant epirubicin/paclitaxel therapy were analyzed as potential risk factors for cTnT positivity. For analysis of sex and concomitant chemotherapy, the $\chi^2$-test was applied. The Mann–Whitney *U*-test was applied for comparison of peak cTnT levels with respect to concomitant chemotherapy. For analysis of LVEF in cTnT+ and cTnT− patients, Wilcoxon's test and logistic regression were used. $P < 0.05$ was considered significant. Calculations were made with SPSS 8.0 (SPSS, Chicago, Illinois, USA). We used a logistic regression analysis to determine whether the cumulative dose of prior anthracyclines was associated with elevated cTnT levels.

## Results

### Patients and cycles

Between January 2002 and March 2004, a total of 20 adult women with chemotherapy-naïve metastatic breast cancer were enrolled and considered evaluable for the study. Median age was 56 years (range 34–75 years). Clinical characteristics of the patients are listed in Table 1. All individuals were treated as outpatients. No concomitant radiation therapy was administered. The

**Table 1 Patient characteristics**

| | |
|---|---|
| Number of enrolled patients | 20 |
| Number of evaluable patients | 20 |
| Overall number of cycles | 352 |
| Median age (years) | 56 |
| range | (34–75) |
| Menopausal status | |
| pre | 2 |
| post | 18 |
| Performance status (ECOG) | |
| 0–1 | 17 |
| 2–3 | 3 |
| Median number of courses | 22 |
| range | (4–24) |
| Estrogen receptors | |
| positive | 17 |
| negative | 3 |
| Dominant metastatic sites | |
| visceral | 9 |
| bone | 6 |
| soft tissue | 5 |
| Number of metastatic sites | |
| 1 | 10 |
| 2 | 5 |
| >2 | 5 |
| Adjuvant chemotherapy | 11 |
| anthracyclines | 7 |
| CMF | 6 |

ECOG, Eastern Cooperative Oncology Group.

**Table 2 Troponin measurement (normal value: 0–0.10 U/l)**

| | Pretreatment median cumulative value (U/l) | Range (U/l) | Posttreatment median cumulative value (U/l) | Range (U/l) |
|---|---|---|---|---|
| Baseline | 0.02 | – | 0.02 | – |
| After 8 weeks | 0.02 | 0.01–0.06 | 0.02 | 0.01–0.05 |
| After 16 weeks | 0.02 | 0.01–0.07 | 0.02 | 0.01–0.07 |
| After 24 weeks | 0.02 | 0.01–0.02 | 0.02 | 0.01–0.02 |

number of courses administered was 352 (median 22, range 4–24). Overall, E median dose given was 600 mg/m$^2$ (range 100–1080) with a median dose intensity of 22.95 (range 15.6–25) mg/m$^2$/week (91.8%). P median dose administered was 1760 mg/m$^2$ (range 320–1920) with a median dose intensity of 73.45 (range 50–80) mg/m$^2$/week (91.8%). In chemotherapy-naïve patients, the E median dose administered was 550 mg/m$^2$ (range 100–600), while in the E-pretreated women (adjuvant setting) it was 930 mg/m$^2$ (range 605–1080).

## Toxicity and activity

Treatment was generally well tolerated (Table 2) and no dose reductions were required. No grade 3 or 4 World Health Organization hematological toxicity was recorded. Five patients experienced grade 2 anemia. Regarding severe nonhematological toxicities, one patient had one episode of grade 3 mucositis, three patients suffered grade 2 neurotoxicity, and nausea and neutropenia occurred in two patients. All patients developed severe alopecia. Eleven patients developed a mild nail toxicity. None of the patients experienced conjunctivitis or ocular disorders requiring treatment. No symptomatic cardiac event was recorded. No serious adverse events requiring hospitalization and no treatment-related deaths were observed. Objective responses were observed in 13 out of 20 patients [65%, 95% confidence interval (CI) 44, 85.9], four of which were complete (20%, 95% CI 2.4, 37.5) and nine partial (45%, 95% CI 23.2, 66.8). Seven patients had stable disease (35%) and one patient progressed (5%). The median duration of the response was 12 months. At a median follow-up of 20 months, median time to progression was 12 months (95% CI 2, 22). The median survival was not reached. One, two and three-year survival rates were 85, 68 and 62.3%, respectively.

## Monitoring of serum levels of cardiac troponin T, myoglobin and other markers

A total of 582 measurements of serum cTnT were performed during 352 treatment cycles in 20 patients. The median baseline cTnT level before initiation of chemotherapy was 0.02 U/l (normal values range 0–0.1 U/l). cTnT never overcame the upper normal limit (UNL), either immediately before or 4 h after E administration (Table 2). One patient experienced cTnT elevation without any clinical or instrumental sign of cardiac failure. In this patient, the maximal level registered was 0.07 U/l after 15 weeks. cTnT levels returned to < 0.01 U/l within 21days after antineoplastic treatment was started, with six further cTnT measurements after subsequent chemotherapy administrations. No concomitantly elevated serum creatinine levels were registered. In total, 582 measurements of serum M were performed during 352 treatment cycles in 20 patients. The median baseline M level before initiation of chemotherapy was 30.8 ng/ml (normal values range 25–58 ng/ml). M never significantly increased, either immediately before or 4 h after E administration (Table 3). One patient experienced M elevation because of the several abdominal fluids. Creatine kinase MB and C-reactive protein never moved outside the UNL. Alanine transaminase, aspartate transaminase and lactic dehydrogenase were always within the normal range except for patients with liver metastases.

## Echocardiografic data

A total of 55 Ecos (88.7% of the theoretical number) were obtained (mean 2.75 Ecos per patient). Ecos obtained at weeks 0, 8, 16 and 24 showed a normal LVEF (respectively 66, 67, 64.5 and 65.5%, median 66%) and diastolic function (diastolic E/A mitral flow pattern,

**Table 3 Myoglobin measurement (normal value: 25–58 ng/ml)**

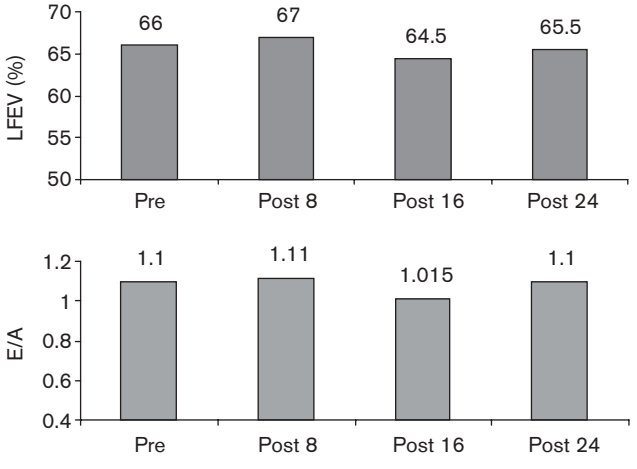
| | Pretreatment median cumulative value (ng/ml) | Range (ng/ml) | Posttreatment median cumulative value (ng/ml) | Range (ng/ml) |
|---|---|---|---|---|
| Baseline | 30.8 | 20–56.4 | 28.6 | 19–49.8 |
| After 8 weeks | 28.1 | 19–174 | 24.1 | 19–126 |
| After 16 weeks | 27.75 | 19–57 | 24.9 | 19–44.9 |
| After 24 weeks | 28.65 | 20–54.7 | 26.65 | 20–83.3 |

respectively, 1.1, 1.11, 1.015 and 1.1, median 1.1). After treatment, at a follow up of 12 months, LVEF (median LVEF 65.5%) and E/A ratio (median E/A 1.1) remained normal (Fig. 1).

## Discussion

Combining an anthracycline with a taxane has become a natural step toward an improved treatment of metastatic breast cancer [6]. Together with the high rate of remission, some authors, however, underlined the unexpectedly high incidence of severe cardiotoxic events when the combination P–DOX was used [7,8]. A strategy for reducing the risk of congestive heart failure (CHF) associated with anthracycline-P regimens includes substituting DOX with E. In fact, at comparable dose levels, E is significantly less cardiotoxic than DOX [9] and P seems to play a minor role on E metabolism when the two drugs are used in combination [10–12], although CHF risk gradually increases along all courses when E cumulative dose reaches 1080 mg/m$^2$, when combined with P [10]. This percentage is significantly higher than that determined for E single agent or in combination with agents other than taxanes [13–15]. A metabolic rather than a pharmacokinetic interference provided by P, when administered after E, is suggested by pharmacokinetic studies, by increasing the plasma exposure of all active E metabolites [16]. Considering that in our series seven patients had received prior adjuvant anthracycline, the absence of CHF or the asymptomatic 20% reduction in LVEF was encouraging. Impaired diastolic or systolic function can occur acutely after DOX treatment [17], although considerably less cTnT is released when myocytes undergo DOX-induced degeneration than when they develop necrosis. Several investigations have already shown severe anthracycline-induced alterations of the contractile apparatus, such as increased loss of myofibrils, rearrangements of the microtubular network and reduced immunohistochemical staining for cTnT [18–21]. Continuous damage of anthracyclines to the cardiac myofibrillar system rather than acute cellular events such as necrosis or apoptosis should be considered of key importance in the pathogenesis of anthracycline-induced cardiac dysfunction. Lipshultz *et al.* [1] used cardiac troponin T as a primary marker of cardiac injury during DOX, instead of Eco measurements as an indicator of myocardial injury, because of the poor sensitivity and specificity of Eco in identifying subclinical abnormalities of left ventricular structure and function in children with cancer who were receiving DOX. The time course of the release of cTnT after each of the individual doses of DOX remains to be evaluated. As the half-life of cTnT in blood is approximately 4 h, it seems likely that this protein is released continuously after DOX-induced myocyte damage has begun to occur [22–24]. The low-level cTnT elevations owing to the cumulative DOX doses might be a result of the prolonged release of cTnT into plasma from initial myocardial injuries and a decrease in the ability to recover [23,25]. Observations made in children [25] demonstrated that cardiomyocyte damage begins early during therapy and can occur even with the initial anthracycline dose. Hence, the cardiac dysfunction observed above the commonly accepted threshold [26,27] seems to result from the accumulation of subclinical toxic events that already begin early during therapy. Preliminary reports suggest that low-level elevations of blood troponin after anthracycline chemotherapy also occur in adults even with normal LVEF [28,29] and continue weeks later [30]. Recent studies have suggested that elevated serum levels of cardiac troponins early after anthracycline therapy at standard doses predict subsequent cardiac dysfunction [19,25,31]. Lipshultz *et al.* [32] observed that low-level increases in serum concentrations of cTnT after the initial dose of DOX were predictive of subsequent risk for left ventricular abnormalities, including dilatation and wall thinning in children. Kilickap *et al.* [4] have recently reported that the measurement of the cTnT level could be useful for the detection of anthracycline cardiotoxicity in the early stages, especially in younger patients. The serum cTnT level after the first dose of anthracycline was, however, elevated in only 4.9% of the patients, although in these patients the E/A ratio was decreased after treatment. The cTnT level after therapy completion was elevated in 34% of the patients (exceeding the upper normal limit in only one case), but the E/A ratio decreased in only 19% of those who had increased levels of serum cTnT. On the contrary, other authors stated that serum cTnT showed no increase in the early stages of anthracycline therapy [29,33]. Serial measurement of serum cTnT revealed delayed subclinical myocardial damage even after minor anthracycline exposure. This is in accordance with a report by Kremer

**Fig. 1**




Median left ventricular ejection fraction (LFEV) (%) and early peak flow/atrial flow velocity (E/A) during treatment.

*et al.* [34] on the low sensitivity of cTnT measurements during the first 24 h after therapy. Our results resembled those reported by these authors, as cTnT never overcame the UNL, either immediately before or 4 h after E administration. Nevertheless, the study by Kilickap shares some limitations with ours: the low number of patients studied, the short mean follow-up and the cumulative anthracycline doses lower than those recognized to be cardiotoxic. Moreover, the difference in cTnT time point measurements between the current study and a previous one could have influenced the negative outcome of the study [3]. Conversely, in our study the patients received the same treatment, whereas in Kilickap's study they were given different chemotherapeutic agents. The tested biochemical parameters are not altered, which could be interesting negative results, but there is no clinical or Eco heart event in our small cohort that may be correlated to this lack of alteration. Thus, no conclusion can be drawn, except the good profile of cardiac tolerance of this schedule and the inability of TnT to capture a likely low level of cardiac injury, resulting from a rather low cumulative exposure to E. We actually have to consider that the selected treatment schedule (weekly E low-dose) is certainly not the most standard way of administering anthracyclines nor the most likely way of generating cardiotoxicity; thus, we are unable to address the value of the proposed surrogate markers in the early detection of anthracycline-induced cardiotoxicity. Despite these findings, there is still little information about the exact mechanisms responsible for the cTnT release, especially with regard to the gradual development of myocardial injury, and the role of cTnT in diagnosing and monitoring cardiac damage remains controversial. Moreover, the lack of evaluation of diastolic heart function represents a negative point in the design of the Lipshultz study [3]. Therefore, the question immediately arises as to whether the diastolic heart function may have a nonnegligible power for the prediction of chronic/late cardiotoxicity of anthracyclines. DOX-induced cardiomyopathy is typically associated with myofibrillar deterioration and intracellular calcium overload, which may trigger the indiscriminate activation of calcium-dependent proteases resulting in the degradation of key myofibrillar proteins. The exclusive use of cTnT as an indicator of disassembly of the myofilament complex could not completely reflect the acute contractile dysfunction associated with doxorubicin-induced myocardial calcium overload and oxidative stress. As a consequence, identification of a reliable serum indicator of early diastolic dysfunction of the cardiac chamber after DOX treatment could be advisable.

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