

Myocardial injury revealed by plasma troponin I in breast cancer treated with high-dose chemotherapy

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Background: High-dose chemotherapy (HDC) has been widely utilized in high-risk breast cancer, but it may induce cardiac toxicity. Cardiac dysfunction may become evident weeks or months after HDC and, to date, no early markers of myocardial injury that are able to predict late ventricular impairment are available. We investigated the role of plasma troponin I (TnI) in this setting.

Patients and methods: We measured TnI plasma concentration after HDC in 211 high-risk breast cancer women (46 ± 11 years, mean \pm SD). According to TnI value (<0.5 or ≥ 0.5 ng/ml), patients were allocated into a troponin positive (TnI⁺; $n = 70$) and a troponin negative (TnI⁻; $n = 141$) group. All patients underwent left ventricular ejection fraction (LVEF, Echo) examination during the following 12 months.

Results: LVEF progressively decreased in the TnI⁺ group but not in the TnI⁻ group. In TnI⁺ patients a close relationship between the TnI increase, as well as the number of positive TnI assays, and the maximal LVEF decrement, was found ($r = -0.92$, $P < 0.0001$ and $r = -0.93$, $P < 0.0001$, respectively).

Conclusions: In our population, the elevation of TnI soon after HDC accurately predicts the development of future LVEF depression. In this setting, TnI can be considered a sensitive and reliable marker of myocardial damage with relevant clinical and prognostic implications.

Key words: breast cancer, cardiotoxicity, high-dose chemotherapy, troponin I

Introduction

Breast cancer is a major cause of cancer death in women. In an attempt to improve the prognosis of patients with high risk breast cancer disease, more and more aggressive chemotherapy schedules have been proposed. High-dose chemotherapy (HDC) has been increasingly used for the treatment of high-risk primary breast cancer [1], although its effects on response rate, response duration and survival are still controversial [2–6]. HDC is frequently complicated by considerable toxic events; in particular, cardiotoxicity represents one of the side effects impacting on patient's survival and quality of life, independent of the cancer stage. Beyond early cardiotoxicity, which occurs during or soon after treatment, the development of cardiac dysfunction many months after the last administration of chemotherapeutic drugs, is increasingly recognized [7–9]. Usually, cardiac involvement becomes clinically manifest as a poor prognosis cardiomyopathy leading to heart failure.

Nevertheless, with the increasing availability of echocardiography, it has become evident that chemotherapy-induced left ventricular impairment is often asymptomatic.

Hence, there is a rapidly growing expectation, in both oncologists and cardiologists, for new non-invasive and cost-effective opportunities for the early identification of patients most likely to develop late ventricular dysfunction. This would allow clinicians to closely monitor cardiovascular function, to prevent cardiac impairment and to support cardiac function with a specific heart failure treatment. In addition, it might be possible to adjust chemotherapeutic dosages or to shift patients with increased cardiac risk toward a less cardiotoxic schedule.

Acute myocardial injury can be sensitively and accurately identified by measurement of plasma concentration of troponin I (TnI), one of the contractile proteins of the myocardium [10]. A rise in the concentration of TnI in the circulation indicates various degrees of myocardial cell damage, and its determination provides the highest diagnostic accuracy for detecting myocardial cell necrosis [11]. The clinical and prognostic significance of TnI in acute coronary syndromes is well appreciated [12–14], but the possible application of this

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Table 1. High-dose chemotherapy schedules

	EC	TEC	ICE	TICE
Patients (<i>n</i>)	51	85	43	32
Patients pre-treated with Ac (%)	0	0	100	100
Drugs and dosages	Epirubicin 200 mg/mq Cyclophosphamide 4 g/mq	Taxotere 85 mg/mq Epirubicin 200 mg/mq Cyclophosphamide 4 g/mq	Ifosfamide 10 g/mq Carboplatin 1.2 g/mq Etoposide 1.2 g/mq	Taxotere 85 mg/mq Ifosfamide 10 g/mq Carboplatin 1.2 g/mq Etoposide 1.2 g/mq

Ac, anthracyclines; EC, epirubicin–cyclophosphamide; TEC, taxotere–epirubicin–cyclophosphamide; ICE, ifosfamide–carboplatin–etoposide; TICE, taxotere–ifosfamide–carboplatin–etoposide.

peptide in the detection of HDC-induced cardiac damage, as well as in the short- and long-term cardiac risk assessment of breast cancer patients undergoing this kind of treatment, has never been investigated.

Patients and methods

Study population

All consecutive women receiving HDC in our institution for poor prognosis breast cancer were enrolled from July 1997 to October 1999. HDC exclusion criteria were as follows: history of ischemic, valvular and hypertensive heart disease; uncontrolled hypertension; left ventricular ejection fraction (LVEF) <50%; acute or chronic renal insufficiency (serum creatinine >1.5 mg/dl); and liver disease (bilirubin >2 mg/dl, aspartate aminotransferase >2× the upper limit of normal). Patients who did not complete the 1-year observation period after HDC because of oncologic death, loss to follow-up or the beginning of a new chemotherapeutic treatment were also excluded. Two hundred and eleven women (age 46 ± 11 years, mean ± SD) were included in the study. Eighteen patients were receiving calcium antagonist agents for mild hypertension; none were receiving β-blockers, angiotensin converting enzyme inhibitors or diuretics. HDC was administered, as adjuvant treatment, in 112 high-risk untreated breast cancer patients (T1–T3, N1 or N2, 10 involved axillary nodes and positive estrogen receptor tumor or five involved axillary nodes and negative estrogen receptor tumor). HDC was also utilized in 99 patients preoperatively treated with neo-adjuvant chemotherapy with residual more than eight involved axillary nodes, or more than five involved and negative estrogen receptor tumor. None of the patients had been previously treated with radiotherapy.

Informed consent was obtained from all patients before participation in the study, and the protocol was approved by the ethics committee of our institution.

Study protocol

Clinical examination, electrocardiogram and chest X-ray were part of the preliminary evaluation. All eligible patients received HDC after breast surgery in different drug combinations, according to our institution's oncological protocols. Patients previously treated with anthracyclines (*n* = 75) were assigned to ICE (ifosfamide–carboplatin–etoposide; *n* = 43) or TICE (taxotere–ifosfamide–carboplatin–etoposide; *n* = 32) regimens. The remaining patients were treated accordingly to EC (epirubicin–cyclophosphamide; *n* = 51) and TEC (taxotere–epirubicin–cyclophosphamide; *n* = 85) schemes (Table 1). All drugs were administered intravenously, via

a central venous catheter. Cycles were delivered at 28-day intervals three times, and each cycle was supported by autologous peripheral blood progenitors cells and filgrastim (granulocyte colony-stimulating factor, G-CSF) [15]. Peripheral blood progenitors cells were mobilized by filgrastim and collected before the beginning of chemotherapy. After completion of HDC, all patients received radiotherapy to the chest wall in the case of mastectomy (*n* = 78; 41 to the left side), or to the residual breast in the case of breast-conserving surgery (*n* = 133; 81 to the left side).

Plasma TnI concentration was measured before and immediately after, and then 12, 24, 36 and 72 h after every single cycle of HDC (18 assays for each patient).

Cardiac assessment was performed by echocardiography and electrocardiography. In all patients, LVEF (biplane method according to Simpson's rule) was evaluated before HDC and 1, 2, 3, 4, 7 and 12 months after the end of the treatment (i.e. starting 3 months after the baseline evaluation).

Electrocardiogram was performed before and after each HDC cycle and then at all follow-up checks. For all patients, the duration of the follow-up was 14 months from the baseline evaluation. During this period, none were treated with other chemotherapeutic drugs and all patients' management decisions were made without the knowledge of their TnI result.

Laboratory methods

The laboratory method employed for TnI determination was an immunoenzymatic fluorescent assay (Stratus II; Dade, International Inc., Miami, FL, USA). This method utilizes two TnI-specific monoclonal antibodies for independent epitopes [16] and has no detectable cross reactivity with skeletal muscle TnI. The lower limit of detection was 0.35 ng/ml; since at levels between 0.4 and 1.5 ng/ml, the coefficient of variation is 9–14%, we set the cut-off level at 0.5 ng/ml. After centrifuging, plasma was stored immediately at –30°C. All samples were analyzed in duplicate, with an interassay variability ≤0.1 ng/ml and all 'positive' samples were immediately re-tested to confirm the result.

Statistical analysis

The LVEF values were analyzed using a repeated measures model taking into account the correlation among the time periods with an unstructured covariance matrix. Time was treated as a factor and the interaction between time, and TnI value was included in the model. All interactions were tested using F tests based on type 3 sums on squares. Least squares means and corresponding confidence intervals of time × TnI value interactions were calculated. All calculations were performed using PROC

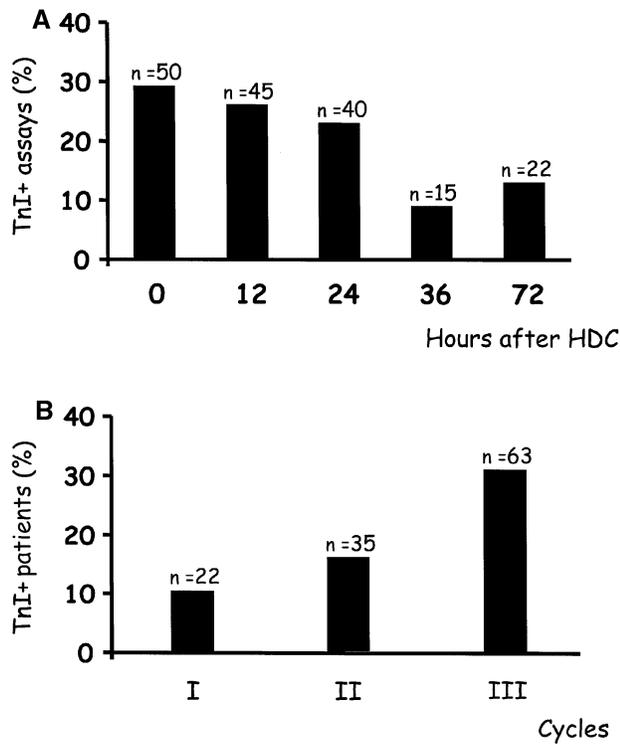


Figure 1. (A) Percentage distribution of TnI⁺ assays after each cycle at the various time points considered. (B) Percentage distribution of TnI⁺ patients after each cycle.

MIXED in SAS. Differences in LVEF values at baseline between the two groups were analyzed using a generalized linear model. Significance was taken at the 5% level. Results are presented as mean \pm SD. Differences in TnI positivity percentage among the different schedules utilized were analyzed according to logistic regression and contrasts method.

Results

All patients underwent HDC without acute cardiological side effects during or soon after the administration of the drugs. Ten patients (4.7%) developed symptoms of heart failure during the follow-up. Seven of them reported only exertional dyspnea; three women developed overt heart failure. In these ten patients heart failure treatment was started and they were not considered in the following period of follow-up.

At the baseline evaluation, as well as before each cycle of HDC, the TnI value was within the normal range in all cases. A rise in the concentration of circulating TnI was detected in 70 of the 211 patients treated (33%) and, when the total number of HDC cycles was considered, in 120 of 633 (19%) cycles. Figure 1 shows the percentage distribution of TnI positive (TnI⁺) assays at the various time points considered and of TnI⁺ patients after each cycle. Fifty-two per cent of TnI⁺ patients had only one positive sample, 11% had two, 8% had three, 7% had four, 8% had five, 7% had six and 4% had seven.

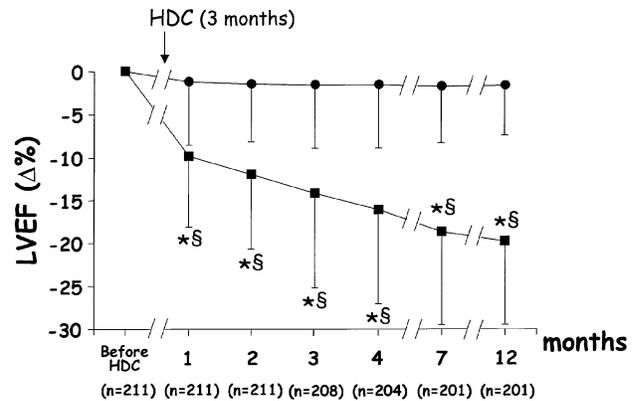


Figure 2. LVEF percentage changes during the follow-up in TnI⁺ (squares) and TnI⁻ (circles) patients. * $P < 0.001$ versus before HDC; $^{\S}P < 0.01$ versus TnI⁻ group.

Taking into consideration the different schedules, the percentage of patients with TnI positivity was 53% in EC, 31% in TEC, 28% in ICE and 16% in TICE. A significant difference was observed among the various schedules, except between TEC and ICE groups. In TnI⁺ group, 17 of 75 (23%) patients had previously received anthracyclines in the neoadjuvant setting.

No electrocardiographic changes were observed in any of the patients, both after HDC and at the follow-up checks.

Patients were grouped according to the maximal TnI value detected after HDC into a negative troponin group (TnI⁻; $n = 141$) and a positive troponin group ($n = 70$). The TnI⁻ group was defined by a value < 0.5 ng/ml in every determination, and the TnI⁺ group was defined by a value ≥ 0.5 ng/ml at least at one of the points of measurement considered (mean value 0.9 ± 0.5 ng/ml; range 0.5–2.3). The two populations were similar with regard to age and other clinical characteristics (incidence of hypertension, smoking habits, kind of surgical intervention, stage of neoplasm).

At the baseline evaluation, LVEF was similar in the two groups, and, in all cases, was within the normal range ($63 \pm 4\%$ and $62 \pm 5\%$ in TnI⁺ and TnI⁻, respectively; $P = 0.23$, not significant). After HDC, there was a different behavior of LVEF in the TnI⁺ group compared with the TnI⁻ group (Figure 2). In the TnI⁺ group, a significant reduction in LVEF was observed after the first month of follow-up. Thereafter, LVEF further worsened during the follow-up period. The TnI⁻ group did not show any significant decrease in LVEF during the entire period of observation.

In TnI⁺ group, a strong relationship between the TnI maximal value detected after HDC and the LVEF maximal reduction observed during the follow-up was found ($r = -0.92$; $P < 0.0001$). A significant correlation was also detected between the number of positive TnI assays per patient and the LVEF maximal decrement ($r = -0.93$; $P < 0.0001$) (Figure 3).

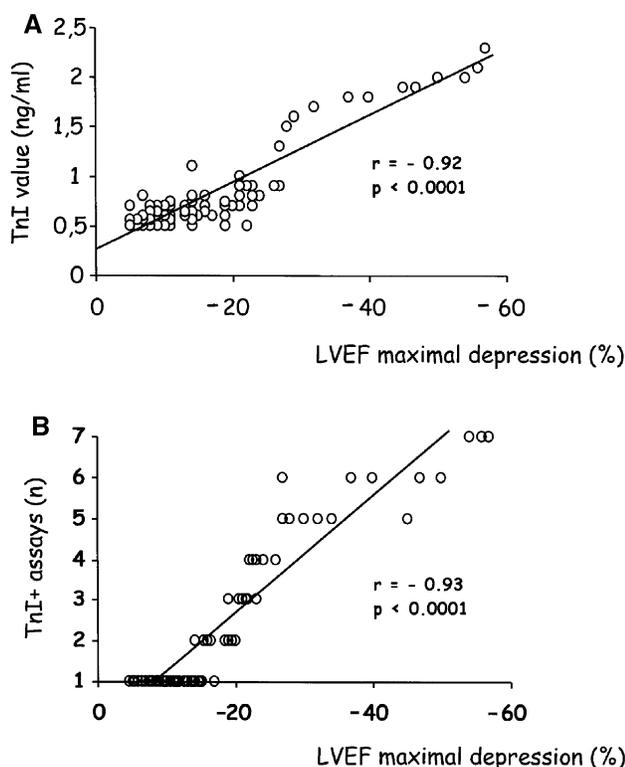


Figure 3. (A) Scatterplot of percent reduction against TnI maximal value in patients with positive TnI. (B) Scatterplot of LVEF against number of positive TnI assays in TnI⁺ group.

The 10 patients which developed symptoms of heart failure during the follow-up, had repeated positive values (range 5–7/18) of TnI after HDC (after EC in four cases, after TEC in two, after ICE in two and after TICE in two). At the time of symptoms onset, LVEF ranged between 30% and 45% in the seven patients having mild heart failure symptoms, and it was <30% in the three women with overt heart failure.

Figure 4 shows the different LVEF pattern in TnI⁺ versus TnI⁻ patients, according to the four different chemotherapy regimens. A similar LVEF course was observed, independently from the HDC schedule.

There was no difference in LVEF reduction between patients who received radiotherapy to the left chest and those in whom it was given to the right chest.

Discussion

HDC has been widely utilized as adjuvant therapy for women with high-risk primary breast cancer [1–6]. However, therapeutic potential of this approach is limited by both acute and chronic toxicity [7–9, 17–19].

While its major acute toxic effects can be largely overcome with hematological growth factors, stem-cell infusion and

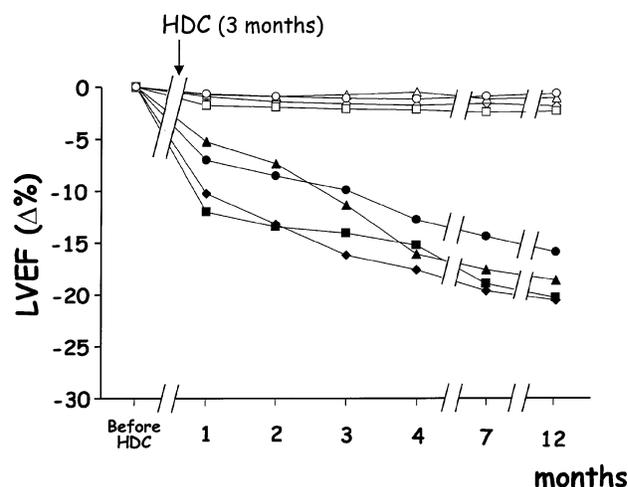


Figure 4. LVEF pattern in TnI⁺ (filled symbols) and TnI⁻ patients (open symbols), according to the four different chemotherapeutic regimens. Diamonds, EC; squares, TEC; triangles, ICE; circles, TICE. For definition of abbreviations see Table 1. Standard deviations were omitted for clarity.

appropriate supportive care, cardiotoxicity remains an unresolved problem. Typically, cardiac involvement becomes manifest late in the course of the natural history of the cardiac disease; it is generally considered to be irreversible and can lead to overt heart failure. Cardiotoxicity is an emerging problem commensurate with the growing number of long-term breast cancer survivors and the increasing number of patients treated with adjuvant chemotherapy.

To detect HDC-induced cardiac damage, regular monitoring of cardiac function is recommended at present [7, 20]. It could be performed during chemotherapy to allow administration of the highest dose without inducing severe cardiac damage. After completion of chemotherapy, monitoring must be carried out again to identify cardiac damage at an early pre-clinical stage, in order to prevent further heart function deterioration by timely medical intervention. The limit of such an approach is that it is too late. In fact, cardiac damage is usually detected when functional impairment has already occurred [17, 19]. Furthermore, the evidence of an unaffected heart function does not exclude the possibility of a future cardiac deterioration [19, 21, 22]. Accordingly, several methods of identifying cardiotoxicity, before it causes irreversible cardiac function depression, have been proposed. Among these are the following: radionuclide imaging tests utilizing monoclonal antimyosin antibodies or metaiodobenzylguanidine [23, 24], study of cardiac autonomic function [25] and endomyocardial biopsy [26]. Although some of these techniques are very sensitive in the detection of heart damage, they have specific limitations and poor positive predictive value.

The early detection of myocardial damage is one of the major challenges to contemporary cardiology. Many recent studies have elucidated the value of both TnT and TnI in the diagnosis and in the risk assessment of acute coronary syn-

dromes [10–14]. Our study addresses the question of whether TnI measurement gives significant information in a population of patients with aggressive breast cancer undergoing HDC, in relation to its possible direct cardiotoxic effect.

Our data demonstrate that TnI is a risk marker for future development of significant LVEF reduction. This information cannot be usually obtained by conventional criteria, such as symptoms, and electrocardiographic and echocardiographic changes. The innovative aspect of this new marker of myocardial damage is that it gives us this information at a very early phase (immediately after the HDC administration), long before a functional impairment can be detected with the other available techniques. The possibility of identifying patients who will develop late myocardial function depression is a golden opportunity for both oncologists and cardiologists. This information can permit oncologists to modify, or to discontinue, the oncologic regimen, or to shift patients toward a less cardiotoxic schedule. In addition, it could allow cardiologists to support cardiac function or to prevent heart dysfunction with cardioprotective agents or with cardiovascular therapy [27, 28]. This issue is particularly important in patients with cancer disease in which onset of cardiac dysfunction, even asymptomatic, importantly limits the therapeutic opportunities and negatively influences the prognosis. On the other hand, the presence of cardiac dysfunction, either symptomatic or not, could be widely counterbalanced by improvement in survival due to enhanced control of the cancer. In contrast, the development of overt heart failure might negatively affect the overall long-term survival, independently from the cancer stage. Compared with other methods of cardiac damage detection, TnI appears to be a more sensitive and specific, and less costly method for the early identification of cardiotoxicity.

The observation that pathological TnI release and significant LVEF reduction can also occur after schedules that are classically considered to have low (TICE) or no (ICE) cardiotoxic properties suggests that their toxic effects probably derive from the combined interaction of different drugs. Indeed, in a previous report, congestive heart failure has been described as occurring when intermediate cumulative doses with multi-agent chemotherapy were utilized [29]. Furthermore, it can be speculated that, in our patients, the previous treatment with anthracyclines might have played either a synergic or a cumulative role.

Evidence of TnT and TnI release after chemotherapy was previously shown in animal studies [30] and in children undergoing anthracycline chemotherapy [31]. In these studies, the elevated troponin levels were correlated with acute clinical toxicity. In addition, Missov et al. [32] described TnI increase during the course of anthracycline chemotherapy in patients with hematological malignancies. However, this is the first study that correlates the presence of TnI increase with the long-term development of cardiac dysfunction, even asymptomatic.

Although the small amount of TnI increase, at least in comparison with that observed in acute coronary syndromes, indicates that only a minimal acute necrosis occurs during HDC, the clinical interest of this increment is quite relevant, as the minor myocardial damage seems to precede left ventricular systolic impairment. Both the underlying mechanism(s) and the time course of the cardiac damage are unclear, and further studies are needed to elucidate whether patients with TnI acute increment and with following LVEF reduction will develop an irreversible dilated cardiomyopathy at a longer observation period. On the other hand, normal TnI values clearly identify patients at lower risk, in which no cardiac damage, at least in the first year after HDC, occurs. In brief, at the present time, TnI result allows separation of patients in which a close monitoring of cardiac function is mandatory from those in which it is not required.

In addition to these prognostic data, TnI also provides us with quantitative information. Indeed, we observed a close relationship between the TnI maximal value after HDC, as well as the number of positive samples after each HDC cycle, and the degree of LVEF reduction (Figure 3). These findings suggest that the HDC-induced myocardial injury, although limited, is an ongoing phenomenon that, once elicited by these drugs, could last for several days or months and results in a cumulative effect reflected by a global left ventricular dysfunction. A more prolonged TnI follow-up could permit us to better understand the time course of the myocardial damage, as well as to better assess the long-term outcome of high-risk patients (TnI⁺). In this regard, the lack of information about TnI behavior after the first 72 h does not allow for obtaining more accurate indications concerning a possible different evolution of cardiac dysfunction among TnI⁺ patients.

Despite this limitation, however, the amplitude of TnI elevation and its release pattern in the days following the oncologic treatment allows us to anticipate, for each patient, the cardiac functional status that will characterize the first year after HDC.

Conclusion

In patients undergoing HDC for aggressive breast cancer, TnI is a sensitive, specific and low cost way to predict the development of ventricular systolic dysfunction in the months to follow, as well as the degree of cardiac impairment. The possibility of carrying out a risk stratification in such a patient population, in a very early phase, has relevant clinical and prognostic implications.

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