

REVIEW ARTICLE

BIOMARKERS OF CARDIAC INJURY IN DETECTION OF CARDIOTOXICITY INDUCED BY CHEMOTHERAPEUTIC AGENTS

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Summary

Cardiotoxicity is a well-known and potentially serious complication of oncology treatment. Anthracyclines and high-dose chemotherapy especially regimens containing high-dose Cyclophosphamide represent the greatest risk. Early detection of cardiotoxicity is crucial for applying preventive and supportive therapeutic strategies. Various methods have been recommended for monitoring of cardiotoxicity. In our conditions, echocardiography and electrocardiography are routinely used. However, this approach shows low sensitivity for the early prediction of cardiomyopathy when the possibilities of appropriate management could still improve the patient's outcome.

Recently, biomarkers of cardiac injury have been investigated in the assessment of chemotherapy-induced cardiotoxicity. Cardiospecific biomarkers, such as cardiac troponins, show high diagnostic efficacy in the early subclinical phase of the disease before the clinical onset of cardiomyopathy. The increase in their concentrations correlates with disease severity. As for natriuretic peptides, some studies, including ours, have shown promising results. Definitive evidence of their diagnostic and prognostic role in this context is still lacking and natriuretic peptides have not been routinely used for monitoring of cardiotoxicity in clinical practice. Other perspective biomarkers of cardiotoxicity in oncology are under study, especially heart-type fatty acid-binding protein (H-FABP) and glycogen phosphorylase BB (GPBB). Our studies using GPBB have brought priority and encouraging results. However, the available data are limited and their practical use in this context cannot be recommended until their clinical efficacy is clearly defined.

The author presents his own experience with multiple biomarkers of cardiac injury in the detection of cardiotoxicity associated with conventional and high-dose chemotherapy for hematological malignancies.

Key words: cardiac biomarkers; cardiotoxicity; chemotherapy; oncology.

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Abbreviations used: ANP – atrial natriuretic peptide; BNP – brain natriuretic peptide; CK-MB – creatine kinase MB; CT – chemotherapy; cTnI – cardiac troponin I; cTnT – cardiac troponin T; GPBB – glycogen phosphorylase BB; HCT – hematopoietic cell transplantation; HD-CT – high-dose

chemotherapy; H-FABP – heart-type fatty acid-binding protein; LV – left ventricular; LVEF – left ventricular ejection fraction; NT-proBNP – N-terminal pro brain natriuretic peptide.

INTRODUCTION

Cardiotoxicity is a well-known and potentially serious complication of oncology treatment that can significantly impair patient's quality of life and also substantially increase health care costs. A wide range of chemotherapeutic agents has been associated with cardiotoxicity as shown in Table 1.

Cytotoxic antibiotics	Antimicrotubule agents
Anthracyclines	Taxanes
Doxorubicin	Paclitaxel
Daunorubicin	Docetaxel
Idarubicin	Etoposide
Epirubicin	Vinca alkaloids
Mitoxantrone	Monoclonal antibodies
Alkylating agents	Trastuzumab
Cyclophosphamide	Rituximab
Ifosfamide	Alemtuzumab
Cisplatin	Tyrosine kinase inhibitors
Mitomycin	Imatinib
Busulfan	Sunitinib
Antimetabolites	Miscellaneous
5-fluorouracil	All-trans retinoic acid (ATRA)
Capecitabine	Interleukin-2
Methotrexate	Interferon-alpha
Fludarabine	
Cytarabine	

Table 1. Chemotherapeutic agents with potential cardiotoxic effects (modified from Dolci et al, 2008)

From chemotherapeutic agents, anthracyclines represent the greatest risk for development of cardiotoxicity. Despite conflicting reports, proposed risk factors for anthracycline cardiotoxicity include: 1. Cumulative dose of the drug (the most important and independent risk factor), 2. Age under 3 years or over 65 years,

3. Irradiation of the mediastinum, 4. Combination with other cardiotoxic chemotherapy (CT), 5. female gender, 6. Heart damage caused by another disease (coronary atherosclerosis, arterial hypertension, diabetes mellitus, valvular heart disease), 7. bolus administration of the drug [1,2]. High-dose chemotherapy (HD-CT) especially regimens containing high-dose Cyclophosphamide [3-6] are also associated with high risk for development of cardiotoxicity.

In general, two forms of CT-induced cardiotoxicity [7] may be distinguished: (1) Acute and subacute cardiotoxicity, found less frequently, can occur anytime from the initiation of CT up to 2 weeks after termination of treatment. In this form, the most common clinical findings range from abnormalities in ventricular repolarization and QT interval changes to supraventricular and ventricular arrhythmias or to acute coronary syndromes, acute heart failure, and pericarditis/myocarditis-like syndromes. (2) Chronic cardiotoxicity, the most frequent cumulative dose-dependent form, may be differentiated in 2 subtypes based on the timing of onset of clinical symptoms: early, within 1 year of the termination of CT, and late, after 1 year. The most typical sign of chronic cardiotoxicity is asymptomatic systolic and/or diastolic left ventricular (LV) dysfunction that leads to severe congestive cardiomyopathy and may eventually lead to death. Incidence of chronic cardiotoxicity depends on presence of risk factors, time of follow-up, criteria used for cardiotoxicity definition and diagnostic methods used for cardiotoxicity identification, ranging in different studies from 5 % to 65 % of patients [7-10]. Anthracycline-induced cardiotoxicity is often divided into 4 subgroups: acute, subacute, chronic (occurring within 1 year of treatment) and late-onset (occurring more than 1 year after the completion of treatment).

DIAGNOSTIC METHODS FOR IDENTIFICATION OF CT-INDUCED CARDIOTOXICITY

Early identification of patients at risk for cardiotoxicity represents a primary goal for cardiologists and oncologists, considering the definition of personalized anticancer therapeutic strategies or interventions [11]. For the detection of subclinical myocardial damage, time and expensive monitoring of cardiac functions is still recommended during and after CT [7,12,13]. Nevertheless, most of

the approaches commonly used in clinical practice – evaluation of left ventricular ejection fraction (LVEF) by echocardiography or radionuclide ventriculography – showed low diagnostic sensitivity and low predictive power in detecting subclinical myocardial injury. The use of some other techniques, such as endomyocardial biopsy, is troublesome in clinical practice owing to the invasiveness of the techniques [7,12-14].

Thus, there is a growing expectation for newer, noninvasive and cost effective diagnostic tools for the early identification of patients susceptible to developing CT-induced cardiotoxicity [15]. The use of easily detectable cardiac biomarkers in blood has been evaluated in animal models and clinical studies [16-21]. Screening of high-risk patients is recommended for the detection of early subclinical cardiotoxicity.

● 1. Conventional methods

At least 3 international consensus guidelines recommend evaluation of LVEF at the beginning of anticancer therapy, after administration of a half of the total anthracycline cumulative dose, and before each of subsequent doses [22-24]. During the follow-up, LVEF evaluation within 3, 6 and 12 months after completion of treatment is recommended [7,14]. A decline in LVEF by more than 10 %, associated with absolute LVEF below 50 %, has been suggested as a criterion for suspending treatment [25]. Following this approach, the risk of development of clinically confirmed heart failure has been reduced in some studies to less than 5 % in patients treated with anthracycline agents [25,26].

However, some major limitations of this approach in clinical practice have been pointed out [15]. Not all patients treated with CT require such frequently repeated LVEF monitoring as suggested by the guidelines because of the negative impact on patient management and cost-effectiveness ratio for the national health system [27]. Moreover, many doubts have been raised about the usefulness of monitoring cardiac function by LVEF evaluation solely because the value of this monitoring seems to be neither sensitive nor specific enough for the early prediction of development of cardiac dysfunction after CT. Therefore, it permits the identification of cardiac damage only after the onset of cardiac dysfunction, not permitting any early interventional strategy capable to prevent future development of cardiomyopathy [9,28].

● 2. Biochemical methods

Evaluation of cardiac biomarkers capable to specifically detect myocardial injury and to predict ventricular dysfunction could represent an alternative diagnostic tool for the early detection of cardiotoxicity [29]. Previous reports consistently laid the theoretical basis for the possible use of cardiac troponins and natriuretic peptides in the early detection of cardiotoxicity in clinical practice, whereas creatine kinase MB (CK-MB) does not seem to be effective owing to a short time window of the serum elevation after myocardial injury and its imperfect cardiac specificity and sensitivity [30,31].

In January 2011, a position statement from the Heart Failure Association of the European Society of Cardiology on “Cardiovascular side effects of cancer therapies” was published [32]. The main recommendations among others include that identification and validation of reliable biomarkers for the prediction and detection of cardiotoxicity of chemotherapeutic agents is urgently required. The use of simple biomarkers such as troponins and natriuretic peptides should be strongly considered but is not a substitute for objective evaluation by echocardiography or similar modalities. When designing clinical trials with potentially cardiotoxic agents, the routine use of currently available biomarkers (e.g. troponins and natriuretic peptides) should be strongly considered and their validation incorporated into the trial design, if possible.

The Expert Working Group on Biomarkers of Drug-Induced Cardiac Toxicity developed the following list of characteristics of “ideal biomarkers”, which includes specificity, sensitivity, kinetics of appearance in accessible media, robust assay, and ability to bridge between preclinical and clinical applications [33].

On this basis, we performed an analysis of the available scientific literature to define the clinical usability of cardiac biomarkers for detection of cardiotoxicity in oncology.

● 2.1. Cardiac troponins as markers of CT-induced cardiotoxicity

The clinical application of cardiac troponins as cardiotoxicity biomarkers was analyzed in 7 clinical studies with a consistent number of subjects (> 40 patients enrolled) monitored by cardiac troponin I (cTnI) or cardiac troponin T (cTnT), for the total number of almost 1 500 adult patients treated with CT for cancer [34-40]. See Table 2.

Author/Year	No. (%) Troponin +	Troponin Type	Cut-offs (µg/L)
Cardinale et al, 2000 [34]	204 (32 %)	cTnI	> 0.50
Cardinale et al, 2002 [35]	211 (33 %)	cTnI	> 0.50
Sandri et al, 2003 [36]	179 (32 %)	cTnI	> 0.08
Auner et al, 2003 [37]	78 (15 %)	cTnT	> 0.03
Cardinale et al, 2004 [38]	703 (30 %)	cTnI	> 0.08
Lipshultz et al, 2004 [39]	76 (32 %)	cTnT	> 0.03
Kilickap et al, 2005 [40]	41 (34 %)	cTnT	> 0.01

Table 2. Clinical studies on cardiac troponins as markers of CT-induced cardiotoxicity (modified from Dolci et al, 2008)

The evidence emerging from these studies is that the percentage of patients with positive troponin values ranges from 15 to 34 %. Thus, the increase in troponin concentrations in the blood underlines the occurrence of irreversible myocardial cell injury in patients treated with potentially cardiotoxic CT.

The agreement in defining the cut-off values for cardiac troponins (concentration measured with an analytic imprecision expressed as the coefficient of variation ≤ 10 %), despite the availability of several methods for troponin determination, would lead to a useful unification of the definition of positive troponin results for the detection of myocardial injury related to cardiotoxicity. This cut-off provides the highest level of sensitivity for detection of myocardial injury at an acceptable level of analytic reliability. Adopting a univocal definition of positivity makes the troponin test very useful in clinical practice to monitor cardiac injury independent of the method used and of the laboratory performing the assay.

On the contrary, the sampling protocol used in different studies is not as homogeneous as expected [18]. It is important to note that the increase of troponin concentrations was detected at different intervals after administration of CT in various studies indicating that it may be necessary to collect several blood samples to demonstrate the possible increase of the marker [41].

Clinical evidence derived from published studies can be summarized as follows: (1) Troponin determination is able to predict the occurrence of a clinically significant LV dysfunction at least 3 months in advance [35,40]. (2) The early increase in the troponin concentrations also predicts the degree and severity of LV dysfunction in the future [35,38]. (3) Among patients with positive troponin

values, persistence of the increase within 1 month after the last CT is related to 85 % probability of major cardiac events within the first year of the follow-up [38,42]. (4) A persistently negative troponin test result can identify patients with the lowest cardiotoxicity risk (negative predictive value of 99 %), who will not encounter cardiac complications at least within the first year after completion of CT.

From this scientific evidence, we can derive the main practical advantages of the use of troponin testing as a biomarker of cardiotoxicity, especially when it is compared with the low efficacy of any other method currently applied in this clinical setting: (1) Troponin determination detects the presence of cardiotoxicity very early, significantly before impairment of cardiac functions can be revealed by any other diagnostic method. (2) Immediately after the last CT, troponin determination allows the discrimination of patients at low risk from patients at high risk for cardiotoxicity requiring more careful long-term cardiac monitoring by imaging techniques.

The role of cardiac troponin determination to stratify the risk of cardiotoxicity is currently based on strong evidence clearly suggesting the routine use of this biomarker [43]. Cardiac troponins have been incorporated into the National Cancer Institute (NCI) classification of cardiotoxicity of anticancer therapy (Common Terminology Criteria for Adverse Events, CTCAE) [44].

• 2.2. Our experience with cardiac troponins as markers of cardiotoxicity in oncology

We evaluated acute and chronic cardiotoxicity of anthracyclines using current immunoassays for cTnT

(Roche Diagnostics, 4th generation) and cTnI (Randox Laboratories Ltd.), and correlated the results with findings on echocardiography.

A total of 23 patients (mean age 47.0 ± 11.1 years, 14 males) with acute leukemia were studied. The patients were treated with 3 – 6 cycles of conventional CT containing anthracycline agent in the total cumulative dose of 472.1 ± 115.0 mg/m²; to calculate the total cumulative dose of anthracyclines, we applied conversion factors derived from the maximum recommended cumulative doses for individual agents used (Idarubicin, Daunorubicin, Mitoxantrone). All patients had normal liver and renal functions during the study. Cardiac evaluation was performed at the baseline (before CT), the day after first CT with anthracyclines (mean cumulative dose 135.8 ± 28.5 mg/m², median 150), the day after last CT with anthracyclines (mean cumulative dose 472.1 ± 115.0 mg/m², median 423) and circa 6 months after completion of CT (6 months after CT).

Concentrations of cardiac troponins diagnostic for cardiotoxicity of oncology treatment have not

been definitely established yet. In our study, values above the reference range recommended by the manufacturer were considered elevated. The cut-off value for cTnT was 0.03 µg/L and for cTnI 0.40 µg/L. Echocardiographic evaluation was performed on Hewlett Packard Image Point machine by an experienced echocardiographer who was blind to the cardiac troponin data. Parameters of systolic and diastolic LV function were assessed. Systolic LV dysfunction was defined as LVEF less than or equal to 55 %. Diastolic LV dysfunction was defined as E/A inversion and E-wave deceleration time above 220 ms on the transmitral Doppler curve (impaired relaxation).

Statistical analysis was performed with the “Statistica” program. Analysis of variance test was used. Correlations were evaluated with normal and Spearman correlation tests. The values are expressed as mean \pm SD. Probability values (p) < 0.01 and lower were considered statistically significant. Similar statistical methods were used in our subsequent studies.

The results are summarized in Table 3.

abnormal cardiac findings	before CT	after first CT	after last CT	6 months after CT
cTnT above 0.03 µg/L	0	0	0	3 (13.0 %)
cTnI above 0.40 µg/L	0	4 (17.4 %)	4 (17.4 %)	6 (26.1 %)
systolic LV dysfunction	0	1 (4.3 %)	3 (13.0 %)	5 (21.7 %)
diastolic LV dysfunction	1 (4.3 %)	4 (17.4 %)	6 (26.1 %)	10 (43.5 %)

Table 3. Abnormal cardiac findings associated with anthracycline-based CT for acute leukemia (n = 23)

From cardiac troponins, only cTnI become positive the day after first and last CT with anthracyclines, in both cases in 4 (17.4 %) patients. Positivity of cTnI correlated with systolic and diastolic LV dysfunction on echocardiography – ($r = 0.712$; $p < 0.00001$) and ($r = 0.591$; $p < 0.0001$), respectively. Patients with cTnI positivity during anthracycline treatment had a significantly greater decrease in LVEF during the follow-up compared to cTnI-negative patients (12.2 ± 7.4 % versus 3.3 ± 4.2 %, $p = 0.003$). Two patients with early cTnI positivity during treatment developed anthracycline-induced cardiomyopathy with symptoms of heart failure during the follow-up. Positivity of cTnT within 6 months after treatment only coincided with LV dysfunction and cardiomyopathy on echocardiography. In asymptomatic patients, abnormal cardiac

findings during and after anthracycline treatment are considered subclinical cardiac toxicity and require further follow-up. In our cohort, we did not find a significant correlation between the total cumulative dose of anthracyclines and elevation of cardiac troponins or LV dysfunction on echocardiography after treatment.

Our results suggest that evaluation of cTnI – in contrast with cTnT – during anthracycline treatment could identify patients at risk for development of anthracycline-induced cardiomyopathy in the future. cTnI seems to be superior to cTnT in the early detection of cardiac injury associated with anthracycline treatment in acute leukemia. The possible explanation could be the difference in the molecular weight and release kinetics of cTnI and cTnT. cTnI is somewhat

smaller than cTnT (23.5 kDa and 38 kDa, respectively) and thus might be released more easily and earlier from the cardiomyocytes injured from anthracycline treatment. The other explanation might be that cTnI unlike cTnT is more prone to degradation and the fragments are detected by the assay used. Based on our preliminary data, a larger prospective and multicenter study would be most desirable.

Our further experience with cardiac troponins in the detection of CT-induced cardiotoxicity is shown in section 2.6.

Since our experience with cardiac troponins in the peritransplant period (HD-CT followed by HCT) is inconclusive, we plan to test the latest immunoassay for cTnT recently introduced by Roche Diagnostics – 5th generation with sensitivity of 0.005 µg/L (limit of detection).

• 2.3. Natriuretic peptides as markers of CT-induced cardiotoxicity

Natriuretic peptides – atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and N-terminal pro brain natriuretic peptide (NT-proBNP) – are produced by myocardium in response to wall strain and pressure overload. ANP is produced mainly in atria, BNP/NT-proBNP predominantly in ventricles. In cardiology, natriuretic peptides are routinely used in diagnostics and management of cardiac dysfunction and heart failure [45,46]. Normal plasma BNP/NT-proBNP concentrations practically exclude heart failure due to high negative predictive value of the test [47,48].

The applicability of natriuretic peptides (ANP, BNP, NT-proBNP) as markers for anthracycline-induced cardiotoxicity has been investigated in a limited number of studies. The results of some studies suggested that natriuretic peptides could be of value in the detection of clinical and subclinical cardiotoxicity of anthracyclines [49-54]. Recently published studies reported significant BNP/NT-proBNP elevations after HD-CT and hematopoietic cell transplantation (HCT) [55-58]. Persistent BNP/NT-proBNP elevations early after HD-CT were observed in 33 – 47 % patients and were associated with the development of cardiac dysfunction during the follow-up. The results suggest that monitoring of BNP/NT-proBNP could identify patients at risk for development of cardiac dysfunction after HD-CT and HCT.

On the other hand, some studies using natriuretic peptides in the detection of CT-induced cardio-

toxicity reported limited clinical usefulness of this method [19,59,60].

The published data are quite heterogeneous and often incomplete, lacking crucial information such as the ratio of patients with increased natriuretic peptide values, the methods used to measure natriuretic peptides and the cut-off values associated with the best diagnostic accuracy. Regarding the methods used to detect the occurrence of cardiac dysfunction, several articles reported the use of echocardiography or radionuclide ventriculography. Some authors studied the association and relationship between natriuretic peptides and diastolic LV dysfunction, and others simply checked systolic LV function. However, only a few studies [61-65] evaluated the potential predictive value of natriuretic peptide concentrations to detect the ongoing development of cardiac dysfunction.

A lack of agreement in the conclusions of different studies is evident. Overall, studies were unable to confirm definitively the clinical usefulness of natriuretic peptides as cardiotoxicity biomarkers.

Even though there are some promising data available, it is not currently possible to recommend the routine use of the natriuretic peptides for monitoring of cardiotoxicity in clinical practice. New prospective studies on large cohorts of patients using validated, commercially available assays and comparing natriuretic peptides with well-established markers of cardiotoxicity are needed.

• 2.4. Our experience with natriuretic peptides as markers of cardiotoxicity in oncology

We evaluated the utility of NT-proBNP (Roche Diagnostics) for monitoring of cardiotoxicity associated with HD-CT followed by HCT and with conventional CT containing anthracyclines.

• 2.4.1. NT-proBNP in the detection of cardiotoxicity associated with HD-CT followed by HCT

A total of 23 adult acute leukemia patients were studied. The patients consisted of 15 males and 8 females with the mean age of 44.5 ± 10.6 years (range: 22 – 60, median 44). Six patients were treated for arterial hypertension; other patients had no pre-existing cardiovascular disease. Renal and liver functions were normal during the study in all patients. The patients were previously treated with 2 – 6 cycles of conventional CT containing anthracyclines in the

total cumulative dose of 452.2 ± 87.9 mg/m² (range: 240 – 609, median 429). Cycles of anthracycline-based CT were administered 2 – 9 months prior to HCT. Preparative regimen consisted of high-dose Cyclophosphamide in the total dose of 120 mg/kg (60 mg/kg/day in a 3-hour intravenous infusion on 2 consecutive days) in all patients, in 17 patients in combination with peroral Busulfan 16 mg/kg (Bu/Cy2) and in 6 patients in combination with fractionated total body irradiation 12 Gy (Cy/TBI). In all cases, cryopreserved peripheral blood stem cells were used as the source for HCT. Thirteen patients were given allogeneic grafts and 10 autologous grafts. Before HD-CT, all patients had normal systolic LV function on echocardiography, 3 patients had echocardiographic signs of diastolic LV dysfunction (impaired relaxation on the transmitral Doppler curve).

Serial measurements of cardiac biomarkers were performed the day before HD-CT (baseline), the day after administration of HD-CT, the day after HCT and 14 days after HCT, i.e. at the time of bone marrow recovery. We measured NT-proBNP

according to the manufacturer's guidelines (Roche Diagnostics; Elecsys analyzer). NT-proBNP concentrations diagnostic for cardiotoxicity of oncology treatment have not been established yet. Thus values above the reference range recommended by the manufacturer and based on a number of studies [47,48] were considered to be elevated in our study. The cut-off values were 100 ng/L for male, 150 ng/L for female (respecting gender). NT-proBNP concentrations above 500 ng/L were considered to be markedly elevated and suggesting functional cardiac injury associated with the treatment.

The day before HD-CT, mean plasma NT-proBNP concentration was 109.9 ± 54.1 ng/L. The mean NT-proBNP concentration increased to 433.4 ± 393.4 ng/L after completion of HD-CT. After HCT, a further increase to 825.6 ± 740.7 ng/L was observed. Fourteen days after HCT, the mean NT-proBNP concentration was 365.5 ± 252.0 ng/L. The differences were statistically significant in comparison with the baseline values ($p < 0.01$). The number of patients with elevated NT-proBNP concentrations is shown in Table 4.

NT-proBNP	1 day before HD-CT	1 day after HD-CT	1 day after HCT	14 days after HCT
above 100/150 ng/L	4 (17.4 %)	14 (60.9 %)	16 (69.6 %)	16 (69.6 %)
above 500 ng/L	0	6 (26.1 %)	9 (39.1 %)	7 (30.4 %)

Table 4. Elevated plasma NT-proBNP concentrations in the peritransplant period in acute leukemia (n = 23)

Correlations between NT-proBNP concentrations and gender, age, history of arterial hypertension, body mass index, febrile episodes, CRP and hemoglobin levels were not significant. Correlation between baseline NT-proBNP or subsequent changes in NT-proBNP concentrations and the baseline parameters of LV function and LV diameters on echocardiography did not reach statistical significance.

In the peritransplant period, one patient (4.3 %) developed manifestation of cardiotoxicity – clinical signs of congestive heart failure, a significant decrease in systolic LV function on echocardiography (decrease in LVEF more than 15 % from the baseline value and LVEF decline to 50 %), NT-proBNP concentrations 659.0 ng/L (after HD-CT) and 2228.0 ng/L (after HCT). The patient was treated with diuretics and ACE inhibitors with a good

response. In this patient, baseline NT-proBNP was 319.9 ng/L, which was by far the highest value in the cohort.

In our study, the patients were pretreated with anthracycline-based CT (median cumulative dose 429 mg/m²), which can explain the slightly elevated NT-proBNP concentrations in 4 (17.4 %) patients even before administration of HD-CT. We found pronounced NT-proBNP elevations (above 500 ng/L) in 6 (26.1 %) patients early after HD-CT and in 9 (39.1 %) patients early after HCT. Renal functions were normal in all patients. Overhydration was avoided by careful monitoring of fluid balance. In our previously published paper, we showed that solely intravenous hydration in acute leukemia patients did not cause a significant increase in NT-proBNP [64]. Since we did not find a correlation with other factors potentially influencing the NT-proBNP

concentrations, we attribute these significant NT-proBNP elevations to acute functional myocardial injury caused by administration of HD-CT and infusion of cryopreserved graft of hematopoietic stem cells. In our cohort, NT-proBNP concentrations remained markedly elevated in 7 (30.4 %) patients 14 days after HCT. These NT-proBNP elevations show persistent neurohumoral activation of cardiac dysfunction and indicate subclinical cardiotoxicity of the undergone treatment which represents a risk for development of heart failure in the future and requires further follow-up.

Administration of preparative regimen containing high-dose Cyclophosphamide (120 mg/kg) may lead to clinical manifestation of cardiac toxicity – in 1 (4.3 %) patient in our cohort. Development of acute heart failure in the patient with the highest baseline NT-proBNP concentration (319.9 ng/L) suggests that implementation of NT-proBNP assay to commonly performed pretransplant cardiac examinations could be useful in the identification of patients at high risk for development of acute heart failure and in the early diagnostics of cardiac dysfunction in the peritransplant period.

• 2.4.2. NT-pro BNP in the detection of cardiotoxicity associated with conventional CT containing anthracyclines

A total of 26 acute leukemia patients (mean age 46.2 ± 12.4 years, 15 males) treated with 2 – 6 cycles of CT containing anthracyclines in the total cumulative dose of 464.3 ± 117.5 mg/m² were studied. Cardiac evaluation, including NT-proBNP testing and echocardiography, was performed at baseline (before CT), after first CT with anthracyclines (cumulative dose 136.3 ± 28.3 mg/m²), after last CT with anthracyclines (cumulative dose 464.3 ± 117.5 mg/m²) and 6 months after completion of CT. Methods and cut-off values for NT-proBNP were the same as mentioned in the previous study.

Mean baseline NT-proBNP concentration was 117.7 ± 46.4 ng/L. After first and last CT, NT-proBNP elevations to 299.7 ± 176.2 ng/L and 287.1 ± 147.4 ng/L were observed, respectively. Six months after CT, mean NT-proBNP concentration was 362.5 ± 304.9 ng/L. Changes in NT-proBNP were significant in comparison with the baseline values ($p < 0.001$).

The number of patients with elevated NT-proBNP concentrations is shown in Table 5.

NT-proBNP	before CT	after first CT	after last CT	6 months after CT
above 100/150 ng/L	3 (11.5 %)	23 (88.5 %)	23 (88.5 %)	16 (61.5 %)
above 500 ng/L	0	5 (19.2 %)	4 (15.4 %)	4 (15.4 %)

Table 5. Elevated plasma NT-proBNP concentrations associated with anthracycline-based CT for acute leukemia (n = 26)

Six months after CT, 2 patients with marked NT-proBNP elevations during CT developed treatment-related cardiomyopathy with symptoms of heart failure. NT-proBNP correlated with systolic and diastolic LV dysfunction on echocardiography ($r = 0.514$; $p < 0.01$) and ($r = 0.587$; $p < 0.01$).

Our study shows that anthracycline treatment is associated with acute and chronic neurohumoral activation of cardiac dysfunction that is manifested by a significant increase in NT-proBNP. NT-proBNP correlated with LV dysfunction on echocardiography. It seems that NT-proBNP could be useful in the early detection of anthracycline-induced cardiotoxicity. Further studies on a larger number of patients and with a longer follow-up will be needed.

• 2.5. Perspective markers of CT-induced cardiotoxicity (under study)

Other potential markers of cardiotoxicity in oncology are under study, especially heart-type fatty acid-binding protein (H-FABP) and glycogen phosphorylase BB (GPBB).

H-FABP and GPBB are newer perspective markers for the early detection of myocardial ischemia and necrosis, recently evaluated in the diagnostics and risk stratification of acute coronary syndromes [66-70]. H-FABP is a relatively small cytoplasmic protein for the oxidation of fatty acids that is quite specific for cardiac muscle. H-FABP is rapidly released from the myocardium after ischemic injury into the

bloodstream. Plasma H-FABP increases above the reference limit within 2 – 3 hours of the onset of myocardial injury and returns to normal values within 18 – 30 hours. GPBB is a glycogenolytic enzyme providing glucose for the heart muscle tissue. During glycogenolysis in ischemic tissue, GPBB is released from the sarcoplasmic reticulum into the cytoplasm and then into the circulation through the damaged cell membrane. GPBB is released into the circulation 2 – 4 hours after myocardial injury, returning to normal values within 24 – 36 hours of damage occurrence. In the acute coronary syndrome setting, both markers are regarded as early markers of cardiac injury due to acute myocardial ischemia. The main mechanism of cardiac injury caused by anticancer therapy is mainly non-ischemic and prior cyclic exposition to anthracycline agents may play a role (chronic and late cardiotoxicity). Therefore, it is difficult to estimate the kinetics of release of these biomarkers from cardiomyocytes in this setting.

Experience with these perspective biomarkers in the assessment of cardiotoxicity of anticancer therapy is very limited.

ElGhandour et al [71] studied H-FABP in 40 non-Hodgkin's lymphoma patients treated with 6 cycles of CT containing Doxorubicin (cumulative dose 300 mg/m²). The authors concluded that H-FABP may serve as a reliable early marker for prediction of cardiomyopathy induced by Doxorubicin.

Since 2007, we have published several papers dealing with multiple biomarkers of cardiac injury, including GPBB and H-FABP, to detect cardiotoxicity associated with CT for hematological malignancies – conventional CT containing anthracyclines and HD-CT followed by HCT [72-78].

• 2.6. Our experience with perspective markers of cardiotoxicity in oncology

We evaluated the utility of GPBB and H-FABP along with other cardiac biomarkers for the detection of cardiotoxicity associated with CT containing anthracyclines and HD-CT followed by HCT. We can summarize our experience with these perspective markers of cardiotoxicity as follows:

• 2.6.1. Multiple biomarkers in the detection of cardiotoxicity associated with conventional CT containing anthracyclines and HD-CT followed by HCT for acute leukemia

We evaluated cardiac toxicity of conventional CT containing anthracyclines and HD-CT followed by HCT with multiple biomarkers of cardiac injury: GPBB, H-FABP, cTnT, cTnI, CK-MB mass, myoglobin.

A total of 47 patients treated for acute leukemia were included in the study. The study population was divided into 2 groups – (1) 24 patients treated with conventional CT containing anthracyclines (3 – 6 cycles of CT, mean total cumulative dose 463.2 ± 114.3 mg/m²), mean age 48.1 ± 10.9 years, 13 males; (2) 23 patients treated with HD-CT (ablative preparative regimen containing high-dose Cyclophosphamide in the total dose of 120 mg/kg in combination with peroral Busulfan 16 mg/kg /BuCy2/ or fractionated total body irradiation 12 Gy /CyTBI/) followed by HCT, mean age of 44.5 ± 10.6 years, 15 males. All patients had normal liver and renal functions during the study.

In the first group, biochemical analysis was performed at the baseline (before CT), the day after first CT with anthracyclines (after first CT; mean

cardiac biomarkers	before CT	after first CT	after last CT
myoglobin above 76.0 µg/L	0	0	0
CK-MB mass above 4.80 µg/L	0	0	0
cTnT above 0.03 µg/L	0	0	0
cTnI above 0.40 µg/L	0	2 (8.3 %)	2 (8.3 %)
H-FABP above 4.50 µg/L	0	0	0
GPBB above 7.30 µg/L	0	4 (16.7 %)	5 (20.8 %)

Table 6. Elevated biomarkers of cardiac injury in association with anthracycline-based CT for acute leukemia (n = 24)

cardiac biomarkers	before HD-CT	after HD-CT	after HCT
myoglobin above 76.0 µg/L	0	0	0
CK-MB mass above 4.80 µg/L	0	0	0
cTnT above 0.03 µg/L	0	0	0
cTnI above 0.40 µg/L	0	0	0
H-FABP above 4.50 µg/L	0	0	0
GPBB above 7.30 µg/L	0	5 (21.7 %)	5 (21.7 %)

Table 7. Elevated biomarkers of cardiac injury in association with HD-CT and HCT for acute leukemia (n = 23)

cumulative dose 130.6 ± 29.8 mg/m²), the day after last CT with anthracyclines (after last CT; mean cumulative dose 463.2 ± 114.3 mg/m²). In the second group, cardiac biomarkers were assessed the day before administration of HD-CT (before HD-CT), the day after completion of HD-CT (after HD-CT) and the day after HCT (after HCT).

Circulating biomarkers of cardiac injury were measured according to the manufacturer's guidelines as follows: cTnT, CK-MB mass, myoglobin (Roche Diagnostics; Elecsys analyzer), GPBB, H-FABP, cTnI (Randox Laboratories Ltd.; Evidence analyzer).

Concentrations of cardiac biomarkers diagnostic for cardiotoxicity of oncology treatment have not been established yet. Therefore values above the reference range based on a number of cardiology studies and recommended by the manufacturers were considered elevated. The cut-off values for cardiac injury were as follows: 7.30 µg/L for GPBB, 4.50 µg/L for H-FABP, 0.40 µg/L for cTnI, 0.03 µg/L for cTnT, 4.80 µg/L for CK-MB mass and 76.0 µg/L for myoglobin. The results are shown in Table 6 and Table 7.

Before CT/HD-CT, all biomarkers of cardiac injury were below the cut-off values in all patients. GPBB concentrations increased above the cut-off in 4 (16.7 %) patients after first CT and in 5 (20.8 %) patients after last CT with anthracyclines. In the second group, GPBB increased above the cut-off in 5 (21.7 %) patients after HD-CT and remained elevated in 5 (21.7 %) patients after HCT. CTnI concentrations became elevated in 2 (8.3 %) patients after first and last CT with anthracyclines. Both patients with cTnI positivity had elevated GPBB. CTnI remained negative after HD-CT and HCT in all patients. Other tested biomarkers (H-FABP, cTnT, CK-MB mass, myoglobin) remained below the cut-offs during conventional CT containing anthracyclines and HD-CT followed by HCT.

In our study on 47 acute leukemia patients, we found significant elevations in GPBB after CT containing anthracyclines (in 16.7 % and 20.8 % patients, respectively) and after HD-CT followed by HCT (in 21.7 % patients). Increased release of GPBB from cardiomyocytes after administration of CT could be considered a sign of acute subclinical cardiotoxicity of this treatment. Positivity of GPBB in patients with negativity of other biomarkers (cTnI, cTnT, H-FABP, CK-MB mass, myoglobin) suggests that GPBB could be a more sensitive marker for detection of acute cardiac injury caused by anticancer therapy, both conventional and HD-CT. Whether these acute changes will predict a development of CT-associated cardiomyopathy in the future is unclear and will be evaluated during a prospective follow-up.

• 2.6.2. Multiple biomarkers in the detection of cardiotoxicity associated with HD-CT followed by HCT for various hematological malignancies

The aim of our study was to assess cardiac toxicity during HD-CT and HCT for various hematological malignancies with multiple biomarkers of cardiac injury: GPBB, H-FABP, cTnI, CK-MB mass, myoglobin.

A total of 53 patients (mean age 49.9 ± 12.3 years, median 54 years, 33 males) undergoing HCT for various hematological malignancies were studied. The diagnoses were multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's lymphoma, acute lymphoblastic leukemia, acute myeloid leukemia, myelodysplastic syndrome and chronic myeloid leukemia. Thirty transplants were autologous, 23 allogeneic.

Cardiac biomarkers were measured on Randox Evidence analyzer the day before administration

of HD-CT (before HD-CT), the day after completion of HD-CT (after HD-CT) and the day after infusion of hematopoietic cell graft (after HCT). Values above the reference range on the basis of a number of cardiology studies and recommended by the manufacturer (Randox

Laboratories Ltd.) were considered elevated. Echocardiographic evaluation of systolic and diastolic LV function was performed in all patients by an experienced echocardiographer who was blinded to the cardiac biomarker data. The results are shown in Table 8.

cardiac biomarkers	before HD-CT	after HD-CT	after HCT
myoglobin above 76.0 µg/L	0	0	0
CK-MB mass above 4.80 µg/L	0	0	0
cTnI above 0.40 µg/L	0	0	0
H-FABP above 4.50 µg/L	0	0	1 (1.9 %)
GPBB above 7.30 µg/L	0	8 (15.1 %)	9 (17.0 %)

Table 8. Elevated biomarkers of cardiac injury in association with HD-CT and HCT for hematological malignancies (n = 53)

Before HD-CT, all biomarkers of cardiac injury were below the cut-off values in all patients. We found significant elevations in GPBB (above 7.30 µg/L) in 8 (15.1 %) patients after HD-CT and in 9 (17.0 %) after HCT. H-FABP increased slightly above the cut-off after HCT in 1 (1.9 %) patient. Other cardiac biomarkers (myoglobin, CK-MB mass, cTnI) remained within the reference range in all patients. No patient manifested clinical cardiotoxicity with symptoms of heart failure in the peritransplant period. We found a significant correlation between elevation in GPBB and diastolic LV dysfunction on echocardiography (defined as impaired relaxation, i.e. E/A inversion and E wave deceleration time above 220 ms on the transmitral Doppler curve): $r = 0.603$; $p < 0.0001$.

Our results on 53 patients suggest that administration of HD-CT followed by HCT for hematological malignancies could be associated with myocardial injury manifested by increased release of GPBB from cardiomyocytes which could correlate with diastolic LV dysfunction on echocardiography. In asymptomatic patients, these findings could be considered a sign of acute subclinical cardiotoxicity. Whether these acute changes will have predictive value for development of treatment-related cardiomyopathy in the future is not clear and should be evaluated during a prospective follow-up. Further studies in a larger number of patients will be needed to confirm our preliminary results and define the potential role of new biomarkers in the assessment of cardiotoxicity in oncology.

CONCLUSIONS

CT is a well-established therapeutic approach for several malignancies, but its clinical efficacy is often limited by CT-related cardiotoxicity which may lead to cardiomyopathy possibly evolving into heart failure. The most frequently adopted diagnostic method for detection of cardiac injury is evaluation of LVEF by echocardiography or radionuclide ventriculography. However, this approach shows low sensitivity for the early prediction of cardiomyopathy when the possibilities of appropriate management could still improve the patient's outcome.

Cardiospecific biomarkers, such as cardiac troponins, show high diagnostic efficacy in the early subclinical phase of the disease, before the clinical onset of cardiomyopathy. The increase in their concentrations correlates with disease severity and may predict the occurrence of major cardiac events during follow-up. Negative troponin concentrations may identify patients with a low risk of cardiomyopathy. The role of cardiac troponin determination to stratify the risk of cardiotoxicity is currently based on strong evidence suggesting the routine use of this biomarker. Recently, natriuretic peptides have been investigated in detection of CT-induced cardiotoxicity. Some studies, including ours, have shown promising results. However, definitive evidence of their diagnostic and prognostic role in this context is still lacking and natriuretic peptides have not been routinely used for monitoring of cardiotoxicity in

clinical practice. Other perspective biomarkers of cardiotoxicity in oncology are under study, especially H-FABP and GPBB. Based on our data, a larger prospective and multicenter study will be needed to define the potential role of GPBB and other proposed biomarkers of cardiac injury in the assessment of cardiotoxicity induced by chemotherapeutic agents.

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