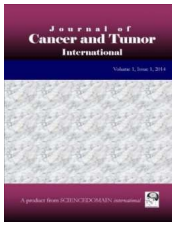

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Evaluation of Cardiac Biomarkers in Detection of Cardiomyopathy Induced by Cardiotoxic Chemotherapeutic Agents

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Authors' contributions

This work was carried out in collaboration between all authors. Author SD designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MP and KG managed the literature searches, analyses of the study performed the spectroscopy analysis and authors RM and MP managed the experimental process and author AKP managed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Background: Doxorubicin is a potent chemotherapeutic drug. The clinical usefulness of doxorubicin has been limited largely by the risk of cardiomyopathy and life-threatening heart failure. Therefore early identification of cardiotoxicity represents a primary goal for cardiologist and oncologist, considering the definition of personalized anticancer therapeutic strategies or intervention. The use of endomyocardial biopsy for detection of myocardial damage and monitoring of cardiac functions is troublesome in clinical practice.
Aim: So, evaluation of different cardiac biomarkers was performed to specifically detect myocardial injury and to predict ventricular dysfunction.

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Methods: Fifty two adult AL patients (mean age 48.9±11.8 years, 25 males) treated with 2–6 cycles of chemotherapy (CT) containing cardiotoxic chemotherapeutic drugs were studied. Cardiac evaluation was performed at baseline, after first and last chemotherapy with cardiotoxic drugs and 6 months after chemotherapy.

Results: Mean baseline NT-proBNP (N-terminal pro brain natriuretic peptide) concentration was 109.3±42.7 pg/ml (slightly elevated in 6 patients). After first and last chemotherapy, NT-proBNP elevations to 317.8±147.6 pg/ml and 302.3±123.9 pg/ml were observed, respectively. Six months after CT, mean NT-proBNP concentration was 412.5±184.2 pg/ml (elevated in 32 patients). Changes in NT-proBNP were significant in comparison with the baseline values ($p < 0.001$). Six months after chemotherapy, four patients with marked NT-proBNP elevations during chemotherapy developed treatment-related cardiomyopathy with symptoms of heart failure. NT-proBNP correlated with systolic and diastolic LV dysfunction on echocardiography ($r = 0.638$; $p < 0.01$) and ($r = 0.412$; $p < 0.01$). hs-cTnT concentrations were negative (<10 pg/ml) during chemotherapy in all patients. Six months after chemotherapy, delayed hs-cTnT positivity occurred in 6 patients. CK-MB mass remained within the reference range in all patients.

Conclusion: The present study suggests that NT-proBNP in blood is better indicator for the detection of doxorubicin induced cardiotoxicity during the treatment and the follow-up than hs-cTnT, cTnI and CK-MB.

Keywords: Cardiotoxicity; chemotherapy; cardiac biochemical markers; NT-proBNP; cTnT; cTnI.

1. INTRODUCTION

Doxorubicin is a potent chemotherapeutic agent and it is the cornerstone of many cytotoxic regimen. It is extensively used for the treatment of haematological malignancies and solid tumours [1]. Despite its therapeutic efficacy, its clinical usage is limited by the development of cumulative dose-dependent life-threatening cardiomyopathy [2] which may occur many years after the cessation of doxorubicin treatment [3]. It precludes some patients from receiving a highly effective treatment. Moreover, once the symptoms of cardiac damage develop, the discontinuation of doxorubicin treatment may not reverse this condition [4]. Therefore early identification of cardiotoxicity is the primary goal for cardiologist and oncologist considering the definition of personalized anticancer therapeutic strategies or intervention. For the detection of myocardial damage, monitoring of cardiac functions is still recommended during and after cardiotoxic chemotherapy. But these mode of investigations are time consuming and expensive [5-7]. Nevertheless, most of the approaches commonly used in clinical practice but evaluation of left ventricular ejection fraction (LVEF) by echocardiography or radionuclide ventriculography which showed low diagnostic sensitivity and low predictive value in detecting subclinical myocardial injury. The use of some other techniques such as endo-myocardial biopsy is troublesome in clinical practice owing to the invasiveness of the techniques [5-8]. Therefore, there is increasing interest in additional newer non-invasive and cost effective diagnostic tools for the early identification of myocardial alternation induced by doxorubicin.

The present study was conducted for evaluation of cardiac biomarkers to specifically detect myocardial injury and predict ventricular dysfunction so that they could represent a non-invasive and cost effective diagnostic tool for the identification of myocardial injury induced by doxorubicin.

B-type natriuretic peptide (BNP) is neurohormone released by the heart. The BNP is synthesized in both the ventricles in response to volume expansion and pressure overload [9,10]. The BNP is a 32 amino acids containing protein, which is the C-terminal part of the pro-BNP molecule and is secreted together with an N-terminal fragment (NT-pro-BNP). These both peptides are present in human plasma [11]. The NT-pro-BNP seems to have a longer plasma half-life than BNP, and NT-pro-BNP might therefore be more stable in plasma [12]. Both peptides are grossly elevated in patients with congestive heart failure (CHF) and diagnosing left ventricular (LV) dysfunction [11,13].

Cardiac troponins like cardiac troponin T (cTnT), cardiac troponin I (cTnI) and myocardial isoenzyme of creatine kinase (CK-MB) are cardiospecific markers that are highly specific for ischemic cardiac injury and may also be a very specific and sensitive marker of myocardial cell injury [14].

2. MATERIALS AND METHODS

2.1 Study Area

The present study was undertaken in the Departments of Biochemistry with the collaboration of the Department of Radiotherapy of Burdwan Medical College, Burdwan, West Bengal, India.

2.2 Selection of Subjects

A total of 52 patients with a diagnosis of acute leukemia, who attended Oncology Department of Burdwan Medical College of Burdwan, were selected by simple random sampling after obtaining informed consent. This prospective and hospital based study was performed between October 2011 and December 2013. The study group consisted of 25 males and 27 females with the mean age of 48.9±11.8 years. They had normal cardiac function, and had not received any treatment affecting the liver, kidneys diseases, or the fluid balance before and throughout the study period. Risks factors that enhance Doxorubicin induced cardiotoxicity such as prior irradiation to mediastinum or had other illnesses such as infections were excluded. No patient was previously treated with chemotherapy or radiotherapy. The study was carried out with the ethics committee approval. [Memo No.BMC/2179/1 (15)].

2.3 Chemotherapy

The patients were given 2–6 cycles of conventional cytotoxic therapy containing idarubicin (Zavedos) 3X10–12 mg/m^2,daunorubicin (Daunoblastina) 2–3X50 mg/m^2, mitoxantrone (Novantrone, Refador) 2–3 X10 mg/m^2 in combination with cytarabine (Cytosar).

The conventional cytotoxic therapy in the total cumulative dose of 464.3±117.5 mg/m^2. To calculate the total cumulative dose of the conventional cytotoxic therapy, conversion factors derived from the maximum recommended cumulative doses for individual agents used (idarubicin, daunorubicin, mitoxantrone) was applied.

2.4 Evaluation of Cardiac Biomarkers

Cardiac evaluation was performed at the baseline (before cytotoxic therapy), the day after first cytotoxic therapy, the day after last cytotoxic therapy and 6 months after completion of cytotoxic therapy.

Venous blood samples for assessment of biochemical markers were obtained from an indwelling catheter after 30 min of rest in supine position. The blood samples were withdrawn into dry tubes containing EDTA. The whole blood was centrifuged at 4℃. Plasma was decanted, immediately frozen and stored at –20℃ until assayed.

Levels of cTnI was measured by an enzyme linked one-step sandwich immunoassay method (TOSOH A1A21 fluorescens Chemistry), and the lowest detectable level was 500 pg/ml [4]. The cut-off values for cTnI was 0.4 µg/L. Plasma concentrations of hs-cTnT (high sensitive cardiac troponin T) was determined by electro-chemiluminescence immunoassay using 4th generation Troponin T high sensitive STAT I kits by utilizing Elecsys 2010 instrument made by Roche Diagnostics. The lower limit of detection was 10 pg/ml. The 99th percentile value of hs-cTnT for a normal reference population was 13.5 ng/L with a CV <10% [15]. Serum CK and CK-MB isoenzyme activities were determined using CK- NAK liquiUV kit and immunoinhibition by monoclonal antibody to CK-MB subunit, Human, Germany [16]. The cut-off values for CK-MB was 4.94 µg/L. Levels of NT-pro-BNP were measured using electrochemiluminescent immunoassay (Roche Moduler Analytics E170, Elecys Module). The cut-off values for NT-pro-BNP (100 ng/L for males, 150 ng/L for females) was used in the present clinical studies [17]. In the present study, values above the reference range based on a number of studies and recommended by the manufacturer were considered elevated and suggesting cardiac injury associated with the treatment.

2.5 Statistical Analysis

The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows. For all tests, the p-value was considered to be significant if it was less than 0.05 at a confidence level of 95 %.

Correlations were evaluated with normal and Spearman correlation tests. The values are expressed as mean±SD.

3. RESULTS

3.1 The Characteristics of the Study Population Are Shown in Table 1.

Personal profile and clinical details of the study population are shown in Table 1.

3.2 Percentage of Study Population Associated with High Concentration Cardiac Biomarkers

In Table 1 it is shown that only NT-pro-BNP is significantly increased after first, last cycle of Doxorubicin therapy and after six months chemotherapy. The percentage of positivity is significantly more with NT-pro-BNP than other cardiac markers from baseline data.

3.3 Plasma Mean NT-pro-BNP, cTnT, cTnI and CPK-MB Mass Concentrations During Treatment and Follow-Up of Chemotherapy

Baseline mean plasma NT-proBNP concentration was 109.3±42.7 pg/ml. After first cycle of Doxorubicin therapy, NT-proBNP was elevated above its cut-off value (Table 3, Fig. 1).

But other cardiac markers donot cross their cut-off value during any cycle or after completion chemotherapy except hs-cTnT (Table 3).

Table 1. Baseline patient's characteristics

Demographic profiles	Mean±SD	N (n = 52)	%
Age (years)	48.9±11.8		
Gender			
Female		27	52
Male		25	48

3.4 Plasma NT-pro-BNP, cTnT, cTnI and CPK-MB Mass Concentrations during Treatment and Follow-up of Chemotherapy - ANOVA test

In the figure .me of chemotherapy follow up from baseline values ($p < 0.001$) but none of the other cardiac markers show so statistical significant change from base-line data above their respective cut-off value.

Table 2. Percentage of study population associated with high concentration cardiac biomarkers associated with cardiotoxic chemotherapy for acute leukemia (n = 52)

	Baseline	After first cycle of doxorubicin therapy	After last cycle of doxorubicin therapy	Six months after doxorubicin therapy
NT-pro-BNP (pg/ml)	6 (11.5)	46 (88.5)	46 (88.5)	32 (61.5)
hs-cTnT (pg/ml)	0 (0)	0 (0)	0 (0)	6 (11.5)
cTnI (pg/ml)	0 (0)	0 (0)	0 (0)	2 (3.8)
CK-MB mass (pg/ml)	0 (0)	0 (0)	0 (0)	0 (0)

Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values ± SD for continuous variables

Table 3. Plasma mean NT-pro-BNP, cTnT, cTnI and CPK-MB mass concentrations during treatment and follow-up of chemotherapy

Different cardiac markers	Baseline	After first cycle of doxorubicin therapy	After last cycle of doxorubicin therapy	Six months after doxorubicin therapy
NT-pro-BNP (pg/ml)	109.3±42.7	317.8±147.6	302.3±123.9	412.5±184.2
hs-cTnT (pg/ml)	3.8±0.5	4.4±0.5	6.3±0.7	12±1.2
cTnI (µg/L)	0.31±0.06	0.28±0.04	0.4±0.07	0.5±0.06
CK-MB mass (µg/L)	1.8±0.2	2.1±0.4	4.2±0.5	3.5±0.5

Table 4. ANOVA with bonferroni correction showing multiple comparisons of different cardiac biomarkers in different times of chemotherapy in AL patients with significance of difference

Dependent variable	Factor (I)	Factor (J)	Mean difference (I-J)	Significance at 95% CI
NT-pro-BNP (pg/ml)	1	2	-208.5	< 0.001*
		3	-193	< 0.001*
		4	-303.2	< 0.001*
	2	1	208.5	< 0.001*
		3	15.5	0.319
		4	-94.7	0.008
	3	1	193	< 0.001*
		2	-15.5	0.319
		4	-110.2	< 0.001*
	4	1	303.2	< 0.001*
		2	94.7	0.008
		3	110.2	< 0.001*
CK-MB mass (µg/L)	1	2	-0.3	0.376
		3	-2.4	0.117
		4	-1.7	0.305
	2	1	0.3	0.376
		3	-2.1	0.392
		4	-1.4	0.528
	3	1	2.4	0.117
		2	2.1	0.392
		4	0.7	0.428
	4	1	1.7	0.305
		2	1.4	0.528
		3	-0.7	0.428
cTnI (µg/L)	1	2	0.03	0.367
		3	-0.09	0.136
		4	-0.19	0.02*
	2	1	-0.03	0.367
		3	-0.12	0.162
		4	-0.22	0.04*
	3	1	0.09	0.136
		2	0.12	0.162
		4	-0.1	0.144
	4	1	0.19	0.02*
		2	0.22	0.04*
		3	0.1	0.144
hs-cTnT (pg/ml)	1	2	-0.6	0.282
		3	-2.5	0.109
		4	-8.2	0.024*
	2	1	0.6	0.282
		3	-1.9	0.124
		4	-7.6	0.032*
	3	1	2.5	0.109
		2	1.9	0.124
		4	-5.7	0.046*
	4	1	8.2	0.024*
		2	7.6	0.032*
		3	5.7	0.046*

** p value significant (p < 0.05) at 95% Confidence interval (CI); 1 = Baseline activity, 2 = After first cycle of Doxorubicin therapy, 3 = After last cycle of Doxorubicin therapy, 4 = Six months after Doxorubicin therapy*

Table 5. Abnormal ECHO findings during treatment and follow-up of doxorubicin therapy (n = 52)

Abnormal ECHO findings	Baseline	After first cycle of doxorubicin therapy	After last cycle of doxorubicin therapy	Six months after doxorubicin therapy
Systolic LV dysfunction	0	2 (3.8%)	2 (3.8%)	4 (7.7%)
Diastolic LV dysfunction	2 (3.8%)	10 (19.2%)	12 (23.1%)	24 (46.2%)
Pericardial effusion	4 (7.7%)	18 (34.6%)	18 (34.6%)	10 (19.2%)

Systolic LV dysfunction – EF less than or equal to 50%; Diastolic LV dysfunction – E/A inversion, E-wave deceleration time above 220 ms (impaired relaxation); pericardial effusion – separation of pericardial leaves at least 2 mm in systole.

3.5 Percentage of Study Population Associated with Changes in ECHO Parameters during the Treatment and the Follow-up

Changes in ECHO parameters during the treatment and the follow-up with chemotherapy are shown in Table 3. Six months after Doxorubicin therapy, 24 (46.2%) patients had ECHO signs of diastolic dysfunction and 4 (7.7%) patients developed systolic LV dysfunction with symptoms of heart failure (Doxorubicin-related cardiomyopathy). Other 3 patients had LVEF equal to 55% and were asymptomatic. In the whole cohort, LVEF decreased from 65.3±4.5% (before CT) to 60.2±5.7% (6 Mo after CT), which was statistically significant ($p < 0.01$).

3.6 Correlation between BLOod NT-proBNP and LVEF Detected by ECHO – Bivarient Correlation

The concentration of NT-proBNP showed a significant ($p < 0.05$) diagnostic value in predicting a low LVEF.

Table 6. Pearson's correlation of LVEF (%) with NT-proBNP, CK-MB, hs-cTNT, cTnI concentration in blood

Cardiac markers	Pearson correlation (r)	p value
NT-proBNP	- 0.859	0.018
CK-MB	-0.127	0.165
hs-cTNT	-0.218	0.089
cTnI	-0.345	0.112

$p < 0.05$ consider statistically significant

4. DISCUSSION

Anthracycline (ANT)-induced cardiotoxicity limits effective cancer chemotherapy by causing early cardiomyopathy, and it can produce late-onset ventricular dysfunction or pericardial effussion years after treatment has ceased that may worsen the patient's outcome. Various methods have been recommended for monitoring of cardiotoxicity in oncology. Endomyocardial biopsy directly measures the presence and extent of fibrosis due to anthracycline cardiotoxicity [18]. However, it is limited by its invasiveness, need for histologic expertise and costs [6,19,20]. Another frequently adopted method for detection of cardiotoxicity is evaluation of LVEF by ECHO or radionuclide ventriculography [19,20]. However, these techniques are only partially reliable, available and have low sensitivity for detection of early cardiac dysfunction that could be reversible with appropriate therapy [21]. Thus, there is a growing expectation for newer, non-invasive and cost-effective diagnostic tools for early identification of patients susceptible to developing cardiotoxic chemotherapy induced cardiotoxicity. Biochemical markers of cardiac injury, especially cardiac troponins and natriuretic peptides, have been recently studied in this context.

The applicability of natriuretic peptides (ANP, BNP, NT-proBNP) as markers for ANT-induced cardiotoxicity has been investigated in a few recent studies. The results suggest that natriuretic peptides could be of value in the detection of clinical and subclinical cardiotoxicity due to ANT [22-27]. They can also be regarded as markers of cardiac toxicity after high-dose CT and hematopoietic stem cell transplantation [22,28-30]. However, definitive clinical evaluation has been missing and natriuretic peptides have not been routinely used for monitoring of cardiotoxicity in clinical practice.

In our study, the baseline NT-proBNP values were slightly elevated in 6 (11.5%) patients. Only in 2 patients, diastolic LV dysfunction on ECHO was found. The other 4 patients had no clinical or ECHO signs of LV dysfunction, which shows that a relatively strict cut-off for NT-proBNP was used. However, these values have been

suggested as the upper limit of the reference interval [17,31,32]. After first and last chemotherapy with cardiotoxic drugs, it was observed that elevated NT-proBNP in 46(88.5%) patients, that means in 40(76.9%) patients NT-proBNP values increased above the cut-off. Administration of chemotherapy with cardiotoxic drugs causes acute myocardial strain of LV and enhanced release of NT-proBNP irrespective of the reached cumulative dose of cardiotoxic drugs. These NT-proBNP elevations could be considered a sign of acute subclinical cardiotoxicity of the drugs. Six months after completion of chemotherapy, neurohumoral activation in sense of NT-proBNP elevation persisted in 32(61.5%) patients, that means NT-proBNP values increased above the cut-off in 26 (50.0%) patients. NT-proBNP concentrations correlated with systolic and diastolic dysfunction on ECHO. Six months after treatment, 4(7.7%) patients with NT-proBNP above 500 ng/L had ECHO signs of LV dysfunction and clinical symptoms of heart failure — these findings represent chronic clinical cardiotoxicity of ANT manifested as cardiomyopathy. These 4 patients with ANT-related cardiomyopathy had NT-proBNP values markedly elevated (above 500 ng/L) already after first and last CT with cardiotoxic drugs. It seems that NT-proBNP could help in identification of patients at risk for development of chemotherapy-related cardiomyopathy. In asymptomatic patients, persistent NT-proBNP elevations signify chronic subclinical cardiotoxicity, which requires a careful follow-up in the future.

Cardiospecific markers, especially cardiac troponins, have been studied in the detection of chemotherapy induced cardiotoxicity. Testing for cTnT and cTnI is equivalent for clinical use. However, only assessment of cTnT is standardized at present [31]. Experimental studies showed significant cTnT elevations that could serve as predictors for ANT-induced cardiomyopathy [32-34]. Clinical studies are limited and the results are ambiguous. In some studies, administration of ANT did not cause any elevation in cardiac troponins [35-37]. In other studies, cardiac troponins became positive after ANT treatment, correlated with the disease severity and might predict subsequent major cardiac events during the follow-up [38-40]. The results of clinical studies are inconsistent and cardiac troponins have not been established in clinical practice for monitoring of cardiotoxicity in oncology.

In our study, no patient had a detectable cTnT concentration early after ANT administration, even in higher cumulative doses. CT with ANT did not lead to detectable acute injury to cardiomyocyte structure. In this respect, it is unlikely that early assessment of cTnT during ANT treatment would be useful for screening for ANT-induced cardiotoxicity. On the other hand, cTnT positivity occurred 6 months after completion of treatment in 3(11.5%) patients. These delayed elevations in cTnT were associated with cardiac dysfunction on ECHO (cardiomyopathy was diagnosed in 2 patients) and indicate chronic cardiotoxicity of ANT. According to our results, negative cTnT concentrations during ANT treatment do not identify patients with a low risk for development of cardiomyopathy in the future.

In our study, we did not find any elevation in CK-MB mass during ANT treatment and the follow-up, which was expected from previously published clinical studies [35,41-44].

ECHO is the most frequently used non-invasive method for evaluation of cardiac function including toxic effect of oncology treatment. In our study, the incidence of systolic and diastolic LV dysfunction on ECHO advanced with increasing cumulative dose of ANT and with time after completion of CT. Impairment of diastolic LV function related to ANT treatment was diagnosed prior to impairment of systolic LV function. This finding is in agreement with the previously published studies.[45-47] Six months after completion of treatment, clinical manifestation of cardiotoxicity in terms of ANT-induced cardiomyopathy with heart failure developed in 2 (7.7%) patients. Asymptomatic changes in ECHO parameters are considered subclinical cardiotoxicity and require regular cardiology check-ups.

Since we did not find significant correlations between abnormal cardiac findings after ANT treatment and the total cumulative dose of ANT and other risk factors for development of cardiotoxicity, we conclude that thorough cardiology follow-up is warranted in all oncology patients treated with these agents. Our findings regarding assessment of ANT-induced cardiotoxicity with biochemical markers need a further prospective follow-up and evaluation in further studies on a larger number of patients.

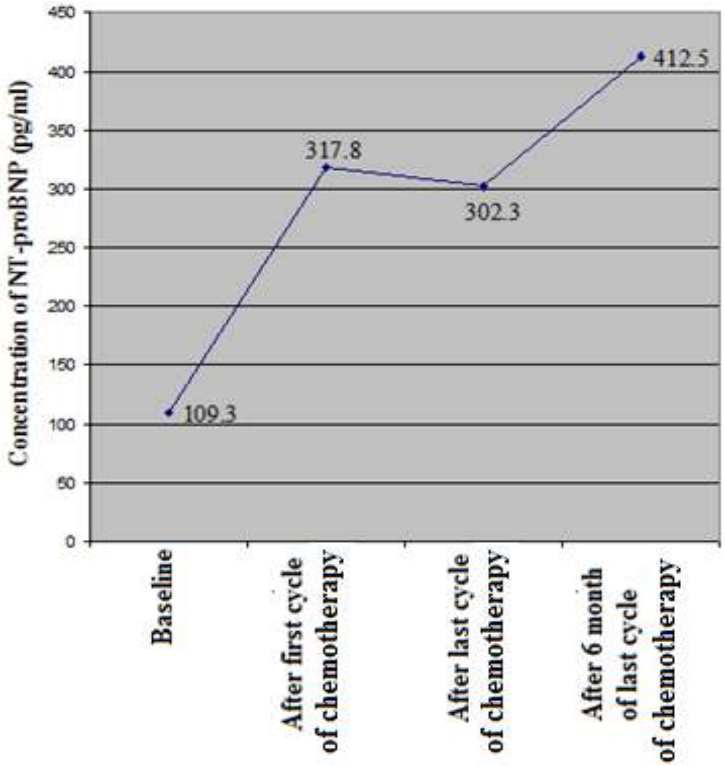

Fig. 1. Plasma NT-proBNP concentrations during treatment and follow-up of AL

5. CONCLUSION

The present study suggests that NT-proBNP in blood is better indicator than other cardiac markers for the detection of doxorubicin induced cardiotoxicity during the treatment and the follow-up.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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