Original Research Article

Evaluation of cardiac biomarkers in detection of cardiomyopathy induced by cardiotoxic chemotherapeutic agents

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. **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 than hs-cTnT, cTnI and CK-MB is better indicator for the detection of doxorubicin induced cardiotoxicity during the treatment and the follow-up.

Key Words: Cardiotoxicity, Chemotherapy, Cardiac biochemical markers, NT-proBNP, cTnT, cTnI.

1.Introduction

Doxorubicin ,a potent chemotherapeutic drug, is the cornerstone of many cytotoxic regimen It is extensively used for the treatment of haematological malignancies and solid tumours.[1], Despite 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 cardio-toxicity 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 cardio-toxic chemotherapy. But these mode of investigations are time consuming and expensive.[5-7] Nevertheless, most of the approaches commonly used in clinical practice – 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 an alternative diagnostic tool for the early detection of cardiotoxicity.

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, and thus elevated end-diastolic pressure.[9,10] The BNP (32 amino acids), which is the C-terminal part of the pro-BNP molecule, is secreted together with an N-terminal fragment (76 amino acids) NT-pro-BNP, and 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). The elevations correlate to the degree of cardiac insufficiency, and the 2 peptides seem to correlate by giving the same information when diagnosing left ventricular (LV) dysfunction.[11,13] Thus, BNP or NT-pro-BNP have a strong prognostic value in

predicting death or future cardiac events.[14] Plasma levels of natriuretic peptides have been shown to be increased in patients with severe congestive heart failure .Moreover testing for natriuretic peptides has been recognized and incorporated in guidelines for diagnosis and management of heart failure,one of the major cardiac event due to doxorubicin chemotherapy.[15]

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.[16]

So, the aim of the present study was to evaluate the cardiac biomarkers capable to specifically detect myocardial injury and to predict ventricular dysfunction.

2.Material and Method

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 (range: 22–61, median 51). 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) 2–3X50mg/m^2, mitoxantrone (Novantrone, Refador) 2–3 X10mg/m^2 in combination with cytarabine (Cytosar).

The conventional cytotoxic therapy in the total cumulative dose of 464.3± 117.5 mg/m^2 (range 240–715, median 426). 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 30min of rest in supine position. The blood samples were withdrawn into dry tubes containing EDTA. The whole blood was centrifuged at 4^{0}C. Plasma was decanted, immediately frozen and stored at –20°C 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] Plasma concentrations of hs-cTnT (high sensitive cardiac troponin T) was determined by electro-chemiluminescence immunoassay, sandwich technique using 4th generation Troponin T high sensitive STAT I kits, Elecsys 2010; Roche Diagnostics. The lower limit of detection was 10 pg/ml. The 99th percentile value of hs-cTnT for a normal reference population was 13.5ng/L, with a CV <10%.[17] Serum CK and CK-MB isoenzyme activities were determined using CK NAK liquiUV kit and immunoinhibition by monoclonal antibody to CK-M subunit, Human, Germany. [18] The cut-off values for individual markers were as follows: for CK-MB mass 4.94 μg/L. Levels of NT-pro-BNP were measured using electrochemiluminescent immunoassay (Roche Moduler Analytics E170, Elecys Module). The upper limit of normal (100ng/L for males,150 ng/L for females) is proper and can be used in clinical studies .[19] 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.

Table 1: Baseline patient's characteristics

Demographic profiles	**Mean ± SD**	**Median**	**N (n = 52)**	**%**
Age (years)	48.9 ± 11.8	51		
Gender				
Female			27	52
Male			25	48

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 and last cycle of Doxorubicin therapy and after six months chemotherapy, percentage of positivity is significantly more with NT-pro-BNP than other cardiac markers from baseline data.

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 (pg/ml)	0 (0)	0 (0)	0 (0)	0 (0)

3.3 Plasma cTnT, cTnI and CPK-MB concentrations during treatment and follow-up of AL – unpaired t test

In the Figure 1, it was observed that though six months after Doxorubicin therapy, hs-cTnT positivity was found in 6 (11.5%) and 2 (3.8%) the mean serum hs-cTnT and cTnI concentrations respectively were bellow the detection limit in all patients during all cycles of Doxorubicin therapy and even after 6 month of last cycle.

CK-MB mass concentrations remained within the reference range (below 4.94 μg/L) in all patients. Changes in CK-MB mass concentrations were not significant in comparison with the baseline values.

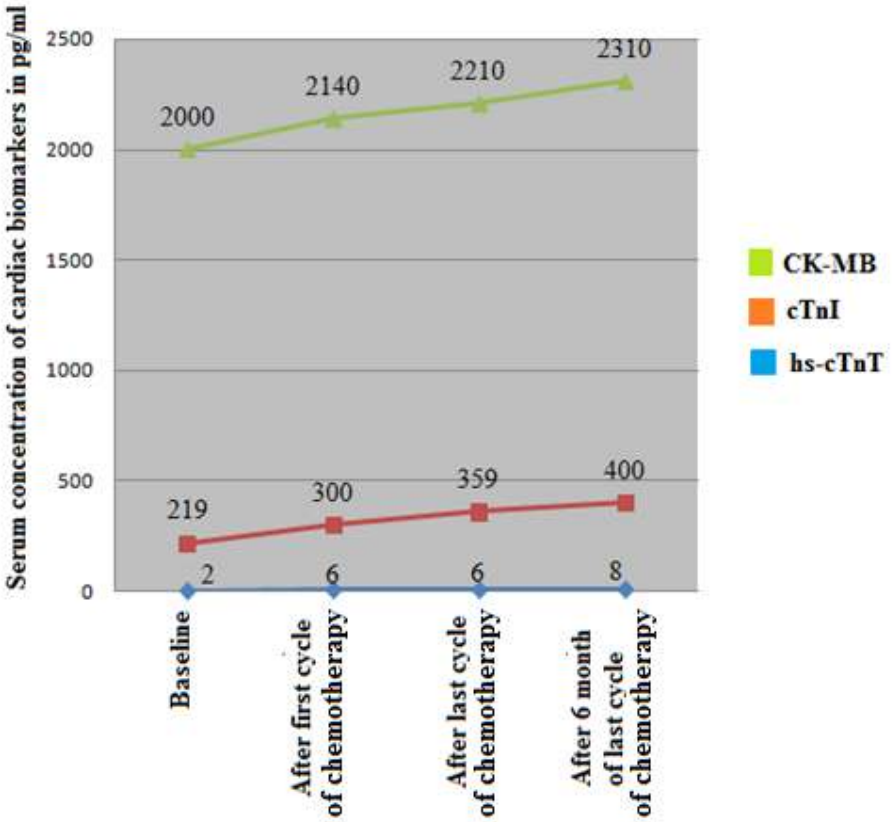

Figure 1: Plasma hs-cTnT, cTnI and CPK-MB concentrations during treatment and follow-up of AL

3.4 Plasma NT-proBNP concentrations during treatment and follow-up of AL – unpaired t test

Baseline plasma NT-proBNP concentration was 109.3 ± 42.7 pg/ml. After first cycle of Doxorubicin therapy, NT-proBNP was elevated to 317.8 ± 147.6 pg/ml. After last cycle, NT-proBNP concentration was 302.3 ± 123.9 pg/ml. Six months after last cycle, the mean plasma NT-proBNP concentration was 412.5 ± 184.2 pg/ml (Figure 2). Changes in NT-proBNP concentrations were significant in comparison with the baseline values ($p < 0.001$).

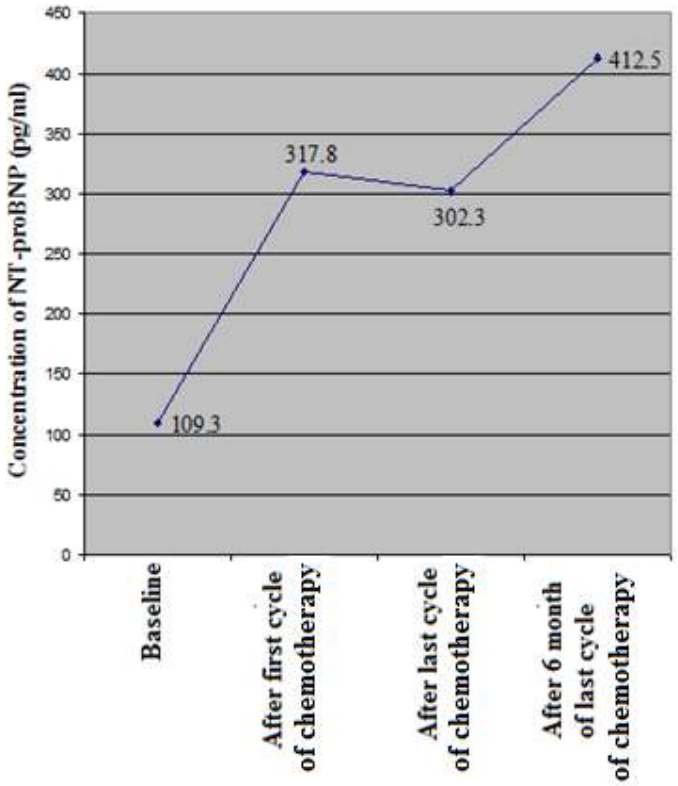

Figure 2: Plasma NT-proBNP concentrations during treatment and follow-up of AL

Changes in evaluated ECHO parameters during the treatment and the follow-up 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 ($p < 0.01$).

Table 3: 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.

Six months after Doxorubicin therapy, NT-proBNP concentrations correlated with systolic and diastolic LV dysfunction on ECHO — ($r = 0.638$; $p < 0.01$) and ($r = 0.412$; $p < 0.01$) respectively.

3.5 Correlation between blood NT-proBNP and LVEF detected by ECHO -

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

Table 5: 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. [20] However, it is limited by its invasiveness, need for histologic expertise and costs.[6,21,22] Another frequently adopted method for detection of cardiotoxicity is evaluation of LVEF by ECHO or radionuclide ventriculography.[21,22] 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.[23] 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 cardio-toxicity due to ANT. [24-29] They can also be regarded as markers of cardiac toxicity after high-dose CT and hematopoietic stem cell transplantation.[24,30-32] 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. [19,33,34] 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 500ng/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.[33] Experimental studies showed significant cTnT elevations that could serve as predictors for ANT-induced cardiomyopathy. [34–36] Clinical studies are limited and the results are ambiguous. In some studies, administration of ANT did not cause any elevation in cardiac troponins. [37–39] 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. [40–42] 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 2patients) 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.[37,43-46]

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.[47-49] 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.

5. Conclusion

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

References

1. Carvalho C, Santos RX, Cardoso S, et al. Doxorubicin: the good, the bad and the ugly effect. Curr Med Chem 2009; 16: 3267 – 3285.
2. Doroshow JH: Doxorubicin-induced cardiac toxicity. N Engl J Med 1991; 324: 843 – 845.
3. Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity cardiotoxicity. Pharmacol Rev 2004;56:185-229.
4. Murat Soker, Mehmet Kervancioglu. Plasma concentration of NT-pro-BNP and cardiac troponin I in relation to Doxorubicin induced cardiomyopathy and cardiac function in childhood malignancy Saudi Med J 2005;26 (8): 1197-1202.
5. Pai VB, Nahata MC. Cardiotoxicity of chemotherapeutic agents: incidence, treatment and prevention. Drug Saf. 2000; 22: 263-302.
6. Gharib MI, Burnett AK. Chemotherapy-induced cardiotoxicity: current practice and prospects of prophylaxis. Eur J Heart Fail. 2002; 4: 235-242.
7. Ganz WI, Sridhar KS, Ganz SS, et al. Review of tests for monitoring doxorubicin induced cardiomyopathy. Oncology 1996; 53: 461-470.
8. Meinardi MT, Van der Graaf WTA, Van Veldhuisen DJ, et al. Detection of anthracyclineinduced cardiotoxicity. Cancer Treat Rev. 1999;25: 237-247.
9. Nousiainen T, Jantunen E, Vanninen E, Remes J, Vuolteenaho O, Hartikainen J. Natriuretic peptides as markers of cardiotoxicity during doxorubicin treatment for non-Hodgkin's lymphoma. Eur J Haematol 1999; 62:135-141.
10. Baughman K. B-type natriuretic peptide a window to the heart. N Engl J Med 2002; 347: 158-159.
11. Hunt PJ, Richards AM, Nicholls MG, Yandle TG, Doughty RN, Espiner EA. Immunoreactive amino-terminal pro-brain natriuretic peptide (NT-PROBNP): a new marker of cardiac impairment. Clin Endocrinol 1997; 47: 287-296.
12. Pemberton CJ, Johnson ML, Yandle TG, Espiner EA. Deconvolution analysis of cardiac natriuretic peptides during acute volume overload. Hypertension 2000; 36:355-359.
13. Hammerer-Lercher A, Neubauer E, Muller S, Pachinger O, Puschendorf B, Mair J. Head-to-head comparison of N-terminal pro-brain natriuretic peptide, brain natriuretic peptide and N-terminal pro-atrial natriuretic peptide in diagnosing left ventricular dysfunction. Clin Chem Acta 2001; 310: 193-197.
14. Tsutamoto T, Wada A, Maeda K, Hisanaga T, Maeda Y, Fukai D, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure. Circulation 1997; 96: 509-516.
15. Swedberg K, Cleland J, Dargie H, *et al*. Guidelines for the diagnosis and treatment of chronic heart failure: executive summary. The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. Eur Heart J 2005; 26: 1115–40.
16. Morandi P, Ruffini PA, Benvenuto GM, La Vecchia L, Mezzena G, Raimondi R. Serum cardiac troponin I levels and ECG/Echo monitoring in breast cancer patients undergoing high-dose (7 g/m(2)) cyclophosphamide. Bone Marrow Transplant 2001; 28: 277-282.
17. Vasile VC, Saenger AK, Kroning JM, Jaffe AS. Biological and Analytical Variability of a Novel High-Sensitivity Cardiac Troponin T Assay. Clin Chem. 2010;56(7):1086-90.

18. Wu AHB, Gornet TG, Bretaudiere JP, Panfili PR. Comparison of enzyme immunoassay and immunoinhibition for creatine kinase MB in diagnosis of acute myocardial infarction. Clin Chem. 1985;31:470-4.
19. Hess G, Runkel S, Zdunek D, et al. Reference interval determination for N-terminal-B-type natriuretic peptide (NT-proBNP): a study in blood donors. Clin Chem Acta 2005; 360: 187–93.
20. Cooper LT, Baughman KL, Feldman AM, Frustaci A, Jessup M, Kuhl U, Levine GN, et al. The role of endomyocardial biopsy in the management of cardiovascular disease: a scientific statement from the American Heart Association, the American College of Cardiology, and the European Society of Cardiology. Endorsed by the Heart Failure Society of America and the Heart Failure Association of the European Society of Cardiology. J Am Coll Cardiol. 2007;50(19):1914-1931.
21. Ganz WI, Sridhar KS, Ganz SS, Gonzalez R, Chakko S, Serafini A. Review of tests for monitoring doxorubicin induced cardiomyopathy. Oncology 1996;53:461-70.
22. Meinardi MT, van der Graaf WT, van Veldhuisen DJ, Gietema JA, de Vries EG, Sleijfer DT. Detection of anthracycline-induced cardiotoxicity. Cancer Treat Rev 1999;25:237-47.
23. Sandri MT, Salvatici M, Cardinale D, et al. N-terminal pro-B-type natriuretic peptide after high-dose chemotherapy: a marker predictive of cardiac dysfunction? Clin Chem 2005; 51: 1405–10.
24. Bauch M, Ester A, Kimura B, et al. Atrial natriuretic peptide as a marker for doxorubicin-induced cardiotoxic effects. Cancer 1992; 69: 1492–7.
25. Suzuki T, Hayashi D, Yamazaki T, et al. Elevated B-type natriuretic peptide levels after anthracycline administration. Am Heart J 1998; 136: 362–3.
26. Okumura H, Iuchi K, Yoshida T, et al. Brain natriuretic peptide is a predictor of anthracycline-induced cardiotoxicity. Acta Haematol 2000; 104: 158–63.
27. Hayakawa H, Komada Y, Hirayama M, et al. Plasma levels of natriuretic peptides in relation to doxorubicin-induced cardiotoxicity and cardiac function in children with cancer. Med Pediatr Oncol 2001; 37: 4–9.
28. Soker M, Kervancioglu M. Plasma concentrations of NT-pro-BNP and cardiac troponin-I in relation to doxorubicin-induced cardiomyopathy and cardiac function in childhood malignancy. Saudi Med J 2005; 26: 1197–202.
29. Ekstein S, Nir A, Rein AJ, *et al*. N-terminal-proB-type natriuretic peptide as a marker for acute anthracycline cardiotoxicity in children. J Pediatr Hematol Oncol 2007; 29: 440–4.
30. Snowden JA, Hill GR, Hunt P, *et al*. Assessment of cardiotoxicity during haemopoietic stem cell transplantation with plasma brain natriuretic peptide. Bone Marrow Transplant 2000; 26: 309–13.
31. Horacek JM, Pudil R, Tichy M, *et al*. Biochemical markers and assessment of cardiotoxicity during preparative regimen and hematopoietic cell transplantation in acute leukemia. Exp Oncol 2007; 3: 243–7.
32. Kuittinen T, Jantunen E, Vanninen E, *et al*. Cardiac effects within 3 months of BEAC high-dose therapy in non-Hodgkin's lymphoma patients undergoing autologous stem cell transplantation. Eur J Haematol 2006; 77: 120–7.
33. Panteghini M. The measurement of cardiac markers: where should we focus? Am J Clin Pathol 2002; 118: 354–61.
34. Herman EH, Lipschultz SE, Rifai N, *et al*. Use of cardiac troponin T levels as an indicator of doxorubicin-induced cardiotoxicity. Cancer Res 1998; 58: 195–7.
35. Koh E, Nakamura T, Takahashi H. Troponin-T and brain natriuretic peptide as predictors for adriamycin-induced cardiomyopathy in rats. Circ J 2004; 68: 163–7.

36. Adamcova M, Simunek T, Kaiserova H, et al. *In vitro* and *in vivo* examination of cardiac troponins as biochemical markers of drug-induced cardiotoxicity. Toxicology 2007; 237: 218–28.
37. Fink FM, Genser N, Fink C, et al. Cardiac troponin T and creatine kinase MB mass concentrations in children receiving anthracycline chemotherapy. Med Pediatr Oncol 1995; 25: 185–9.
38. Kismet E, Varan A, Ayabakan C, et al. Serum troponin T levels and echocardiographic evaluation in children treated with doxorubicin. Pediatr Blood Cancer 2004; 42: 220–4.
39. Clark SJ, Pippon M, Hemsworth S, et al. Cardiac troponin T following anthracycline chemotherapy in children and adolescents. J Chemother 2007; 19: 332–4.
40. Auner HW, Tinchon C, Linkesch W, *et al*. Prolonged monitoring of troponin T for the detection of anthracycline cardiotoxicity in adults with hematological malignancies. Ann Hematol 2003; 82: 218–22.
41. Cardinale D, Sandri MT, Colombo A, *et al*. Prognostic value of troponin I in cardiac risk stratification of cancer patients undergoing high-dose chemotherapy. Circulation 2004; 109: 2749–54.
42. Kilickap S, Barista I, Akgul E, *et al*. CTnT can be a useful marker for early detection of anthracycline cardiotoxicity. Ann Oncol 2005; 16: 798–804.
43. Missov E, Calzolari C, Davy JM, et al. Cardiac troponin I in patients with hematologic malignancies. Coron Artery Dis 1997; 8: 537–41.
44. Herman EH, Zhang J, Lipshultz SE, Rifai N, Chadwick D, Takeda K, Yu ZX, Ferrans VJ. Correlation between serum levels of cardiac troponin T and the severity of the chronic cardiomyopathy induced by doxorubicin. J Clin Oncol 1999;17:2237-43.
45. Koh E, Nakamura T, Takahashi H. Troponin T and brain natriuretic peptide as predictors for adriamycin-induced cardiomyopathy in rats. Circ J 2004;68:163-7.
46. Kral M, Skoloudik D, Sanak D, Veverka T, Bartkova A, Dornak T, Hutyra M, Vindis D, Ulehlova J, Slavik L, Svabova M, Kubickova V, Herzig R, Kanovsky P. Assessment of relationship between acute ischemic stroke and heart disease - protocol of a prospective observational trial. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 2012;156:284-9.
47. Sorensen K, Levitt GA, Bull C, *et al*. Late anthracycline cardiotoxicity after childhood cancer: a prospective longitudinal study. Cancer 2003; 97: 1991–8.
48. Lipshultz SE, Lipsitz SR, Sallan SE, *et al*. Chronic cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. J Clin Oncol 2005; 23: 2629–36.
49. Scully RE, Lipshultz SE. Anthracycline cardiotoxicity in long-term survivors of childhood cancer. Cardiovasc Toxicol 2007; 7:122–8.