

# Plasma concentrations of NT-pro-BNP and cardiac troponin-I in relation to doxorubicin-induced cardiomyopathy and cardiac function in childhood malignancy

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## ABSTRACT

**Objective:** Anthracyclines are well established as highly efficacious antineoplastic agents for childhood malignancy, but they frequently cause dose-related cardiotoxicity. For this reason, children who have received anthracyclines need periodical cardiac evaluation. The plasma levels of B-type natriuretic peptide (BNP) have been shown to increase in proportion to severity of cardiac dysfunction. N-terminal BNP (NT-pro-BNP) is secreted from the cardiac ventricles in response to volume expansion and pressure overload. The aim of our study was to investigate whether plasma levels of NT-pro-BNP and cardiac troponin I (cTnI) can be used as specific markers for doxorubicin-induced cardiotoxicity in children with malignancy.

**Methods:** We performed the study in Dicle University Hospital, Pediatric Hematology and Oncology clinic. Were measured plasma NT-pro-BNP and cTnI in 31 patients (14 boys and 17 girls) who received doxorubicin-containing chemotherapy for their malignancy at cumulative doses of 30-600 mg/m<sup>2</sup>, between October 2000 and December 2004. Cardiac

evaluation of the patients included recording of electrocardiography and assessment of systolic and diastolic functions of the heart by echocardiography.

**Results:** Of the 31 patients, 4 (12.9%) had left ventricular dysfunction as assessed by echocardiography. Plasma NT-pro-BNP levels in these patients were significantly elevated in comparison with healthy controls ( $p<0.001$ ). Plasma NT-pro-BNP levels were significantly elevated in patients with cardiac dysfunction when compared with normal cardiac function ( $p<0.008$ ). The cTnI levels were found under normal value in all patients.

**Conclusion:** Measurement of NT-pro-BNP level may be an easy and practical tool, and during treatment may allow earlier-identification of individuals at risk for monitoring cardiac damage. Plasma NT-pro-BNP concentration may be a useful and sensitive indicator of cardiac dysfunction in children receiving doxorubicin therapy.

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**A**nthracyclines are effective antineoplastic drugs for pediatric cancer with dose limiting acute and chronic cardiotoxicity. Several reports have indicated that a significant percentage of children who have received anthracyclines have at least

subclinical cardiac abnormalities at late follow-up and that these appear to be progressive with time. The late cardiomyopathy observed at higher cumulative doses of anthracycline is serious and clinically important due to subsequent irreversible

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congestive heart failure (CHF) and death. Once symptoms of CHF develop, the discontinuation of anthracycline treatment may not reverse this condition. Therefore, there is a need to detect cardiotoxicity earlier.<sup>1</sup> Noninvasive techniques for the identification of patients who are at high risk of developing anthracycline cardiomyopathy are critically important for the prevention and management of this complication. Both echocardiography and radionuclide ventriculography lack the sensitivity required to detect the early stages of the cardiomyopathy, in which deterioration of cardiac function has not yet developed. Therefore, there is increasing interest in additional noninvasive methods for the detection of early myocardial alterations induced by anthracyclines.<sup>2</sup> Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are cardiac neurohormones released by the heart. The ANP is mainly synthesized in the atria.<sup>3</sup> The BNP is synthesized in both the atria and ventricles, but is mainly released from the ventricles in response to volume expansion and pressure overload, and thus elevated end-diastolic pressure.<sup>4,5</sup> 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.<sup>6</sup> 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.<sup>7</sup> Both peptides are grossly elevated in patients with 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.<sup>6,8</sup> Thus, BNP or NT-pro-BNP have a strong prognostic value in predicting death or future cardiac events.<sup>9</sup> Plasma levels of natriuretic peptides have been shown to be increased in patients with severe CHF, BNP is more sensitive and specific than ANP in the detection of LV dysfunction.<sup>10,11</sup> There are few studies on the changes in natriuretic peptides during anthracycline therapy.<sup>4,12,13</sup> These studies have shown either no association between the impairment of LV function or a correlation between impaired LV systolic function and increased secretion of natriuretic peptides.<sup>12,13</sup> However, only a few studies have been undertaken to investigate the correlation between plasma levels of natriuretic peptides and cardiac function in cancer children receiving anthracycline therapy.<sup>1,14</sup> We were unable to find any a Medline search of the English-language medical literature on the correlation between plasma levels of NT-pro-BNP, and cardiac function in cancer children treated with anthracycline. Recently, it was found that cardiac troponin I (cTnI) is a marker that is highly specific for ischemic cardiac injury, and may also be a very specific and sensitive marker of myocardial cell injury.<sup>15</sup> We report here, the

incidence of echocardiographically diagnosed doxorubicin-induced cardiac dysfunction in children with cancer, and their association with the secretion of NT-pro-BNP and cTnI.

**Methods.** This prospective study was performed in the Pediatric Hematology and Oncology unit of Dicle University Hospital, Turkey, between October 2000 and December 2004. Of the 31 patients, 27 had acute lymphoblastic leukemia (ALL) (3 relapsed ALL), 2 had acute myeloblastic leukemia (AML), one Hodgkin disease (HD) and one non-Hodgkin lymphoma (NHL). Thirty-one patients received combination chemotherapy including doxorubicin. Patients who had received their last dose of anthracycline more than one month previously, and continued to be in complete remission were enrolled in this study. Patients who received radiation therapy to mediastinum, or had other illnesses such as, infections were excluded. The median age of patients was  $8.16 \pm 3.48$  years (range 4-15 years). The control group consisted of 30 (16 boys and 14 girls) healthy volunteers having no abnormality on routine examination who were in the same age and gender distribution. They had normal cardiac function, and had not received any treatment affecting the heart, kidneys, or the fluid balance before and throughout the study period. Approval for the study was obtained from the patients who provided written informed consent.

**Chemotherapy.** The TRALL-BFM 2000 chemotherapy regimen was administered in ALL and standard doses of doxorubicin 30 mg/m<sup>2</sup>. The total cumulative dose of doxorubicin was 180 mg/m<sup>2</sup>, 240 mg/m<sup>2</sup>, and 300 mg/m<sup>2</sup> in standard, medium, and high risk group ALL. The AML-BFM 93 chemotherapy regimen was administered in patients with AML. In this protocol standard doses of doxorubicin 30 mg/m<sup>2</sup>, and a total cumulative dose of 300 mg/m<sup>2</sup> was administered. The Adriamycin, Bleomycin, Vinblastine, and Dacarbazine chemotherapy regimen was administered in HD and standard doses of doxorubicin 25 mg/m<sup>2</sup>, where given on day one and 15. The cycle was repeated every 2 weeks to the maximum of 6 cycles. The NHL-BFM 90 chemotherapy regimen was administered in NHL. In this protocol, doxorubicin was used as 25 mg/m<sup>2</sup>, total cumulative dose of 150 mg/m<sup>2</sup>. All 31 patients underwent chemotherapy, and the total cumulative dose of doxorubicin ranged between 30-600 mg/m<sup>2</sup> (mean  $\pm$  SD,  $227.25 \pm 111.09$  mg/m<sup>2</sup>, median 240 mg/m<sup>2</sup>).

**Measurement of cTnI and NT-pro-BNP.** Three milliliters of blood were drawn into tubes containing sodium ethylene diamino tetra acetate (EDTA) and aprotinin while the patient was resting in the supine position. Immediately, the blood was centrifuged at 4°C and plasma was separated. Plasma then was stored at -20°C until additional processing. Levels of

cTnI were measured by an enzyme linked one-step sandwich immunoassay method (TOSOH A1A21 fluorescens Chemistry), and the lowest detectable level was 0.50 ng/ml. Levels of NT-pro-BNP were measured using electrochemiluminescent immunoassay (Roche Moduler Analytics E170, Elecys Module). No hemolyzed or EDTA-treated samples were included.

**Echocardiography.** Patients were simultaneously evaluated with 2-dimensional echocardiogram and biochemical investigations. Pulsed-wave Doppler and M-mode echocardiography were performed by one experienced pediatric cardiologist, with a Hewlett Packard Sonos 1000 ultrasound system using 2.5-MHz and 3.5-MHz transducers. M-mode measurements were performed according to the recommendations of the American Society of Echocardiography.<sup>16</sup> The LV fractional shortening (FS) and ejection fraction (EF) were calculated by Teich formula. The LV systolic functions were considered abnormal when EF was less than 60% or FS less than 30% based on a previous report.<sup>1</sup> Doppler signals were recorded from the apical 4-chamber view with a 3-5 mm sample volume placed at the level of mitral leaflet tips parallel to maximal mitral inflow. The midpoints of the darkest portion of the Doppler velocity waveforms were digitized, and the following indices were calculated: 1. Peak flow velocity of early LV filling (peak E); 2. Peak flow velocity of late (atrial) LV filling (peak A); 3. Ratio of peak early to peak late flow velocities (E/A ratio), and 4. Isovolumic relaxation time (IVRT).

**Statistical analysis.** We used the SPSS computer package (SPSS Inc., Chicago). Values were expressed as mean  $\pm$  SD. The unpaired Student t-test was used to assess differences between each group. The correlations between variables were found using Pearson's correlation test. A  $p < 0.05$  was considered as statistically significant.

**Results.** Out of 31 patients, 27 patients (aged between 4-15 years; mean age 7.8 years) showed normal cardiac function by echocardiography assessment. All of the patients had normal chest x-rays, electrocardiograms, and 4 patients (aged between 7-14 years; mean age 10.5 years) had abnormal systolic cardiac function parameters. The time past from the last doxorubicin dose was 1-42 months ( $9.39 \pm 12.3$ ). The median cumulative doxorubicin doses of the patients with normal and abnormal echocardiographic parameters were 240 and 270 mg/m<sup>2</sup>. The mean cumulative anthracycline dose was  $217.04 \pm 111.52$  mg/m<sup>2</sup> in the patients with normal cardiac function (normal group). Four patients (one of AML and 3 of ALL) had cardiac dysfunction on echocardiography. The mean cumulative anthracycline dose in this group with dysfunction was  $296.25 \pm 77.82$  mg/m<sup>2</sup>, which was

Table 1 - The mean values of NT-pro-BNP level in patients (n=31) and control groups (n=30).

Group	n	NT-pro-BNP level (mean $\pm$ SD)	pg/ml
<b>Patients with</b>			
Cardiac dysfunction	4	299.03 $\pm$ 264.97	62.2 - 550
Normal cardiac function	27	107.55 $\pm$ 131.82	5 - 501
<b>Total</b>	<b>31</b>	<b>135.92 <math>\pm</math> 166.16</b>	<b>5 - 550</b>
Normal healthy controls	30	47.17 $\pm$ 19.48	15 - 95.1

significantly higher than that in normal group ( $p < 0.01$ ). The mean values of NT-pro-BNP level in patients and control groups are summarized in **Table 1**. The NT-pro-BNP plasma levels in patients with cardiac dysfunction were elevated significantly, compared with control group ( $p < 0.001$ ) and the patients with normal cardiac function ( $p < 0.008$ ). The changes in systolic and diastolic echocardiographic parameters of patients are summarized in **Table 2**. The left ventricular ejection fraction (LVEF) decreased from  $68.16 \pm 4.43$  to  $55.72 \pm 3.63\%$  and FS from  $37.24 \pm 3.43$  to  $27.30 \pm 1.77\%$  in patients with normal and abnormal cardiac function (**Table 2**). There were no statistically significant changes in the LV systolic indices ( $p > 0.05$ ). And there were no statistically significant changes in peak A and peak E wave velocities, or E/A ratio ( $p > 0.05$ ). No significant correlations were found between any of the echocardiographic parameters with natriuretic peptides and cumulative doxorubicin dose. Serum cTnI values of all patients were below the detection limit ( $< 0.50$  ng/ml). There was no difference between serum cTnI levels of the patients with normal and abnormal echocardiographic findings. Two of 4 patients with LV systolic dysfunction had clinical findings. A 7-year-old girl with mild LV systolic dysfunction, who had AML, received a total cumulative anthracycline dose of 300 mg/m<sup>2</sup>. Her cardiac function was abnormal (LVEF: 56.3%, FS: 28%, Peak E: 89 and Peak A: 68.6), and she was treated with digoxin. She had mild of CHF, and her plasma NT-pro-BNP level was 505 pg/ml. Another 10-year-old boy with mild mitral failure, who had relapsed ALL, received a total cumulative anthracycline dose of 405 mg/m<sup>2</sup>. His cardiac function was abnormal (LVEF: 57.4%, FS: 27%, peak E: 82.6 and peak A: 47.1) and his plasma NT-pro-BNP level was 550 pg/ml, one month after receiving the last anthracycline dose.

**Discussion.** Anthracycline-induced cardiotoxicity limits effective cancer chemotherapy by causing early cardiomyopathy, and it can produce late-onset ventricular dysfunction years after

Table 2 - The changes in systolic and diastolic echocardiographic parameters of patients group.

Group	LVEF	FS	Peak E filling velocity (cm/s)	Peak A filling velocity (cm/s)	E/A ratio	IVRT (s)	LVED (cm)
<b>Patients with normal cardiac function (n=27)</b>							
Mean	68.16	37.24	94.38	53.16	1.81	0.06	3.50
SD	4.43	3.43	18.79	10.99	0.38	0.01	0.48
Min	62.30	33	72	40.4	1.1	0.05	2.5
Max	80.60	47.2	128	85.2	2.8	0.08	4.2
Median	67.85	36.35	93	50.80	1.98	0.06	3.48
<b>Patients with cardiac dysfunction (n=4)</b>							
Mean	55.72	27.30	86.22	50.85	1.77	0.08	3.92
SD	3.63	1.77	9.79	12.38	0.50	0.01	0.35
Min	50.70	25	75.1	39.8	1.3	0.07	3.4
Max	59.40	29.2	98.2	68.6	2.5	0.09	4.2
Median	56.40	27.50	85.80	47.50	1.66	0.08	4.07
<b>Total Patients (n=31)</b>							
Mean	66.25	35.71	93.17	51.33	1.80	0.07	3.57
SD	6.25	4.86	17.85	10.99	0.39	0.01	0.48
Min	50.70	25	72	39.8	1.1	0.05	2.5
Max	80.60	47.2	128	85.2	2.8	0.09	4.2
Median	66.35	36	93	50	1.82	0.06	3.60
EF - ejection fraction, FS - fractional shortening, SD - standard deviation, IVRT - isovolumic relaxation time, LVED - left ventricular end diastolic, LVEF - left ventricular ejection fraction							

treatment has ceased. Three distinct types of anthracycline-induced cardiotoxicity have been described.<sup>17</sup> First, acute or subacute injury can occur immediately after treatment. This rare form of cardiotoxicity may cause transient arrhythmias, a pericarditis myocarditis syndrome, or acute failure of the left ventricle.<sup>18,19</sup> Second, anthracyclines can induce chronic cardio-toxicity resulting in cardiomyopathy. This is a more common form of damage and is clinically the most important.<sup>20</sup> Finally, late-onset anthracycline cardio-toxicity causing late-onset ventricular dysfunction and arrhythmias, which manifest years to decades after anthracycline treatment has been completed, is increasingly recognized.<sup>21,22</sup> The diagnosis of early subclinical cardiac dysfunction is very important in patients taking anthracycline. The most serious long-term side effect of doxorubicin therapy is CHF. Conventional doses of anthracycline often lead to permanent myocardial damage and reduced functional reserve. At total dose of less than 400 mg/m<sup>2</sup> body surface area, the incidence of CHF is 0.14%, this incidence increases to 7% at a dose of 550 mg/m<sup>2</sup> body surface area, and to 18% at a dose of 700 mg/m<sup>2</sup> body surface area, but the occurrence of CHF is unpredictable.<sup>23</sup> The exact pathophysiological mechanisms leading to the development of cardiomyopathy are unclear. Underlying patho-

genetic mechanisms may include free-radical mediated myocyte damage, adrenergic dysfunction, intracellular calcium overload, and the release of cardiotoxic cytokines. The histologic findings in anthracycline-induced cardiomyopathy include myofibrillar loss, myocyte vacuolization, and eventually death of the myocyte.<sup>24</sup> Singal and Ilikskovic,<sup>25</sup> suggested that there is evidence of free radical formation and increased oxidative stress leading to lipid peroxidation and cell membrane injury. The overall incidence of doxorubicin induced cardiac dysfunction in children was approximately 23% in some investigations.<sup>1,22</sup> Our findings were similar to that of other studies.

Various methods have been recommended for the early identification of anthracycline cardiotoxicity. Two-dimensional echocardiography is the other primary noninvasive technique used to monitor anthracycline cardiotoxicity, particularly in children.<sup>26</sup> Detection of subclinical anthracycline induced cardiomyopathy through resting LVEF or echocardiographic FS is suboptimal. However, as with radionuclide measurements, cardiac compensation in the face of substantial anthracycline induced cardiac injury often maintains normal LVEF until the cardiomyopathic changes are relatively well established. Fractional shortening has been reported to be more sensitive than diastolic

parameters in children with early anthracycline induced cardiomyopathy.<sup>27</sup> In contrast, a recent smaller prospective study has shown the value of diastolic indices in detecting early anthracycline induced cardiomyopathy in asymptomatic children.<sup>28</sup>

Biochemical methods to diagnose and monitor cardiac function in doxorubicin-induced cardiotoxicity would be most useful. Nousiainen et al,<sup>29</sup> reported that doxorubicin-induced cardio-myopathy is a model to prospectively investigate the relationship between the secretion of natriuretic peptides, and the impairment of LV function. They showed that the development of doxorubicin-induced LV dysfunction in adult cancer patients was followed by neurohumoral activation characterized by a significant increase in plasma ANP, NT-pro-ANP and BNP. In addition, they found that the increased secretion of natriuretic peptides is related to the impairment of LV diastolic function rather than to the impairment of systolic function. They also found an inverse correlation between the decrease in FS and the increase in plasma NT-pro-ANP and plasma BNP. Previous studies suggested that plasma ANP and BNP levels of some cancer children with cardiac dysfunction were significantly higher than those of normal control subjects and patients with normal cardiac function.<sup>1</sup> The ANP is secreted mainly from the atria and BNP from the ventricles.<sup>10</sup> 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.<sup>7</sup> The NT-pro-BNP level in cancer children who have taken doxorubicin was found higher than the control group in our study. The LVEF, FS, peak E, peak A wave velocity and E/A ratio decreased, IVRT increased in patients with cardiac dysfunction. However, the differences in the systolic and diastolic echocardiographic indices between 2 groups were not statistically significant. Recently, cardiac troponin T (cTnT), particular cTnI, have been found to represent a uniquely sensitive and specific marker of myocyte membrane integrity, and therefore to increase in response to minimal myocardial cell damage in different settings, including doxorubicin-induced cardiotoxicity.<sup>15</sup> Kismet et al,<sup>30</sup> reported the usefulness of serum cTnT to detect doxorubicin related cardiotoxicity as a non-invasive and reliable method. This study included 24 patients who received doxorubicin for their solid tumors at cumulative doses of 400 mg/m<sup>2</sup> or higher. In their study, the serum cTnT values of 21 patients were below the detection limit (< 0.010 ng/ml), and no correlation was found between serum cTnT values, cumulative doxorubicin doses, and systolic or diastolic cardiac functions. There was no statistical difference between serum cTnT levels of the patients with normal and abnormal echocardiographic findings. They concluded that

echocardiographic follow-up is more reliable than serum cTnT levels for detecting subclinical cardiac toxicity.<sup>30</sup>

Cardiac troponin-I is an isoform of a thin filament contractile protein present in high concentrations in the myocardium, but usually not expressed in regenerating skeletal muscle or in other tissues. It is the only known molecular marker of myocardial injury and is detectable within 6 hours of the damage.<sup>31</sup> We did not find any human studies on cTnI and cardiotoxicity, except one animal study<sup>32</sup> which, suggested that cTnI was not a reliable marker of doxorubicin-induced cardiotoxicity in rats. In our study, all of the patients cTnI values were below the detection limit (<0.5 ng/ml). For this reason, we do not speculate a correlation between cTnI and doxorubicin-induced cardiotoxicity.

In summary, our study shows that the serum NT-pro-BNP measurements seemed to represent a useful contribution in the long-term cardiac follow-up of doxorubicin-induced cardiotoxicity in children with cancer. Plasma NT-pro-BNP level could be a marker for doxorubicin-induced cardiotoxicity in children, and it can readily be included in laboratory follow-up, it is reasonably inexpensive, and may decrease the need for more laboratory tests of cardiac function. Further experimental and clinical studies are required on the correlation between cTnI and cardiotoxicity.

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