

## Long-term cardiac effects of treatment for childhood leukemia.

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Late cardiac complications in cancer survivors may develop from subclinical myocardial damage. Biochemical correlates of minimal myocardial changes can be analyzed using a commercially available rapid assay. Biomarkers are considered more sensitive markers of subclinical cardiotoxicity than conventional electrocardiographic and echocardiographic methods.

The aim of this study was to determine the values of plasma N-terminal pro brain natriuretic peptide (NT-pro-BNP) and cardiac troponin T (cTnT) in asymptomatic childhood leukemia survivors after anthracycline therapy in comparison with healthy volunteers. The survivors also underwent a detailed echocardiography.

Twenty six survivors of leukemia previously treated with anthracyclines with total cumulative dose 95-600 (median 221) mg/m<sup>2</sup> were evaluated. Analyses of cTnT and NT-proBNP from blood samples and echocardiography were performed 5–25 years after completion of therapy for childhood leukemia. Control group for biochemical analyses consisted of 22 age- and gender- matched apparently healthy volunteers.

Values of NT-proBNP were significantly elevated in ANT group compared to controls ( $35.1 \pm 37.8$  vs.  $9.6 \pm 6.7$  pg/ml,  $P < 0.010$ ). cTnT remained below the diagnostic cut-off values in both groups. All echocardiographic parameters of patients remained normal.

In conclusion, differences in NT-proBNP values between patients treated with anthracyclines and healthy volunteers might signal an initial stage of anthracycline-induced myocardial damage. The potential of this biomarker to detect subclinical anthracycline-induced myocardial alterations before development of echocardiographic and clinical changes is promising.

*Key words: cardiotoxicity, natriuretic peptides, cardiac troponins, anthracyclines, echocardiography*

Long-term cardiac complications may occur several years after completion of anticancer treatment [1–6]. In the past year report from the Childhood Cancer Survivor Study (the largest cohort of childhood cancer survivors in the world) showed that survivors of childhood cancers are five to ten times more likely than their healthy siblings to develop heart

disease even 30 years after diagnosis - such as congestive heart failure, myocardial infarction, pericardial disease, valvular heart disease [2].

Minimal potentially progressive myocardial changes cannot be diagnosed using the standard 12-leads electrocardiography or echocardiography [7].

Biochemical cardiomarkers that reflect cardiomyocyte degeneration (serum levels of cardiac troponin T - cTnT) and functional myocardial changes (serum levels of N-terminal fragment of the brain natriuretic peptide precursor - NT-proBNP) of cancer patients treated with potentially cardiotoxic therapy have been evaluated in several clinical and experimental studies [8–24]. However only little information is available about the usefulness of biomarkers cTnT and NTproBNP in detection of late anthracycline cardiotoxicity (more than 5 years after diagnosis) in survivors of pediatric leukemia.

**Abbreviations:** ANT group – patients previously treated with anthracyclines; cTn – cardiac troponins; cTnT – cardiac troponinT; NPs – natriuretic peptides; NT-proBNP – N-terminal fragment of the brain natriuretic peptide precursor; LV – left ventricular; LVEDD – left ventricular end-diastolic diameter; LVESD – left ventricular end-systolic diameter; LA – left atrium dimension; RV – right ventricular dimension; EF – ejection fraction; E – early diastolic flow velocity; A – late diastolic flow velocity; DT – deceleration time; IVRT – left ventricular isovolumetric relaxation time; TDI – tissue Doppler imaging; Ea – early diastolic velocity of the medial mitral annulus; Em; Sa – systolic velocity; Am – late diastolic velocity

**Table 1. Baseline characteristics of both study populations and control group.**

|  | ANT Group*<br>(N=26)  | Control<br>Group<br>(N=40) |
|--|-----------------------|----------------------------|
| Sex M/F                                  | 18/8                  | 30/10                      |
| Diagnosis<br>(number of patients)        | ALL‡ (21)<br>AML§ (5) |                            |
| Age at diagnosis (years)                 | 10 (1-16)             |                            |
| Years after completion of chemotherapy   | 10.5 (5-19)           |                            |
| Age at study (years)                     | 22.5 (18-27)          | 23 (20-27)                 |
| ANT cumulative dose (mg/m <sup>2</sup> ) | 221 (95-600)          |                            |
| CNS radiation                            | 17                    |                            |
| Dose of CNS radiation (Gy)               | 18 (12-24)            |                            |

Values are presented as median (range).

\* patients previously treated with anthracyclines

‡ acute lymphoblastic leukemia

§ acute myeloblastic leukemia

## Patients and methods

**Study population.** A total of 26 consecutive survivors of childhood acute leukemia at complete remission were enrolled in this study. All patients were treated in Children's University Hospital, their therapy containing anthracyclines (doxorubicin and daunorubicin) was completed 5–25 years ago. They were followed at out-patient clinic of National Cancer Institute, Bratislava, Slovak Republic from January 2006 to September 2008. Medical history, physical examination, standard electrocardiography and measurement of blood pressure did not reveal any signs of cardiac impairment in patients and controls.

No other concomitant diseases (e.g. renal insufficiency, liver disease) that could potentially modify study results were observed at the time of cardiac evaluation. The control group consisted of 22 healthy, age- and sex- matched apparently healthy volunteers - medical students (Table 1). We measured NT-proBNP repeatedly at two weeks interval in eighteen of them, altogether 40 blood samples were analyzed in controls.

Patients as well as healthy volunteers were enrolled into the study after written informed consent was obtained. The study was approved by the Ethics Committee of the Faculty of Medicine, Comenius University in Bratislava, Slovak Republic. All patients were examined by general cardiologist, echocardiographic recordings were single blinded and assessed by the same cardiologist. A blood samples were drawn for immunochemical analysis of cTnT and NT-proBNP at the same time as echocardiography. Analyses of blood for biomarker estimation were performed in the reference laboratory of National Institute of Cardiovascular Diseases in Bratislava, Slovak Republic.

**Biochemical analysis.** EDTA-anticoagulated blood (5 mL) was collected by venous puncture. Fasting was not prerequisite before sampling. The whole blood was centrifuged for 10 minutes (3500 rpm.) within 2 h after sampling. 500 µL of centrifuged plasma was aliquoted to labeled eppendorf tubes before freezing and stored at –20°C until assayed. Cardiomarkers (cTnT, NT-proBNP) were measured within 6 month after the collection at the Clinical Biochemistry Department, National Cardiovascular Institute, Bratislava, Slovak Republic. Hemolyzed samples were excluded. Plasma concentrations of cardiomarkers were assayed on Elecsys 2010 Immunoassay analyzer (Roche Diagnostics). The sandwich electrochemiluminescence (ECLIA) Elecsys proBNP (Roche Diagnostics) and Elecsys Troponin T STAT Immunoassay (Roche Diagnostics) immunoassay was used for quantitative determination of studied biomarkers. The detection limit for NT-proBNP was 5.0 pg/ml. The cTnT immunoassay had a sensitivity of 0.01 ng/ml.

No cut-off values of NT-pro-BNP were used, but we compared levels of NT-pro-BNP between the study group and the our age- and gender-matched control group.

**Echocardiography.** Echocardiography using GE VIVID 7 machine (GE Ultrasound Europe) was performed in all patients included into the study. Assessment was done by single experienced cardiologist. Standard techniques were used to obtain M-mode, two-dimensional, and Doppler (color, pulse, continuous, tissue) measurements.

Left ventricular (LV) end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD) and left atrium dimension were measured using standard M-mode methods from parasternal LV long axis images. LV ejection fraction (EF) was calculated by Teichholz formula (M-mode) as well as by the Simpson method from apical view (two-dimensional echocardiography). Pulsed Doppler traces of the mitral valve inflow were used to extract the ratio of early to late diastolic flow velocity (E/A), deceleration time (DT), left ventricular isovolumetric relaxation time (IVRT) and were assessed as standard parameters of left ventricular diastolic function. The tissue Doppler imaging (TDI) of the mitral annulus from apical four-chamber view provided additional parameters reflecting the global systolic and diastolic function of the LV. Early diastolic velocity (Ea) of the mitral annulus was postulated a good indicator of LV myocardial relaxation and diastolic function, as well as the ratio of Em and late diastolic velocity (Am). Systolic velocity (Sa) was used to assess the longitudinal systolic function. The ratio of early diastolic LV inflow velocity (E) to early diastolic velocity (Ea) of the medial mitral annulus (E/Ea) was used for the estimation of LV filling pressure.

**Statistical analysis.** Comparison of the biomarkers values between studied groups were performed with the Kruskal-Wallis test. A probability value P<0.05 was considered statistically significant.

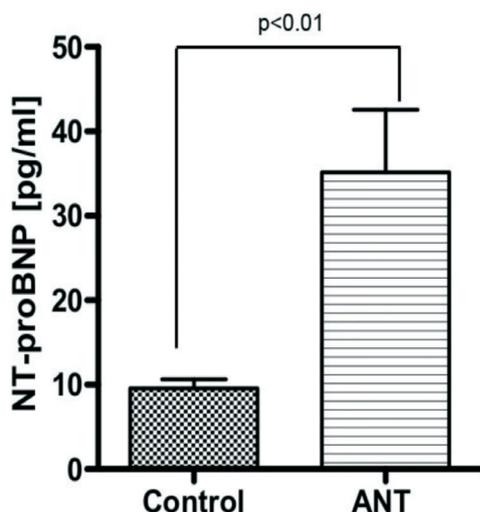


Figure 1. Comparison of NT-proBNP plasmatic concentrations in control group, and in ANT Group.

Values are presented as mean  $\pm$  SD. Significance was assumed at  $P < 0.050$ .

## Results

**Biochemical analysis. Cardiac Troponin T.** Plasma values of cTnT remained below the diagnostic cut-off values of the assay ( $< 0.01$  ng/ml) in all patients as well as in controls.

**NT-proBNP.** The mean plasma NT-proBNP concentration in ANT group was  $35.1 \pm 37.8$  pg/ml (from detection limit to 123.5 pg/ml). Healthy volunteers had the mean value of NT-proBNP  $9.6 \pm 6.7$  pg/ml (detection limit – 25.5 pg/ml). NT-proBNP values were significantly elevated in leukemia survivors ( $P < 0.01$ ) (Figure 1).

**Echocardiographic parameters.** All measured echocardiographic results in 26 asymptomatic leukemia survivors are presented as mean  $\pm$  SD:

LVEF (%)  $64.4 \pm 4.5$ , E/A  $1.8 \pm 0.5$ , DT (ms)  $193.8 \pm 37.5$ , IVRT (ms)  $73.2 \pm 10.4$ , E/Ea  $6.3 \pm 1.2$ , Em/Am  $2.0 \pm 0.6$ , LVEDD (mm)  $46.9 \pm 4.3$ , LVESD (mm)  $29.9 \pm 3.1$ , LA (mm)  $33.5 \pm 3.9$ , RV (mm)  $27.7 \pm 3.3$ .

We did not find any abnormalities in these systolic, diastolic and morphologic parameters.

## Discussion

Conventional methods used until now in detection of cardiotoxicity, such as 12-lead electrocardiography and echocardiography, have several limitations, mainly low sensitivity and specificity. Echocardiographic abnormalities are seen only when critical amount of microstructural changes are accumulated.

Better understanding of pathophysiology of late cardiotoxicity as well as early detection of cardiomyocyte degeneration

and minimal functional abnormalities is necessary for better management of long-term survivors of cancer many years after cardiotoxic therapy. Currently there is no optimal method that can be used for early detection of subclinical late cardiotoxicity. The role of biochemical cardiomarkers that reflect cardiomyocyte degeneration (serum levels of cTnT) and functional myocardial changes (serum levels of NT-proBNP) in early detection of cardiotoxicity of cytostatics have been evaluated in many clinical and experimental studies [8-25].

The clinical value of cTn and NPs measurement in diagnosis of heart failure during and after the anticancer treatment has been evaluated in several studies with adults and children [7, 9, 13, 14, 22-25].

Plasma values of cTnT were not elevated in asymptomatic leukemia survivors and healthy subjects. Most of the clinical trials were designed to assess cTn levels during or soon after completion of the cardiotoxic chemotherapy with the further follow up of cardiac performance. The study of Cardinale et al. confirmed a strong relation between the value of cTnI and reduction of left ventricular ejection fraction (LVEF) seven months after high-dose chemotherapy [10]. Early increase of cTnI concentrations was able to predict left ventricular dysfunction in this study (10). Elevations of cTnT associated with LV diastolic dysfunction were observed by Kilickap et al. [11]. Lipshultz et al. shown increase of cTnT concentrations during anticancer therapy before development of echocardiographic abnormalities [7]. So far no studies have reported cTn elevations as markers of late cardiotoxicity.

We found higher NT-proBNP values after therapy with anthracyclines in comparison with controls. Since NT-proBNP cut-off values are age- and sex-dependent, we compared parameters in young survivors with those from our control group of 22 apparently healthy volunteers.

NT-proBNP elevations in these patients might reflect late subclinical cardiotoxicity. Rosenberg et al. in a large group of 5875 cardiac patients showed significant relation of elevated NT-proBNP in a range 83 – 118 pg/ml with a 90% increased risk for cardiovascular mortality within three years of follow-up. Elevations above 83 pg/ml were found in 7,7% of our survivors, none of our volunteers had elevated values of this marker [26].

So far, only a limited number of studies have investigated NTproBNP as marker or predictor of late-cardiotoxicity. Lipshultz et al. (2003) showed proBNP as a marker of late cardiotoxicity in survivors of childhood cancer. Pro-BNP levels were higher in survivors previously treated with cardiotoxic therapy (anthracyclines and/or mediastinal radiation), as well as in survivors treated with noncardiotoxic agents) than in their healthy siblings [9].

Germanakis et al. found association between LV mass reduction and alteration of plasma NT-proBNP concentrations in asymptomatic children, 3.9 years after anthracycline treatment. The increase in NT-proBNP levels correlated with the decrease in LV mass [14].

Assessment of plasma NT-proBNP concentrations may be a useful tool for monitoring of late anthracycline cardiotoxicity in asymptomatic patients after completion of potentially cardiotoxic therapy (even after low doses of anthracyclines). These changes might reflect an initial stage of anthracycline cardiotoxicity before development of echocardiographic changes [27, 28]. However continued follow-up is required to bring more insight into their predictive value.

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## References

- [1] SIMBRE VC, DUFFY SA, DADLANI GH, MILLER TL, LIPSHULTZ SE. Cardiotoxicity of cancer chemotherapy: implications for children. *Paediatr Drugs*. 2005; 7: 187–202. [doi:10.2165/00148581-200507030-00005](https://doi.org/10.2165/00148581-200507030-00005)
- [2] MULROONEY DA, DOVER DC, LI S, YASUI Y, NESS KK et al. Childhood Cancer Survivor Study. Cardiovascular disease in adult 1. survivors of childhood and adolescent cancer: a report from the Childhood Cancer Survivor Study (CCSS). Presented at the 44th Annual Meeting of the American Society of Clinical Oncology; May-June 2008; Chicago, IL.
- [3] PIHKALA J, SAARINEN UM, LUNDSTRÖM U, VIRTANEN K, VIRKOLA K, et al. Myocardial function in children and adolescents after therapy with anthracyclines and chest irradiation. *Eur J Cancer*. 1996; 32A: 97–103. [doi:10.1016/0959-8049\(95\)00555-2](https://doi.org/10.1016/0959-8049(95)00555-2)
- [4] KREMER LC, VAN DALEN EC, OFFRINGA M, OTTENKAMP J, VOUTE PA. Anthracycline-induced clinical heart failure in a cohort of 607 children: long-term follow-up study. *J Clin Oncol*. 2001; 19: 191–196.
- [5] GIANNI L, HERMAN HE, LIPSHULTZ SE, MINOTTI G, SARVAZYAN N, SAWYER DB. Anthracycline Cardiotoxicity: From Bench to Bedside. *J Clin Oncol*. 2008, 26: 3777–3784. [doi:10.1200/JCO.2007.14.9401](https://doi.org/10.1200/JCO.2007.14.9401)
- [6] MLADOSIEVICOVA B, FOLTINOVA A, LUPTAK I, PETRASOVA H, HULIN I. Frequency-domain analysis of the QRS complex after treatment of childhood cancer with anthracycline cytostatics. *Pediatr Cardiol*. 2001; 22: 478–482. [doi:10.1007/s002460010278](https://doi.org/10.1007/s002460010278)
- [7] LIPSHULTZ SE, RIFAI N, SALLAN SE, LIPSITZ SR, DALTON V, et al. Predictive value of cardiac troponin T in pediatric patients at risk for myocardial injury. *Circulation*. 1997; 96: 2641–2648.
- [8] HERMAN EH, ZHANG J, LIPSHULTZ SE, RIFAI N, CHADWICK D, et al. Correlation between serum levels of cardiac troponin- T and the severity of the chronic cardiomyopathy induced by doxorubicin. *J Clin Oncol*. 1999; 17: 2237–2243.
- [9] LIPSHULTZ SE, HINKLE AS, FRENCH CA, KOZLOWSKI AM, CONSTINE LS, et al. Accelerated atherosclerosis contributes to elevated global risk for premature symptomatic cardiovascular disease in survivors of childhood cancer. Abstract No: 3203, 39th Annual Meeting of the American Society of Clinical Oncology (ASCO); 2003 May 31 – June 3; Chicago, IL.
- [10] CARDINALE D, SANDRI MT, MARTINONI A, TRICCA A, CIVELLI M, et al. Left ventricular dysfunction predicted by early troponin I release after high-dose chemotherapy. *J Am Coll Cardiol*. 2000; 36: 517–522. [doi:10.1016/S0735-1097\(00\)00748-8](https://doi.org/10.1016/S0735-1097(00)00748-8)
- [11] KILICKAP S, BARISTA I, AKGULE E, AYTEMIR K, AKSOYEK S, et al. CTnT can be a useful marker for early detection of anthracycline cardiotoxicity. *Ann Oncol*. 2005; 16: 798–804. [doi:10.1093/annonc/mdj152](https://doi.org/10.1093/annonc/mdj152)
- [12] DAUGAARD G, LASSEN U, BIE P, PEDERSEN EB, JENSEN KT, et al. Natriuretic peptides in the monitoring of anthracycline induced reduction in left ventricular ejection fraction. *Eur J Heart Fail*. 2005; 7: 87–93. [doi:10.1016/j.ejheart.2004.03.009](https://doi.org/10.1016/j.ejheart.2004.03.009)
- [13] 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: 1197–1202.
- [14] GERMANAKIS I, KALMANTI M, PARTHENAKIS F, NIKITOVIC D, STIAKAKI E, et al. Correlation of plasma N-terminal probrain natriuretic peptide levels with left ventricle mass in children treated with anthracyclines. *Int J Cardiol*. 2006; 108: 212–215. [doi:10.1016/j.ijcard.2005.05.006](https://doi.org/10.1016/j.ijcard.2005.05.006)
- [15] BRYANT J, PICOT J, BAXTER L, LEVITT G, SULLIVAN I, et al. Use of cardiac markers to assess the toxic effects of anthracyclines given to children with cancer: a systematic review. *Eur J Cancer*. 2007; 43: 1959–1966. [doi:10.1016/j.ejca.2007.06.012](https://doi.org/10.1016/j.ejca.2007.06.012)
- [16] DOLCI A, DOMINICI R, CARDINALE D, SANDRI MT, PANTEGHINI M. Biochemical markers for prediction of chemotherapy-induced cardiotoxicity: systematic review of the literature and recommendations for use. *Am J Clin Pathol*. 2008; 130: 688–695. [doi:10.1309/AJCPB66LRHVMQDR](https://doi.org/10.1309/AJCPB66LRHVMQDR)
- [17] URBANOVA D, URBAN L, DANOVA K, SIMKOVA I. Natriuretic peptides: biochemical markers of anthracycline cardiac toxicity? Review. *Oncol Res*. 2008; 17: 51–58.
- [18] KATUS HA, REMPPIS A, SCHEFFOLD T, DIEDERICH KW, KUEBLER W. Intracellular compartmentation of cardiac troponin T and its release kinetics in patients with reperfused and nonreperfused myocardial infarction. *Am J Cardiol*. 1991; 67: 1360–1367. [doi:10.1016/0002-9149\(91\)90466-X](https://doi.org/10.1016/0002-9149(91)90466-X)
- [19] ADAMCOVA M, STERBA M, SIMUNEK T, POTACOVA A, POPELOVA O, et al. Troponin as a marker of myocardial damage in drug-induced cardiotoxicity. *Expert Opin Drug Saf*. 2005; 4: 457–472. [doi:10.1517/14740338.4.3.457](https://doi.org/10.1517/14740338.4.3.457)
- [20] BERTINCHANT JP, POLGE A, JUAN JM, OLIVA-LAURAIRE MC, GIULIANI I, et al. Evaluation of cardiac troponin I and T levels as markers of myocardial damage in doxorubicin-induced cardiomyopathy rats, and their relationship with echocardiographic and histological findings. *Clin Chim Acta*. 2003; 329: 39–51. [doi:10.1016/S0009-8981\(03\)00013-5](https://doi.org/10.1016/S0009-8981(03)00013-5)
- [21] KOH E, NAKAMURA T, TAKAHASHI H. Troponin-T and brain natriuretic peptide as predictors for adriamycin-induced cardiomyopathy in rats. *Circ J*. 2004; 68: 163–167. [doi:10.1253/circj.68.163](https://doi.org/10.1253/circj.68.163)
- [22] LIPSHULTZ SE, RIFAI N, DALTON VM, LEVY DE, SILVERMAN LB, et al. The effect of dexrazoxane on myocardial

- injury in doxorubicin-treated children with acute lymphoblastic leukemia. *N Engl J Med.* 2004; 351: 145–53.
- [23] MAVINKURVE-GROOETHIUS AM, KAPUSTA L, NIR A, GROOT-LOOWEN J, et al. The role of biomarkers in the early detection of anthracycline-induced cardiotoxicity in children. Review. *Pediatr Hematol Oncol* 2008,25: 655–664. [doi:10.1080/08880010802244001](https://doi.org/10.1080/08880010802244001)
- [24] MAVINKURVE-GROOETHIUS AM, GROOT-LOONEN J, BELLERSEN L, POURIER MS, FEUTH T et al.: Abnormal NT-pro-BNP levels in asymptomatic long-term survivors of childhood cancer treated with anthracyclines. *Pediatr Blood Cancer* 2009, published online DOI 10.1002/pbc
- [25] KISMET E, VARAN A, AYABAKAN C, ALEHAN D, PORTAKAL O, et al. Serum troponin T levels and echocardiographic evaluation in children treated with doxorubicin. *Pediatr Blood Cancer.* 2004; 42: 220–224. [doi:10.1002/pbc.10368](https://doi.org/10.1002/pbc.10368)
- [26] ROSENBERG J, SHOU M, GUSTAFSSON F, BADSKJAER J, HILDEBRANDT P et al. Prognostic threshold levels of NTproBNP testing in primary care. *Eur Heart J.* 2009 30 (1): 66–73. [doi:10.1093/eurheartj/ehn525](https://doi.org/10.1093/eurheartj/ehn525)
- [27] HORACEK JM, PUDIL R, TICHY M, JEBAVY L, STRASSOVA A et al. The use of biochemical markers in cardiotoxicity monitoring in patients treated for leukemia. *Neoplasma.* 2005; 52: 430–4.
- [28] HORACEK JM, TICHY M, PUDIL R, JEBAVY L, ZAK P et al. Multimarker approach to evaluation of cardiac toxicity during preparative regimen and hematopoietic cell transplantation. *Neoplasma.* 2008; 55: 532–7.