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ORIGINAL REPORT

Changes in Cardiac Biomarkers During Doxorubicin Treatment of Pediatric Patients With High-Risk Acute Lymphoblastic Leukemia: Associations With Long-Term Echocardiographic Outcomes

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A B S T R A C T

Purpose
Doxorubicin causes cardiac injury and cardiomyopathy in children with acute lymphoblastic leukemia (ALL). Measuring biomarkers during therapy might help individualize treatment by immediately identifying cardiac injury and cardiomyopathy.

Patients and Methods
Children with high-risk ALL were randomly assigned to receive doxorubicin alone (n = 100; 75 analyzed) or doxorubicin with dexrazoxane (n = 105; 81 analyzed). Echocardiograms and serial serum measurements of cardiac troponin T (cTnT; cardiac injury biomarker), N-terminal pro-brain natriuretic peptide (NT-proBNP; cardiomyopathy biomarker), and high-sensitivity C-reactive protein (hsCRP; inflammatory biomarker) were obtained before, during, and after treatment.

Results
cTnT levels were increased in 12% of children in the doxorubicin group and in 13% of the doxorubicin-dexrazoxane group before treatment but in 47% and 13%, respectively, after treatment (P = .005). NT-proBNP levels were increased in 89% of children in the doxorubicin group and in 92% of children in the doxorubicin-dexrazoxane group before treatment but in only 48% and 20%, respectively, after treatment (P = .07). The percentage of children with increased hsCRP levels did not differ between groups at any time. In the first 90 days of treatment, detectable increases in cTnT were associated with abnormally reduced left ventricular (LV) mass and LV end-diastolic posterior wall thickness 4 years later (P < .01); increases in NT-proBNP were related to an abnormal LV thickness-to-dimension ratio, suggesting LV remodeling, 4 years later (P = .01). Increases in hsCRP were not associated with any echocardiographic variables.

Conclusion
cTnT and NT-proBNP may hold promise as biomarkers of cardiotoxicity in children with high-risk ALL. Definitive validation studies are required to fully establish their range of clinical utility.

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INTRODUCTION

Doxorubicin treats hematologic malignancies and solid tumors[1] but is limited by cardiotoxicity.[2-6] Doxorubicin cardiotoxicity is dose limiting during treatment and causes long-term morbidity and early mortality among cancer survivors.[4-6] Because doxorubicin-induced cardiac damage is progressive and often irreversible, minimizing cardiac injury during treatment to prevent late cardiomyopathy is critical.[7-10] For example, the concomitant administration of dexrazoxane substantially reduces cardiotoxicity.[9-11]

Identifying risk factors has helped characterize groups at high risk for cardiac injury,[2-6] but tolerance of chemotherapy and predisposition to cardiac damage vary substantially among patients. Although cardiac imaging techniques can assess heart structure and function during and after therapy, they lack the sensitivity to detect early treatment-induced cardiac injury and the specificity to distinguish myocardial injury that may have late

implications in long-term survivors from short-term changes in left ventricular (LV) loading conditions or transient cytokine-mediated myocardial depressant effects.[8] Serial endomyocardial biopsies are impractical.[12]

Serial monitoring of circulating biomarker levels can evaluate cardiotoxicity during and after therapy accurately and efficiently.[13-16] Increases in cardiac troponin T (cTnT) and cardiac troponin I (cTnI) reflect cardiac damage and often indicate irreversible cardiomyocyte necrosis.[17-21] Chronic increases in N-terminal pro-brain natriuretic peptide (NT-proBNP) indicate increased LV wall stress associated with pressure and volume overload and increased diastolic pressure.[22-33]

As a global inflammatory marker, high-sensitivity C-reactive protein (hsCRP) is used to assess patient health.[34] Increases in serum hsCRP may indicate cardiac stress because inflammation is important in heart disease.[35,36]

Biomarkers may improve cardiotoxicity monitoring during and after doxorubicin treatment.[5,6,9,13-16,18,19] Thus, in this study, we characterized trends in cTnT, NT-proBNP, and hsCRP levels in children with high-risk acute lymphoblastic leukemia (ALL) before, during, and after treatment with doxorubicin to examine the effect of doxorubicin with or without dexrazoxane on biomarker levels and to identify the relationships between these biomarkers and echocardiographic variables over time.

PATIENTS AND METHODS

Patients

This study was part of the Dana-Farber Cancer Institute Childhood ALL Consortium Protocol 95-01, which enrolled patients younger than age 18 years with newly diagnosed ALL between January 1996 and September 2000.[10] The institutional review board at each participating institution approved the protocol before patient enrollment. Informed consent was obtained from patients, parents, or guardians before starting therapy.

Serial serum samples and echocardiograms were obtained in children with high-risk ALL, that is, those who at diagnosis were younger than 12 months or more than 10 years of age and had any of the following: a leukocyte count of $\geq$ 50,000 cells/μL, a T-cell immunophenotype, an anterior mediastinal mass, or CNS disease.

Therapy and Data Collection

In addition to multiagent chemotherapy and CNS radiation, all children with high-risk ALL received two doses of doxorubicin (30 mg/m^2/dose) during remission induction and then eight more doses (30 mg/m^2/dose) every 3 weeks during postremission intensification to a cumulative dose of 300 mg/m^2.[10] No doxorubicin was given after about 9 months of therapy. In addition, patients were randomly assigned to receive doxorubicin alone or doxorubicin immediately preceded by dexrazoxane (300 mg/m^2/dose).

Serum for biomarker assays was collected at diagnosis (before doxorubicin therapy), daily after induction doses of doxorubicin for days 1 through 7, 7 days after a doxorubicin dose during the consolidation phase, and at the end of doxorubicin therapy. Serum samples were sent to a central laboratory, frozen immediately, and stored at $-70°C$ until assayed. Hemolyzed samples or samples with insufficient material could not be assayed and were excluded. Investigators performing serum assays and echocardiographic evaluations remained blinded throughout the study. An independent data safety monitoring committee reviewed accrual, toxicity, and troponin results every 6 months and released the troponin results for publication after all children had completed doxorubicin treatment.[9]

Biomarker Assays

Levels of hsCRP were determined by using the Tina-quant C-reactive protein [latex] high-sensitive assay (Roche Diagnostics, Indianapolis, IN; sen-

sitivity, 0.03 mg/L). Levels of NT-proBNP were measured by using the Elecsys immunoanalyzer (Roche Diagnostics; sensitivity, 5 pg/mL). Levels of cTnT were measured by using the Elecsys Troponin-T STAT Immunoassay (Roche Diagnostics; sensitivity, 0.01 ng/mL).[18] cTnI levels were measured on 40 samples from 18 children selected by cTnT level and available volume; 20 samples with increased cTnT levels and 20 without were analyzed for cTnI levels by using the Singulex ERENNA System (Singulex, Alameda, CA; sensitivity, 0.0002 ng/mL).[37] Abnormal values were defined as follows: for cTnT and cTnI, any detectable amount; for NT-proBNP, levels $\geq$ 150 pg/mL in infants younger than 1 year or $\geq$ 100 pg/mL in children age 1 year or older; and for hsCRP, levels $\geq$ 1.9 mg/L.

Echocardiographic Measurements

Echocardiograms were obtained at diagnosis, after doxorubicin therapy, and every 2 years thereafter. LV status was assessed with systolic and diastolic dimensions; mass; end-diastolic posterior wall thickness; thickness-to-dimension ratio; and fractional shortening, an index of LV systolic performance influenced by heart rate, preload, afterload, and contractility.[9-11] Echocardiograms were obtained at local treatment sites and remeasured at a single facility by blinded study staff.[9] Children were eligible for cardiac follow-up throughout their first continuous complete remission.

Statistical Methods

Means of normally distributed outcome variables (eg, echocardiographic measurements) were compared with t tests; means of non-normally distributed variables (eg, age) were compared with Wilcoxon rank sum tests. Differences in proportions of dichotomous values (eg, abnormal cTnT) between groups were compared with Fisher's exact tests.

To adjust for growth-related changes, echocardiographic data were standardized by age (LV fractional shortening and blood pressure) or body surface area (LV mass, LV dimension, and LV wall thickness). A Z score was then calculated from normal predicted values from a regression model by using values from 285 normal children[38] in whom body surface area ranged from 0.2 to 2.2 m^2, and who had normal height, weight, height-for-weight percentiles, and blood pressure and no evidence of cardiac or other disorders.

We did not assume that all follow-up biomarker and echocardiographic data would be collected from all patients at predetermined time points. Thus, although we collected blood samples from as many patients as possible until 2 months after doxorubicin treatment, we could not calculate the proportion of patients with abnormal values at each time point (eg, monthly).

Each time a patient was observed, we created a binary outcome for each biomarker (abnormal or normal). The probability of an abnormal outcome was then estimated from logistic regression models as a linear, quadratic, and cubic function of the time of measurement by using all data from all time points on all patients. Then we estimated the probability of an abnormal value at each month for 9 months from this logistic regression model (using months 0 through 9 in the model).

Given repeated measures on the same patient, we fit a logistic regression model for the probability of abnormal outcomes occurring over time by repeated measures (generalized estimating equations) logistic regression models in SAS PROC GLIMMIX (SAS Institute, Cary, NC) that modeled the probability of an abnormal outcome as a linear, quadratic, and cubic function of time. (See Appendix [online only] for more details on the modeling methods.) We modeled the correlation between outcomes measured at a pair of times on the same child with an autoregressive structure. We plotted the probability of abnormal cardiac biomarkers from this logistic regression against time to estimate the probability of an abnormal biomarker level at a given time point. A similar mixed model was used to model echocardiographic Z scores after doxorubicin treatment as a function of time, treatment, and increased abnormal cardiac biomarkers during therapy (dichotomized as abnormal or normal). From this mixed model, we estimated the mean echocardiographic Z scores at 4 years as a function of abnormal cardiac biomarker levels during therapy. We calculated 95% CIs for the predicted probabilities by transforming a 95% CI for the logit at each time point (calculated as the logit $\pm$ 1.96 times the SE of the logit).

Although the number of dropouts did not differ between groups, our approach protects against biases caused by patients on one treatment being
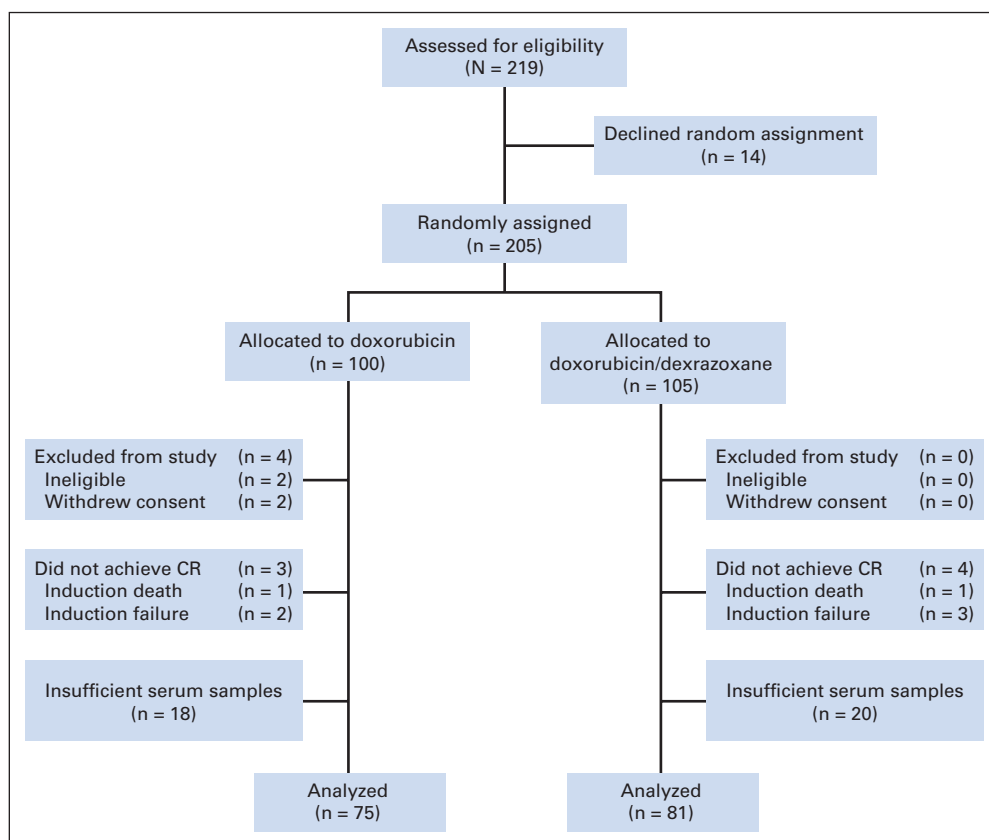
Fig 1. CONSORT diagram shows allocation and disposition of patients with high-risk acute lymphoblastic leukemia in the Dana-Farber Cancer Institute Childhood Acute Lymphoblastic Leukemia Consortium Protocol 95-01 randomized controlled trial. CR, complete remission.

seen more often and followed for longer periods than patients on the other treatment, as might occur in patients with poorer heart function on that treatment. The logistic regression model formulation in SAS PROC GLIMMIX adjusts for this type of missing follow-up data[38-40] and ensures that data from patients with poor cardiac function who came back most often for repeat testing were not weighted excessively and did not disproportionately influence the tails of the plots.

RESULTS

Patient Characteristics

Between 1996 and 2000, 491 children were enrolled on Protocol 95-01, of whom 219 were high risk. Of these, 14 declined assignment. Of the remaining 205, 100 (49%) were randomly assigned to receive doxorubicin alone and 105 (51%) to receive doxorubicin with dexrazoxane. Of the 205 patients, 75 (75%) in the doxorubicin group and 81 (77%) in the doxorubicin-dexrazoxane group had evaluable serum samples, providing a sample size of 156 patients (Fig 1).

In children with any serum samples evaluated during treatment, the percentage of girls was similar in both groups. The median cumulative dose of doxorubicin was 300 mg/m^2 for both groups. The percentage of children contributing evaluable samples did not differ between groups on any characteristic (data not shown). The mean number of samples per child was also similar between groups (about 15) and among specific biomarkers by study intervals (Table 1). Patients with biomarkers measured before doxorubicin therapy were similar between groups ($P > .3$) on all observed baseline characteristics.

cTnT

At baseline, cTnT was detected in approximately 12% of children in both groups (Table 2). During treatment, the cumulative percentage of children with detectable cTnT levels increased to 47% (35 of 75) in the doxorubicin group and to 20% (16 of 80) in the doxorubicin-dexrazoxane group ($P < .001$; Table 2). After therapy ended, detectable levels were found in 47% (14 of 30) of children in the doxorubicin group but only in 13% (four of 32) of the doxorubicin-dexrazoxane group ($P = .005$; Table 2). Similarly, the percentage of children with multiple detections at any study interval was also higher in the doxorubicin group (40% [30 of 75] v 13% [11 of 81]; $P < .001$; Table 2).

A repeated measure model revealed that there were no significant treatment differences at baseline, but then 1.8 months after assignment, the percentage of samples with detectable cTnT levels in the doxorubicin group became significantly higher than that in the doxorubicin-dexrazoxane group and remained so until the end of treatment (Fig 2A). Further, overall, the repeated measures model found significant differences in cTnT ($P < .001$) between the groups in Figure 2A.

NT-proBNP

At baseline, the percentages of children with increased NT-proBNP levels did not differ between groups (Table 2). During treatment, the percentage of children with increased NT-proBNP levels decreased in the doxorubicin-dexrazoxane group but increased in the doxorubicin group ($P = .005$; Table 2). After therapy, the

Table 1. Characteristics of 156 Children With High-Risk Acute Lymphoblastic Leukemia Treated With Doxorubicin, With or Without Dexrazoxane

Characteristic	Doxorubicin* No.	Doxorubicin* %	Dexrazoxane-Doxorubicin* No.	Dexrazoxane-Doxorubicin* %
No. of children	75		81	
Females	47	35	35	28
Mean age at diagnosis, years	7.7		8.2	
Median doxorubicin cumulative dose, mg/m²	300		300	
Median No. of samples per patient	14.7		14.8	
Echocardiograms (No. of echocardiograms/No. of patients)				
0 to 4 years	127/58		140/62	
> 4 years	73/44		75/46	
Overall	200/66		215/68	
cTnT measurements by time since assignment (No. of samples/No. of patients)				
0 to 3 months	592/74		629/78	
3 months to end of doxorubicin therapy	439/64		456/65	
After doxorubicin therapy	50/30		46/30	
Entire duration of protocol	1,161		1,285	
NT-proBNP measurements by time since assignment (No. of samples/No. of patients)				
0 to 3 months	491/68		487/70	
3 months to end of doxorubicin therapy	345/57		343/58	
After doxorubicin therapy	36/25		29/20	
Entire duration of protocol	1,090		1,184	
hsCRP measurements by time since assignment (No. of samples/No. of patients)				
0 to 3 months	592/74		629/78	
3 months to end of doxorubicin therapy	439/64		456/65	
After doxorubicin therapy	50/30		46/30	
Entire duration of protocol	1,092		1,187	

Abbreviations: cTnT, cardiac troponin T; hsCRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide.
*The groups did not differ significantly on any variable.

Table 2. Frequency of Biomarker Elevations in 156 Children With High-Risk Acute Lymphoblastic Leukemia, by Treatment Group and Study Interval

Biomarker and Study Interval	Doxorubicin %	Doxorubicin No. of Patients With Elevations/No. of Patients	Dexrazoxane-Doxorubicin %	Dexrazoxane-Doxorubicin No. of Patients With Elevations/No. of Patients	P*
cTnT†					
Any increase	50.7	38/75	27.1	22/81	.003
Before doxorubicin therapy	11.7	6/51	12.7	7/55	.99
During doxorubicin therapy	46.6	35/75	20.0	16/80	< .001
After doxorubicin therapy	46.6	14/30	12.5	4/32	.005
Multiple increases	40.0	30/75	13.4	11/81	< .001
NT-proBNP‡					
Any increase	100	70/70	86.3	63/73	.001
Before doxorubicin therapy	88.8	32/36	91.8	34/37	.71
During doxorubicin therapy	98.5	68/69	84.9	62/73	.005
After doxorubicin therapy	48.0	12/25	20.0	4/20	.07
Multiple increases	90.4	66/73	78.0	57/73	.007
hsCRP§					
Any increase	95.7	67/70	89.0	65/73	.20
Before doxorubicin therapy	83.3	30/36	81.0	30/37	.99
During doxorubicin therapy	95.6	66/69	87.6	64/73	.13
After doxorubicin therapy	45.8	11/24	42.1	8/19	.99
Multiple increases	88.5	62/70	76.7	56/73	.08

Abbreviations: cTnT, cardiac troponin T; hsCRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide.
*Fisher's exact test.
†Increased cTnT defined as > 0.01 ng/mL.
‡Increased NT-proBNP defined as ≥ 100 pg/mL in patients ≥ 1 year old and ≥ 150 pg/mL in patients < 1 year old.
§Increased hsCRP defined as ≥ 1.9 mg/L.

hsCRP

The percentage of children with increased hsCRP levels did not differ significantly between groups before, during, or after treatment, nor did the percentage with multiple increases (Table 2). In the repeated measure model, the percentage of samples with increased hsCRP levels did not differ significantly between groups at baseline (Fig 2C). During treatment, the percentage fell in both groups, with the doxorubicin group reaching a nadir of 16% (from 79%) 3.6 months after starting therapy before increasing toward the end of treatment and becoming significantly greater than that in the doxorubicin-dexrazoxane group (Fig 2C). Further, overall, the repeated measures model found significant differences in hsCRP (P = .04) between the groups in Figure 2C.

cTnI

During treatment, cTnI levels were measured in a subset of samples. The median cTnI level during the first month of treatment was 1.75 pg/mL for the doxorubicin group and 4.42 pg/mL for the doxorubicin-dexrazoxane group (P = .26). By the end of treatment, the median value for the doxorubicin group (41.65 pg/mL) was significantly higher than that for the doxorubicin-dexrazoxane group (1.44 pg/mL; P < .001; Fig 3).

Association of Biomarker Levels That Increased During Treatment With Echocardiographic Findings 4 Years Later

A total of 415 echocardiograms were obtained from 134 children (200 from 66 children in the doxorubicin group and 215 from 68

percentage decreased in both groups but was lower in the doxorubicin-dexrazoxane group (48% [12 of 25] v 20% [four of 20]; P = .07; Table 2). The percentage of children with any increase in NT-proBNP was also significantly lower in the doxorubicin-dexrazoxane group (86% [63 of 73] v 100% [70 of 70]; P = .001; Table 2). The doxorubicin group also had a significantly higher percentage of children with multiple NT-proBNP increases (P = .007, Table 2).

In the repeated measure model, the percentage of samples with increased NT-proBNP levels was similar between the groups at baseline (Fig 2B). During treatment, the percentage fell to 35% (from 83%) in the doxorubicin group and to 16% (from 87%) in the doxorubicin-dexrazoxane group. After the end of treatment, the percentage of samples with increases rose in the doxorubicin group but continued to decrease in the doxorubicin-dexrazoxane group. The percentage of samples with increased NT-proBNP was significantly lower in the doxorubicin-dexrazoxane group during treatment (P < .001; Fig 2B). Further, overall, the repeated measures model found significant differences in NT-proBNP (P < .001) between the groups in Figure 2B.
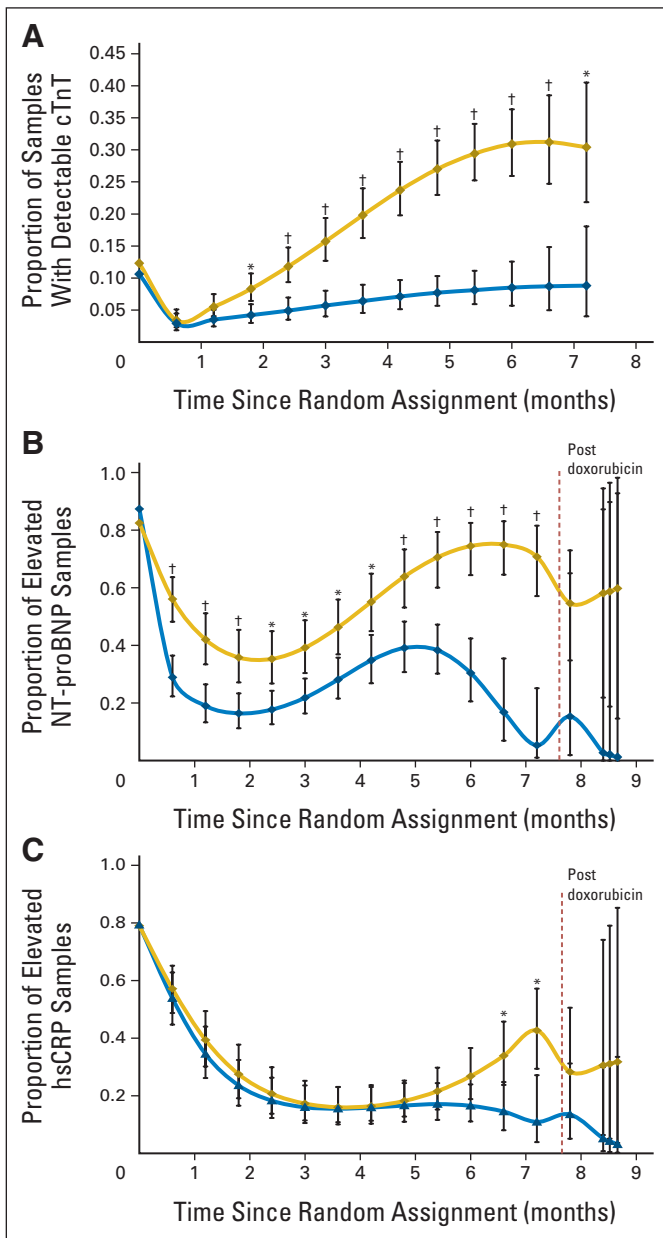
Fig 2. (A) Model-based estimated probability of having an increased cardiac troponin T (cTnT) level at each depicted time point in patients treated with doxorubicin, with or without dexrazoxane. The doxorubicin-dexrazoxane group is indicated by the blue line, the doxorubicin group by the gold line. Vertical bars show 95% CIs. Increased cTnT is defined as a value greater than 0.01 ng/mL. *P value versus dexrazoxane group ≤ .05; †P value versus dexrazoxane group ≤ .001. An overall test for dexrazoxane effect during treatment was significant (P < .001). (B) Model-based estimated probability of having an increased N-terminal pro-brain natriuretic peptide (NT-proBNP) level at each depicted time point in patients treated with doxorubicin with or without dexrazoxane. The dexrazoxane group is indicated by the blue line, the doxorubicin group by the gold line. Vertical bars show 95% CIs. Increased NT-proBNP is defined as a value ≥ 150 pg/mL for children younger than 1 year old and a value ≥ 100 pg/mL for children age ≥ 1 year. *P value versus dexrazoxane group ≤ .05; †P value versus dexrazoxane group ≤ .001. An overall test for dexrazoxane effect during treatment was significant (P < .001) and after treatment was not significant (P = .24). (C) Model-based estimated probability of having an increased high-sensitivity C-reactive protein (hsCRP) level at each depicted time point in patients treated with doxorubicin with or without dexrazoxane. The dexrazoxane group is indicated by the blue line, the doxorubicin group by the gold line. Vertical bars show 95% CIs. Increased hsCRP is defined as a level ≥ 1.9 mg/L. *P value versus dexrazoxane group ≤ .05. Overall tests for dexrazoxane effect during treatment (P = .08) and after treatment (P = .49) were not significant.
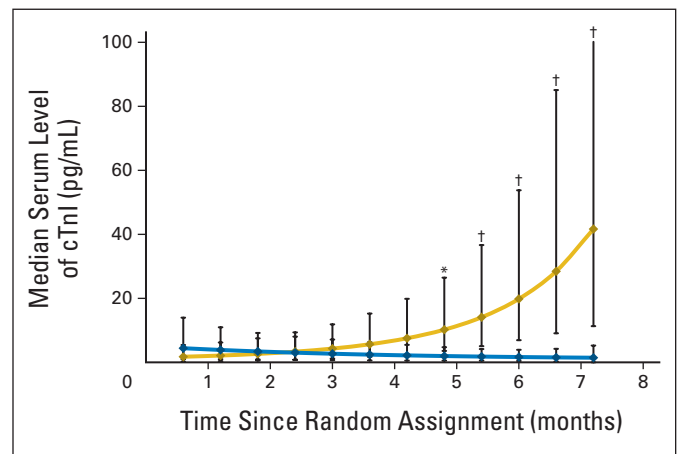

Fig 3. Median serum cardiac troponin I (cTnI) levels during doxorubicin therapy of a 40-sample subset from 18 children with high-risk acute lymphoblastic leukemia. The dexrazoxane group is indicated by the blue line, the doxorubicin group by the gold line. Vertical bars show 95% CIs. *P value versus dexrazoxane-doxorubicin group ≤ .05; †P value versus dexrazoxane group ≤ .001.

children in the doxorubicin-dexrazoxane group). Mean follow-up time was 4.1 years (range, 1.5 to 7.9 years). Increased cTnT levels during the first 90 days of doxorubicin therapy were significantly associated with lower LV mass and LV end-diastolic posterior wall thickness 4 years later; they were marginally significantly associated with lower LV thickness-to-dimension ratios (Table 3). Increases in NT-proBNP detected in the first 90 days of treatment were significantly associated with changes in the LV thickness-to-dimension ratio 4 years later (Table 3).

During the last 90 days of treatment, increased levels of cTnT, hsCRP, or NT-proBNP were not significantly related to any echocardiographic outcome 4 years later (Table 4).

DISCUSSION

Our results suggest the utility of assessing cardiac status with biomarkers such as cTnT and NT-proBNP in children receiving doxorubicin for ALL. Serum cTnT levels increased during the first 90 days of therapy, indicating cardiomyocyte damage or death; they were significantly associated with reduced LV mass and LV end-diastolic posterior wall thickness and were marginally associated with a reduced LV thickness-to-dimension ratio 4 years later. Similarly, abnormal NT-proBNP levels during the first 90 days of therapy were associated with an abnormal LV thickness-to-dimension ratio, suggesting pathologic LV remodeling 4 years later. In addition, before, during, and after treatment, a higher percentage of children had increased levels of NT-proBNP (indicating increased LV wall stress) than had abnormal cTnT levels (indicating cardiomyocyte death), suggesting that NT-proBNP may detect cardiac stress before irreversible cardiomyocyte death.

There are some well-defined trends in biomarkers throughout doxorubicin treatment. Immediately after doxorubicin treatment began, the proportion of children with increased levels of NT-proBNP, hsCRP, and cTnT decreased substantially. Many children with high-risk ALL are quite ill at presentation. With supportive care and intensive multidrug induction chemotherapy, these children often

Table 3. Associations Between Biomarkers Measured in the First 90 Days of Doxorubicin Treatment and Echocardiographic Outcomes 4 Years Later in 156 Doxorubicin-Treated Children With High-Risk Acute Lymphoblastic Leukemia

Left Ventricular Characteristic	Mean Z Score	SE	P v 0	P for Equal Means
Fractional shortening				
Increased cTnT*	−0.84	0.26	.001	
Normal cTnT	−0.91	0.20	< .001	.82
Mass				
Increased cTnT	−1.36	0.19	< .001	
Normal cTnT	−0.68	0.14	< .001	.004
End-diastolic dimension				
Increased cTnT	−0.34	0.25	.17	
Normal cTnT	−0.06	0.17	.74	.35
End-systolic dimension				
Increased cTnT	0.13	0.26	.60	
Normal cTnT	0.37	0.18	.04	.46
End-diastolic posterior wall thickness				
Increased cTnT	−1.51	0.24	< .001	
Normal cTnT	−0.74	0.17	< .001	.008
Thickness-to-dimension ratio				
Increased cTnT	−1.14	0.26	< .001	
Normal cTnT	−0.52	0.18	< .001	.054
Fractional shortening				
Increased NT-proBNP†	−0.70	0.17	< .001	
Normal NT-proBNP	−1.43	0.55	.01	.19
Mass				
Increased NT-proBNP	−0.87	0.12	< .001	
Normal NT-proBNP	−0.37	0.49	.44	.33
End-diastolic dimension				
Increased NT-proBNP	0.02	0.12	.85	
Normal NT-proBNP	−0.69	0.65	.29	.27
End-systolic dimension				
Increased NT-proBNP	0.37	0.13	.006	
Normal NT-proBNP	0.02	0.63	.98	.58
End-diastolic posterior wall thickness				
Increased NT-proBNP	−1.02	0.15	< .001	
Normal NT-proBNP	−0.20	0.49	.68	.10
Thickness-to-dimension ratio				
Increased NT-proBNP	−0.87	0.16	< .001	
Normal NT-proBNP	0.32	0.44	.47	.01
Fractional shortening				
Increased hsCRP‡	−0.82	0.18	< .001	
Normal hsCRP	−0.60	0.47	.20	.67
Mass				
Increased hsCRP	−0.85	0.12	< .001	
Normal hsCRP	−0.39	0.60	.52	.47
End-diastolic dimension				
Increased hsCRP	−0.06	0.13	.68	
Normal hsCRP	−0.24	0.70	.73	.79
End-systolic dimension				
Increased hsCRP	0.35	0.15	.019	
Normal hsCRP	0.05	0.62	.93	.64
End-diastolic posterior wall thickness				
Increased hsCRP	−0.95	0.16	< .001	
Normal hsCRP	−0.57	0.51	.27	.48
Thickness-to-dimension ratio				
Increased hsCRP	−0.78	0.16	< .001	
Normal hsCRP	−0.16	0.58	.78	.23

Abbreviations: cTnT, cardiac troponin T; hsCRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide.
*Increased cTnT defined as > 0.01 ng/mL.
†Increased NT-proBNP defined as ≥ 100 pg/mL in patients ≥ 1 year old and ≥ 150 pg/mL in patients < 1 year old.
‡Increased hsCRP defined as ≥ 1.9 mg/L.

Table 4. Associations Between Increased Biomarkers Measured in the Last 90 Days of Doxorubicin Treatment and Echocardiographic Outcomes 4 Years Later in 156 Doxorubicin-Treated Children With High-Risk Acute Lymphoblastic Leukemia

Left Ventricular Characteristic	Mean Z Score	SE	P v 0	P for Equal Means
Fractional shortening				
Increased cTnT*	−1.04	0.33	.001	
Normal cTnT	−0.81	0.24	.001	.59
Mass				
Increased cTnT	−1.02	0.21	< .001	
Normal cTnT	−0.81	0.17	< .001	.45
End-diastolic dimension				
Increased cTnT	0.06	0.18	.73	
Normal cTnT	0.06	0.20	.78	.67
End-systolic dimension				
Increased cTnT	0.54	0.24	.02	
Normal cTnT	0.33	0.20	.10	.50
End-diastolic posterior wall thickness				
Increased cTnT	−1.12	0.26	< .001	
Normal cTnT	−0.86	0.18	< .001	.38
Thickness-to-dimension ratio				
Increased cTnT	−1.02	0.27	< .001	
Normal cTnT	−0.61	0.20	.002	.20
Fractional shortening				
Increased NT-proBNP†	−0.97	0.23	< .001	
Normal NT-proBNP	−0.66	0.30	.033	.40
Mass				
Increased NT-proBNP	−0.93	0.14	< .001	
Normal NT-proBNP	−0.53	0.37	.15	.32
End-diastolic dimension				
Increased NT-proBNP	0.01	0.16	.97	
Normal NT-proBNP	−0.46	0.30	.13	.14
End-systolic dimension				
Increased NT-proBNP	0.46	0.18	.01	
Normal NT-proBNP	−0.03	0.32	.93	.16
End-diastolic posterior wall thickness				
Increased NT-proBNP	−0.92	0.17	< .001	
Normal NT-proBNP	−0.68	0.32	.04	.51
Thickness-to-dimension ratio				
Increased NT-proBNP	−0.80	0.18	< .001	
Normal NT-proBNP	−0.09	0.37	.80	.09
Fractional shortening				
Increased hsCRP§	−0.94	0.24	< .001	
Normal hsCRP	−0.96	0.31	.002	.95
Mass				
Increased hsCRP	−0.79	0.24	.001	
Normal hsCRP	−0.88	0.18	< .001	.77
End-diastolic dimension				
Increased hsCRP	0.21	0.19	.28	
Normal hsCRP	−0.31	0.21	.14	.06
End-systolic dimension				
Increased hsCRP	0.61	0.20	.003	
Normal hsCRP	0.21	0.25	.40	.21
End-diastolic posterior wall thickness				
Increased hsCRP	−0.95	0.21	< .001	
Normal hsCRP	−0.75	0.21	< .001	.50
Thickness-to-dimension ratio				
Increased hsCRP	−0.92	0.21	< .001	
Normal hsCRP	−0.41	0.23	.08	.11

Abbreviations: cTnT, cardiac troponin T; hsCRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal pro-brain natriuretic peptide.
*Increased cTnT defined as > 0.01 ng/mL.
†Increased NT-proBNP defined as ≥ 100 pg/mL in patients ≥ 1 year old and ≥ 150 pg/mL in patients < 1 year old.
§Increased hsCRP defined as ≥ 1.9 mg/L.

markedly improved, consistent with rapidly improved NT-proBNP levels. However, after 2 months, the proportion of children in the doxorubicin-only group with abnormal cTnT and NT-proBNP levels began to rise. This pronounced increase after doxorubicin therapy, which exceeded pretreatment levels by month 3, indicates cardiomyocyte death.[9,41] Furthermore, the percentage of children with detectable cTnT levels continued to rise, even after doxorubicin treatment had ended, illustrating the persistent, progressive nature of doxorubicin-related cardiac damage.

Among children who received concomitant dexrazoxane, the percentage of samples with detectable cTnT remained stable. Further, throughout treatment, the percentage of samples with increased NT-proBNP was consistently lower in the dexrazoxane-treated group. The ability of dexrazoxane to chelate iron and thus lower oxygen-radical-producing doxorubicin-iron complexes explains not only the reduction in cardiomyocyte death, indicated by reduced cTnT levels, but also the reduction in ventricular stress and inflammation, indicated by decreased NT-proBNP and hsCRP levels.

In contrast, the percentage of samples with increased hsCRP levels in the doxorubicin-only group did not increase until 6 months after starting treatment. By the end of treatment, this percentage was significantly greater than that in the dexrazoxane-treated group. Although hsCRP in adults predicts clinical cardiac events, its use in children deserves attention because increased hsCRP levels are likely associated with worse cardiac characteristics in children with heart failure.[42]

We also measured cTnI levels in 40 samples, 20 with and 20 without increased cTnT levels. Median cTnI levels did not differ significantly between groups until 5 months after assignment, when levels in the doxorubicin-only group progressively increased, becoming significantly higher than those in the dexrazoxane-treated group.

Circulating cardiomyocyte proteins, such as cTnI, have been related to LV dysfunction and to increased cardiovascular risk in adults receiving high-dose chemotherapy.[19,21] Adults with increased cTnI levels at the start of chemotherapy have an increased risk of cardiac events, and this risk is greater among those with increases persisting until the end of treatment.[19] In this pilot analysis, increased cTnI levels were strongly related to doxorubicin therapy without dexrazoxane.

Our study found associations between cardiac biomarkers and echocardiograms 4 years after treatment. NT-proBNP, an indicator of cardiomyopathy,[26] was increased in a higher percentage of children before, during, and after therapy than was cTnT in both groups. In addition, NT-proBNP may be associated with echocardiographic measurements.[42] We found that NT-proBNP and cTnT levels that increase in the first 90 days of treatment, when patients are most ill, may predict an abnormal LV thickness-to-dimension ratio consistent with LV remodeling years later, suggesting a time when their hearts may be more vulnerable to the cardiotoxic effects of doxorubicin. Our finding of the importance of the first 90 days of cardioprotective therapy suggests that current recommendations to add dexrazoxane later in the course of doxorubicin therapy might require re-evaluation.

Similarly, serum NT-proBNP and cTnT levels were highly associated, suggesting that monitoring NT-proBNP in these children might predict which children are at higher risk for cardiotoxicity, allowing for treatment modification before irreversible cardiomyocyte damage.

This study has some limitations. It was not designed to determine sensitivity or specificity or to establish cutoffs to guide clinical decisions. However, our results should inform clinical trials and prospective studies assessing the trade-off between cancer treatment and cardioprotective efficacy and the associated biomarker-guided clinical decisions.

In conclusion, these results suggest that monitoring NT-proBNP and cTnT in children receiving doxorubicin helps assess later echocardiographic status. The results underscore the importance of dexrazoxane in reducing anthracycline cardiotoxicity. A comprehensive panel of biomarkers to assess cardiac status in these patients should be developed.

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