

## Screening for Cardiac Dysfunction in Anthracycline-Exposed Childhood Cancer Survivors

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### Abstract

**Purpose:** To examine the utility and reliability of obtaining early echocardiographic measurements of left ventricular (LV) remodeling as well as blood biomarkers of cardiac injury in asymptomatic childhood cancer survivors at risk for LV dysfunction and congestive heart failure due to past exposure to anthracycline chemotherapy.

**Experimental Design:** Using a cross-sectional design, anthracycline-exposed childhood cancer survivors with preserved ejection fraction (EF;  $\geq 50\%$ ) were evaluated using early echocardiographic indices and blood biomarkers of LV dysfunction. Survivors treated with  $\geq 300$  mg/m<sup>2</sup> anthracyclines [high risk (HR):  $n = 100$ ] were compared with those treated with  $< 300$  mg/m<sup>2</sup> anthracyclines [low risk (LR):  $n = 50$ ] and matched healthy controls (HC:  $n = 50$ ). All echocardiograms were interpreted by an institutional cardiologist and a study cardiologist blinded to risk status.

**Results:** Time from diagnosis was comparable for HR (12.0 years) and LR (13.2 years,  $P = 0.8$ ) survivors. Echocardiograms: HR had lower LV thickness-dimension ratio (Z-score: HR:  $-0.62$ , LR:  $-0.03$ , HC:  $-0.02$ ;  $P < 0.001$ ), increased LV wall stress (HR:  $66.7$  g/cm<sup>2</sup>, LR:  $56.6$  g/cm<sup>2</sup>, HC:  $54.2$  g/cm<sup>2</sup>;  $P < 0.01$ ), and higher myocardial performance index (HR:  $0.51$ , LR:  $0.46$ , HC:  $0.46$ ;  $P < 0.01$ ). Interobserver correlation (clinical/blinded reading) for all echocardiographic indices was excellent (range:  $R = 0.76$ – $0.97$ ,  $P < 0.001$ ). Blood biomarkers: With the exception of NT-proBNP ( $r = 0.28$ ,  $P < 0.01$ ), there was no correlation between blood biomarkers (B-type natriuretic peptide, Troponin-T, ST-2, Galectin-3) and LV dysfunction.

**Conclusion:** Childhood cancer survivors with preserved EF 10+ years from anthracycline exposure had dose-dependent changes in echocardiographic markers of LV dysfunction. *Clin Cancer Res*; 20(24); 6314–23. ©2014 AACR.

### Introduction

Anthracyclines are widely used in the treatment of childhood cancer (1). However, there is clear evidence for a strong dose-dependent association between anthracyclines and congestive heart failure (CHF); it is estimated that 1 in 10 children treated with high-dose anthracyclines

( $\geq 300$  mg/m<sup>2</sup>) will develop CHF (2–6). Outcome following CHF is poor; 5-year survival rates are  $< 50\%$  (7, 8). Detection of anthracycline-related cardiotoxicity has conventionally relied upon echocardiographic screening using resting left ventricular (LV) ejection fraction (EF) and fractional shortening (SF; ref. 9). These parameters are derived from crude measurements of ventricular volume (EF) and size (SF), are load dependent, may demonstrate intra-patient and inter-observer variability, and have increasingly been recognized as inadequate for detecting subtle changes in cardiac function (9). Most importantly, at the point when changes in EF and SF are detected, functional deterioration is often irreversible, despite pharmacologic intervention (10).

Previous research on anthracycline-related cardiotoxicity has revealed that cardiac remodeling after anthracycline exposure is associated with thinning of the LV wall, enlargement of LV diameter, and subsequent increase in LV end-systolic wall stress (ESWS; refs. 2, 4). Other than EF and SF, ESWS is the best-studied echocardiographic index in childhood cancer survivors, and is a well-recognized precursor to anthracycline-related CHF (2, 4, 11). However, the clinical application of ESWS is limited because of concerns about its reproducibility across

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Prior presentations: This work was presented, in part, at the International Conference on Long Term Complications of Treatment of Children and Adolescents for Cancer and the American Society of Clinical Oncology, 2013.

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doi: 10.1158/1078-0432.CCR-13-3490

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### Translational Relevance

Cardiovascular complications such as anthracycline-related congestive heart failure (CHF) have emerged as a leading cause of morbidity and mortality in survivors of childhood cancer. Outcome following CHF is poor, emphasizing the importance of early detection, before onset of symptomatic heart disease. Screening for asymptomatic disease using conventional echocardiographic indices such as left ventricular ejection fraction (EF) precludes intervention, because EF decline is a late event in the progression to CHF. The current study describes how novel echocardiographic indices of early cardiac dysfunction can be reliably obtained from long-term survivors of childhood cancer, using routine echocardiograms that can be ordered as part of clinical care. Importantly, we found clear dose-dependent changes in these echocardiographic indices by anthracycline dose. These findings may facilitate the development of early detection strategies as well as interventions aimed at progression or potential reversal of cardiac injury, and therefore, prevention of overt CHF in these survivors.

different settings. Other echocardiographic indices such as Doppler-derived myocardial performance index (MPI) have been shown to prognosticate CHF in nononcology populations (12, 13), but have not been adequately studied in long-term survivors of childhood cancer. These gaps in knowledge are especially evident in childhood cancer survivors at the highest risk of CHF (anthracycline dose  $\geq 300$  mg/m<sup>2</sup>; refs. 1, 2, 11), and have limited the use of these indices as much-needed early and robust endpoints for secondary prevention trials.

Established blood biomarkers of acute cardiac injury (i.e., cardiac troponins) and heart failure (i.e., natriuretic peptides) have been studied in nononcology populations, but less is known about their ability to detect asymptomatic LV dysfunction years after completion of cardiotoxic cancer therapy. Emerging biomarkers of cardiac dysfunction (galectin-3 and protein ST-2) have not been evaluated in this population (1, 9). The current study examined the utility and reliability of early echocardiographic indices of LV remodeling as well as blood biomarkers of cardiac injury in long-term childhood cancer survivors exposed to anthracyclines, using a recruitment strategy that enriched for patients at the highest risk of CHF.

## Materials and Methods

### Study participants

Study participants included cancer survivors and healthy controls (HC). The survivor population was recruited from patients seen between October 2010 and September 2012 at the Childhood Cancer Survivorship Clinics at City of Hope (COH; Duarte, CA) and the Children's Hospital Los Angeles (CHLA; Los Angeles, CA). Eligibility criteria includ-

ed: (i) cancer diagnosis before 22 years of age, irrespective of current age; (ii) 2 or more years since completion of cancer treatment; and (iii) exposure to anthracyclines. Survivors at high risk (HR: cumulative anthracycline dose  $\geq 300$  mg/m<sup>2</sup>) and low risk (LR: 1–300 mg/m<sup>2</sup>) of developing CHF per the Children's Oncology Group Long Term Follow-up Guidelines (14) were targeted for recruitment. We excluded survivors who were actively being treated for cardiomyopathy, as echocardiographic indices or blood biomarkers in these individuals could have been altered by pharmacologic therapy. HCs without a history of cancer or cardiomyopathy were recruited from the general population, matched to HR cancer survivors on sex and age at study participation. Patient recruitment disproportionately (2:1) targeted HR survivors over those who were LR or controls. The study was approved by the respective Institutional Review Boards. All study participants or their parents/legal guardians provided written informed consent.

### Cardiac evaluation

Study participants underwent a clinical cardiac assessment by their healthcare provider within the respective Survivorship Clinics. The evaluation included a detailed physical examination, with special attention to signs and symptoms of CHF. Echocardiograms were performed on the day of clinical evaluation by a designated study technician, and consisted of complete two-dimensional (2D), M-mode, and Doppler evaluations, as per the American Heart Association/American College of Cardiology (AHA/ACC) task force practice guidelines for the clinical application of echocardiography (15). Identical ultrasound machines (General Electric Vivid-7 echocardiography machine; General Electric) were used for all study-related echocardiographic evaluations at the two institutions. LV end-diastolic/systolic diameters (LVEDD, LVESD) and LV posterior wall thickness in diastole and systole (LVPWD, LVPWS) were measured on M-mode recordings obtained from a standard LV parasternal view. LV ESWS was calculated using the formula  $(1.35 \times \text{MAP} \times \text{LVESD}) / ((4 \times \text{LVPWS}) (1 + \text{LVPWS}/\text{LVESD})$ ; refs. 16, 17), where MAP is the mean arterial pressure as obtained by Dinamap blood pressure machine. LV EF was calculated from the apical 4- and 2-chamber views using a modified Simpson biplane method (15). LV mass was calculated from the recommended AHA/ACC formula (15) that takes into consideration LV dimension, posterior wall and intraventricular septal thickness, and correction factors derived from regression analysis, divided by body surface area. Corrected velocity of circumferential fiber shortening (VCfC) was calculated using the following formula:  $(\text{LVEDD} - \text{LVESD}) / (\text{LVEDD} \times \text{ETc})$ , where ETc is the heart rate corrected ejection time measured from the onset to the end of LV outflow velocity pattern (18).

Measurements of LV diastolic function included pulsed Doppler measurement of mitral valve inflow, peak velocities of the early filling (E-wave) and filling during atrial systole (A-wave), and mitral E/A ratio. LV isovolumic contraction time (IVCT) was measured as the interval between

mitral valve closing and aortic valve opening (10). LV isovolumic relaxation time (IVRT) was measured as the interval between aortic valve closure and the onset of mitral flow (10). MPI (19) was calculated using the following formula:  $(IVCT+IVRT)/ET$ . All Doppler measurements were the average of three consecutive cycles. Study echocardiograms were first interpreted by a designated cardiologist at each institution (COH, K. Venkataraman; CHLA, J.-D. Mentzer). Then, an anonymized copy of the digitized echocardiogram was sent to the study core cardiology laboratory (University of Michigan, Ann Arbor, MI) where all study endpoints were remeasured by a single cardiologist (S.K. Gelehrter) who was blinded to the risk or control status of the study participant.

### Blood biomarkers

Blood samples were collected on the day of the echocardiographic assessment. Troponin-T was determined using the Elecsys 2010 system (Roche Diagnostics; Elecsys Troponin sandwich immunoassay); measurable range: 0.01 to 25.00 ng/mL. B-type natriuretic peptide (BNP) was measured using the Triage BNP test (Beckman Coulter); measurable range: 2.0 pg/mL to 5,000 pg/mL. NT proBrain Natriuretic Peptide (NT-proBNP) was measured using the Elecsys proBNP II test (Roche Diagnostics); lower limit of detection: 5 pg/mL. Galectin-3 was measured using a research use only assay (BG Medicine), which is a sandwich ELISA that measures quantitative galectin-3 in plasma or serum samples; measurable range: 1.35 to 79.9 ng/mL. Protein ST2 was measured using the Presage ST2 ELISA (Critical Diagnostics) test; lower limit of detection: 2.0 U/mL.

### Clinical data collection

Self-reported questionnaires were used to obtain baseline data on demographics. Medical records provided the following information: date of diagnosis, type of cancer, stage of disease (if applicable), cumulative dose of anthracycline exposure, and receipt of chest-directed radiation therapy. Lifetime cumulative anthracycline dose was calculated by multiplying the total dose of each anthracycline (doxorubicin, daunorubicin, epirubicin, idarubicin, and mitoxantrone) by a factor that reflects the cardiotoxic potential of each drug, and then summing the individual doses (9).

### Statistical analysis

The primary endpoint for this study was LV dysfunction, defined as abnormal ESWS ( $>2SD$  norm). ESWS was selected because of the preexisting clinical experience with this index in anthracycline-exposed childhood cancer survivors. Median ESWS was projected to be  $50 \pm 10$  g/cm<sup>2</sup> for HC,  $60 \pm 10$  g/cm<sup>2</sup> for the LR survivors, and  $70 \pm 10$  g/cm<sup>2</sup> for HR survivors (2, 11). Assuming a type I error of 0.025 (controlling for conducting two *t* tests: HR vs. HC, and HR vs. LR), enrolling approximately 100 HR survivors, 50 LR survivors, and 50 HCs provided 80% power to detect a significant difference in ESWS between the groups.

To standardize LV chamber size and wall thickness data by age or body surface area, we expressed LV diastolic (LVEDD, LVEWD) and systolic (LVESD, LVEWS) dimensions in Z-scores, using established normative data for children and adults (20, 21). The remaining echocardiographic indices are independent of age and body growth (20); their raw values were used in the analyses.

Descriptive statistics for clinical variables, echocardiographic indices, and blood biomarkers were generated for all three groups. Categorical variables were compared using  $\chi^2$  tests. Continuous variables were compared using independent two-sample *t* tests or ANOVA. Interobserver variability for each echocardiographic index was reported as the mean of the difference between values obtained from readings at the primary site (COH or CHLA) and the core cardiology lab (University of Michigan). However, only the measurements obtained from the core cardiology lab were used in our comparison of echocardiographic indices across the three groups. Pearson correlation was calculated between continuous variables as well as for assessment of interobserver variability. Multivariable unconditional logistic regression was used to identify variables that were associated with LV dysfunction. The dependent variable was LV dysfunction ( $>2SD$  ESWS). The independent variables included clinical and treatment-related factors that differed between the three groups ( $P < 0.1$ ), as well as variables thought to impact ESWS, and included: sex, age at examination ( $\leq 18$  years, 19–29 years,  $\geq 30$  years), ethnicity/race (non-Hispanic White, Hispanic, other), chest radiation exposure (yes/no), and risk status (HC, LR, HR); a separate regression model was created to evaluate the association between ESWS and anthracycline dose (0, 1–99 mg/m<sup>2</sup>, 100–299 mg/m<sup>2</sup>, 300–399 mg/m<sup>2</sup>,  $\geq 400$  mg/m<sup>2</sup>). Data were analyzed using SPSS Version 18.0 (IBM). All statistical tests were two sided, and  $P < 0.05$  was considered statistically significant.

## Results

### Patient characteristics

Two hundred and three individuals were approached to participate in the study. One individual (HR group) refused participation, and 2 others (1 HR, 1 control) did not complete the full battery of echocardiographic and blood biomarker testing. The current report includes results from 200 study participants who completed all study measurements (participation rate: 98.5%). Table 1 summarizes the participants' characteristics. Because of previously established matching criteria, there were no statistically significant differences by sex, race/ethnicity, and age at study enrollment between HR participants and HCs. Because of study design, HR survivors were more likely to have received a higher anthracycline dose than LR survivors. In addition, HR survivors were significantly more likely to have been treated for a nonhematologic malignancy, received chest radiation, and were older at cancer diagnosis as well as at the time of study enrollment when compared with LR survivors.

**Table 1.** Patient and treatment characteristics

Characteristics	HR (N = 100)	LR (N = 50)	P	Controls (N = 50) <sup>a</sup>	P
Sex, No. (%)					
Male	55 (55.0)	30 (60.0)		27 (54.0)	
Female	45 (45.0)	20 (40.0)	0.56	23 (46.0)	0.91
Race/ethnicity, No. (%)					
Non-Hispanic White	40 (40.0)	14 (28.0)		20 (40.0)	
Hispanic	46 (46.0)	27 (54.0)		16 (32.0)	
Other	14 (14.0)	9 (18.0)	0.35	14 (28.0)	0.08
Age at examination, y					
Median, range	26.6 (6.7–55.8)	20.8 (10.2–44.4)	<0.01	28.6 (14.9–57.9)	0.07
BMI at examination, kg/m <sup>2</sup>					
<25	57 (57.0)	29 (58.0)		29 (59.2)	
≥25	43 (43.0)	21 (42.0)	0.91	20 (40.8)	0.80
On antihypertensive medications at the time of assessment, No. (%) <sup>b</sup>					
Any	10 (10%)	3 (6%)	0.42	1 (2%)	0.08
Diagnosis, No. (%) <sup>c</sup>					
Hematologic malignancy	49 (49.0)	46 (92.0)		—	
Acute lymphoblastic leukemia	13 (13.0)	40 (80.0)		—	
Acute myeloid leukemia	18 (18.0)	0		—	
Lymphoma	18 (18.0)	6 (12.0)		—	
Solid tumor	51 (51.0)	4 (8.0)	<0.01	—	
Ewing sarcoma	18 (18.0)	1 (2.0)		—	
Osteosarcoma	13 (13.0)	1 (2.0)		—	
Soft tissue sarcoma	16 (16.0)	0		—	
Other	4 (4.0)	2 (4.0)		—	
Age at diagnosis, y					
Median, range	13.1 (0.4–21.7)	6.1 (1.2–21.9)	<0.01	—	
Date of diagnosis					
≤1994	31 (31.0)	17 (34.0)			
1995–2001	36 (36.0)	19 (38.0)			
≥2002	33 (33.0)	14 (28.0)	0.83		
Lifetime Anthracycline, mg/m <sup>2</sup>					
Median, range	375 (300–642)	120 (25–225)	<0.01	—	
Chest radiation, No. (%)					
Any	16 (16.0)	0	<0.01	—	
Time since diagnosis, Years					
Median, range	12.0 (2.6–37.9)	13.2 (5.3–28.6)	0.76	—	

<sup>a</sup>Matched to HR: sex, age at examination.

<sup>b</sup>Angiotensin converting enzyme inhibitor or  $\beta$ -blocker.

<sup>c</sup>Analyzed as hematologic malignancy versus solid tumor.

There were no differences by sex, race/ethnicity, treatment era, and time from cancer diagnosis to study enrollment between the two survivor populations. Importantly, none of the survivors had clinical signs or symptoms of cardiac dysfunction at the time of study evaluation.

#### Echocardiographic assessment

As seen in Table 2, interobserver correlation was excellent across all echocardiographic indices ranging from  $R = 0.76$ ,  $P < 0.001$  [IVRT, mean difference: 2.55 (–55–38)] to  $R = 0.97$ ,  $P < 0.001$  [LVEDD, mean difference –0.09 (0.55–0.81)]. A comparison of the echocardiographic measure-

ments of LV systolic and diastolic function between HR and LR survivors as well as HCs is presented in Table 3. All study participants had a normal EF ( $\geq 50\%$ ) at the time of study enrollment. However, HR survivors were significantly more likely to have an enlarged LV diameter in diastole/systole, as well as decreased wall thickness in diastole/systole, resulting in a significantly decreased LV thickness-dimension ratio. In addition, median ESWS and MPI were significantly higher for HR survivors when compared with the other two populations. There were no differences in MAP, LV mass, VCFc, and indices of diastolic function such as E/A ratio and IVRT between the three groups.



**Table 2.** Interobserver correlation for LV echocardiographic indices

Characteristics	Mean difference <sup>a</sup> (range)	R	P
EDD, systole	-0.05 (-0.61-0.29)	0.91	<0.001
EDD, diastole	-0.09 (-0.55-0.81)	0.97	<0.001
PWS	0.00 (-0.31-0.46)	0.89	<0.001
PWD	0.02 (-0.23-0.41)	0.83	<0.001
Thickness-dimension ratio	0.00 (-0.04-0.07)	0.90	<0.001
ESWS	0.40 (-24.60-25.10)	0.85	<0.001
LV Mass	0.12 (-12.30-18.60)	0.94	<0.001
IVRT	2.55 (-55.00-38.00)	0.76	<0.001
IVCT	1.81 (-44.00-27.00)	0.78	<0.001
Ejection time	1.62 (-46.00-34.80)	0.88	<0.001
MPI	2.11 (-8.20-12.30)	0.82	<0.001
E wave	0.03 (-0.07-0.44)	0.95	<0.001
A wave	0.01 (-0.19-0.11)	0.96	<0.001
E/A ratio	0.02 (-0.62-0.90)	0.93	<0.001

<sup>a</sup>Calculated as the mean difference between values obtained from echocardiographic reading at the clinical site (COH or CHLA) and the core cardiology lab (University of Michigan).

Figures 1 and 2 depict the dose-dependent association between LV echocardiographic indices and cumulative anthracycline dose. There was a significant increase in median LV diameter in diastole/systole, ESWS, and MPI by increasing anthracycline dose; there was a corresponding decrease in LV wall thickness and thickness-dimension ratio by anthracycline dose as well. There were no dose-dependent changes in other echocardiographic indices such as LV mass, VCFc, E/A ratio, and IVRT (data not shown). In multivariable logistic regression analysis adjusting for sex, age at examination, race/ethnicity, and chest radiation exposure, HR survivors had a greater than 8-fold risk of having LV dysfunction (controls, referent group with no exposure to anthracyclines or chest radiation): HR, OR = 8.15 ( $P < 0.01$ ); LR, OR = 2.13 ( $P = 0.36$ ). When examining this association by anthracycline dose, there was a dose-dependent increasing risk by cumulative exposure: 1-99 mg/m<sup>2</sup>, OR = 1.43; 100-299 mg/m<sup>2</sup>, OR = 2.71; 300-399 mg/m<sup>2</sup>, OR = 4.13;  $\geq 400$  mg/m<sup>2</sup>, OR = 12.81;  $P = 0.01$  (trend).

### Blood biomarkers

Candidate biomarkers of cardiac injury and remodeling were compared across all three groups (Table 1). HR survivors had significantly higher serum NT-proBNP levels (71 pg/dL) when compared with LR survivors (37 pg/dL,  $P = 0.02$ ) and controls (26 pg/dL,  $P < 0.01$ ). There were no differences in BNP, Troponin-T, galectin-3, and ST-2 levels among the three groups. With the exception of NT-proBNP ( $r = 0.28$ ,  $P < 0.01$ ), there was no correlation between the blood biomarkers and ESWS: BNP ( $R = 0.16$ ,  $P = 0.06$ ),

Troponin-T ( $R = 0.03$ ,  $P = 0.66$ ), ST-2 ( $-0.13$ ,  $P = 0.24$ ), and Galectin-3 ( $0.14$ ,  $P = 0.10$ ).

### Discussion

It is well recognized that there is a clear dose-response relation between anthracyclines and CHF, and that there is often a long latency between exposure to anthracyclines and clinically overt disease (1, 4). Several randomized clinical trials conducted mostly in adults with cancer have found that empiric intervention (during cancer treatment) with a  $\beta$ -blocker and/or ACE-inhibitor (21-24), use of liposomal formulation of anthracyclines (25), or iron chelators such as dexrazoxane (26) can decrease the risk of anthracycline-related LV dysfunction. However, a growing concern among oncologists is whether the cardioprotective effects of these agents will interfere with the tumoricidal activity of anthracyclines (26). This concern is especially pervasive in the pediatric oncology community where, despite data supporting the cardioprotective efficacy of dexrazoxane, the drug has yet to be incorporated as standard of care (27). As a result, current clinical practice includes echocardiographic screening after treatment completion, and initiation of pharmacologic therapy (ACE inhibition and/or  $\beta$ -blockade) only after abnormal EF or SF are identified (8). Furthermore, although data from adult nononcology populations support efficacy of pharmacologic therapy in this setting (28), the beneficial effects of these treatments in childhood cancer survivors have been shown to be short lived (9). This is thought to be due to the progressive irreversible chronic cardiac remodeling and fibrosis which inevitably follows detectable change in EF or SF, highlighting the importance of early echocardiographic biomarkers of LV dysfunction and remodeling that precede detectable change in EF or SF.

Early intervention with after load reduction, before decrease in EF or SF, has been an effective strategy for CHF risk reduction in children with progressive neuromuscular disorders such as Duchene muscular dystrophy (22, 23). There is a paucity of information on early markers of LV dysfunction that can be used reliably in secondary prevention studies for childhood cancer survivors at HR of CHF. To date, studies in these survivors have relied on time- and labor-intensive echocardiographic measurements to describe asymptomatic cardiac dysfunction (11, 24, 25). These measurements have generally been obtained relatively soon after completion of cardiotoxic cancer therapy (26, 27), and often include small numbers of survivors exposed to high anthracycline doses and therefore at the highest risk of CHF (11, 16, 28). The current study overcomes these limitations by reporting on echocardiographic measurements that can be obtained reliably from routine 2D, M-mode, and Doppler-based echocardiograms, long after completion of cardiotoxic therapy, in a population enriched for survivors at the highest risk for CHF. Overall, interobserver correlation for these echocardiographic indices was excellent, with the highest correlation seen for LV chamber diameter in diastole ( $R = 0.97$ ) and the lowest for IVRT ( $R = 0.76$ ). These findings demonstrate that despite

**Table 3.** Cardiac echocardiographic and blood biomarkers

Characteristics	HR(N = 100)	LR(N = 50)	P <sup>a</sup>	Controls (N = 50) <sup>a</sup>	P <sup>b</sup>
Echocardiographic indices					
LVESD					
Z-score, median	0.80	0.36	<0.01	0.02	<0.01
Range	-2.0-5.7	-1.8-3.3		-2.3-2.7	
LVEDD					
Z-score, median	0.75	0.25	0.01	0.01	<0.01
Range	-2.8-5.0	-1.8-3.25		-2.5-2.5	
LVPWS					
Z-score, median	-0.58	-0.08	<0.01	0.01	<0.01
Range	-2.4-1.7	-2.1-2.5		-2.4-2.5	
LVPWD					
Z-score, median	-0.63	-0.04	<0.01	0.03	<0.01
Range	-3.1-2.2	-2.0-3.8		-2.5-2.6	
LV thickness-dimension ratio					
Z-score, median	-0.62	-0.03	0.02	-0.02	<0.01
Range	-3.0-2.4	-1.8-2.7		-1.4-2.9	
Mean arterial pressure (mm Hg)					
Median	82 (63-135)	81 (58-122)	0.23	83 (68-108)	0.45
ESWS (g/cm <sup>2</sup> )					
Median, range	66.7 (34-116)	56.6 (28-82)	<0.01	54.2 (34-109)	<0.01
LV mass (g/m <sup>2</sup> )					
Median, range	56.7 (25-121)	53.4 (34-110)	0.19	56.0 (30-82)	0.28
Velocity of circumferential shortening (c/s)					
Median, range	1.11 (0.69-1.69)	1.16 (0.86-1.48)	0.16	1.16 (0.77-1.52)	0.11
MPI					
Median, range	0.51 (0.26-0.81)	0.46 (0.24-0.67)	<0.01	0.46 (0.32-0.68)	<0.01
IVRT, ms					
Median, range	80 (20-123)	72 (44-106)	0.07	78 (49-109)	0.36
IVCT, ms					
Median, range	66 (20-123)	61 (37-100)	0.18	62 (37-88)	0.09
Ejection time, ms					
Median, range	290 (241-355)	298 (241-350)	<0.01	304 (250-343)	<0.01
E/A ratio					
Median, range	1.5 (0.7-2.7)	1.6 (0.9-2.5)	0.10	1.5 (0.8-2.6)	0.41
E Wave, ms					
Median, range	0.9 (0.5-2.0)	1.1 (0.7-1.5)	0.08	1.0 (0.6-1.5)	0.13
A Wave, ms					
Median, range	0.6 (0.3-1.5)	0.6 (0.3-0.9)	0.39	0.6 (0.4-1.0)	0.90
Blood biomarkers					
BNP (pg/dL)					
Median, range	15.8 (0-225)	13.7 (0-168)	0.06	18 (0-92)	0.17
NT-proBNP (pg/dL)					
Median, range	71 (6-804)	37 (6-552)	0.01	26 (8-151)	<0.01
Troponin-T (ng/mL)					
Median, range	0 (0-0.02)	0 (0-0.03)	0.37	0 (0-0.02)	0.45
Galectin-3 (ng/mL)					
Median, range	10.8 (5.2-20.6)	9.2 (5.4-19.7)	0.11	9.7 (6.8-18.4)	0.40
ST-2 (U/mL)					
Median, range	26 (11.1-59.0)	25.6 (13.8-60.0)	0.69	26.1 (12.0-62.0)	0.78

<sup>a</sup>HR versus standard risk.<sup>b</sup>HR versus controls.

excellent correlation across the measured indices, there may be variance in reproducibility, suggesting that certain indices are more reliably obtained than others in a multicenter setting (29).

Anthracycline cardiotoxicity is thought to be due to formation of free radicals and cardiotoxic alcohol metabolites that result in direct cardiac injury (1). With enough

cardiac damage, the heart expands in size and the chamber walls become thinner, creating a clinical picture similar to dilated cardiomyopathy (4). The current study confirms these findings, by reporting on clear dose-dependent changes in LV chamber diameter and wall thickness, resulting in an increase in ESWS. Measurement of ESWS had, until recently, relied on simultaneous acquisition of an M-mode

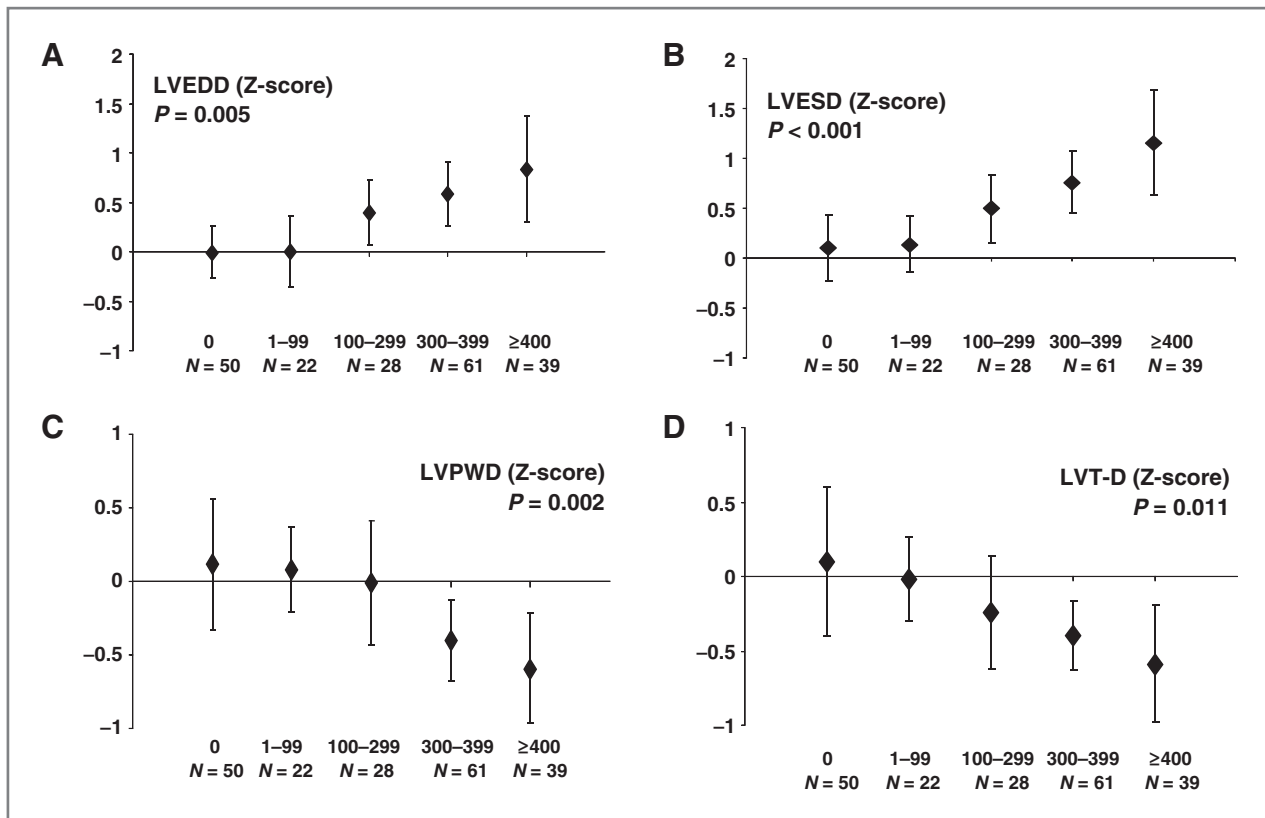


Figure 1. LV echocardiographic indices by cumulative anthracycline dose (mg/m<sup>2</sup>). A, LVEDD. B, VESD. C, LVPWD. D, thickness-dimension ratio (LV T-D).

echocardiogram, carotid pulse tracing, phonocardiogram, and blood pressure measurement (11, 12). Obtaining a carotid artery tracing can be especially difficult in the pediatric population due to the shortness of the neck, discomfort, and heart rate variability. Aggarwal and colleagues (17) found that substitution of MAP for carotid pulse tracing-based measurements had excellent diagnostic accuracy (sensitivity 95%, specificity 96%) for ESWS, which is the approach utilized in the current study. We successfully

obtained complete echocardiographic measurements, including ESWS, for nearly all study participants, with excellent interobserver correlation, demonstrating clearly the feasibility of using these indices to monitor anthracycline-related LV dysfunction.

In addition to ESWS, we found a dose-dependent increase in MPI, a Doppler-derived global marker of cardiac function that is independent of heart rate, intravascular volume, and blood pressure. MPI does not rely on geometric

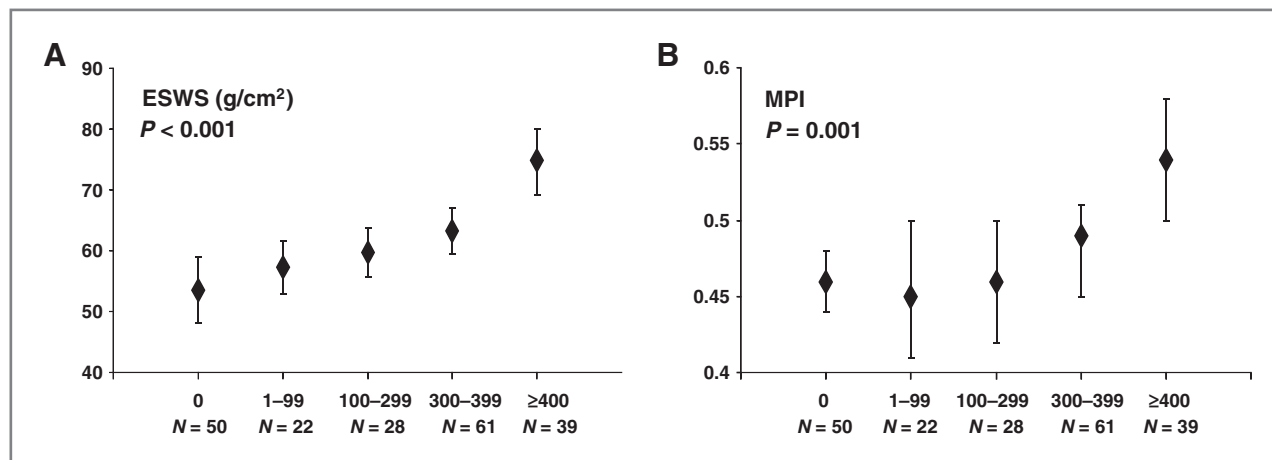


Figure 2. LV ESWS and MPI by cumulative anthracycline dose (mg/m<sup>2</sup>).

assumptions, and has been shown to be predictive of CHF and cardiac death in nononcology populations, irrespective of other echocardiographic indices of CHF risk such as depressed EF (13, 30, 31). Studies in small numbers of childhood cancer survivors treated with anthracyclines have shown that prolongation of MPI can be seen soon after completion of cancer therapy (16, 32, 33). The current study builds on these findings by demonstrating elevation in MPI within a large population of survivors, several years after completion of anthracycline therapy. It is important to note, however, that the long-term prognostic implications of persistent elevation in MPI as well as other indices such as ESWs in anthracycline-exposed childhood cancer survivors are not known. Longitudinal studies are needed to evaluate the association between these echocardiographic indices and subsequent CHF risk.

Serum cardiac troponins are sensitive and specific biomarkers for cardiac cell injury, and have successfully been used to monitor cardiac injury during and shortly after anthracycline administration (1, 9). However, in the current study, all participants were  $\geq 2$  years from exposure to cardiotoxic therapies, making it unlikely that there would be significant elevation in cardiac troponins in the setting of chronic LV dysfunction. Unlike the situation with cardiac troponins, studies have been more consistent about the diagnostic value of natriuretic peptides in anthracycline-related CHF monitoring (1, 9). Several studies have documented that BNP and NT-proBNP levels, if persistently elevated, correlate well with echocardiographic indices of cardiac dysfunction (34–36). In the current study, even though there were detectable differences in median NT-ProBNP by risk group, for the most part, the association between ESWs and natriuretic peptides (BNP, NT-ProBNP), as well as newer biomarkers such as galectin-3 and ST-2 was weak. It remains to be seen what role, if any, blood biomarkers may play in screening for cardiac dysfunction after anthracycline therapy. Current long-term follow-up guidelines for survivors of childhood cancer (9, 37) do not recommend routine surveillance using these blood biomarkers. This is largely due to the wide interpatient variability of these biomarkers in individuals with asymptomatic LV dysfunction as measured by EF, as well as difficulties with establishment of an appropriate cutoff for abnormal values in the asymptomatic setting (34–36).

Unlike previous studies (16, 28, 36), we did not find a significant association between anthracycline dose and spectral Doppler echocardiographic indices of LV diastolic dysfunction. This may be because diastolic dysfunction can be transient, occurring shortly after anthracycline exposure, and is largely due to exposure of cardiac tissues to radiation (4). Our study included survivors who were on average 12 years from diagnosis and only 10% of the population had received

chest-directed radiation therapy. Finally, there is emerging data that newer imaging modalities such as cardiac magnetic resonance imaging, "speckle tracking," and 3-D echocardiography may facilitate early identification of cardiac dysfunction before changes in EF (38, 39). However, these modalities are not uniformly available across cancer follow-up centers, and lack of longitudinal follow-up studies in childhood cancer survivors precludes their routine use for primary surveillance at the current time. The current study relied on measurements that can be obtained from routine 2D, M-mode, and Doppler echocardiograms and included indices with clear prognostic implications in pediatric as well as adult populations at risk for cardiac compromise.

In summary, comprehensive profiling of anthracycline-exposed childhood cancer survivors with preserved EF revealed dose-dependent changes in LV function more than 10 years from cancer diagnosis. These indices were readily obtained from standard echocardiograms performed as part of clinical care, and there was excellent correlation between the clinical reading and the established core cardiology laboratory. The findings from the current study may facilitate the development of early pharmacologic interventions aimed at progression or potential reversal of cardiac remodeling, and therefore, prevention of overt CHF in anthracycline-exposed childhood cancer survivors.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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#### Grant Support

This work was supported by NIH/NCI 2 K12 CA001727-14 and STOP Cancer Foundation.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 2, 2014; revised April 22, 2014; accepted June 3, 2014; published OnlineFirst June 19, 2014.

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