

Pilot Study of Vascular Health in Survivors of Osteosarcoma

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Background. Cardiovascular-related toxicities have been reported among survivors of osteosarcoma. **Methods.** Fasting blood samples from 24 osteosarcoma survivors were analyzed for high-sensitivity C-reactive protein (hsCRP), triglycerides, total cholesterol, high-density lipoprotein (HDL), apolipoprotein-β, lipoprotein (a), fibrinogen, circulating endothelial cells (CECs), and surface expression of vascular cell adhesion molecule-1 (VCAM-1). Values were compared to subjects in the natural history Coronary Artery Risk Development in Young Adults (CARDIA) cohort study except for CECs and VCAM-1 expression, which were compared to controls studied at the University of Minnesota Lillehei clinical trials unit. **Procedure.** Survivors (54.2% male), median age 18 years (9–32) at diagnosis, 36.5 years (20–56) at evaluation were treated with a variety of chemotherapeutic exposures, all but one were exposed to

doxorubicin (median dose 450 mg/m²; range: 90–645 mg/m²), 14 (58.3%) received cisplatin, and 3 (12.5%) were exposed to carboplatin. Two survivors (8.3%) received radiation therapy for disease relapse. Compared to CARDIA subjects, mean hsCRP (3.0 mg/L ± 2.0 vs. 1.6 ± 2.3), triglycerides (151 mg/dl ± 81.7 vs. 95.4 ± 101.3), lipoprotein (a) (34.9 mg/dl ± 17.7 vs. 13.8 ± 22.0), and fibrinogen (315.0 mg/dl ± 49.3 vs. 252.4 ± 61.7) were significantly elevated. The number of CECs (0.47 cells/ml ± 2.5 vs. 0.92 ± 2.5) did not differ while surface expression of VCAM-1 (86.4% ± 34.0 vs. 42.1 ± 33.8) was significantly elevated compared to controls. **Conclusions.** Among survivors of osteosarcoma, assessed a median of 14 years from diagnosis, there is evidence of vascular inflammation, dyslipidemia, and early atherogenesis. *Pediatr Blood Cancer* 2013;60:1703–1708. © 2013 Wiley Periodicals, Inc.

Key words: osteosarcoma; survivorship; vascular late effects

INTRODUCTION

Improvements in survival rates among children with cancer remain encouraging but are tempered by recognition of the increased risks for excessive morbidity and early mortality as these survivors progress into adulthood. Estimates suggest that there are over 350,000 survivors of childhood or adolescent cancer alive in the United States [1], two-thirds of whom report lasting effects of their prior diagnosis and therapy, ranging from mild to life-threatening [2]. An eightfold increased risk of death, up to 35 years from diagnosis, has been reported among 5-year childhood cancer survivors compared to the age and gender matched US population [3].

While end-organ toxicities of chemotherapy and radiation therapy among cancer survivors have been well described, effects upon the circulatory system have not been as thoroughly studied and may significantly contribute to end-organ disease. Increased risks have been reported for myocardial ischemia, transient ischemic attacks, and stroke [4–6]. Additionally, survivors exposed to CNS directed radiation therapy are known to suffer neurocognitive sequelae, potentially related to underlying cerebrovascular injury. However, even long-term survivors of Hodgkin lymphoma have been found to have neurocognitive impairments, leukoencephalopathy, and cerebrovascular injury despite not typically being exposed to CNS directed radiation [7]. These outcomes may be unified by injury to the vascular endothelium, resulting in chronic dysfunction, leading to progressive atherosclerosis, and eventual end-organ disease.

The vascular effects of cancer therapy are becoming recognized as a potential contributor to many of the sequelae that cancer survivors experience [8]. Radiation-induced vascular injury has been described in animal models [9] and autopsy studies have identified ionizing radiation as a potential cause for the development of early plaques, medial fibrosis, and adventitial thickening [10]. Two studies suggest that vascular dysfunction is possible in childhood cancer survivors. Both reported decreased brachial artery reactivity among survivors either treated with

300 mg/m² or more of anthracyclines (N = 14) or treated with multi-agent chemotherapy (with or without cranial radiation) for ALL (N = 75) compared to healthy controls [11,12]. The complex mechanisms of cardiovascular injury remain poorly defined and additional study is needed to help guide the testing of therapeutic interventions.

The objective of this study was to assess markers of vascular injury among survivors of osteosarcoma, a population with intensive, potentially vascular toxic, chemotherapy exposures, typically without radiation exposure. We previously collected the same biomarkers among survivors of Hodgkin lymphoma and found evidence suggestive of vascular inflammation, dyslipidemia,

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Grant sponsor: Children's Cancer Research Fund, Karen Wyckoff Rein in Sarcoma Fund, Vikings Children's Fund; Grant numbers: 1K12RR023247; 5PO1HL055552-13; 01DK072124-01A3; Grant sponsor: Minnesota Medical Foundation, Minneapolis, MN. Cancer Center Support (CORE); Grant number: CA21765; Grant sponsor: National Institutes of Health, Bethesda, MD; Grant sponsor: American Lebanese Syrian Associate Charities (ALSAC), Memphis, TN

Conflict of interest: Nothing to declare.

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Received 27 March 2013; Accepted 30 April 2013

and potential early atherogenesis [13]. We hypothesized that survivors of osteosarcoma would also have increased markers of inflammation and endothelial cell injury compared to controls.

METHODS

Study Population and Data Collection

Survivors of osteosarcoma 18 years of age or older, 5 or more years from diagnosis were recruited from the University of Minnesota Pediatric Oncology and long-term follow-up clinic databases. Survivors reporting a history of heart disease, stroke, taking cardiovascular medications, or a subsequent malignant neoplasm were excluded. The study protocol and documents were reviewed and approved by the Human Subjects Review Committee at the University of Minnesota.

Anthropomorphic measurements, fasting blood samples, and a current health questionnaire were obtained for each participant by a home health agency. Blood was analyzed for high-sensitivity C-reactive protein (hsCRP), triglycerides, cholesterols, apolipoprotein β , lipoprotein (a), and fibrinogen by the University of Minnesota Medical Center, Fairview Diagnostic Laboratory.

Measurements of circulating endothelial cells (CEC) and their surface expression of vascular cell adhesion molecule-1 (VCAM-1) were performed by the University of Minnesota Vascular Biology Center. One milliliter of whole blood was centrifuged and the buffy coat smear stained with PIH12 antibody (anti-CD146) and an alkaline phosphatase labeled secondary antibody [14]. Cells were manually counted under the microscope. Assessment of surface CEC phenotype required enrichment of blood samples and isolation of PIH12-positive cells by immunomagnetic bead method and subsequent staining with PIH12 and anti-VCAM-1 antibodies [14].

A medical history questionnaire was used to assess current health status, family and social histories, and medications. Chemotherapy doses were abstracted from medical records.

In order to compare the inflammatory markers from osteosarcoma survivors to a relevant population, we used the Coronary Artery Risk Development in Young Adults (CARDIA). Study dataset from the National heart, lung, and blood institute (NHLBI). Initiated in 1984, CARDIA is a prospective cohort study of risk factors for coronary artery disease among young adults. Originally 5,115 participants aged 18–30 years were enrolled from four geographic centers: Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA and followed for nearly 30 years [15]. The CARDIA dataset included individual participant data for hsCRP, fibrinogen, and lipids as well as demographic information. Circulating endothelial cells and VCAM-1 expression were compared to a healthy young adult population (N = 129) collected in the University of Minnesota Lillehei Clinical Research Unit (mean age 36.4 years (18–54), 42% male).

Statistical Analysis

Descriptive statistics were calculated for demographic and treatment variables. Likely due to the demographics of our institution, all study participants were Caucasian. Observed means and standard deviations for inflammatory markers were compared between cancer survivors and Caucasian CARDIA participants in general linear regression models adjusted for age, gender, BMI, and smoking status. Similarly, means and standard deviations for CECs and VCAM-1 expression were compared between cancer survivors

and controls in general linear models, adjusted for age, gender, and BMI (smoking status was not available for these controls). The distribution of CECs and their VCAM-1 expression may not necessarily be normal; therefore, we also compared these markers between survivors and controls using a non-parametric test, the Wilcoxon signed-rank test. Pearson's correlation coefficients determined for osteosarcoma survivors, adjusted for age, gender, BMI, and smoking status were calculated to evaluate potential correlations between inflammatory biomarkers, CECs, and VCAM-1 expression.

RESULTS

Characteristics of cancer survivors are shown in Table I. Just over half of the participants were male (54.2%) and diagnosed in the adolescent to young adult age range (median 18 years, range 9–32), as might be expected given the typical age distribution of osteosarcoma. Median age at evaluation was 36.5 years (20–56) and 14.2 years (6–42) from diagnosis. All but one survivor had a history of anthracycline exposure (median dose 450 mg/m²; 90–645 mg/m²). One survivor, diagnosed in 1966, was initially treated with surgical resection alone followed 18 months later by staged thoracotomies, 5 days of mitomycin-C infusion, and whole lung radiation for a bilateral pulmonary relapse. Another participant initially treated with chemotherapy and surgical resection, received

TABLE I. Characteristics of Study Population

	Osteosarcoma survivors ^a (N = 24)	
	N	%
Gender		
Male	13	54.2
Female	11	45.8
Median age in years (range)		
At diagnosis	18	9–32
At survey	36.5	20–56
Time from diagnosis	14.2	6–42
Treatment (not exclusive)		
Chemotherapy		
Doxorubicin (adriamycin)	23	95.8
Cisplatin	14	58.3
Carboplatin	3	12.5
Methotrexate—IV	22	91.7
Ifosfamide	14	58.3
Cyclophosphamide—IV	6	25.0
VP 16 (Etoposide)—IV	3	12.5
Bleomycin	5	20.8
Mitomycin C	1	4.2
Radiation	2	8.3
Smoke		
Yes	6	25.0
No	15	62.5
Not reported	3	12.5
	Median	Range
BMI in kilograms per m ²	27	22–52
Heart rate in beats per minute	73	60–104
Systolic BP in mmHg	120	98–140
Diastolic BP in mmHg	79	52–95

^aAll participating survivors were Caucasian.

whole brain radiation for a central nervous system relapse approximately 1 year following completion of therapy. This older cohort was also exposed to a variety of other chemotherapies as shown in Table I.

Heart rate and blood pressure measurements at the time of evaluation were not outside normative ranges, except for one individual at 140/95. Median BMI (27 kg/m²; 22–52) was in the overweight range with 9 (37.5%) survivors considered overweight (25–29 kg/m²) and 6 (25%) obese (≥30 kg/m²). Six (25%) survivors reported being smokers.

Only two participants reported recent illnesses, one with bronchitis and one recovering from a viral illness. However, three survivors reported taking an angiotensin converting enzyme inhibitor (ACE-I) for hypertension, three female survivors were on thyroid replacement, and four were taking antacid therapy. Additionally, one survivor reported taking 400–600 mg of ibuprofen daily and another reported daily aspirin therapy, both of which have anti-inflammatory properties. Reasons for these therapies were not provided. Finally, one survivor, also for unknown reasons, reported taking a long acting cardioselective beta-adrenergic blocker and a thiazide diuretic.

Mean values for the various biomarkers are shown in Tables II and III. High-sensitivity CRP, triglycerides, lipoprotein (a), and fibrinogen were greater among osteosarcoma survivors compared to 2,476 Caucasian CARDIA participants. Total cholesterol level, while not statistically different, tended to be higher among survivors compared to CARDIA controls, potentially influenced by a corresponding trend in HDL-cholesterol. Apolipoprotein-β levels did not differ between the two groups.

CEC cells with staining for VCAM-1 expression are shown in Figure 1. The number of CECs did not differ between survivors and controls (0.47 cells/ml ± 2.5 vs. 0.92 cells/ml ± 2.5, *P* = 0.38,

respectively). However, surface expression of VCAM-1 was elevated (86.4% ± 34.0 vs. 42.1% ± 33.8. *P* < 0.001) (Table III) among survivors compared to healthy controls. There was an inverse association between number of CECs and triglyceride levels (*P* = 0.01). Expression of VCAM-1 was positively correlated with hsCRP (*P* = 0.02).

DISCUSSION

Chronic vascular dysfunction may be a significant contributor to the cardio- and cerebro-vascular toxicities experienced by survivors of childhood cancer. In this pilot study among adults formerly treated for osteosarcoma, we used a panel of biomarkers to evaluate vascular health and potential endothelial injury. We found significant elevations in hsCRP, triglycerides, lipoprotein (a), and fibrinogen when compared to participants in the CARDIA Study. Additionally, while the number of CECs did not differ significantly between survivors and controls, expression of VCAM-1 was elevated and correlated with hsCRP levels, suggestive of endothelial activation and emerging atherogenesis. Most of our population was not exposed to radiation. Vascular injury in cancer has been previously associated with exposure to radiation but few studies have suggested potential chemotherapy-induced vascular toxicity.

Osteosarcoma survivors were intentionally selected for this study given their intensive chemotherapy exposures, particularly to anthracycline containing regimens, typically without radiation therapy. The long-term cardiac toxicities of anthracyclines have been described; however, specific toxicity to the endothelium, potentially a contributor to end-organ disease [16], has been less fully explored. Animal models have demonstrated in vitro and in vivo anthracycline-induced endothelial apoptosis [17,18] as well as

TABLE II. Cardiovascular Biomarkers in Survivors of Osteosarcoma (N = 24) Compared to CARDIA Participants*

	OS survivors		CARDIA subjects ^a		<i>P</i>
	Mean	SD	Mean	SD	
hsCRP ^b (mg/L)	3.0	2.0	1.6	2.3	<0.001
Triglycerides (mg/dl)	151	81.7	95.4	101.3	<0.001
Total cholesterol (mg/dl)	191.9	33.2	179.9	41.2	0.08
HDL ^c (mg/dl)	55.6	12.0	51.0	14.9	0.06
Apolipoprotein β (mg/dl)	89.7	23.6	90.2	30.0	0.91
Lipoprotein (a) (mg/dl)	34.9	17.7	13.8	22.0	<0.001
Fibrinogen (mg/dl)	315.0	49.3	252.4	61.7	<0.001

^aCaucasian CARDIA participants N = 2,476; ^bHigh-sensitivity C-reactive protein; ^cHigh-density lipoprotein; *Adjusted for age, gender, body mass index, and smoking status.

TABLE III. Circulating Endothelial Cells (CEC) and VCAM-1 Expression in Survivors of Osteosarcoma (N = 24) Compared to Controls*

	OS survivors		Controls ^a		<i>P</i>
	Mean	SD	Mean	SD	
CECs (cells/ml)	0.47	2.5	0.92	2.5	0.42
VCAM-1 (%)	86.4	34.0	42.1	33.8	<0.001

^aNormative CEC and VCAM-1 values collected from population controls (n = 129); *Adjusted for age, gender, and body mass index.

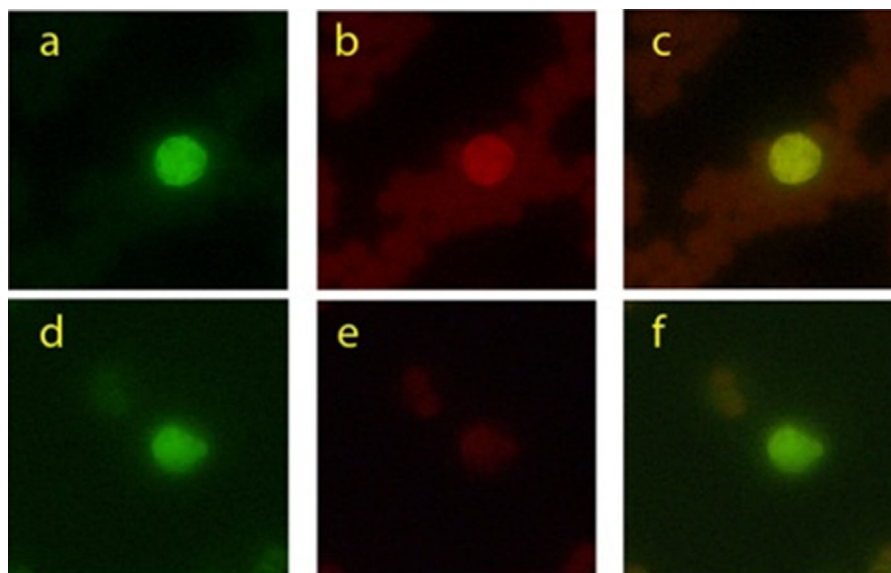


Fig. 1. Example of circulating endothelial cells (CECs) and surface VCAM-1 expression. Row 1—CEC expressing surface VCAM-1. **a:** CEC stained with fluorescein isothiocyanate—conjugated PIH12 (green), **b:** Positive VCAM-1 expression detected with Cy3 labeled anti-VCAM-1 antibody (red), **c:** merged images (yellow). Row 2—CEC not expressing surface VCAM-1. **d:** CEC stained with fluorescein isothiocyanate—conjugated PIH12 (green), **e:** no VCAM-1 expression detected with Cy3 labeled anti-VCAM-1 antibody, **f:** merged images showing only PIH12 staining (green).

impaired vascular relaxation after chronic exposure [18]. Few clinical studies of vascular toxicity have been performed among cancer survivors, fewer yet among those treated for a childhood cancer. Dengel et al. [11] measured brachial artery reactivity among a group of childhood acute lymphoblastic leukemia (ALL) survivors. Diminished reactivity was reported among those treated with chemotherapy and cranial radiation ($7.1 \pm 2.6\%$) and those treated with chemotherapy only ($6.5 \pm 2.6\%$), compared to an age, gender, and weight matched healthy control group ($9.5 \pm 2.9\%$). A smaller study assessed endothelial dysfunction among 14 survivors of childhood cancer of varying diagnoses and treatments. All had high dose ($\geq 300 \text{ mg/m}^2$) anthracycline exposures and demonstrated decreases in brachial artery reactivity when compared to a healthy control group. More recently, Jenei et al. [19] studied endothelial function among childhood cancer survivors exposed to anthracyclines ($n = 67$) as well as a group treated with non-anthracycline containing regimens ($n = 29$) and healthy controls ($n = 72$). Using ultra sound to measure brachial artery reactivity and aortic stiffness, investigators found the lowest flow mediated dilation and highest aortic stiffness among those treated with anthracyclines, significantly different from both the non-anthracycline exposed survivors and controls. None of the survivors in the Jenei et al. study were exposed to more than 350 mg/m^2 of anthracyclines, substantially lower than the mean in our study. Similar to our study, triglyceride levels were higher among survivors compared to healthy controls. However, unlike the members of our cohort, their population did not have higher levels of hsCRP and fibrinogen levels compared to controls. It is possible that biomarkers of vascular inflammation and hemostatic function increase with higher anthracycline exposures and potentially, while not measured in our study, vascular function declines even further. These findings of a potential association between anthracyclines and vascular health are also suggested by evidence from the Childhood Cancer Survivor Study where anthracycline exposure $\geq 250 \text{ mg/m}^2$

was found to be associated with self-reported cardiac valve abnormalities (HR 2.3, 95% CI 1.6–3.3) even after adjustment for cardiac radiation [20]. While typically thought to be related to radiation, chemotherapy injury to the endocardial tissue (endothelial cells) covering the cardiac valves may contribute to valvular dysfunction.

It is difficult to identify a single chemotherapeutic agent potentially responsible for endothelial injury. In our pilot study survivors were exposed to a variety of chemotherapies. Particularly pertinent for survivors of osteosarcoma might be exposure to platinum containing agents. A number of studies have reported adverse cardiovascular outcomes among survivors of gonadal germ cell tumors. Cisplatin and bleomycin-containing regimens have been associated with adverse vascular outcomes among testicular cancer survivors [21], with an increase in the risk for cardiac events among those treated with chemotherapy alone (RR 2.6 95% CI 1.2–5.8), radiation alone (RR 2.4 95% CI 1.0–5.5), and combined modalities (RR 2.8 95% CI 1.1–7.1) [22]. Elevated markers of endothelial injury, microalbuminuria, have been identified among testicular cancer survivors as well as among young women treated for ovarian cancer [23,24]. While each chemotherapeutic agent interfaces with the pervasive endothelium, the effects may be individual or, more likely, multi-factorial.

Vascular research has increasingly supported the theory that atherogenesis, previously thought to result solely from excessive lipid deposition, is the result of a chronic inflammatory response to an unknown underlying injurious insult, with endothelial dysfunction being the earliest evidence of the disease trajectory. Moreover, subclinical atherosclerosis begins in the pediatric age range with progression into adulthood, when clinical disease becomes evident [25]. Numerous serum biomarkers have been measured in a variety of populations and found to be predictive of primary and/or secondary cardiovascular events. Thus far, hsCRP has been

the most studied and found to be associated with cardiovascular outcomes independent of gender, age, smoking, cholesterol levels, blood pressure, and diabetes [26]. Guidelines for cardiovascular risk assessment in asymptomatic adults from the American Heart Association recommend consideration of hsCRP testing among asymptomatic men (≤ 50 years) and women (≤ 60) deemed at intermediate-risk for developing cardiovascular events (Class IIb) [27]. Risk stratification models are weighted for age and typically do not apply to a young population. However, it is unknown if cancer therapy early in life contributes to later cardiovascular risk. In fact, participants in our study had a mean hsCRP level double that found among participants in the CARDIA study, suggestive of an element of underlying chronic inflammation. This cross-sectional analysis does not allow us to predict future risk; however, the impact of chronic inflammation at an early age will likely only be compounded by the accumulation of risk factors with increasing age, potentially contributing to the adverse cardiovascular outcomes seen among adult survivors of childhood cancer.

Similar to our previous study among survivors of Hodgkin lymphoma, we again found mild elevation in triglycerides and a more significant increase in lipoprotein (a) levels (34.9 mg/dl vs. 13.8 mg/dl, $P < 0.001$) compared to controls. The atherogenic mechanisms of lipoprotein (a) remain unclear but may be related to dysfunction of the hemostatic system. The molecule is linked to apolipoprotein (a), which shares significant structural homology with plasminogen [28]. A single amino acid substitution of arginine for a serine moiety prevents plasminogen activation, potentially contributing to a pro-thrombotic state on the endothelial surface. A large meta-analysis has identified significant associations between lipoprotein (a) and vascular outcomes including coronary artery disease and stroke but no association with non-vascular mortality [29]. Bostom et al. [30] reported a relative risk of 1.9 (95% CI 1.2–2.9) for incident coronary heart disease among men (mean age 36.3 years, range 20–54) with lipoprotein (a) levels above 30 mg/dl after adjusting for age, BMI, smoking, glucose intolerance, hypertension, total cholesterol (>240 mg/dl), and HDL-C (<35 mg/dl).

We also found elevated fibrinogen levels in our study population. Hemostatic dysfunction has been associated with atherosclerosis and elevated fibrinogen levels associated with adverse vascular events.[31] In the atherosclerosis risk in communities (ARIC) study a one standard deviation increase in fibrinogen level was associated with a 1.8 and 1.5 fold increased risk of coronary heart disease in men and women, respectively [32]. Mean fibrinogen levels in the ARIC study among both men and women who had a vascular event were 320 ± 65 and 346.5 ± 65 mg/dl, respectively, not dissimilar to the 315 mg/dl among survivors in our study, nearly one standard deviation higher than the CARDIA participants.

The endothelial monocellular layer, the interface between the blood and interstitium, is central to maintaining vascular homeostasis and loss of its normal function; as evidenced by inflammation, dyslipidemia, and hemostatic anomalies is believed to be the initiating factor in the atherosclerotic disease process.

CECs are believed to be shed from the vessel wall in response to an injurious event and elevations have been reported in a variety of pathologic states including cardiovascular disease, sickle cell disease, rheumatologic conditions as well as some active adult cancers [33]. Other than our previous study among survivors of Hodgkin lymphoma, CECs have only been measured in a small population of testicular cancer survivors and found to be elevated

among young men treated with cisplatin-based chemotherapy regimens compared to those not treated with chemotherapy [34]. We identified mild elevations among Hodgkin lymphoma survivors treated with radiation therapy but no difference in surface expression of VCAM-1. Interestingly, among osteosarcoma survivors we found the reverse; no difference in enumerated CECs but a statistically significant elevation in cellular expression of VCAM-1 compared to controls. This may be due to the lack of radiation exposure or perhaps the more varied chemotherapy exposures in this group. In our study only a portion were exposed to platinum containing agents (58% cisplatin, mean dose = 347 mg/m²; 12.5% carboplatin, mean dose = 1987 mg/m²). Comparison to the testicular cancer survivors (mean cisplatin dose = 300 mg/m²) is difficult since CECs were enumerated in only a random subset of the study population and by flow cytometry, rather than the immunemagnetic bead method used in this study.

Assessment of VCAM-1 expression is unique to our study and may indicate an activated (dysfunctional) endothelial layer. VCAM-1 is known to be expressed over developing atheromatous plaques and binds the $\alpha_4\beta_1$ integrins expressed on monocytes and T-lymphocytes, inhibiting leukocyte rolling and mediating adhesion and transmigration into the vascular intima. In mice with reduced VCAM-1 expression, the quantified area of atherosclerotic lesions in the aorta was significantly reduced compared to the wild type, despite comparable cholesterol, lipoprotein, and leukocyte levels [35]. In its inactivated state, the endothelium resists prolonged leukocyte contact but when inflamed VCAM-1 transcription is rapidly up-regulated and expressed on the endothelial surface. Pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) contained within atherosclerotic plaques mediate this process [36]. While IL-1 β or TNF- α were not measured in this study, we did note a significant correlation between inflammation, measured by hsCRP, and VCAM-1 expression. The elevated expression in our study suggests an activated endothelial layer without significant cellular detachment from the vessel wall, the opposite of what we found among irradiated HL survivors.

Results from this pilot study should be interpreted in light of a number of limitations. With the aim of studying subclinical disease, we attempted to recruit participants from our clinical databases without a history of known cardiovascular disease, thus limiting our ability to assess overall prevalence of vascular disease in this population. Despite not reporting a cardiovascular event, several survivors did report taking a number of anti-hypertensive medications and two were on anti-inflammatories (a non-steroidal and aspirin). It is possible this may have influenced measurement of serum biomarkers and results may be attenuated slightly based on these medications. We carefully selected a number of common biomarkers for this study which have previously been associated with primary events. However, numerous biomarkers have been studied and associated with cardiovascular risks in a variety of populations. It is impractical to assess all potential disease markers, particularly given the limited sample size in this initial pilot study. A larger population of survivors will allow more detailed analyses including assessments of various therapeutic exposures and lifestyle factors as well as correlation with other measures of cardiovascular function. Finally, conclusions regarding the development and progression of endothelial injury over time are limited in this cross-sectional analysis. It is challenging to study a chronic, progressive disease such as atherosclerosis at an arbitrary time point

and within the confines of most study cycles. Characterization of vascular health in a well-defined cohort of cancer patients followed from diagnosis into survivorship would provide a more robust assessment of vascular injury following cancer therapy, insight into potential mechanisms, and guidance for the design of clinical trials directed at ameliorating or preventing adverse late outcomes.

Vascular dysfunction plays a role in many disease processes and may be a significant contributor to the late-effects of cancer therapy. Using a panel of established and novel biomarkers in a cohort of survivors of osteosarcoma, we found evidence suggestive of subclinical vascular injury and atherogenesis. An improved understanding of the effects of cancer therapy on the circulatory system will help identify at-risk survivors and lead the way to improved prevention and treatment.

REFERENCES

- Mariotto AB, Rowland JH, Yabroff KR, et al. Long-term survivors of childhood cancers in the United States. *Cancer Epidemiol Biomarkers Prev* 2009;18:1033–1040.
- Oeffinger KC, Mertens AC, Sklar CA, et al. Chronic health conditions in adult survivors of childhood cancer. *N Engl J Med* 2006;355:1572–1582.
- Mertens AC, Liu Q, Neglia JP, et al. Cause-specific late mortality among 5-year survivors of childhood cancer: The Childhood Cancer Survivor Study. *J Natl Cancer Inst* 2008;100:1368–1379.
- Hancock SL, Donaldson SS, Hoppe RT. Cardiac disease following treatment of Hodgkin's disease in children and adolescents. *J Clin Oncol* 1993;11:1208–1215.
- Bowers DC, Liu Y, Leisenring W, et al. Late-occurring stroke among long-term survivors of childhood leukemia and brain tumors: A report from the Childhood Cancer Survivor Study. *J Clin Oncol* 2006;24:5277–5282.
- Bowers DC, McNeil DE, Liu Y, et al. Stroke as a late treatment effect of Hodgkin's disease: A report from the Childhood Cancer Survivor Study. *J Clin Oncol* 2005;23:6508–6515.
- Krull KR, Sabin ND, Reddick WE, et al. Neurocognitive function and CNS integrity in adult survivors of childhood Hodgkin lymphoma. *J Clin Oncol* 2012;30:3618–3624.
- Mulrooney DA, Blaes AH, Duprez D. Vascular injury in cancer survivors. *J Cardiovasc Transl Res* 2012; 5:287–295.
- Tribble DL, Barcellos-Hoff MH, Chu BM, et al. Ionizing radiation accelerates aortic lesion formation in fat-fed mice via SOD-inhibitable processes. *Arterioscler Thromb Vasc Biol* 1999;19:1387–1392.
- Brosius FC III, Waller BF, Roberts WC. Radiation heart disease. Analysis of 16 young (aged 15 to 33 years) necropsy patients who received over 3,500 rads to the heart. *Am J Med* 1981;70:519–530.
- Dengel DR, Ness KK, Glasser SP, et al. Endothelial function in young adult survivors of childhood acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2008;30:20–25.
- Chow AY, Chin C, Dahl G, et al. Anthracyclines cause endothelial injury in pediatric cancer patients: A pilot study. *J Clin Oncol* 2006;24:925–928.
- Mulrooney DA, Ness KK, Solovey A, et al. Pilot study of vascular health in survivors of Hodgkin lymphoma. *Pediatr Blood Cancer* 2012;59:285–289.
- Solovey A, Lin Y, Browne P, et al. Circulating activated endothelial cells in sickle cell anemia. *N Engl J Med* 1997;337:1584–1590.
- Friedman GD, Cutter GR, Donahue RP, et al. CARDIA: Study design, recruitment, and some characteristics of the examined subjects. *J Clin Epidemiol* 1988;41:1105–1116.
- Camici PG, Crea F. Coronary microvascular dysfunction. *N Engl J Med* 2007;356:830–840.
- Wu S, Ko YS, Teng MS, et al. Adriamycin-induced cardiomyocyte and endothelial cell apoptosis: In vitro and in vivo studies. *J Mol Cell Cardiol* 2002;34:1595–1607.
- Murata T, Yamawaki H, Yoshimoto R, et al. Chronic effect of doxorubicin on vascular endothelium assessed by organ culture study. *Life Sci* 2001;69:2685–2695.
- Jenei Z, Bardi E, Magyar MT, et al. Anthracycline causes impaired vascular endothelial function and aortic stiffness in long term survivors of childhood cancer. *Pathol Oncol Res* 2012; DOI 10.1007/s12253-102-9589-6
- Mulrooney DA, Yeazel MW, Kawashima T, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: Retrospective analysis of the Childhood Cancer Survivor Study cohort. *BMJ* 2009;339:b4606.
- Meinardi MT, Gietema JA, van der Graaf WT, et al. Cardiovascular morbidity in long-term survivors of metastatic testicular cancer. *J Clin Oncol* 2000;18:1725–1732.
- Huddart RA, Norman A, Shahidi M, et al. Cardiovascular disease as a long-term complication of treatment for testicular cancer. *J Clin Oncol* 2003;21:1513–1523.
- Nuver J, Smit AJ, Sleijfer DT, et al. Microalbuminuria, decreased fibrinolysis, and inflammation as early signs of atherosclerosis in long-term survivors of disseminated testicular cancer. *Eur J Cancer* 2004; 40:701–706.
- de Vos FY, Nuver J, Willemse PH, et al. Long-term survivors of ovarian malignancies after cisplatin-based chemotherapy: cardiovascular risk factors and signs of vascular damage. *Eur J Cancer* 2004;40:696–700.
- Charakida M, Deanfield JE, Halcox JP. Childhood origins of arterial disease. *Curr Opin Pediatr* 2007;19:538–545.
- Ridker PM. High-sensitivity C-reactive protein: Potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 2001;103:1813–1818.
- Greenland P, Alpert JS, Beller GA, et al. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: Executive summary: A report of the American college of cardiology foundation/American heart association task force on practice guidelines. *Circulation* 122:2748–2764.
- Bermudez V, Arraiz N, Aparicio D, et al. Lipoprotein(a): From molecules to therapeutics. *Am J Ther* 2010;17:263–273.
- Erqou S, Kaptoge S, Perry PL, et al. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA* 2009;302:412–423.
- Bostom AG, Cupples LA, Jenner JL, et al. Elevated plasma lipoprotein(a) and coronary heart disease in men aged 55 years and younger. A prospective study. *JAMA* 1996;276:544–548.
- Borissoff JJ, Spronk HM, Ten Cate H. The hemostatic system as a modulator of atherosclerosis. *N Engl J Med* 2011;364:1746–1760.
- Folsom AR, Wu KK, Rosamond WD, et al. Prospective study of hemostatic factors and incidence of coronary heart disease: The Atherosclerosis risk in communities (ARIC) study. *Circulation* 1997;96: 1102–1108.
- Blann AD, Woywodt A, Bertolini F, et al. Circulating endothelial cells. Biomarker of vascular disease. *Thromb Haemost* 2005;93:228–235.
- Vaughn DJ, Palmer SC, Carver JR, et al. Cardiovascular risk in long-term survivors of testicular cancer. *Cancer* 2008;112:1949–1953.
- Cybulsky MI, Iiyama K, Li H, et al. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. *J Clin Invest* 2001;107:1255–1262.
- Libby P. Inflammation in atherosclerosis. *Nature* 2002;420:868–874.