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Pilot Study of Vascular Health in Survivors of Hodgkin Lymphoma

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Abstract

Background—Vascular-related toxicities have been reported among survivors of Hodgkin lymphoma (HL), but their genesis is not well understood.

Procedure—Fasting blood samples from 25 previously irradiated HL survivors were analyzed for biomarkers that can reveal underlying inflammation and/or endothelial cell activation: high-sensitivity C-reactive protein (hsCRP), triglycerides, total cholesterol, high-density lipoprotein (HDL), apolipoprotein B, lipoprotein (a), fibrinogen, circulating endothelial cells (CECs) and vascular cell adhesion molecule-1 (VCAM-1) expression. Values were compared to subjects in the Coronary Artery Risk Development in Young Adults (CARDIA) study. CECs and VCAM-1 were compared to healthy controls.

Results—Survivors (76% male), median age 17.6 yrs (5-33) at diagnosis, 33.0 yrs (19-55) at follow-up, included stages IA (n=6), IIA (n=10), IIB (n=2), IIIA (n=4), and IVA (n=3) patients. Twenty-four received at least chest radiation therapy (RT) (median dose 3,150 cGy; range: 175-4,650 cGy), one received neck only; 14 (56%) had a history of anthracycline exposure (median dose: 124 mg/m² range: 63-200 mg/m²). Compared to CARDIA subjects, mean hsCRP (3.0 mg/L ± 2.0 vs. 1.6 ± 1.9), total cholesterol (194.1 mg/dl ± 33.2 vs. 179.4 ± 32.9), lipoprotein (a) (34.2 mg/dl ± 17.5 vs. 13.8 ± 17.5), and fibrinogen (342.0 mg/dl ± 49.1 vs. 252.6 ± 48.4) were significantly elevated. CECs (2.3 cells/ml ± 1.5 vs. 0.34 ± 1.4) were significantly elevated compared to controls. No difference in VCAM-1 expression (51.1% ± 36.8 vs. 42.3 ± 35.6) was detected.

Conclusion—HL survivors exposed to RT have evidence of vascular inflammation, dyslipidemia, and injury suggestive of early atherogenesis.

Keywords

Survivorship; Hodgkin lymphoma; Vascular late effects

INTRODUCTION

Cancer survival rates continue to improve. This improvement is credited to multi-modal and risk-based therapies developed and tested through cooperative group research protocols. While encouraging, enthusiasm is tempered by emerging recognition of potential long-term morbidity and early mortality among cancer survivors. Chronic adverse outcomes of cancer therapy are significant and may include cardiovascular disease [1], subsequent neoplasms [2], and metabolic abnormalities [3,4]. An 8-fold increased risk of death, persisting up to 35 years from diagnosis, has been reported among 5-year childhood cancer survivors compared to an age and gender matched population [5].

Cardiac toxicity is a significant contributor to late morbidity and mortality following cancer therapy. Dilated cardiomyopathies are the most common cardiac late effect; however pericardial disease, conduction abnormalities, valvular disorders, and premature atherosclerotic coronary vascular disease have also been reported.[6-8] Cardiovascular disease is the leading non-malignant cause of death among these survivors, resulting in a 7-fold higher risk of death compared to age-matched peers.[5]

The impact of chemotherapy and/or radiation therapy on the vascular system has not been fully elucidated. Animal models and autopsy studies suggest ionizing radiation as a potential cause for the development of early vascular plaques, medial fibrosis, and adventitial thickening.[9,10] Additionally, radiation induced coronary lesions appear more proximal in the arterial tree, likely influenced by the prior radiation field.[11] While survivors from several diagnostic groups have been reported to have premature cardiovascular disease, the risk is particularly high among survivors of Hodgkin Lymphoma (HL).[12,13]

The objective of this pilot study was to assess markers of vascular injury among HL survivors exposed to radiation. As atherosclerosis has become recognized as a complex inflammatory process involving endothelial injury, we selected a panel of vascular biomarkers which have been studied in other non-cancer populations and associated with primary and secondary cardiovascular events. To our knowledge, this is the first study to assess vascular biomarkers among survivors of HL, with or without a history of radiation exposure. For this analysis we specifically chose biomarkers associated with primary cardiovascular events and survivors with a history of radiation therapy, given their higher risk of late adverse cardiovascular outcomes. We hypothesized that biomarker levels would be increased in HL survivors compared to controls. These data will provide a foundation to support a broader assessment of vascular health among cancer survivors.

METHODS

Study Population and Data Collection

A convenience sample of HL survivors with a history of radiation exposure, 5 years from diagnosis, and currently age 18 were recruited from the University of Minnesota Pediatric Oncology and Long-Term Follow-Up Clinic databases. Survivors who reported any history of heart disease, stroke, taking cardiovascular medications, or a subsequent 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, cholesterol, 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 vascular cell adhesion molecule-1 (VCAM-1) expression were performed by the University of Minnesota's Vascular Biology Center. One milliliter of whole blood was centrifuged and the buffy coat smear stained with P1H12 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 P1H12-positive cells by immunomagnetic bead method and subsequent staining with P1H12 and anti-VCAM-1 antibodies.[14]

A medical history questionnaire was used to record potentially inflammatory chronic conditions, assess family and social histories, and current medications. Chemotherapy doses and radiation exposures were abstracted from the medical records. Radiation volumes were calculated for each participant by a radiation oncologist (CKKL).

In order to compare our cancer survivors to a relevant population group for inflammatory markers, we requested use of the Coronary Artery Risk Development in Young Adults (CARDIA) Study dataset from the National Heart, Lung, and Blood Institute's (NHLBI) Biologic Specimen and Data Repository Information Coordinating Center. The CARDIA study is a prospective cohort initiated in 1984 to study risk factors for coronary artery disease in young adults. Initially 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 over 20 years.[15] CECs and VCAM-1 expression were compared to a young adult population (N=134) collected in the University of Minnesota Lillehei Clinical Research Unit (mean age 37.6 years (36-45), 48% male).

Statistical Analysis

Descriptive statistics were calculated for demographic and treatment variables. 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, body mass index (BMI), and smoking status. Similarly, means and standard deviations for CECs and VCAM-1 expression were compared between cancer survivors and controls in age, gender, BMI, and smoking adjusted general linear models. 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. Age, gender, BMI, and smoking adjusted Pearson's correlation coefficients were calculated to evaluate potential correlations between inflammatory biomarkers, CECs, VCAM-1 expression, and radiation and anthracycline doses.

RESULTS

Characteristics of cancer survivors are shown in Table 1. Most (76%) were male and diagnosed in the adolescent to young adult age range (median 17.6 years, range 5-33) reflective of the typical HL age distribution. Over half presented with stage IA or IIA disease and all received radiation therapy by study design, though one participant only received radiation to the neck and 9 also received abdominal radiation. Doxorubicin was the only anthracycline exposure (median dose 124 mg/m²; 63 – 200 mg/m²).

Five survivors (20%) reported thyroid replacement therapy. Two reported a known inflammatory condition (inflammatory bowel disease and asthma) that could potentially result in an elevated hsCRP. Neither participant was having an exacerbation at the time of evaluation.

Heart rate and blood pressure measurements at the time of evaluation were not outside normative ranges, except for one hypertensive individual at 160/105. Median BMI was 27 kg/m² with 16 (64%) survivors considered overweight (≥ 25 kg/m²) and 6 (24%) obese (≥ 30 kg/m²).

Mean values for the various biomarkers are shown in Tables 2 and 3. High-sensitivity CRP, total cholesterol, lipoprotein (a), and fibrinogen were statistically significantly elevated compared to subjects in the CARDIA study. Triglyceride levels also were elevated but did not reach statistical significance. HDL-cholesterol and apolipoprotein- β levels did not differ from those reported by CARDIA.

The number of CECs was significantly elevated ($2.3 \text{ cells/ml} \pm 1.5$ vs. $0.34 \text{ cells/ml} \pm 1.4$, $p < 0.001$) compared to controls while surface expression of VCAM-1 did not significantly differ ($51.1\% \pm 36.8$ vs. $42.3\% \pm 35.6$, $p = 0.29$) (Table 3). Additionally, no significant correlations were identified between biomarkers, CECs, and VCAM-1 expression with radiation or anthracycline dose exposure.

DISCUSSION

Cardiovascular disease, the leading cause of death in the developed world, contributes to significant premature morbidity and mortality among cancer survivors. Using a panel of biomarkers, we evaluated vascular health among a small cohort of survivors of HL previously exposed to radiation therapy. Significant elevations in hsCRP, total cholesterol, lipoprotein (a), and fibrinogen suggest vascular inflammation.[16] Additionally, the number of CECs was significantly elevated compared to controls, suggestive of underlying vascular injury. However, expression of the activation marker, VCAM-1, was not significantly elevated on these cells. These data are consistent with the notion that chronic vascular injury, inflammation, and progression of subclinical atherosclerosis could explain some of the late cardiovascular pathology experienced by survivors of HL.

Among cancer survivors, those with a history of HL are at particularly high risk for late adverse vascular outcomes. Hancock et al. first reported an increased risk of myocardial infarction (MI) among adults and children formerly treated for HL,[12,13] while more recent reports have similarly found an increased incidence of and death from MI extending at least 25 years from treatment.[17,18] The risk of stroke, analyzed in the Childhood Cancer Survivor Study, is also significantly elevated, potentially related to radiation of the carotid vessels.[19] Carotid intima-media thickness and plaque formation has been observed following neck radiation.[20,21] While our study cannot localize an injured vessel, our data are suggestive of early vascular injury that likely precedes the development of clinical events.

Atherosclerosis is understood as the outcome of a chronic, subclinical inflammatory state.[22] It is relevant that endothelial dysfunction is currently believed to be the earliest detectable evidence of the atherosclerotic disease trajectory, and it has predictive value for future development of atherosclerotic morbidity and mortality. Indeed, a variety of biomarkers have been studied to predict primary and secondary events. High-sensitivity CRP has been the most studied and is predictive of adverse cardiovascular events, [23,24] independent of traditional risk factors such as gender, age, smoking, cholesterol, blood pressure, and diabetes. Recently, elevated markers of inflammation and coagulation have also been identified among HIV infected persons, potentially associated with the early cardiovascular disease reported among these patients.[25] Survivors in this study had hsCRP levels nearly twice as high as that measured among CARDIA participants. Additionally, survivors had an increase in total cholesterol but no significant difference in HDL

cholesterol. Cholesterol deposition leads to endothelial dysfunction and stimulates a cytokines cascade contributing to the inflammatory response.[22] It is not possible in this cross-sectional analysis to determine if hsCRP preceded hyperlipidemia or the reverse. However, hsCRP has been shown to be independent of lipid and non-lipid risk factors[23] as well as additive to the predictive value of cholesterol levels.[26]

Triglyceride and lipoprotein (a) measurements were higher among survivors compared to age, gender, BMI and smoking status adjusted values from the CARDIA population. Triglycerides, while elevated, did not statistically differ from subjects in CARDIA and remained within a normal range. Several clinical studies have reported elevated Lp(a) levels as an independent risk factor for atherosclerotic-related events among young adults. Bostom et al. reported a two-fold risk of incident coronary heart disease among men 55 years old (mean 36.3 ± 8.7 years) with lipoprotein (a) levels above 30 mg/dl when compared to those with lower levels after adjustment for age, BMI, total cholesterol, HDL, smoking, glucose intolerance, and hypertension.[27] The etiology of elevated Lp(a) levels is unclear but generally thought to be related to increased production (likely hepatic) rather than decreased catabolism.[28] The objective of this pilot study was to study atherosclerotic biomarkers in an asymptomatic cancer survivor population thought to be at risk for premature vascular disease. One could speculate that the etiology of an increased Lp(a) level might be related to effects upon the liver, however, further investigation of this interesting finding and its predictive value in this population is needed.

Chronic vascular inflammation may lead to dysfunction of the hemostatic system resulting in thrombus formation and growth of arteromatous plaques. Fibrinogen levels have been associated with primary and secondary vascular events. Among men and women ages 45-64 in the Atherosclerosis Risk in Communities (ARIC) study, the risk of coronary heart disease was elevated 1.8 and 1.5 times, respectively, per each standard deviation increase in fibrinogen level.[29] The mean fibrinogen level among those who had an event was 320 mg/dl, similar to the mean (340.5 mg/dl) observed in our study. In this study fibrinogen among HL survivors was approximately 1.5 standard deviations higher than that reported in the CARDIA population.

Circulating endothelial cells (CECs), believed to have sloughed from the vessel wall in response to injury have been associated with a variety of conditions believed to involve vascular wall injury/dysfunction, such as: cardiovascular disease[30], sickle cell disease[14], connective tissue and other inflammatory disorders [31], and cancer.[32] Markers of endothelial cell activation, such as VCAM-1 can be identified on the endothelial cell surface and are suggestive of thrombogenicity and atherogenicity.

While the number of CECs compared to controls was elevated in our pilot study, and may indicate a degree of underlying vascular injury, the total number was quite small. Interestingly, a striking finding is that statistically significant elevations of CEC number that accompany atherosclerotic and other vascular conditions are quantitatively small if gold-standard methods are employed for the enumeration.[33] Rarely found in peripheral blood, the number of CECs reported among study and healthy subjects has been quite varied,[34] and few studies have examined CECs among cancer patients and none in survivors of childhood cancer. Among a variety of adult cancers, CECs have been reported to be 3.6 to 5 times higher than healthy controls.[32,35] In allogeneic stem cell transplant patients, the number of CECs at baseline was elevated compared to controls and increased further following the preparative regimen, both myeloablative and reduced intensity conditioning. [36] The increase was more rapid among those that received total body irradiation but higher and more sustained among those conditioned with chemotherapy only. In a survivor population, CECs have only been reported in a cohort of men treated for testicular cancer

and found to be significantly elevated in survivors treated with cisplatin-based chemotherapy compared to a chemotherapy naïve control group.[37]

Expression of the VCAM-1 adhesion molecule is known to be elevated on endothelial cells overlying nascent atheromas and is responsible for monocyte and T-lymphocyte recruitment into the vessel intima and growing plaque.[38] Animal models suggest VCAM-1 expression is the major adhesion molecule implicated in the early development of atherosclerotic lesions.[39] We did not identify increased VCAM-1 expression on the surface of the CECs in this study compared to controls. This may be the result of a limited sample size in this pilot or indicative of apoptosis of the sloughed, injured endothelial cells. Soluble VCAM-1 can also be measured in the plasma following release from the endothelial cell surface and measurement of both surface and soluble forms in a larger follow-up study could be informative.

This pilot study may be limited by use of a convenience sample recruited from our clinical databases and our selection of biomarkers. While we excluded survivors with a history of cardiovascular disease or taking cardiovascular medications, it is possible that subjects recruited from our Long-Term Follow-Up Clinic were more likely to participate or potentially suffer from other chronic medical conditions, potentially inflammatory, than those who chose not to participate.[40] Furthermore, we carefully selected a limited number of biomarkers, concentrating on those associated with primary prediction of vascular events. However, numerous biomarkers, in a variety of populations, have been associated with cardiovascular risks, but it is impractical to assess all potential biomarkers. Definitive conclusions regarding long-term mechanistic changes to the vascular endothelium are not possible from this cross sectional analysis. However, results suggest that planned longitudinal studies that will include vascular functional assessments in addition to measurement of cardiovascular biomarkers, will perhaps better quantify the effects of cancer therapy on the endothelial layer. These studies will help elucidate the pathophysiology of adverse vascular events in survivors of childhood cancer, allowing for the development of appropriately directed preventive measures.

Using established and novel biomarkers predictive of atherosclerotic disease, we found evidence suggestive of chronic vascular inflammation, dyslipidemia, and injury in a small cohort of survivors of childhood HL. This may play a role in the pathogenesis of the vascular disease observed among these cancer survivors, and longitudinal assessments are needed to study the predictive value of biomarkers in this at-risk population. An improved understanding of which survivors are at greatest risk will better guide screening practices and future therapeutic interventions.

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Table I

Characteristics of study population

	Participants (N=25)	
	N	(%)
Gender		
Male	19	(76)
Female	6	(24)
Median age in years (range)		
At diagnosis	17.6	(5-33)
At survey	33.0	(19-55)
Time from diagnosis	12.3	(4-34)
Stage of disease		
IA	6	(24.0)
IIA	10	(40.0)
IIB	2	(8.0)
IIIA	4	(16.0)
IVA	3	(12.0)
Radiation	25	(100)
Mantle	24	(96)
Median dose in cGy (range)	3,150	(175-4,650)
Dose per cubic centimeter (range)	1.3	(0.03-7.2)
Anthracycline chemotherapy	14	(56)
Median dose doxorubicin in mg/m ² (range)	124	(63-200)
Chronic Conditions		
Inflammatory bowel disease	1	(4)
Hypothyroidism	5	(20)
Asthma	1	(4)
Iron Overload	1	(4)
Hyperlipidemia	2	(8)
Smoke	3	(12)
	Median	Range
BMI in kilograms per m²	27	(19-50)
Heart rate in beats per minute	70	(53-98)
Systolic BP in mmHg	118	(102-160)
Diastolic BP in mmHg	74	(55-105)

Table II
Cardiovascular Biomarkers in Survivors of Hodgkin Lymphoma (N=25) Compared to CARDIA Participants*

	<u>HL Survivors</u>		<u>CARDIA Subjects</u> [#]		<u>(p)</u>
	<u>Mean</u>	<u>(SD)</u>	<u>Mean</u>	<u>(SD)</u>	
hsCRP [†] (mg/L)	3.0	(2.0)	1.6	(1.9)	0.003
Triglycerides (mg/dl)	123.1	(81.6)	95.4	(81.2)	0.09
Total cholesterol (mg/dl)	194.1	(33.2)	179.4	(32.9)	0.03
HDL [‡] (mg/dl)	54.8	(12.0)	51.0	(12.0)	0.13
Apolipoprotein B (mg/dl)	89.8	(23.2)	90.3	(23.5)	0.92
Lipoprotein (a) (mg/dl)	34.2	(17.5)	13.8	(17.5)	<0.001
Fibrinogen (mg/dl)	342.0	(49.1)	252.6	(48.4)	<0.001

* adjusted for age, gender, body mass index, and smoking status,

[†] High-sensitivity C-reactive protein,

[‡] High density lipoprotein,

[#] Caucasian CARDIA participants N=2476

Circulating Endothelial Cells (CEC) and VCAM-1 Expression in Survivors of Hodgkin Lymphoma (N=25) Compared to Controls*

Table III

	HL Survivors		Controls	
	Mean	(SD)	Mean	(SD)
CECs (cells/ml) **	2.3	(1.5)	0.34	(1.4)
VCAM-1 (%) **	51.1	(36.8)	42.3	(35.6)

* adjusted for age, gender, body mass index, and smoking status,
** normative CEC and VCAM-1 values collected from population controls (n=19)