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Accelerated atherosclerosis, hyperlipoproteinemia and insulin resistance in long-term survivors of Hodgkin lymphoma during childhood and adolescence

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Long-term survivors of Hodgkin lymphoma during childhood or adolescence (HL survivors) are at high risk of developing treatment-related late cardiovascular sequelae. In our study we evaluated the presence of modifiable cardiovascular risk factors (hypertension, hyperlipoproteinemia, hyperinsulinemia, obesity), endothelial and inflammatory markers (E-selectin, PAI-1, hs-CRP) and atherosclerotic changes in the common carotid arteries. Assessment was performed in 80 young adult Hodgkin lymphoma long-term survivors at more than 10 years after the potentially cardiovascular toxic anticancer treatment (median age at evaluation 34.7 years; range 24.1-40.9 years). The HL survivors were compared with 83 age- and gender-matched healthy volunteers. The HL survivors showed unfavorable lipid profiles compared to those of healthy controls: triglycerides ($p=0.01$), total cholesterol ($p=0.0004$), low density lipoprotein cholesterol ($p=0.005$). In HL survivors, we found a higher prevalence of hypertension ($p=0.004$) and insulin resistance – HOMA-IR ($p=0.0002$). Ultrasonographic examination of both common carotid arteries revealed a higher prevalence of atherosclerotic plaques ($p=0.0009$) and higher carotid intima-media thickness ($p<0.0001$) in HL survivors. Markers of oxidative stress (advanced oxidation protein products, oxidized low-density lipoprotein), inflammation (hs-CRP) and endothelial dysfunction (E-selectin, PAI-1) were also higher in HL survivors ($p<0.0001$, $p=0.0002$, $p=0.0031$, $p=0.0087$, $p=0.004$, respectively). Adult survivors of Hodgkin lymphoma during childhood and adolescence need closer follow-up with screening of metabolic syndrome components, unfavorable lifestyle factors and early management of these risk factors.

Key words: Hodgkin lymphoma, long-term survivors, hyperlipoproteinemia, insulin resistance, metabolic syndrome, intima-media thickness

The survival of children and adolescents with Hodgkin lymphoma has improved dramatically over the last decades. Over 90% have become current long-term survivors, who are unfortunately at increased risk of developing severe, even life-threatening sequelae many years after the end of a treatment.

Therapy-related cardiovascular disease is established as a primary cause of noncancer morbidity and early mortality in childhood cancer survivors [1, 2]. The estimated cumulative cardiovascular mortality is 5-fold higher than the expected rate in the general population [3], and many of these deaths are related to atherosclerotic cardiovascular diseases, such as stroke, myocardial infarction and other vascular diseases [4, 5].

Radiation therapy to the neck and mediastinum increases the risk of carotid artery disease and stroke in patients with HL [6, 7]. The initial event in the development of premature atherosclerotic changes is endothelial cell damage, which leads to smooth muscle cell proliferation and migration. In the general population, increased intima-media thickness (IMT) of the carotid artery, which can be measured noninvasively by ultrasound, is considered an early marker for atherosclerosis and an independent predictor of cardiovascular events [8, 9]. Exposure to chemotherapy and/or radiotherapy is also associated with the development of insulin resistance [10], lipid abnormalities and obesity [11].

The most of published data concerning insulin resistance and metabolic syndrome prevalence in childhood cancer survivors have been focused on childhood acute leukemia survivors [12–15], only few studies also included small numbers of lymphoma and solid tumor patients [16, 17]. Therefore, the aim of this study was to assess in HL survivors not only the presence of modifiable cardiovascular risk factors, endothelial and inflammatory markers but also to evaluate atherosclerotic changes in the common carotid arteries.

Patients and methods

The project was approved by the Ethics Committee of University Hospital in Motol and University Hospital Kralovske Vinohrady. All HL survivors and volunteers provided written informed consent for participation in the study.

A total of 630 children and adolescents were treated for Hodgkin lymphoma at University Hospital in Motol between February 1977 and December 2017, and approximately 350 patients attended the follow-up clinic regularly. All HL survivors attending routine follow-up visits at the Late Effects Clinic in the Department of Pediatric Hematology and Oncology were enrolled in the study between May 2015 and December 2017 if they fulfilled the following inclusion criteria: 24–40 years old and disease-free for a minimum of 10 years after the end of their treatment. HL survivors with symptomatic cardiovascular disease were not eligible for enrollment. Healthy volunteers were recruited by newspaper advertisements.

In our prospective observation study, between May 2015 and May 2018, we included eighty long-term survivors of Hodgkin lymphoma in childhood or adolescence and eighty-three age- and gender-matched healthy controls.

Clinical examination and questionnaires. During the clinical visit, a medical history was obtained, and a physical examination was performed. Anthropometric parameters – height, weight and waist circumference – were measured. Body mass index (BMI) was computed as weight in kilograms divided by height in square meters. Overweight was defined as BMI ≥25 kg/m^2 and obesity BMI ≥30 kg/m^2. The waist to hip ratio was calculated – in women, the ratio should be 0.8 or less, and in men, it should be 1.0 or less. Systolic/diastolic blood pressure (BP) was determined from the average of two measurements of resting systolic/diastolic blood pressure. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg or the use of anti-hypertension drugs at the time of the examination. Smoking status, alcohol and illicit drug abuse were ascertained on the basis of a self-reported history of cigarette smoking, illicit drug experience and usual amount of alcohol intake per week. Nonsmokers were defined as persons who had never smoked. Smokers were a combined group of past and current smokers. Other conventional cardiovascular risk factors (family history of cardiovascular diseases, diabetes and obesity, eating habits) were assessed by questionnaire. Positive family history was defined as parent or sibling with a history of myocardial infarction, stroke or sudden cardiac death before the age of 55 years in men and 65 years in women. Physical activity was assessed by the Baecke physical activity questionnaire.

The treatment details of HL survivors were obtained by retrospective analysis of their medical records – chemotherapy regimen with cumulative doses of all chemotherapeutic agents, dose and fields of radiation therapy were recorded.

Ultrasonographic examination. High-resolution B-mode ECG-gated duplex ultrasonography of both common carotid arteries was performed in the supine position after 10 minutes of rest. All measurements were performed by one qualified sonographer to avoid interobserver variability. The sonographer was not aware of the subject´s medical history. The extracranial carotid arteries were examined bilaterally using a Philips iU22 ultrasound system equipped with a 7.5 MHz linear array transducer and analyzed by QLAB Quantification software (Philips). Both common carotid arteries were examined along their full visible length for the eventual presence of plaques. The IMT was measured in diastole on the far wall of both common carotid arteries at a distance of 10 mm from the carotid bulb [18], and the pulsatility index and resistance index obtained from the Doppler examination were recorded. In addition, parameters of arterial elasticity (carotid artery compliance – CAC, stiffness index β – SI, and Young´s elastic modulus YEM) were calculated [19, 20].

Transverse scanning was performed to measure the maximum visceral fat thickness (VFT) and subcutaneous fat thickness (SFT) using a 3.5-MHz abdominal transducer. The measurements were carried out at 1 cm above the umbilicus. VFT was defined as the distance between the anterior wall of the aorta and the internal face of the rectoabdominal muscle perpendicular to the aorta. SFT was measured at the same location and was defined as the distance between the cutaneous surface and linea alba. Images were obtained immediately after a calm and complete expiration to avoid the influence of respiratory status or abdominal wall tension [21]. All ultrasound measurements were performed three times, and the results were averaged.

Laboratory measurements. Overnight fasting blood samples were collected from every HL survivor and volunteer to perform biochemical analyses, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG). LDL-C was calculated by the Friedewald equation, and other parameters were measured by standard automated diagnostic laboratory methods. Dyslipidemia was determined by the presence of at least one of three factors: high LDL-C (>3.0 mmol/l), high TG (>1.7 mmol/l) and low HDL-C (<1.0 mol/l).

The homeostasis model assessment insulin resistance (HOMA-IR) index was calculated with fasting insulin and glucose values using the equation HOMA-IR = [fasting insulin (μU/ml) * fasting glucose (mmol/l)] / 22.5. Hyperinsulinemia was defined as fasting insulin levels of 20 μU/ml or greater.

Markers of endothelial dysfunction – E-selectin and plasminogen activator inhibitor-1 (PAI-1) – were measured by ELISA by using a R&D Systems ELISA kit (BioTechne brand, Minneapolis, Minnesota, USA). Advanced oxidation protein products (AOPP) were measured by using a Cloud-Clone Corp. ELISA kit (Katy, Texas, USA), and oxidized

low-density lipoprotein (oxLDL) was measured by using a Mercodia ELISA kit (Uppsala, Sweden).

Metabolic syndrome was defined according to the Modified Adult Treatment Panel III (ATP III) criteria [22] by three or more of the following components: 1. waist circumference ≥90 cm in men or ≥85 cm in women; 2. triglyceride level ≥150 mg/dl (≥1.7 mmol/l); 3. HDL-C level ≤40 mg/dl (≤1.0 mmol/l) in men or ≤50 mg/dl in women (≤1.3 mmol/l); 4. blood pressure ≥130/85 mmHg; and 5. fasting glucose level ≥100 mg/dl (≥5.6 mmol/l).

Statistical analysis. Continuous variables are expressed as the means±standard deviation and compared using the t-test if normally distributed. Continuous variables in skewed distribution are expressed as the median (interquartile range) and compared by the Mann-Whitney (Wilcoxon) test. Categorical variables are expressed as proportions (%) and analyzed using cross-tabulation and the χ^2 test or the Fischer exact test where appropriate. Statistical significance was defined as a p-value less than 0.05. Statistical analyses were performed using R software version 3.4.4 (R Core Team, 2018).

Results

The clinical characteristics of HL survivors and healthy volunteers are shown in Table 1. The median age of the HL survivors at the time of Hodgkin's lymphoma diagnosis was 14.9 years (range, 4.5–19.3 years), and the median time of follow-up was 19.2 years (range, 10–34.6 years). The chemotherapy regimens differed according to the period of treatment; 12.5% of patients received more than one regimen for refractory/relapsed HL. All patients with HL received external beam photon radiotherapy as an essential part of their treatment. All HL survivors, except one, were irradiated to the neck, fifty-one HL survivors were irradiated to the mediastinum and twenty-nine HL survivors received radiotherapy to the retroperitoneal lymph nodes. The doses ranged from 15–40 Gy in the first-line treatment; in refractory/relapsed HL patients, reirradiation was applied at a total dose 55–70 Gy to the site of relapse/refractory disease. Radiotherapy fields varied from mantle field to involved field radiotherapy.

The comparison of the characteristics between the included HL survivors and those who were eligible but not included is presented in Table 2.

Hypertension was documented in thirteen HL survivors (16.25%); five of these survivors had regularly received anti-hypertension drugs. Additionally, seven HL survivors (8.75%) on anti-hypertension medication had systolic and diastolic pressure values within normal limits at the time of examination. Hypertension was newly detected in seven (8.4%) healthy volunteers, and none of the healthy volunteers had been previously diagnosed or treated for hypertension. We found an increased heart rate in HL survivors compared to that in healthy volunteers (76.1±8.48/min vs. 70.0±7.9/min, p<0.0001).

The mean fasting insulin concentrations in HL survivors were approximately twice those of healthy controls (12.56±9.32 μU/ml vs. 6.86±3.27 μU/ml, p<0.0001). In seven HL survivors, the insulin levels were above the arbitrary cut-off for hyperinsulinemia of 20 μU/ml, whereas no hyperinsulinemia was detected in healthy volunteers (p=0.0059). Hyperglycemia was detected in twenty HL survivors and only two healthy volunteers (5.38±1.04 mmol/l vs. 4.9±0.35 mmol/l, p<0.0001). HOMA-IR was also significantly higher in HL survivors (3.24±3.87 vs. 1.51±0.79, p=0.0002).

The lipid profiles of HL survivors were unfavorable compared to those of healthy controls (TG 1.48±1.19 mmol/l vs. 1.09±0.58 mmol/l, p=0.01; TC 5.20±1.08 mmol/l vs. 4.67±0.74 mmol/l, p=0.0004; LDL-C 3.03±0.92 mmol/l

Table 1. Clinical characteristics of HL survivors and healthy volunteers.

	HL survivors n = 80	Healthy volunteers n = 83	p-value
Age, years, mean (SD)	33.87±4.13	33.22±4.34	0.3278
Age, years, median (range)	34.7 (24.1-40.9)	33.33 (24-40.3)	
Gender, male, n (%)	45 (57.2)	50 (60.2)	0.72
BMI, mean (SD)	25.20±5.43	24.60±4.20	0.4355
Overweight, n (%)	23 (28.8)	23 (27.7)	
Obesity, n (%)	8 (10)	9 (10.8)	
Waist circumference, cm, mean (SD)	83.8±14.74	86.3±12.73	0.4599
Waist to hip ratio, mean (SD)	0.87±0.07	0.85±0.09	0.088
Waist to height ratio, mean (SD)	0.49±0.07	0.47±0.06	0.11
Visceral fat thickness, cm, mean (SD)	4.09±1.99	3.76±1.47	0.2357
Subcutaneous fat thickness, cm, mean (SD)	2.01±1.11	1.88±1.02	0.4489
Systolic blood pressure, mm Hg, mean (SD)	123.5±12.8	120.8±12.5	0.2441
Diastolic blood pressure, mm Hg, mean (SD)	77.2±10.7	76.2±8.8	0.5296
Heart rate, per minute, mean (SD)	76.1±8.48	70.0±7.9	<0.0001

vs. 2.57±0.67 mmol/l, p=0.005). HDL-C levels did not differ between HL survivors and healthy volunteers (1.54±0.52 mmol/l vs. 1.60±0.42 mmol/l, p=0.3429). The relative rate of subjects with isolated hypertriglyceridemia (p=0.943) and isolated hypercholesterolemia (p=0.399) did not differ between HL survivors and healthy volunteers, but combined hyperlipoproteinemia was found in 16.25% of HL survivors and only in 4.8% of healthy volunteers (p=0.017).

AOPP levels in HL survivors were three times higher compared to those in healthy volunteers (23.71±13.78 μg/ml vs. 8.00±3.46 μg/ml, p<0.0001). OxLDL levels were also higher in HL survivors (71.93±29.50 U/l vs. 56.88±19.41 U/l, p=0.0002).

The mean PAI-1 levels were higher in HL survivors than in healthy controls (5.52±3.99 ng/ml vs. 3.83±3.30 ng/ml, p=0.004). E-selectin levels were also higher in HL survivors (36.19±16.95 ng/ml vs. 30.12±11.24 ng/ml, p=0.0087).

In our study, the prevalence of metabolic syndrome as defined by ATP III was almost three times higher in HL survivors (21.3% of HL survivors vs. 7.2% of healthy volunteers, p=0.01). In HL survivors and volunteers with metabolic syndrome, we found higher levels of AOPP (19.98±13.98 μg/ml vs. 15.04±12.38 μg/ml, p=0.051) and PAI-1 (7.56±5.05 ng/ml vs. 4.20±3.29 ng/ml, p=0.0007) compared with those without metabolic syndrome in both cohorts. The prevalence of cardiovascular risk factors is presented in Table 3.

We also found a significant difference in the median high-sensitivity C-reactive protein (hs-CRP) of HL survivors at 1,308 (interquartile range 740.7–3,016.7) μg/ml compared to that in healthy volunteers at 746.7 (336.4–1,589.7) μg/ml (p=0.0031). The laboratory parameters are summarized in Table 4.

In our study, the prevalence of subclinical thyroid dysfunction among HL survivors was very high, and 82.5% of these individuals had been undergoing long-term substitution with thyroid hormones. None of the HL survivors had TSH levels above 10 mIU/l. We also did not show any significant correlation of TSH or free T4 levels with TC, LDL-C and TG levels or HOMA-IR.

Elevated follicle-stimulating hormone (FSH) levels above 18.1 IU/l were found in fourteen men (31.1% of male HL survivors). In three women, an irregular menstrual cycle was recorded (two of these women received systemic hormonal replacement therapy for this reason). Decreased levels of anti-Müllerian hormone (AMH) below the third percentile were found in nine women (25.7% of HL female survivors). In our study, we did not show any correlation of FSH or AMH levels with TC, LDL-C, HDL-C and TG. We did not find increased TG or LDL-C levels in male HL survivors with

Table 2. Description of HL survivors study cohort and comparison with eligible but not included HL survivors.

	Study HL survivors n=80	Eligible but not included HL survivors n=183
Age at diagnosis, years, median (range)	14.9 (4.5-19.3)	14.1 (2.2-19.3)
Age, years, median (range)	34.7 (24.1-40.9)	33.0 (25.0-40.9)
Gender, male, n (%)	45 (57.2)	106 (57.9)
Histology subtype		
NLPHL, n (%)	7 (8.8)	16 (8.7)
Nodular sclerosis, n (%)	45 (56.2)	101 (55.2%)
Mixed cellularity, n (%)	20 (25)	49 (26.8)
Lymphocyte rich, n (%)	2 (2.5)	3 (1.6)
Not specific, n (%)	6 (7.5)	14 (7.7)
Stage according to Ann Arbor		
Stage I, n (%)	17 (21.3)	41 (22.4)
Stage II, n (%)	31 (38.7)	74 (40.4)
Stage III, n (%)	22 (27.5)	45 (24.6)
Stage IV, n (%)	10 (12.5)	23 (12.6)
Chemotherapy regimen		
CVPP, n (%)	10 (12.5)	17 (9.3)
ABVD, n (%)	10 (12.5)	24 (13.1)
CVPP/ABVD, n (%)	15 (18.8)	24 (13.1)
VEPA, n (%)	5 (6.3)	6 (3.3)
VAMP, n (%)	7 (8.8)	20 (10.9)
DBVE, n (%)	11 (13.8)	21 (11.5)
DBVE-PC, n (%)	12 (15)	65 (35.5)
multiple regimens, n (%)	10 (12.5)	15 (8.2)

Table 3. Prevalence of cardiovascular risk factors in HL survivors and healthy volunteers.

	HL survivors n (%)	Healthy volunteers n (%)	p-value
Hypertension	20 (25)	7 (8.4)	0.004
Hypertriglyceridemia	20 (25)	11 (13.3)	0.056
Decreased HDL-cholesterol	14 (17.5)	11 (13.3)	0.452
Hyperglycemia	20 (25)	2 (2.4)	< 0.0001
Increased waist circumference	33 (41.3)	31 (37.3)	0.610
Presence of three or more metabolic syndrome components	17 (21.3)	6 (7.2)	0.01
Smoking, current smokers, n (%)	12 (15.0)	17 (20.5)	0.0846
Smoking, past smokers, n (%)	9 (11.3)	18 (21.7)	
Alcohol abuse, abstainers, n (%)	56 (70)	62 (74.7)	0.782
Physical activity (Baecke), mean (SD)	7.97±1.24	8.34±1.35	0.069
Family history of early onset CVD, n (%)	17 (21.3)	18 (21.7)	0.937
Family history of diabetes, n (%)	15 (18.8)	11 (13.3)	0.4772
Family history of hypertension, n (%)	43 (53.8)	37 (44.6)	0.3467
Family history of obesity, n (%)	31 (38.8)	24 (28.9)	0.2678

Table 4. Summary of laboratory findings.

	HL survivors n=80	Healthy volunteers n=83	p-value
Glycemia, mmol/l (SD)	5.38±1.04	4.9±0.35	0.0002
Insulin, μU/l (SD)	12.56±9.32	6.86±3.27	<0.0001
HOMA IR (SD)	3.24±3.87	1.51±0.79	0.0002
Total cholesterol, mmol/l (SD)	5.2±1.07	4.67±0.7	0.0004
LDL-C, mmol/l (SD)	3.03±0.92	2.57±0.67	0.0005
HDL-C, mmol/l (SD)	1.53±0.51	1.60±0.42	0.3429
TG, mmol/l (SD)	1.48±1.19	1.09±0.58	0.0101
AOPP, μg/ml (SD)	23.71±13.78	8.00±3.46	<0.0001
oxLDL, U/l (SD)	71.93±29.50	56.88±19.41	0.0002
PAI-1, ng/ml (SD)	5.52±3.99	3.83±3.30	0.004
E-selectin, ng/ml (SD)	36.19±16.95	30.12±11.24	0.0087
hs-CRP, ug/ml (interquartile range)	1,308 (740.7–3,016.7)	746.7 (336.4–1,589.7)	0.0031

testosterone levels less than 10 nmol/l compared to those in male HL survivors with normal testosterone levels, but HOMA-IR was significantly higher in male HL survivors with testosterone levels below 10 nmol/l (p=0.0172).

Ultrasonographic findings on common carotid arteries are presented in Table 5. HL survivors showed higher IMT (5.44±0.89 vs. 4.74±0.66, p<0.01) as well as increased arterial stiffness – higher YEM (1,044.27±612.67 vs. 779.00±297.36, p=0.0007), higher SI (5.57±2.68 vs. 3.76±1.48, p<0.0001) and lower CAC (0.0022±0.0009 vs. 0.003±0.0012, p<0.0001). In our study, 12.5% of HL survivors had plaques in common carotid artery, whereas no carotid plaques were found in healthy volunteers (p=0.0009). The correlation between YEM and the radiotherapy dose administered to the neck was not statistically significant (p=0.0778). We found correlation of YEM with systolic blood pressure (p=0.0021) and lipid parameters – TC, TG, LDL-C (p=0.0012, p=0.0062 and p=0.0088, respectively). In our study, IMT was significantly correlated with visceral obesity (waist circumference, p=0.0002, visceral fat thickness, p=0.003) and insulin resistance (HOMA-IR, p<0.0001, glycemia, p<0.0001).

Discussion

In this study, we demonstrate a higher prevalence of modifiable cardiovascular risk factors, namely hyperlipoproteinemia and metabolic syndrome, as well as atherosclerotic changes in carotid arteries among young adult survivors of Hodgkin lymphoma during childhood or adolescence.

In the general population, the contribution of modifiable risk factors to the development of cardiovascular disease is well defined [23]. In childhood cancer survivors, several

Table 5. Carotid ultrasound findings.

	HL survivors n=80	Healthy volunteers n=83	p-value
Intima-media thickness, mm, mean (SD)	5.44±0.89	4.74±0.66	<0.0001
Young´s elastic modulus, mean (SD)	1,044.27±612.67	779.00±297.36	0.0007
CAC, mean (SD)	0.0022±0.0009	0.003±0.0012	<0.0001
SI, mean (SD)	5.57±2.68	3.76±1.48	<0.0001
Atherosclerotic plaques, n (%)	10 (12.5)	0 (0)	0.0009

conditions increase the prevalence of some cardiovascular risk factors. Neoplasms likely cause changes in insulin sensitivity, lipid metabolism and inflammatory mediators [24]. Prolonged catabolism and malnutrition during the treatment of childhood cancer may induce changes in body composition with abnormal fat distribution and lower lean body mass. Specific cancer therapies may also contribute to these changes in several ways: by damaging the endocrine organs, by inducing endothelial and adipose tissue dysfunction [25]. Irradiation has been shown to alter mitochondrial function [26] by generating reactive oxygen species. Subsequent abnormalities in muscle, liver and pancreas play a role in the development of insulin resistance, which is considered to be the central pathophysiological mechanism of metabolic syndrome [27]. Insulin resistance promotes atherogenesis from the early stages to advanced plaque progression by the involvement of systemic factors, such as dyslipidemia, hypertension and proinflammatory state, as well as local factors in the arterial wall [28]. In our study, in four HL survivors among seventeen with metabolic syndrome, atherosclerotic plaques were found by ultrasonographic examination of the carotid arteries. Nevertheless, plaques were also found in another six HL survivors without metabolic syndrome. The development of insulin resistance is more strongly associated with abdominal adiposity than with increased BMI [29, 30]. However, in our study, we did not observe any difference in BMI, visceral adiposity or waist circumference between HL survivors and healthy volunteers, and the prevalence of overweight and obesity was the same in both cohorts.

The overt hypothyroidism adversely affects serum lipid levels, can lower basal metabolism and induce weight gain, which can lead to metabolic syndrome [31]. Severe subclinical hypothyroidism, with thyroid stimulating hormone (TSH) levels of 10 mIU/l and greater, have been associated with an increased cardiovascular risk and exacerbation of carotid atherosclerosis [32–34], however none of HL survivors in our study exceeded these levels. The effect of subtle subclinical thyroid dysfunction on vascular atherosclerosis remains uncertain [35–37]. Hypogonadism, mainly as a consequence of gonadal damage by alkylating drugs, procarbazine or platinum compounds, is associated with visceral obesity, insulin resistance, dyslipidemia and changes in vascular tone and blood pressure [38]. In our study, we did not show any correlation of FSH or AMH levels with lipid levels, but in male HL survivors with testosterone levels below 10 nmol/l HOMA-IR was significantly higher compared to male HL survivors with normal testosterone levels. Hypertension has been previously reported in 13–19% adult survivors of childhood malignancies [16, 39]. In our study, we documented hypertension in 25% of HL survivors. Radiotherapy to the neck or mediastinum can alter blood pressure or vascular tonus by influencing baroreceptors in the glomus caroticum or in the aortic arch. Abdominal radiotherapy is also associated with an increased risk of hypertension due to induced renal artery stenosis or radiation nephropathy [40], also treatment with some anticancer medications can directly cause renal damage (ifosfamide, cisplatin used mainly in HL salvage therapy), leading to hypertension [41]. The increased heart rate in HL survivors in our study can be explained by increased sympathetic nervous activity.

In our study, compared to healthy volunteers, HL survivors had unfavorable lipid profiles and a higher prevalence of metabolic syndrome. We found no association of lipid parameters or insulin resistance with a given chemotherapy regimen. We report a high rate of carotid artery plaques in HL survivors together with increased carotid IMT and markers of arterial stiffness. In a published study of childhood cancer survivors who received radiotherapy as an integral part of their treatment, increased carotid IMT and higher PAI-1 levels were also found [42].

Hyperinsulinemia could, by its effects on antioxidative enzymes and on free radical generators, enhance oxidative stress, and through the increased bioavailability of insulin-like growth factor-1 could also promote carcinogenesis [43]. We found significantly higher levels of AOPP and oxLDL in HL survivors. Significantly higher concentrations of AOPP are observed in metabolic syndrome [44, 45]. HL survivors and volunteers with metabolic syndrome had higher levels of AOPP (and PAI-1 compared to those without metabolic syndrome in both cohorts. We cannot conclude the etiology of the elevated AOPP concentration, as we do not have any previous information about insulin, AOPP and oxLDL levels in our HL survivors before enrolment into the study. Prospective studies will be necessary to clarify whether enhanced oxidative stress is the predisposition to metabolic syndrome in this specific population or one of its multiple manifestations.

In our study, HL survivors showed significantly higher hs-CRP than controls. Hyperlipoproteinemia and insulin resistance in HL survivors could be a result of chronic inflammation, as observed in adult survivors of other hematologic malignancies [46, 47]. Inflammation and oxidative stress are closely related, and one can be induced by the other. Reactive species, by activation of NFkB, can enhance the expression of genes involved in inflammation [48]; on the other hand, reactive oxygen and nitrogen species are produced by inflammatory cells [49, 50].

Compared to healthy volunteers, HL survivors suffered more endothelial damage as measured by the endothelial marker PAI-1 and E-selectin. PAI-1 has been implicated in the control of insulin signaling in adipocytes but also in angiogenesis and atherogenesis [51]. In our study, we confirmed a strong association between PAI-1 and visceral adiposity ($p<0.0001$) [52] and an association with carotid intima-media thickness ($p=0.012$) [53]. We found no association between the given chemotherapy regimen and vascular damage. In our study, we could not determine whether insulin resistance is a cause or consequence of endothelial dysfunction.

In HL survivors as well as in the general population, modifiable risk factors related to aging, hereditary predisposition and unhealthy lifestyle behaviors also play important roles. We did not find any difference between HL survivors and healthy volunteers in family history of early onset of cardiovascular disease, hypertension, diabetes or obesity. Regarding lifestyle parameters, there was no significant difference in smoking status, physical activity or eating habits between the groups. Few limitations of this study should be considered when interpreting the findings. First, smoking, physical activity levels, eating habits and family history were assessed using self-reported data, which is certainly subject to bias and imprecision. Our findings reflect therapy in the 1970s, 1980s and 1990s. Another limitation is the small number of included HL survivors, but only multicentric studies could recruit a larger cohort. Despite the fact that the comparison of the reported group of Hodgkin lymphoma survivors with the nonincluded HL survivors of the same age did not show any significant difference, we cannot elucidate a larger proportion of the nonincluded patients treated in the late 1990s with the DBVE/DBVE-PC regimen and involved field radiotherapy. Additionally, selection bias may play a role, although in both cohorts, only persons interested in their health status participated in the current study. The strengths of our study are the long follow-up of HL survivors and the assessment of modifiable cardiovascular risk factors together with vascular structure and markers of oxidative stress, inflammation and endothelial damage.

In view of the excellent prognosis of Hodgkin lymphoma in HL survivors, the impact of vascular damage and metabolic abnormalities caused by cancer treatment on the lifetime risk of cardiovascular morbidity has become increasingly important. Exercise may suppress inflammatory responses and improve insulin sensitivity and antioxidant activity [54–56]. Therefore, HL survivors with cardiovascular risk factors or unfavorable lifestyle factors should receive early support for lifestyle improvement and optimal management of these cardiovascular risk factors together with adequate hormonal substitution in thyroid and gonadal deficiencies. When these interventions (optimization of energy balance by increasing physical activity and reducing caloric intake) are insufficient, drug therapy is necessary. Since LDL-C represents the primary goal in hypolipidemic therapy, statins should be considered as drug of first choice. The majority of adult HL survivors receive their follow-up care from primary care physicians rather than from long-term follow-up specialists at cancer centers, therefore the general awareness of the surveillance recommendations and appropriate interventions has to be increased.

More research is needed to define how the modification of these factors affects the progression of atherosclerotic changes and the manifestation of cardiovascular events to identify individuals most likely to benefit from intervention strategies.

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References

[1] MERTENS AC, LIU Q, NEGLIA JP, WASILEWSKI K, LEISENRING W 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. https://doi.org/10.1093/jnci/djn310

[2] ARMSTRONG GT, LIU Q, YASUI Y, NEGLIA JP, LEISENRING W et al. Late mortality among 5-year survivors of childhood cancer: a summary from the Childhood Cancer Survivor Study. J Clin Oncol 2009; 27: 2328–2338. https://doi.org/10.1200/JCO.2008.21.1425

[3] TUKENOVA M, GUIBOUT C, OBERLINE O, DOYON F, MOUSANNIF A et al. Role of cancer treatment in long-term overall and cardiovascular mortality after childhood cancer. J Clin Oncol 2010; 28: 1308–1315. https://doi.org/10.1200/JCO.2008.20.2267

[4] MULROONEY DA, YEAZEL MW, KAWASHIMA T, MERTENS AC, MITBY P et al. Cardiac outcomes in a cohort of adult survivors of childhood cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. BMJ 2009; 339: b4606. https://doi.org/10.1136/bmj.b4606

[5] LIPSHULTZ SE, ADAMS MJ, COLAN SD, CONSTINE LS, HERMAN EH et al. Long-term cardiovascular toxicity in children, adolescents, and young adults who receive cancer therapy: pathophysiology, course, monitoring, management, prevention, and research directions: a scientific statement from the American Heart Association. Circulation 2013; 128: 1927–1995. https://doi.org/10.1161/CIR.0b013e3182a88099

[6] DE BRUIN ML, DORRESTEIJN LD, VAN´T VEER MB, KROL AD, VAN DER PAL HJ et al. Increased risk of ischemic stroke and transient ischemic attack in 5-year survivors of Hodgkin lymphoma. J Natl Cancer Inst 2009; 101: 928–937. https://doi.org/10.1093/jnci/djp147

[7] BOWERS DC, LIU Y, LEISENRING W, YASUI Y, STOVALL M 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. https://doi.org/10.1200/JCO.2005.15.107

[8] LORENTZ MW, MARKUS HS, BOTS ML, ROSVALL M, SITZER M. Prediction of clinical cardiovascular events with carotid intima-media thickness: A systematic review and meta-analysis. Circulation 2007; 115: 459–467. https://doi.org/10.1161/CIRCULATIONAHA.106.628875

[9] POLAK JF, PENCINA MJ, PENCINA KM, O'DONNELL CJ, WOLF PA et al. Carotid-Wall Intima-Media Thickeness and Cardiovascular Events, N Engl J Med 2011; 365: 213–221. https://doi.org/10.1056/NEJMoa1012592

[10] NUVER J, SMIT AJ, POSTMA A, SLEIJFER DT, GIETEMA JA. The metabolic syndrome in long-term cancer survivors, an important target for secondary preventive measures. Cancer Treat Rev. 2002; 28: 195–214. https://doi.org/10.1016/S0305-7372(02)00038-5

[11] OEFFINGER KC, MERTENS AC, SKLAR CA, YASUI Y, FEARS T et al. Obesity in adult survivors of childhoo acute lymphoblastic leukemia: a report from the childhood cancer survivor study. J Clin Oncol 2003; 17: 1359–1365. https://doi.org/10.1200/JCO.2003.06.131

[12] OEFFINGER K, ADAMS-HUET B, VICTOR R, CHURCH TS, SNELL PG et al. Insulin resistance and risk factors for cardiovascular disease in young adult survivors of childhood acute lymphoblastic leukemia. J Clin Oncol 2009; 27: 3698–3704. https://doi.org/10.1200/JCO.2008.19.7251

[13] GURNEY J, NESS K, SIBLEY S, O'LEARY M, DENGEL DR et al. Metabolic syndrome and growth hormone deficiency in adult survivors of childhood acute lymphoblastic leukemia. Cancer 2006; 107: 1303–1312. https://doi.org/10.1002/cncr.22120

[14] STEINBERGER J, SINAIKO AR, KELLY AS, LEISENRING WM, STEFFEN LM et al. Cardiovascular risk and insulin resistance in childhood cancer survivors. J Pediatr 2012; 160: 494–499. https://doi.org/10.1016/j.jpeds.2011.08.018

[15] SAULTIER P, AUQUIER P, BERTRAND Y, VERCASSON C, OUDIN C et al. Metabolic syndrome in long-term survivors of childhood acute leukemia treated without hematopoietic stem cell transplantation: an L.E.A. study. Haematologica 2016; 101: 1603–1610. https://doi.org/10.3324/haematol.2016.148908

[16] NEVILLE KA, COHN RJ, STEINBECK KS, JOHNSTON K, WALKER JL. Hyperinsulinemia, impaired glucose tolerance, and diabetes mellitus in survivors of childhood cancer: prevalence and risk factors. J Clin Endocrinol Metab 2006; 91: 4401–4407. https://doi.org/10.1210/jc.2006-0128

[17] TALVENSAARI KK, LANNING M, TAPANAINEN P, KNIP M. Long-term survivors of childhood cancer have an increased risk of manifesting the metabolic syndrome. J Clin Endocrinol Metab 1996; 81: 3051–3055. https://doi.org/10.1210/jcem.81.8.8768873

[18] STEIN JH, KORCARZ CE, HURST RT, LONN E, KENDALL CB et al. Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American society of echocardiography carotid intima-media thickness task force. Endorsed by the society for vascular medicine. J Am Soc Echocardiogr 2008; 21: 93–111. https://doi.org/10.1016/j.echo.2007.11.011

[19] CAVALLINI MC, ROMAN MJ, BLANK SG, PINI R, PICKERING TG et al. Association of the auscultatory gap with vascular disease in hypertensive patients. Ann Intern Med 1996; 124: 877–883. https://doi.org/10.7326/0003-4819-124-10-199605150-00003

[20] MITSUMURA H, SAKUTA K, BONO K, YAMAZAKI M, SENGOKU R et al. Stiffness parameter β of cardioembolism measured by carotid ultrasound was lower than other stroke subtypes. J Stroke Cerebrovasc Dis 2014; 23: 1391–1395. https://doi.org/10.1016/j.jstrokecerebrovasdis.2013.11.015

[21] DE LUCIA ROLFE E, SLEIGH A, FINUCANE FM, BRAGE S, STOLK RP et al. Ultrasound measurements of visceral and subcutaneous abdominal thickness to predict abdominal adiposity among older men and women. Obesity (Silver Spring) 2010; 18: 625–631. https://doi.org/10.1038/oby.2009.309

[22] NATIONAL CHOLESTEROL EDUCATION PROGRAM (NCEP) EXPERT PANEL ON DETECTION, EVALUATION, AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS (ADULT TREATMENT PANEL III). Third report of the National Cholesterol Education Program (NCEP) expert panel on the detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002; 106: 3143–3421.

[23] BERRY JD, DYER A, CAI X, GARSIDE DB, NING H et al. Lifetime risks of cardiovascular disease. N Engl J Med 2012; 366: 321–329. https://doi.org/10.1056/NEJMoa1012848

[24] SAINI A, AL-SHANTI N, STEWART CE. Waste management – cytokines, growth factors and cachexia. Cytokine Growth Factor Rev 2006; 17: 475–486. https://doi.org/10.1016/j.cytogfr.2006.09.006

[25] KAVEY RE, ALLADA V, DANIELS SR, HAYMAN LL, MCCRINDLE BW et al. Cardiovascular risk reduction in high-risk pediatric patients: a scientific statement from the American Heart Association Expert Panel on Population and Prevention Science; the Councils on Cardiovascular Disease in the Young, Epidemiology and Prevention, Nutrition, Physical Activity and Metabolism, High Blood Pressure Research, Cardiovascular Nursing, and the Kidney in Heart Disease; and the Interdisciplinary Working Group on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. Circulation 2006; 114: 2710–2738. https://doi.org/10.1161/CIRCULATIONAHA.106.179568

[26] KAWAMURA S, TAKAI D, WATANABE K, HAYASHI J, HAYAKAWA K et al. Role of mitochondrial DNA in cells exposed to irradiation: Generation of reactive oxygen species (ROS) is required for G2 checkpoint upon irradiation. J Health Sci 2005; 51: 385–393. https://doi.org/10.1248/jhs.51.385

[27] SARTI C, GALLAGHER J. The metabolic syndrome: prevalence, CHD risk, and treatment. J Diabetes Compl 2006; 20: 121–132. https://doi.org/10.1016/j.jdiacomp.2005.06.014

[28] BORNFELDT KE, TABAS I. Insulin resistance, hyperglycemia, and atherosclerosis. Cell Metab 2011; 14: 575–585. https://doi.org/10.1016/j.cmet.2011.07.015

[29] WAJCHENBERG BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000; 21: 697–738. https://doi.org/10.1210/edrv.21.6.0415

[30] VEGA GL, ADAMS-HUET B, PESHOCK R, WILLETT D, SHAH B et al. Influence of body fat content and distribution on variation in metabolic risk. J Clin Endocrinol Metab 2006; 91: 4459–4466. https://doi.org/10.1210/jc.2006-0814

[31] BIONDI B, KLEIN I. Hypothyroidism as a risk factor for cardiovascular disease. Endocrine 2004; 24: 1–13. https://doi.org/10.1385/ENDO:24:1:001

[32] RODONDI N, DEN ELZEN WP, BAUER DC, CAPPOLA AR, RAZVI S et al. Subclinical hypothyroidism and the risk of coronary heart disease and mortality. JAMA 2010; 304: 1365–1374. https://doi.org/10.1001/jama.2010.1361

[33] SUN J, YAO L, FANG Y, YANG R, CHEN Y et al. Relationship between subclinical thyroid dysfunction and the risk of cardiovascular outcomes: A systematic review and meta-analysis of prospective cohort studies. Int J Endocrinol 2017; 2017: 8130796. https://doi.org/10.1155/2017/8130796

[34] FLORIANI C, GENCER B, COLLET TH, RODONDI N. Subclinical thyroid dysfunction and cardiovascular diseases: 2016 update. Eur Heart J 2018; 39: 503–507. https://doi.org/10.1093/eurheartj/ehx050

[35] DELITALA AP, FILIGHEDDU F, ORRU M, ALGHATRIF M, STERI M et al. No evidence of association between subclinical thyroid disorders and common carotid intima medial thickness or atherosclerotic plaque. Nutr Metab Cardiovasc Dis 2015; 25: 1104–1110. https://doi.org/10.1016/j.numecd.2015.09.001

[36] KIM H, KIM TH, KIM HI, PARK SY, KIM YN et al. Subclinical thyroid dysfunction and risk of carotid atherosclerosis. PLoS One 2017; 12: e0182090. https://doi.org/10.1371/journal.pone.0182090

[37] ROOS A, BAKKER SJ, LINKS TP, GANS RO, WOLFFENBUTTEL BH. Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. J Clin Endocrinol Metab 2007; 92: 491–496. https://doi.org/10.1210/jc.2006-1718

[38] RUTTER MM, ROSE SR. Long-term endocrine sequelae of childhood cancer. Curr Opin Pediatr 2007; 19: 480–487. https://doi.org/10.1097/MOP.0b013e3282058b56

[39] HADDY TB, MOSHER RB, REAMAN GH. Hypertension and prehypertension in long-term survivors of childhood and adolescent cancer. Pediatr Blood Cancer 2007; 49: 79–83. https://doi.org/10.1002/pbc.20886

[40] JONES DP, SPUNT SL, GREEN D, SPRINGATE JE. Renal late effects in patients treated for cancer in childhood: a report from the Children's Oncology Group. Pediatr Blood Cancer. 2008; 51:724–31. https://doi.org/10.1002/pbc.21695

[41] MEACHAM LR, CHOW EJ, NESS KK, KAMDAR KY, CHEN Y et al. Cardiovascular risk factors in adult survivors of pediatric cancer--a report from the childhood cancer survivor study. Cancer Epidemiol Biomarkers Prev 2010; 19: 170–181. https://doi.org/10.1158/1055-9965.EPI-09-0555

[42] BROUWER CAJ, POSTMA A, HOOIMEIJER HLH, SMIT AJ, VONK JM et al. Endothelial damage in long-term survivors of childhood cancer. J Clin Oncol 2013; 31: 3906–3913. https://doi.org/10.1200/JCO.2012.46.6086

[43] FACCHINI FS, HUA NW, REAVEN GM, STOOHS RA. Hyperinsulinemia: the missing link among oxidative stress and age-related diseases? Free Radic Biol Med 2000; 29: 1302–1306. https://doi.org/10.1016/S0891-5849(00)00438-X

[44] ROBERTS CK, SINDHU KK. Oxidative stress and metabolic syndrome. Life Sci 2009; 84: 705–712. https://doi.org/10.1016/j.lfs.2009.02.026

[45] HOPPS E, NOTO D, CAIMI G, AVERNA MR. A novel component of the metabolic syndrome: The oxidative stress. Nutr Metab Cardiovasc Dis 2010; 20: 72–77. https://doi.org/10.1016/j.numecd.2009.06.002

[46] SULICKA J, SURDACKI A, MIKOLAJCZYK T, STRACH M, GRYGLEWSKA B et al. Elevated markers of inflammation and endothelial activation and increased counts of intermediate monocytes in adult survivors of childhood acute lymphoblastic leukemia. Immunobiology 2013; 218: 810–816. https://doi.org/10.1016/j.imbio.2012.09.003

[47] ARIFFIN H, AZANAN MS, ABD GHAFAR SS, OH L, LAU KH et al. Young adult survivors of childhood acute lymphoblastic leukemia show evidence of chronic inflammation and cellular aging. Cancer 2017; 123: 4207–4214. https://doi.org/10.1002/cncr.30857

[48] SIES H, BERNDT C, JONES DP. Oxidative stress. Annu Rev Biochem 2017; 86: 715–748. https://doi.org/10.1146/annurev-biochem-061516-045037

[49] ANDERSON MT, STAAL FJT, GITLER C, HERZENBERG LA, HERZENBERG LA. Separation of oxidant-initiated and redox-regulated steps in the NF-κB signal transduction pathway. Proc Natl Acad Sci U S A 1994; 91: 11527–11531. https://doi.org/10.1073/pnas.91.24.11527

[50] KHOVIDHUNKIT W, KIM MS, MEMON RA, SHIGENAGA JK, MOSER AH et al. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. J Lipid Res 2004; 45: 1169–1196. https://doi.org/10.1194/jlr.R300019-JLR200

[51] SCHAFER K, MULLER K, HECKE A, MOURNIER E, GOEBEL J et al. Enhanced thrombosis in atherosclerosis-prone mice is associated with increased arterial expression of plasminogen activator inhibitor-1. Arterioscler Thromb Vasc Biol 2003; 23: 2097–2103. https://doi.org/10.1161/01.ATV.0000097766.36623.DF

[52] MERTENS I, VAN GAAL LF. Obesity, haemostasis and the fibrinolytic system. Obes Rev 2002; 3: 85–101. https://doi.org/10.1046/j.1467-789X.2002.00056.x

[53] ADLY AAM, ELBARBARY NS, ISMAIL EAR, HASSAN SR. Plasminogen activator inhibitor-1 (PAI-1) in children and adolescents with type 1 diabetes mellitus: relation to diabetic micro-vascular complications and carotid intima-media thickness. J Diabetes Complic 2014; 28: 340–347. https://doi.org/10.1016/j.jdiacomp.2014.01.011

[54] CARNETHON MR, GULATI M, GREENLAND P. Prevalence and cardiovascular disease correlates of low cardiorespiratory fitness in adolescents and adults. JAMA 2005; 294: 2981–2988. https://doi.org/10.1001/jama.294.23.2981

[55] DUNCAN GE, PERRI MG, THERIAQUE DW, HUTSON AD, ECKEL RH et al. Exercise training, without weight loss, increases insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. Diabetes Care 2003; 26: 557–562. https://doi.org/10.2337/diacare.26.3.557

[56] DE LEMOS ET, OLIVEIRA J, PINHEIRO JP, REIS F. Regular physical exercise as a strategy to improve antioxidant and anti-inflammatory status: benefits in type 2 diabetes mellitus. Oxid Med Cell Longev 2012; 2012: 741545. https://doi.org/10.1155/2012/741545