Pathol. Oncol. Res. (2013) 19:375–383
DOI 10.1007/s12253-012-9589-6

RESEARCH

Anthracycline Causes Impaired Vascular Endothelial Function and Aortic Stiffness in Long Term Survivors of Childhood Cancer

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Received: 28 June 2012 / Accepted: 7 November 2012 / Published online: 16 December 2012

Abstract Vascular and endothelial functions were investigated in long term survivors of childhood cancer exposed to anthracycline treatment. We enrolled 96 long-term survivors (57 males and 39 females, mean age 14.9±5.3 year) of different childhood cancers and 72 age-, sex-, bodyweight- and blood pressure matched controls (39 males and 33 females, mean age 13.7±4.9 year). Aortic stiffness was characterized by echocardiography. Brachial artery endothelial function was assessed by flow-mediated dilatation (FMD%) and nitrate-mediated dilatation (NTG%). Results were compared between three subgroups: anthracycline treated, only chemotherapy treated and control subgroups. The cumulative anthracycline dose was less than 350 mg/m^2. The healthy control subgroup had a significantly greater FMD response (13.13±2.40 %), and lower stiffness index (2.08±0.6) than both the anthracycline (7.12±6.28 % and 6.45±3.25, respectively) and only chemotherapy treated (10.17±4.23 % and 4.12±2.32, respectively) subgroups. In the anthracycline treated subgroup a significantly ($p<0.01$) lower FMD% response, and higher stiffness index were detected than in the only chemotherapy treated subgroup. Higher triglyceride level, higher cumulative anthracycline dose and lower age at the start of treatment were found to be associated independently with impairment of FMD% response and aortic stiffness. We found a significant negative correlation between FMD and aortic stiffness ($p<0.001$) and a positive correlation between FMD and distensibility ($p<0.0001$) Childhood cancer long term survivors exposed to anthracycline treatment exhibit a marked preclinical vasculopathy, characterized by endothelial dysfunction and increased arterial stiffness, contributing to a deteriorated cardiovascular function.

Keywords Childhood cancer · Survivors · Endothelial dysfunction · Stiffness

Introduction

Improving cure rates of children with cancer prompted the scientific community to focus more on late effects of chemotherapy. Approximately 2 of every 3 childhood cancer survivor will experience at least one complication, including higher rates of precocious cardiovascular morbidity and mortality [1, 2]. In almost 40 % of patients the condition may be severe, disabling, or life threatening within 30 years after the diagnosis of cancer [1, 2].

Among cytostatic agents, cardiotoxicity of anthracyclines and cyclophosphamide has been well documented [3, 4]. Among long-term survivors of childhood acute lymphoblastic leukemia impairment of endothelial-dependent flow-mediated dilatation (FMD%) of the brachial artery has been observed [5, 6]. Recent studies have demonstrated that anthracyclines damage the functional integrity of vascular endothelium and impair vasodilatatory responses [7–11]. However, adverse effects on earliest markers of cardiovascular diseases (CVD), such as endothelial function, and aortic stiffness have not yet been characterized simultaneously in survivors of different types of childhood cancer.

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Our hypothesis was that long term survivors of childhood cancer, who were treated with chemotherapy, containing anthracyclines may be at risk for developing preclinical vascular abnormalities. To test this hypothesis we measured endothelial-dependent and independent vascular functions and arterial stiffness simultaneously in individuals who received chemotherapy, containing anthracyclines, chemotherapy only without antracyclines and in healthy age-and sex-matched individuals.

Material and Methods

Patients

Ninety-six long term survivors of childhood cancer (57 boys and 39 girls) treated at the Department of Hematology-Oncology of the Institute of Pediatrics, Medical and Health Science Center of the University of Debrecen (MHSCUD) between 1989 and 2008 were involved in the study. All survivors were in complete remission for at least 5 years.

All patients were treated according to standard protocols as applied by the Hungarian Pediatric Oncology Group (HPOG). During the 19 years between 1989 and 2008, different protocols were used [12].

Patients with leukemia and lymphoma (LL) (63 cases) were treated according to the BFM protocols consisting of, among others, anthracycline antibiotics, high dose methotrexate (MTX), cyclophosphamide (CYC), and ifosfamide (IFO) (high risk patients) [12–16]. Twenty-one patients received cranial irradiation treatment (CRT) with less than 24 Gy. Wilms tumor (WT) patients (14 cases) underwent heminephrectomy, while polar resection was performed in 1 case. One WT patient received local (flank) irradiation. Standard and medium risk WT patients (12 cases) were treated with vincristine and D-actinomycine, and high risk patients (2 cases) received IFO and carboplatin (CARBO), in addition [17]. Five WT patients received anthracycline treatment. Patients with other solid tumors (ST) (19 cases) received either IFO or platinum derivates or high dose MTX or combinations of these agents with or without additional cytostatic drugs (Table 1), [18–20]. Fourteen ST patients received anthracycline treatment.

Among long-term cancer survivors 67 patients received anthracyclines as part of combination chemotherapy. Twenty-nine individuals were treated with chemotherapy without anthracycline. Anthracycline doses were converted to doxorubicin equivalents using conversion factors of 0.83 and 5.0 for daunorubicin and idarubicin, respectively.

We calculated the sum of the cumulative antracycline (doxorubicin, daunorubicin and idarubicin) dose given to the patients to be less than 350 mg/m^2. The mean time between the last anthracycline dose and the date of study was 10.2 years. Anthracycline was administered in parallel with dexrazoxane.

Exclusion criteria were: acute or chronic renal dysfunction, diabetes mellitus, metabolic syndrome, impaired glucose tolerance, heart failure, valvular heart disease, asthma, pregnancy or taking of oral contraceptives, concurrent therapy with medications that might affect blood pressure, history of smoking, aortic diseases, and connective tissue disorders. Vascular measurements of female patients were conducted within 72 h before or after completion of menstruation.

The healthy comparison group comprised of 39 males and 33 females was matched to be similar in blood pressure- and bodyweight, with a mean age of 13.7±4.9 years and was recruited from among school-children.

Informed consent was obtained from the parents and/or guardians. The study protocol was accepted by the Ethical Committee of MHSCUD, and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP)/International Conference on Harmonization (ICH) guidelines.

Methods

Physical examination, blood pressure (BP) measurement, complete blood count (CBC) and measurement of serum electrolytes were performed according to standard methods at every check-up. Blood samples for determination of triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), glucose (BG), total homocysteine (Hcys), highly sensitive C-reactive protein (CRP), and fibrinogen were collected between 8:00–9:00 am after overnight fasting. TG and HDL-C were assayed by enzymatic methods (Roche Diagnostics, Mannheim, Germany). LDL-C level was calculated by the Friedewald's equation. Hcys levels were measured by fluorescence polarisation immunoassay (Abbott Laboratories, Abbott Park, Illinois, U.S.A.). Fibrinogen was determined by the Clauss method (Diagnostico Stago, Asnieres-sur-Seine, France). CRP was measured by latex sensitized immunturbidimetry (Roche Diagnostics, Mannheim, Germany).

Adult reference ranges were as follows: TG<1.7 mmol/L, LDL-C<3.4 mmol/L, HDL-C > 1 mmol/L, Hcys < 12.5 μmol/L, fibrinogen 1.5–4.0 g/L, CRP<4.6 mg/L, BG =3.5–5.5 mmol/L.

Vascular Reactivity

Evaluation of vascular function of the brachial artery was performed non-invasively, as described previously [3, 21, 22]. Briefly, high-resolution ultrasound was used to measure changes in arterial diameter in response to reactive

Table 1 Diseases characteristics and treatment modalities of patients (n=96)

Diagnosis	Number of patients	Anti-cancer treatment
Acute lymphoblastic leukemia	49	ALL-BFM [11]
Acute myeloid leukemia	2	AML-BFM [12]
Hodgkin/Non-Hodgin's lymphoma	12	DAL HD/NHL-BFM [13, 14]
Wilms tumor	14	SIOP [15]
Osteosarcoma	5	COSS [16]
Ewing sarcoma	2	CWS [17]
Neuroblastoma	11	OPEC/OJEC [18]
Schwannoma	1	CWS [17]

hyperaemia as endothelium-dependent, flow-mediated vasodilatation (FMD%) as well as to glyceryl-trinitrate (NTG), as endothelium-independent, nitrate-mediated vasodilatation (NTG%) with a 7.0 MHz linear array transducer (Philips HDI-5000 system, Philips Medical Systems, Bothell, USA). FMD% and NTG% were assessed by measuring the percent change in the brachial artery diameter during reactive hyperemia and after NTG. The intra- and interobserver variability of artery diameters were 0.09± 0.10 mm and 0.08±0.13 mm, respectively. Subjects rested in the supine position for 10 min before the first scan and remained in that position throughout the study. The target artery (brachial artery, 2–15 cm above the elbow) was scanned in longitudinal section and the center of the vessel was identified when the clearest images of anterior and posterior walls of the artery were obtained. The transmit zone was set to the level of the anterior vessel wall. Depth and gain settings were optimized to identify the lumen-to-vessel wall interface. Settings were kept constant during each study. Flow increase was induced by inflation of a blood pressure tourniquet placed around the forearm distal to the target artery to 250 mmHg. The cuff was released after 5 min and after cuff deflation the artery was scanned continuously for 90 s. Then, 15 min was allowed for vessel recovery, sublingual NTG (0.5 mg) was administered, and 4–5 min later the last scan was measured. Parallel electrocardiogram was monitored continuously. Vessel diameter was measured by two observers, unaware of clinical details and the stage of the experiment. Arterial diameter was measured at a fixed distance from an anatomical marker, such as a bifurcation, with ultrasonic calipers. Measurements were taken from the anterior to the posterior 'm' line at end diastole, incident with the R-wave on the electrocardiogram. The mean diameter was calculated from four cardiac cycles. For the hyperemia scan, vessel diameter was measured 45–60 s after cuff release. Diameter changes were derived as percentage change relative to the first baseline scan (100 %). Baseline blood flow (measured during the first baseline scan) was estimated by multiplying angle-corrected, pulsed Doppler, recordings of the flow velocity integral and the cross-sectional area of the artery. All vascular measurements were conducted in a quiet, temperature-controlled environment [21, 22]. Participants were in fasting state, without caffeine-containing drinks. There was no physical activity 12 h prior to study.

Blood Pressure Measurements

Blood pressure was measured three times for each patient with a standard mercury validated sphygmomanometer on the right arm in sitting position following 10 min of rest. Phase I and V Korotkoff sounds were used to determine systolic and diastolic blood pressure measurements. Patient measurements were performed by the same investigator, in the same room, and at the same time of the day. The average of three measurements was used for the analyses.

Echocardiography

Transthoracic echocardiography was performed by using Philips HDI-5000 system (Philips Medical Systems, Bothell, USA) 2.5 Mhz- probe at the left lateral decubitus position in a standard manner. Echocardiographic measurements were made on the screen by two independent investigators who were unaware of the patients' clinical data. M mode tracings of ascending aorta were obtained in the parasternal long axis views at a speed of 50 mm/s. Five consecutive cardiac cycles were averaged for every echocardiographic measurement. With M mode, aortic tracing was recorded at the level of approximately 3 cm above the aortic valve. From the M mode recordings, aortic systolic and diastolic diameters (Aos and Aod, respectively) were measured. Aos was determined at the time of the full opening of the aortic valve and Aod was determined at the peak of QRS. All parameters were measured in five consecutive cardiac cycles and averaged. Simultaneously, cuff brachial artery systolic (SBP) and diastolic (DBP) blood pressures were measured with and sphygmomanometer [23].

The Measurements of Aortic Elasticity Parameters

Aortic elasticity was assessed using the following indices: [23–25]

Aortic strain (%) = 100 × (Aos-Aod)/Aod.
Aortic distensibility index (cm^{-2} dyn^{-1} 10^{-6}) = 2 × aortic strain × (SBP-DBP).
Aortic stiffness index [beta] = ln (SBP/DBP)/aortic strain.

Statistical Analysis

All statistical analyses were performed by using the SAS for Windows (8.2 cary/nc SAS® Institute Inc. USA) statistical package. Continuous data are expressed as mean±standard deviation. Relationships between the continuous variables were evaluated by Pearson's or Spearman's correlation analysis. Comparisons between healthy and treatment groups for continuous variable were made by one way ANOVA and post-hoc Turkey's test to adjust these comparisons for age and gender. To improve the normality of the data distribution, TG values were log-transformed for significance test and correlation analysis. To investigate the independent effect of the different factors on FMD and stiffness, a multiple stepwise linear regression model was performed. The multivariate model consisted of FMD%, distensibility and stiffness index as dependent variables and independent variables that had significant correlation with FMD% and stiffness parameters in the simple linear regression analysis. $P<0.05$ was considered statistically significant.

Results

Of the original cohort of 112 childhood cancer survivors, we were not able to perform our study on 16 patients: 8 patients were unable to visit our clinic for personal reasons, 6 patients had died from childhood cancer and 2 patients were lost to follow up.

Baseline characteristics of the patients and controls groups are presented in Table 2. Cancer survivors did not differ statistically from the control group by age, sex, body weight, body mass index (BMI) and BP. The healthy control group had significantly lower TG levels than the only chemotherapy and the anthracycline treated groups. There was no difference in HDL-C, BG, fibrinogen, CRP, and LDL-C between the healthy controls and the survivor groups (Table 3). Significant differences between male and female survivors and controls were found only in brachial artery diameter (data not shown).

Due to gender differences in brachial artery diameter all FMD% and NTG% measurements were adjusted for the brachial artery diameter. As demonstrated in Table 4, the healthy control group had a significantly greater FMD response (13.13 %±2.40 %), aortic distensibilty (14.57 cm^{-2} dyn^{-1}±4.16 $cm^{-2}dyn^{-1}$), strain 18.2 %±5.20 %, and lower stiffness index (2.08±0.6) than both the anthracycline treated (7.12 %±6.28 %, 6.94 $cm^{-2}dyn^{-1}$±7.7 cm^{-2} dyn^{-1},11.89 %±7.78 % and 6.45±3.25 respectively) and only chemotherapy treated (10.17 %±4.23 %, 8.7 cm^{-2} dyn^{-1}±3.87 $cm^{-2}dyn^{-1}$, 13.08 %±10.08 % and 4.12±2.32 respectively) subgroups. In the anthracycline treated subgroup a significantly ($p<0.01$) lower FMD% response,

Table 2 Demographic data and treatment characteristics for cancer survivors and control group

	Chemotherapy + anthracycline (N=67)	Chemotherapy (N=29)	Control (N=72)	P
Gender (N,%)				
Male	39(59 %)	18(63 %)	42(58 %)	NS
Female	28(41 %)	11(37 %)	30(42 %)	NS
Age at treatment, years (mean, SD)	4.78±2.32	4.91(3.11)	–	NS
Age at examination, years (mean, SD)	15.11±4.21	14.73±5.11	13.7±4.9	NS
Years of survival	11.21±6.33	10.81±4.98	–	NS
Diagnosis (N)				
lymphoma/leukemia	60	3	–	
Wilms tumor	1	13	–	
Solid tumor	6	13	–	
Cumulative anthracycline dose mg/m^2				
<200 mg/m^2	8	–	–	
200–250 mg/m2	18	–	–	
251–300 mg/m2	33	–	–	
>300 mg/m2	8	–	–	

Overall P values were calculated by one-way analysis of variance (ANOVA). Intergroup differences were calculated by Turkey's significant

test: *NS* Non-significant

Table 3 Comparison of baseline characteristics of Control and chemotherapy treated patients

Variable	Controls n=72	chemotherapy + anthracycline n=67	chemotherapy n=29	P
BMI (Kg/m^2)	20.4±2.9	21.8±4.8	20.13±4.5	0.45
BPsyst (mmHg)	120±5	121±3.56	122±4.12	0.21
BPdiast (mmHg)	80±3.1	81±2.1	82±1.45	0.12
BG(mmol/L)	4.7±0.5	4.5±0.9	4.5±0.8	0.5
LDL-C (mmol/L)	2.2±0.6	2.3±0.7	2.1±0.3	0.45
HDL-C(mmol/L)	1.6±0.3	1.4±0.6	1.5±0.9	0.31S
TG(mmol/L)	0.8±0.3	1.24±0.6*	1.13±0.7*	0.01
Fibrinogen(g/L)	3.6±0.6	3.3±0.6	3.0±0.7	0.2
Hcyst(μmol/L)	10.3±2.2	9.2±5.6	8.5±2.4	0.35
hsCRP(mg/L)	1.1±0.1	1.13±0.1	1.13±0.1	0.38

BMI Body mass index; *BP* Blood pressure; *BG* Glucose; *LDL-C* Low-density lipoprotein cholesterol: *HDL-C* High-density lipoprotein cholesterol; *TG* Triglyceride; *Hcys* Total homocysteine; *hsCRP* highly sensitive C-reactive protein; Overall P values were calculated by one-way analysis of variance (ANOVA). Intergroup differences were calculated by Turkey's significant test: * P<0.05 vs. control group

distensibility and higher stiffness index were detected compared to the only chemotherapy treated subgroup.

There were no differences in NTG% between the healthy controls and the survivors. Females had a greater peak FMD% than did males in the anthracycline treated subgroup (8.12 % vs. 6.12 %, p=0.02), in the only chemotherapy treated subgroup (11.25 % vs. 9.09 %, p=0.015) and in the control (14.13 % vs 12.13 %, P=0.01) subgroup. In NTG% and in stiffness parameters significant differences with respect to gender were not found (data not shown).

In the total patients group 21 patients received CRT (<24 Gy), however significant difference in FMD and in other stiffness parameters between the CRT subgroup and chemotherapy only subgroup was not found (data not shown).

Applying a simple regression analysis, a significant negative correlation was found between FMD% and TG (R^2= 0.39, p=0.02), Hcyst (R^2=0.45, p=0.01), age (R^2=0.44, p= 0.01) and cumulative anthracycline dose (R^2=0.41, P= 0.001), and positive correlations between FMD% and age at start of treatment (R^2=0.40, p=0.01). A significant negative correlation was found between distensibility and TG (R^2=0.38, p=0.02), and cumulative anthracycline dose (R^2=0.42, p=0.001) and a positive correlation between distensibility and age at start of treatment (R^2=0.40, p= 0.02). A positive correlation was found between stiffness index and TG (R^2=0.43, p=0.0015) and cumulative anthracycline dose (R^2=0.41, p=0.001), and a negative correlation between age at start of treatment (R^2=0.40, p=0.01), (Table 5).

When all these characteristics as predictors were examined in a multivariable model only TG, cumulative anthracycline dose and the age at start of treatment were found to be associated independently with FMD%, distensibility and stiffness index (Table 6).

We found a significant negative correlation between FMD and aortic stiffness (p<0,001, R^2=0.48), (Fig. 1), and a positive correlation between FMD and distensibility (p<0,0001, R^2=0.42), (Fig. 2).

Discussion

Our study had four new outcomes. First, that long-term survivors of childhood cancer who received anthracyclines

Table 4 Peak flow-mediated dilatation (FMD%), nitrate-mediated, endothelium- independent dilation (NTG%), aortic diameters and aortic elastic properties in healthy controls and survivor groups

Groups	Control n=72	Chemotherapy + anthracycline n=67	Chemotherapy only n=29	P value
Aortic diameters (mm)				
Systolic	26.1±0.27	25.6±0.23*	25.3±0.29*	0.03(0.044)†
Diastolic	21.7±0.27	23.9±0.31*	22.5±0.25*	0.02(0.032) †
Distensibility (cm^{-2}dyn^{-1})	14.57±4.16	6.94±7.7*§	8.7±3.87*	0.02(0.037) †
Aortic strain (%)	18.2±5.20	11.89±7.78*	13.08±10.08*	0.01(0.019) †
Stiffness index β	2.08±0.6	6.45±3..25*§	4.12±2.32*	0.03(0.043) †
Brachial artery diameter (mm)	3.45±0.32	3.37±0.28	3.61±0.34	0.21(0.321) †
FMD (%)	13.13±2.40	7.12±6.28*§	10.17±4.23*	0.01(0.024) †
NTG (%)	26.32±6.12	25.86±4.36	25.95±5.67	0.41(0.432) †

Data are mean (SD). *FMD(%)* Flow mediated dilatation; NTG (%): glyceryl-trinitrate. Overall P values were calculated by one-way analysis of variance (ANOVA). Intergroup differences were calculated by Turkey's significant test: * P<0.01 vs. control group, § p<0.01 vs. chemotherapy group, † adjusted age and gender

Table 5 Correlation between FMD%, aortic distensibility, stiffness index and other determinants by univariate analysis

	Patient group (n=96)		
	FMD%	Distensibility	Stiffness index
Determinants			
Sex	0.10	0.09	0,17
Age (years)	−0.30*	0.12	0,21
Glu (μmol/l)	−0.08	0.06	0,10
TG (mmol/l)	−0.38*	−0.34*	0,37*
LDL-C (mmol/L)	−0.03	0.03	0,12
HDL-C (mmol/l)	0.05	0.02	0,06
Hcyst (μmol/L)	−0.30*	−0.25	0,25
Age at start of treatment (years)	0,34*	0.28*	−0,29*
Time of survivor (years)	0.24	0.22	0.26
Cumulative anthracycline dose (mg/m^2)	−0.41*	−0.38*	0.40*

Numbers represent correlation coefficients assessed with simple linear regression analysis (Pearson and Spearman) *Glu* Glucose; *TC* Total cholesterol;*TG* Triglyceride; *LDL-C* Low-density lipoprotein cholesterol; *HDL-C* High-density lipoprotein cholesterol; *Hcys* Total homocysteine; *FMD(%)* Flow mediated dilatation; * p<0.05

had poorer endothelial function and aortic stiffness compared with both those of age, sex matched healthy individuals who did not receive cancer therapy, and those of age, sex matched survivors treated only with chemotherapy. Second, that the decrease in FMD% and increase in aortic stiffness persisted long (more than 10 years) after anthracycline treatment, suggesting that it may be clinically relevant and play an important role in the progression of cardiovascular diseases. Third, that cumulative anthracycline dose, and age at the beginning of chemotherapy are independently associated with a decrease in FMD% and increase in aortic stiffness. Fourth, that as far as we know, this is the first study to demonstrate an association between aortic stiffness and endothelial dysfunction in long term survivors of childhood cancer.

To the best of our knowledge this is the first study that simultaneously examined, by non-invasive ultrasound technique, the endothelial function and aortic stiffness defined by FMD, distensibility and stiffness index in long term survivors of childhood cancer treated with anthracycline and without anthracycline. FMD% and aortic stiffness parameters are intermediate markers of endothelial and vascular dysfunction, and they can be used as earliest preclinical indicators of atherosclerosis and other cardiovascular diseases [5, 8, 23]. Impaired endothelial-dependent vasodilatation has been observed in patients with risk factors for development of atherosclerosis without clinically evident disease and in patients not receiving cancer treatment, aortic stiffness is also associated with future risk of adverse cardiovascular events even after accounting for the Framingham risk score [26, 27].

The spectrum of the patient population was broad with respect to the underlying malignancy. However, the advantage of such a study is to obtain a cross sectional view on potential hazards that might hamper long-term quality of life of paediatric patients cured from cancer [12].

Our data are consistent with the results of a few previous studies reporting on an impairment of endothelial-dependent dilatation responses in childhood cancer survivors [5, 11]. We also found that the survivor group, especially the anthracycline treated subgroup had a significantly lower peak FMD% than the healthy control subgroup. However, this difference in FMD% disappeared in NTG% indicating that a decreased FMD% response was not due to smooth muscle dysfunction but to pure endothelial dysfunction.

Table 6 Multivariate linear regression analysis of clinical and biochemical variables associated with FMD% and stiffness in survivors

Dependent variables	Independent variables	β Coefficient	95 % CI	P value	R^2
FMD%	Age	−0.25	−0.38 to −0.11	0.12	0.28
	TC (μmol/l)	−0.36	−0.48 to −0.21	0.02	0.34
	Hcyst (μmol/l)	−0.28	−0.33 to −0.13	0.08	0.28
	Age at start treatment (years)	0.33	0.15 to 0.48	0.01	0.36
	Cumulative anthracycline dose (mg/m2)	−0.40	−0.54 to −0.31	0.005	0.32
Distensibility	TC (μmol/l)	−0.32	−0.48 to −0.21	0.02	0.34
	Age at start treatment (years)	0.30	0.15 to 0.48	0.01	0.36
	Cumulative anthracycline dose (mg/m2)	−0.36	−0.54 to −0.31	0.005	0.32
	TC (μmol/l)	0.36	0.22 to 0.44	0.01	0.32
	Stiffness index Age at start treatment (years)	−0.28	−0.38 to −0.11	0.03	0.30
	Cumulative anthracycline dose (mg/m2)	0.38	0.24 to 0.54	0.015	0.39

TC Total cholesterol; *TG* Triglyceride; *LDL-C* Low-density lipoprotein cholesterol; *Hcys* Total homocysteine; *FMD%* Flow mediated dilatation

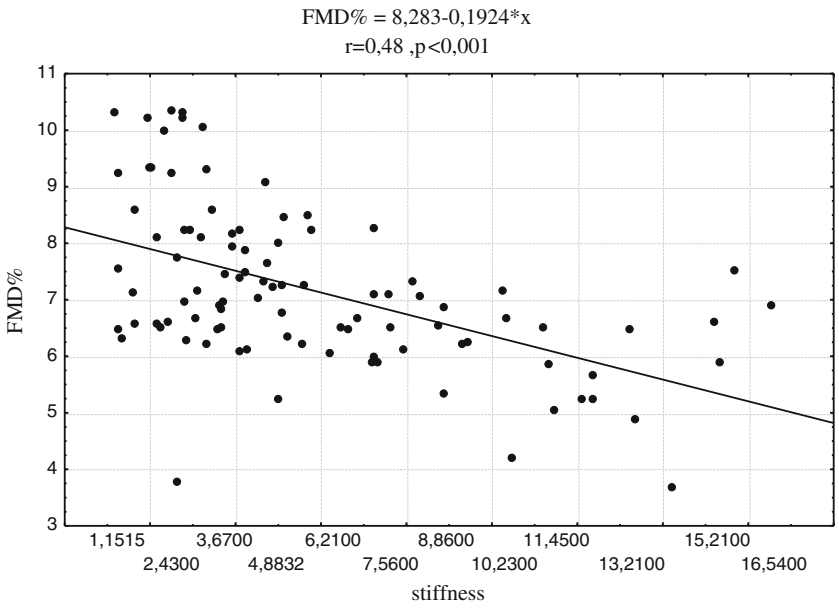

Fig. 1 Simple regression analysis to evaluate correlation of FMD with aortic stiffness

Females examined in this study had significantly greater FMD% than their male counterparts in all groups. The greater peak FMD% in females has been reported previously, and is known to be caused by elevated levels in serum estradiol in follicular and luteal phases of menstrual cycle [28]. This greater peak FMD is still present even after correcting for the smaller brachial artery diameter. However, those females who received chemotherapy had significantly lower FMD% than their healthy female controls.

The exact mechanism by which chemotherapy induces endothelial dysfunction and impaired vascular stiffness is unknown; however, in vitro and in vivo studies have shown that doxorubicin causes apoptosis of vascular endothelial cells [9, 10]. It has been suggested that this process may result in the permanent damage of endothelial cells. Another possible mechanism by which chemotherapy may impair endothelial function is by altering TG levels. All survivor groups had significantly higher serum TG levels compared with the control group. A number of studies have demonstrated that elevated TG levels in young adults and patients with type-2 diabetes were associated with reduced endothelial function [29–33]. The possible explanation of unfavourable lipid alteration might be the presence of growth hormone (GH) deficiency caused by chemotherapy or CRT [34]. Metabolic syndrome and other lipid abnormalities, including increased TC, LDL-C, TG and decreased

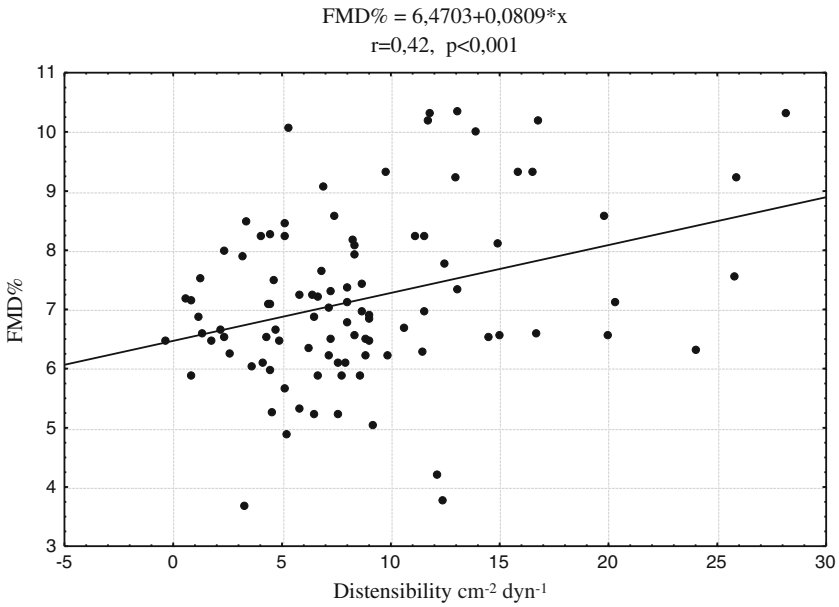

Fig. 2 Simple regression analysis to evaluate correlation of FMD with aortic distensibility

HDL-C were found in GH deficient patients [34–36]. However, similar to a recent study, only elevated triglyceride levels of cancer survivors were found compared with healthy controls [34]. Moreover, CRT in our study was not associated with increased metabolic risk and did not influence the endothelial function and stiffness parameters. This can be explained by a relatively low (<24 Gy) irradiation protocol [5, 37, 38].

Similar to previous studies, our results demonstrated that patients treated with chemotherapy consisting of anthracyclines, or chemotherapy without antracyclines, also had greater aortic stiffness compared to healthy controls [27]. Impairment of stiffness parameters are associated with significantly lower FMD%. Anthracyclines may influence aortic stiffness through one of several mechanisms. Anthracyclines increase oxygen free radicals and induce oxidative stress, thereby increasing arterial stiffness by causing structural changes within the vascular matrix and interfering with endothelial regulation of vascular smooth muscle tone [27]. Vascular endothelial damage diminishes nitric oxide synthesis and promotes endothelial cell dysfunction that increases vascular stiffness [39].

We found that patients treated with cumulative doses of anthracycline of 242±56 mg/m^2 showed greater aortic stiffness than age and gender matched controls and only chemotherapy treated patients. Thus, at this cumulative dose, anthracycline may be associated with preclinical cardiac and vascular consequences that consist of vascular stiffness The increase in stiffness observed more than 10 years after treatment suggest that it may be clinically relevant as an independent predictor of cardiovascular events of long time survivors of childhood cancer.

In our study we have shown that the impairment of endothelial function and aortic stiffness are related to the younger age at which the treatment regimen starts, higher cumulative anthracycline dose, and TC level.

One potential limitation of the study is that the influence on endothelial function and on aortic stiffness of other chemotherapeutic agents used as part of the chemotherapy regimen in conjunction with anthracycline is unknown. On the other hand, patients were treated with various drug combination for different previous diseases, and in some cases modified therapeutic protocols were applied for the same disease over time.

Conclusion

In conclusion, in long term survivors of childhood cancer treated with mean cumulative anthracycline doses of 242±56 mg/m^2, impairment in endothelial and vascular functions were found. These unfavourable changes can be considered not only as risk factors but as earliest subclinical markers of development of clinically relevant CVD also.

It is suggested also, that the anthracycline dose, age at beginning of the treatment, and TG level have additional negative effects on endothelial function and stiffness. Long term survivors at high risk for developing CVD should be identified and subjected to life-long clinical check-up programs to detect, prevent and manage their cardiovascular complications. Further studies are needed to identify the in vivo effects of several chemotherapeutic drugs on vascular function, and to evaluate the incidence of CVD among childhood cancer survivors.

Acknowledgments The help of Zsolt Karányi in performing statistical analysis is acknowledged. This work was supported by the grant of the Health Science Council of the Ministry of Education Republic of Hungary (ETT) No. 396/2006, 01-478/2009 and by the "For the leukemic children" foundations. The publication is supported by the TÁMOP 4.2.1./B-09/1/KONV-2010-0007 project. The project is co-financed by the European Union and the European Social Fund.

Conflict of Interest None declared.

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