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Premature atherosclerosis after treatment for acute lymphoblastic leukemia in childhood

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Abstract

Introduction. Late cardiovascular complications are the leading causes of morbidity and mortality in patients treated for common malignancies of childhood. Late cardiotoxicity include increased development of atherosclerosis and atherosclerosis – related diseases. An evaluation of the endothelium can be made based on the measurement of endothelium-derived blood vasoactive factors, such as cytokines and adhesion molecules. Their elevated serum levels may serve as sensitive indicators of early atherosclerotic lesions in high risk patients. Currently, assessment of common carotid intima-media thickness has emerged as one of the more powerful tools for evaluation of subclinical atherosclerosis. The purpose of this study was to compare these parameters between patients after antineoplatic treatment compared to persons not exposed to such factors.

Methods. Early progression of atherosclerotic disease was evaluated in 64 survivors treated for Acute Lymphoblastic Leukaemia (ALL) in childhood, and in a control group of 36 healthy volunteers. Blood serum concentrations of selected new biomarkers, indicative of endothelial damage and inflammatory activity, were measured, including intercellular adhesion molecule-1 (sICAM-1), endothelial leukocyte adhesion molecule-1 (E-selectin), thrombomodulin (TM), interleukin 6 (IL-6), and high-sensitivity C-reactive protein (hs-CRP). The common carotid intima-media thickness (IMT) was also assessed via ultrasound examination.

Results. Significantly higher blood concentrations of sICAM-1 adhesive molecule (229.3±62.2 ng/mL vs. 199.9 ± 63.3 ng/mL, p=0.0072) and IL-6 (2.1 ± 2.7 pg/mL vs. 1.9 ± 3.6 pg/mL, p=0.0414) were found in ALL survivors compared with control subjects. Concentration of hs-CRP was also higher in the ALL group: 1.3 ± 2.2 ug/mL vs. 0.6 ± 0.9 ug/mL. This difference was close to statistical significance (p=0.0599). The mean IMT values for right and left carotid arteries were higher in ALL patients after antineoplastic therapy, compared with healthy subjects (IMT-R 0.056±0.008 mm vs. 0.052±0.003 mm; p=0.0021; IMT-L 0.057±0.009 mm vs. 0.052±0.003 mm; p=0.0051).

Conclusion. Survivors of childhood ALL in the examined group demonstrated elevated concentrations of selected new biomarkers and increased IMT values, compared to controls, which may confirm the occurrence of endothelial injuries in blood vessels. This study indicates that subjects treated for childhood malignancy are at a higher risk of prematurely developing atherosclerosis.

Key words

nthracycline cardiotoxicity, leukemia, survivors, atherosclerosis, biomarkers, intima-media thickness

INTRODUCTION

Prognosis in common malignancies of childhood, including acute lymphoblastic leukemia (ALL), has improved significantly in recent years, primarily due to curative therapy. Successful treatment increases survival but it also places the patients at risk of side effects [1–3]. Possible treatment-related cardiovascular complications are serious and may be life- threatening. Late cardiac effects include increased development of atherosclerosis and atherosclerosis-related diseases. The results of the Childhood Cancer Survivor Study (SSCC) show that within 30 years following antineoplastic treatment, the risk of CVD-related death in cancer survivors is 8 times higher than in the general population: the coronary artery disease occurs more than 10 times more frequently, cerebral strokes are 9 times more frequent, and the likelihood of congestive heart failure is as much as 15 times higher than in the general population [4, 5]. The risk of ischemic disease and cardiovascular events after childhood cancer therapy is the highest in patients treated for Hodgkin's disease, non-Hodgkin's lymphoma, CNS tumours, acute lymphoblastic leukaemia (ALL), and post-bone marrow transplantation [5–7]. Taking into account the frequency and severity of adverse cardiovascular outcomes reported in childhood cancer survivors, there is an urgent need to look for the best methods for preventing, screening and treating such complications.

In recent years, a series of new attractive strategies for the detecting and monitoring of cardiotoxicity have been proposed. An evaluation of the endothelium can be made based on the measurement of endothelium-derived blood vasoactive factors, such as cytokines and adhesion molecules. Their elevated serum levels may serve as sensitive indicators of early atherosclerotic lesions in high risk

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patients [8, 9]. Currently, assessment of common carotid intima-media thickness (cIMT) with high-resolution B-mode ultrasonography has emerged as one of the more powerful tools for evaluation of subclinical atherosclerosis. Longitudinal studies have demonstrates that increased cIMT in young adults is associated with the presence of cardiovascular risk factors in childhood, and is predictive of future cardiovascular events, including stroke and myocardial infarction [10, 11].

Selected biochemical markers of endothelial dysfunction and carotid IMT measurements were used to assess the progress of early atherosclerotic lesions in the patients.

The study indicates that patients treated in childhood for ALL are at a higher risk of prematurely developing atherosclerosis. Therefore, these patients should be encouraged to lead a healthy lifestyle and undergo thorough, periodic follow-up cardiovascular evaluations

OBJECTIVE

The purpose of this study was to compare the serum level of selected biochemical markers of endothelial dysfunction and carotid IMT measurements between patients with a history of childhood treatment for acute lymphoblastic leukaemia (ALL) and persons not exposed to such factors.

MATERIALS AND METHHOD

One hundred randomly selected children, adolescents, and young adults, from among 253 patients who had been treated for ALL in the Clinic of Haematology and Paediatric Oncohematology at the Medical University in Lublin, Poland, during 1992–2006 were informed in writing of this study and invited to participate. Of the 100 cases, 36 were excluded because of refusal (distance, lack of time or interest), or non- response to the invitation. Finally, 64 patients who gave their written informed consent and remained in complete remission after their first or subsequent chemotherapy course, were enrolled in the study. The time interval from exposure to anticancer therapy was a minimum of 5 years. None of the subjects had previously been diagnosed with congenital heart disease or cardiomyopathy, and had not been treated for diabetes, hypertension, or growth hormone deficiency. The data regarding treatment details of patients with ALL was obtained by retrospective analysis of their medical records.

All patients were diagnosed, treated and as classified into the specific risk groups according to the protocols which had been approved by the Polish Paediatric Leukaemia/ Lymphoma Study Group. The ALL treatment differs with the doses of cytostatics, depending on the risk of relapse. The patients in the standard risk (SR) and medium risk (MR) groups, treated with BFM (87, 90, 95) and ALLIC2002 protocols, received 120 mg/m^2 of DNR (daunorubicin) as part of the induction treatment, and 60 or 120 mg/m^2 of DOX (doxorubicin) as the part of the consolidation treatment. The cumulative dose of cyclophosphamide was 2g/m^2 in the SR group and 3 g/m^2 in the MR group. Patients in the high risk (HR) group received120 mg/m^2 of DNR for the induction treatment, and 150 mg/m^2 or 180 mg/m^2 of DOX for the consolidation treatment, according to the BFM 95 and ALLIC 2002 protocols, respectively. The cumulative dose of anthracyclines in the New York protocol, which had also been used for the high risk group, was 500 mg/m^2. The cumulative dose of cyclophosphamide in HR group was 5 g/m^2. More details about the risk group definition and treatment are found in the literature [12, 13]. The characteristics of the enrolled patients are presented in Table 1.

Table 1. Characteristics of study patients

All patients n (%)	64 (100)
Male, n (%)	29 (45.3)
Female, n (%)	35 (54.7)
Age at diagnosis – years, mean (SD), median (range)	6.3 (±3.9); 5.0 (1–17)
Age at time study – years, mean (SD), median (range)	15.5 (5.5); 15.0 (7–29)
Years since anthracycline treatment, mean (SD), median (range)	9.2 (4.4); 7.0 (5–19)
Protocols of chemotherapy received	
BFM 87, 90, 95, n (%)	21 (32.8)
ALLIC 2002, n (%)	38 (59.4)
New York, n (%)	5 (7.8)
Type of treatment	
Chemotherapy only, n (%)	45 (70.3)
Chemotherapy + CNS radiotherapy, n (%)	19 (29.7)
Dose of radiotherapy	
12 Gy, n (%)	14 (21.9)
18 Gy, n (%)	5 (7.8)
Anthracycline treatment, n (%)	64 (100)
Cumulative dose of anthracyclines ≤240 mg/m2, n (%)	44 (68.7)
180 mg/m^2, n (%)	4 (6.2)
240 mg/m^2, n (%)	40 (62.5)
Cumulative dose of anthracyclines >240 mg/m2, n (%)	20 (31.3)
270 mg/m^2, n (%)	3 (4.7)
300 mg/m^2, n (%)	12 (18.8)
500 mg/m^2, n (%)	5 (7.8)
Relapse n(%)	3 (4.7)
BMT n(%)	5 (7.8)

BMT – bone marrow transplantation

The control group was composed of 36 healthy subjects, matched for gender and age, and recruited randomly from pupils and students living in the city of Lublin in eastern Poland. All patients and volunteers underwent a physical examination, height, weight and arterial blood pressure measurements, plus ultrasound carotid IMT measurements. Venous blood was drawn from every patient and volunteer to perform biochemical determinations, including total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG), as well as quantification of new atherosclerotic markers, including sICAM-1, TM, IL-6, E-selectin and hs-CRP. The conventional risk factors for cardiovascular diseases, such as obesity/overweight, dyslipidemia, physical inactivity, family history of cardiovascular disease and smoking, were also assessed.

All patients and volunteers or their guardians expressed written informed consent for participation in this study. The study protocol was approved by the Institutional Review Board at the Medical University of Lublin.

Laboratory studies. Participants were asked to fast for 12 hours before their examination. Venous blood samples were obtained for plasma lipoprotein analysis and measurements

of selected, new biomarkers of atherosclerosis. TC, LDL-C, HDL-C and TG were measured using enzymatic, colorimetric methods on a Cobas Integra plus analyzer (Roche Diagnostics, Mannheim, Germany) with reagents from Roche Diagnostics. Commercial enzyme-linked immunosorbent assay (ELISA) kits (Quanticine Human sICAM/CD54 Immunoassay, Quanticine Human sE-selectin/CD62E Immunoassay, Quanticine Human Thrombomodulin/BDCA-3 Immunoassay and Quanticine Human IL-6 Immunoassay, R&D Systems) were used for the quantitative determination of human sICAM -1, E-selectin, TM, and IL-6 levels in plasma samples. The ELISA Reader VictorM^3 (PerkinElmer, USA) was used. Detection limits for BDCA-3, sICAM, IL-6, and sE-selectin were: 7.82 pg/ml, 3.12 pg/ml, 0.096 ng/ml, and 0.009 ng/ml, respectively. For the assessment of acute inflammation, the Demeditec CRP ELlSA ref DE 740001 was used, according to the manufacturer's protocols.

Cardiovascular risk factors were analyzed in all survivors and controls. Obesity/ overweight, dyslipidaemia, physical inactivity, family history of cardiovascular disease, and history of smoking were defined and cutoff points established in agreement with the Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (14). Following the recommendation of the Expert Panel, children and adolescents ages 2–18 years with a BMI ≥ 95th percentile were classified as 'obese' and children with a BMI that fell between the 85th – 95th percentiles were classified as 'overweight'. For adults, the criteria were established as follows: for obesity BMI ≥ 30 and for overweight BMI≥ 25. The predominant dyslipidaemic pattern in childhood was a combined pattern associated with obesity, with moderate to severe elevation in triglycerides (TG), normal to mild elevation in LDL-C and reduced high density lipoprotein cholesterol (HDL-C). The cut-off points for children and adolescents were as follows: total cholesterol (TC) ≥ 200 mg/dL, low density lipoprotein cholesterol (LDL-C) ≥ 130mg/dL, high density lipoprotein cholesterol (HDL-C) < 40mg/dL. The cut-off points for young adults (20–24 years) were, respectively: TC ≥225 mg/dL, LDL-C ≥160 mg/dL, HDL-C< 40mg/dL. The cut-off points for triglycerides (TG) in different age groups were: 0–9 years- ≥ 100 mg/dL, 10–19 years-≥ 130 mg/dL, 20–24 years- ≥150 mg/dL. For adults, a positive family history was defined as a parent and/or sibling with a history of treated angina, myocardial infarction, percutaneous coronary catheter interventional procedure coronary artery bypass grafting, stroke or sudden cardiac death before the age of 55 in men or 65 years in women. Because the parents and siblings of children and adolescents are usually young themselves, it was the panel consensus that when evaluating family history in a child, history should also be ascertained for the occurrence of cardiovascular disease in grandparents, aunts and uncles, although the evidence supporting this is insufficient to-date.

The Expert Panel recommends at least 1 hour of moderate to vigorous activity every day of the week for children over 5 years of age. In agreement with the 'Physical Activity Guidelines Advisory Committee Report, 2008' from the Department of Health and Human Services, the Expert Panel recommends that activity should be vigorous on 3 days/week [14].

Intima-media measurements. Ultrasound examinations of the intima-media complex (IMT) of the common carotid artery (CCA) were performed with the use of a Philips IU22 ultrasound scanner, with a 5–12 MHz linear transducer with vascular preset. Patients were in the supine position with the head slightly deflected backwards. IMT measurements were performed on the far wall of the CCA, 1 cm from its division. The intima-media complex is a structure between the lumen of the vessel/intima and media. Ultrasonic complex of intima-media is created by 2 echogenic lines parallel to each other, visualized in longitudinal section. For each artery, 3 measurements of IMT were taken and the mean values established separately for left and right arteries. Intima-media measurements were completed on each patient and control subject by a single observer to avoid inter-observer variability. The observer was unaware of each patient's treatment protocol, cumulative doses of anthracyclines, and potential risk factors.

Statistical analysis. The values of analyzed parameters measured by rated scale were characterized using sample size and percentage, whereas those by ratio scale were characterized with the assistance of mean value, standard deviation, median, minimal, and maximal value. Contingency tables and χ^2 homogeneity or independence tests were used for evaluation of differences or relationships in analyzed, non-measurable parameters. Due to the slanting distribution of studied measureable parameters and assessed based on Shapiro-Wilk test, non-parametric tests were used to analyze the differences in the studied groups. The Mann-Whitney U test was used to compare the 2 independent groups. A significance test of the Spearman's Rank Correlation Coefficient (R) was used to investigate the relationships between 2 measurable parameters.

A 5% inference error and related $p<0.05$ significance level indicating the presence of statistically significant differences or relationships was assumed. Computer-assisted statistical analyses were performed with STATISTICA v.10.0 software (StatSoft, Poland, 2011).

RESULTS

Clinical characteristics of both patients and control subjects are presented in Table 2. The 2 groups did not differ in gender and age distribution. Also, no differences in systolic

Table 2. Clinical characteristics and cardiovascular risks factors in study groups

Variable	Patients n = 64	Controls n = 36	P value
Age, mean (SD)	15.5 (±5.5)	16.2 (±5.1)	0,4129
Median (range)	15.0 (7.0–29.0)	16.0 (7.0–27.0)	
Male, n (%)	29 (45.3)	18 (50.0)	0,6521
Female, n (%)	35 (54.7)	18 (50.0)	
Systolic blood pressure [mm Hg], mean (SD)	120.0 (±13.7)	121.1(±13.9)	0,7932
Diastolic blood pressure [mm Hg], mean (SD)	71.2 (±9.7)	69.7 (±9.8)	0,3523
BMI [kg/m^2], mean (SD), median (range)	21.0 (±4.6) 20.8 (13.4–34.5)	20.9 (±3.7) 20.1 (14.0–31.7)	0,8801
Risk Factors			
Obese / overweight, n (%)	18 (28.1)	10 (27.8)	0,5917
Dyslipidaemia, n (%)	16 (25)	4 (11.1)	0,1596
Smoking history, n (%)	1 (1.6)	2 (5.6)	0,6080
Physical inactivity, n (%)	26 (40.6)	8 (22.2)	0,0622
Family history of CVD, n (%)	22 (34.4)	12 (33.3)	0,9159

Table 3. Comparison of biochemical parameters and IMT measurements between patients and controls

Variable	Patients n= 64	Controls n = 36	P value
TC [mg/dl], mean (SD)	161.1 (±27.1)	162.1 (±26.6)	0,6253
HDL-C [mg/dl], mean (SD)	53.1 (±13.6)	58.5 (±18.3)	0,1690
LDL-C [mg/dl], mean (SD)	91.1(±24.4)	90.1(±27.0)	0,9284
TG [mg/dl], mean (SD), median (range)	86.5 (±53.3) 69.5 (27.0–282.0)	74.9(±38.3) 62.13(25.8–211.06)	0,5088
IL-6 [pg/mL], mean (SD), median (range)	2.1(±2.7) 1.19 (0.004–17.89)	1.9(±3.6) 0.71 (0.007–18.17)	0,0414
hsCRP [ug/mL], mean (SD), median (range)	1.3 (±2.2) 0.58 (0.024–10.64)	0.6 (± 0.9) 0.35 (0.037–5.27)	0,0599
TM [pg/mL], mean (SD), median (range)	5285.1(±1122.3) 5056.36(3173.63–8277.23)	4873.7(±887.9) 4964.15(3083.1–6908.27)	0,1174
E-selectin [ng/mL] mean (SD), median (range)	46.2(±19.08) 42.38(11,757–98.43)	50.0(±20.0) 47.62(17.75–99.13)	0,3617
sICAM[ng/mL] mean (SD), median (range)	229.3(±62.2) 222.92(120.3–403.45)	199.9(±63.3) 190.95(123.7–477.55)	0,0072
Intima-media thickness			
IMT – CCA-R, mean (SD), median (range)	0,056 (±0,008), 0,053(0.04–0,082)	0,052 (±0,003), 0,052(0,042–0,056)	0,0021
IMT- CCA-L, mean (SD), median (range)	0,057 (±0,009), 0,053(0.04–0,090)	0,052 (±0,003), 0,052(0,042–0,059)	0,0051

Significant p value <0.05 in bold

and diastolic arterial blood pressure or BMI were found between them. Likewise, analysis of conventional risk factors, such as obesity/overweight, dyslipidemia, physical inactivity, family history of cardiovascular disease and smoking, did not reveal any significant differences. Comparison of biochemical parameters and IMT measurements between patients and controls (Tab. 3) indicated that concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides did not differ significantly between the groups. The mean values of IMT index for both the right and left carotid arteries were statistically higher in patients after antineoplastic therapy relative to the control group, and were: IMT-R 0.056 ± 0.008 mm vs. 0.052 ± 0.003 mm; p=0.0021; IMT-L 0.057 ± 0.009 mm vs. 0.052 ± 0.003 mm; p=0.0051.

Significantly higher blood serum concentrations of sICAM-1 and IL-6 were found in young patients following ALL therapy, as follows: 229.3 ± 62.2ng/mL vs.199.9 ± 63.3 ng/mL; p=0.0072, and 2.1 ± 2.7 pg/mL vs. 1.9 ± 3.6 pg/mL; p=0.0414. The concentration of hs-CRP was also higher in the ALL group: 1.3 ± 2.2 ug/mL vs. 0.6 ± 0.9 ug/mL. This difference was close to statistical significance (p=0.0599). Serum blood concentrations of the soluble fraction of TM and E-selectin did not differ significantly between the groups.

A positive correlation was observed between IL-6 concentrations and IMT complex thickness for the right (R=0.38; p=0.0015) and the left carotid arteries (R=0.33; p=0.0082), and IL-6 concentration and hs-CRP (R=0.45; p=0.0002) concentrations (Fig. 1). A positive correlation was also obtained between IL-6 concentrations and E-selectin levels (R= 0.29; p=0.0162) and between E-selectin concentrations and sICAM-1 levels (R=0.47; p=0.0001).

In analyzing the impact of previous antineoplastic therapy on the accelerated development of artherosclerosis, no significant differences were found in the analyzed parameters, compared to patients treated exclusively with chemotherapy or treated with chemotherapy and CNS radiotherapy concurrently. Similarly, no differences in studied parameters were noted between patients treated with a dose of anthracyclines ≤240 mg/m^2, compared to those treated with an anthracycline dose of >240 mg/m^2 (Tab. 4).

DISCUSSION

Arterial hypertension, cigarette smoking, lipid metabolism disturbances, diabetes, positive family history, and lack of physical activity are acknowledged risk factors for the

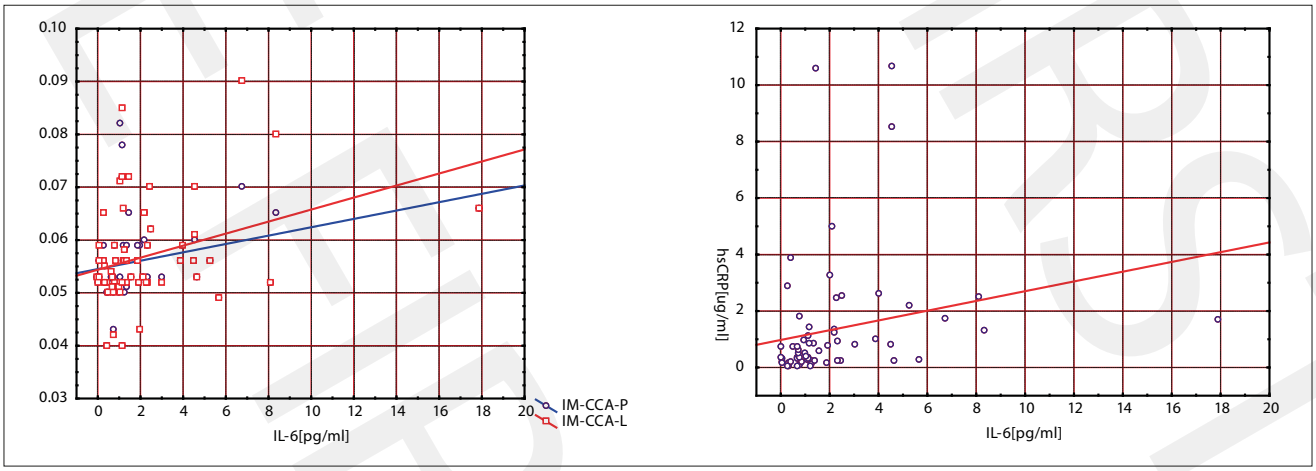

Figure 1. Observed positive correlations between studied: IL-6, IMT, hsCRP
IL-6 – interleukin 6
IM-CCA – P-intima media complex of the rght common carotid artery
IM-CCA-L – intima media complex of left common carotid artery

IL-6 – interleukin 6
hsCPR – high sensivity; C – Reactive Protein

Table 4. Influence of type of received treatment on study parameters in survivors of childhood ALL

	Treatment received		P value	Anthracycline dose		P value
	chemotherapy	chemotherapy + CNS radiotherapy		≤ 240 mg/m²	>240 mg/m²	
No. of patients (n)	45	19		44	20	
Age at diagnosis, Mean ±SD	6.09 ± 3.97	6.94 ± 3.70	0,2900	5.93 ± 3.75	7.25±4.10	0,1415
Time from diagnosis. Mean ±SD	9.31 ± 4.40	9.0 ± 4.53	0,6225	9.5 ± 4.6	8.6 ± 3.99	0,4869
Time from completing the treatment. Mean ±SD	7.13±3.99	6.68± 4.03	0,6172	7.2 ± 4.08	6.55±3.81	0,4298
FS [%]. Mean ±SD	34.79 ±3.69	34.07±4.61	0,6593	35.02±3.82	33.59±4.17	0,2292
EF [%].Mean ±SD	63.92 ±5.05	62.96±6.32	0,6915	64.3±5.14	62.18±5.87	0,2051
RR syst [mmHg]. Mean ±SD	119.48±14.45	121.26±12.11	0,8084	119.05±13.49	122.15±14.34	0,3968
RR diast [mmHg]. Mean ±SD	71.91±8.92	69.47±11.28	0,3701	71.43±9.35	70.65±10.53	0,7390
BMI. Mean ±SD	20.36± 4.37	22.6±4.72	0,1158	20.61±4.28	21.92±5.10	0,4470
Cholesterol [mg/dl]. Mean ±SD	160.13 ±29.32	163.26 ±21.38	0,4760	162.77±29.71	157.3±20.29	0,6957
HDL [mg%]. Mean ±SD	53.29± 12.60	52.65±16.03	0,4990	53.26±14.25	52.75±12.3	0,7721
LDL [mg%]. Mean ±SD	91.03 ±26.84	91.38 ±18.17	0,7298	93.19±26.26	86.59±19.73	0,3577
Trigicerids [mg%]. Mean ±SD	80.22 ±49.55	101.41± 59.93	0,1860	82.68±50.07	94.94±60.17	0,4257
IM-CCA-P. Mean ±SD	0,056 ±0,008	0,057±0,006	0,2282	0.057±0,008	0.054±0.006	0,6121
IM-CCA-L. Mean ±SD	0,056 ±0,009	0,059±0,009	0,1175	0.058±0,009	0.055±0.01	0,4960
IL-6 [pg/ml]. Mean ±SD	2,018 ±3.05	2.45 ±1.88	0,0673	2.16±3.11	2.1 ±1.79	0,4341
hsCRP [ug/ml]. Mean ±SD	1,049 ±1.72	2.05±29.4	0,2547	1.51±2.49	0.97±1.19	0,6957
trombM [pg/ml]. Mean ±SD	5324.2±1062.1	5192.54±1280.04	0,4669	5288.59±1117.03	5277.49±1163.12	0,9884
E-selectin [ng/ml]. Mean ±SD	45.54±19.77	47.6 ±17.74	0,5226	46.29±19.14	45.85± 19.42	0,9307
sICAM [ng/ml]. Mean ±SD	233.87±68.32	218.51±44.20	0,5226	228.77± 63.60	230.51± 60.55	0,9884

Significant p value < 0.05

development of atherosclerosis and cardiovascular diseases. Although past antineoplastic therapy is not considered a conventional risk factor, since it only refers to a small percentage of people among a large population of patients with symptoms of cardiovascular diseases, then taking into consideration a growing population of long-term childhood cancer survivors and a substantial risk for treatment-related cardiovascular complications, this problem seems to be important.

According to the literature, development of atherosclerotic lesions in patients treated for common malignancies of childhood is attributed mainly to radiotherapy and anthracycline-based chemotherapy [15, 16], although other treatment regiments, including mitoxantrone, a high dose of cyclophosphamide, bleomycin, vincristine and tyrosine kinase inhibitors may also participate in this process [1, 2, 7]. The theory of vascular endothelial injury describes the post-radiation pathogenesis of coronary disease. Reactive forms of oxygen are produced in response to radiation-induced injuries to endothelial cells and local inflammatory reactions develop. This leads to a reduction in the local bioavailability of vasodilative nitric oxide, which causes augmented aggregation of platelets and proliferation of vascular cells in smooth muscle. Radiation-induced damage accumulates and intensifies with time, leading to programmed cell death (PCD) [17, 18]. Previous *in vitro* and *in vivo* studies prove that anthracyclines can also cause endothelial injuries in blood vessels [19, 20], and simultaneous use of both these therapies increases the risk for developing cardiac events [21].

It is generally believed that antineoplastic therapy primarily accelerates the development of atherosclerotic lesions in the presence of known risk factors [2, 7]. Moreover, according to some authors, CNS radiotherapies used in patients with ALL can induce obesity and other conventional risk factors; these can also cause pituitary gland dysfunction, growth hormone deficiency, and development of metabolic syndrome [22, 23].

The results of the presented study indicate that antineoplastic therapy applied in young patients with ALL is a significant risk factor of atherosclerosis. Survivors of childhood ALL in the examined group demonstrated elevated concentrations of the soluble adhesive molecule sICAM and IL-6, as well as hsCRP, which may confirm the occurrence of endothelial injuries in blood vessels. The study also revealed that atherosclerotic lesions may even develop in young patients following ALL therapy, irrespective of coexisting cardiovascular risk factors, which is consistent with some reports [2, 7]. The elevated values of the carotid IMT complex, also shown in the current study, additionally confirm this observation. However, there are important limitations in interpreting the results, such as case control study design and the small studied sample. These aspects could have influenced the results obtained (low statistical power), although the statistical differences in blood biomarkers of adhesive and inflammatory molecules, as well as statistical differences in common carotid IMT study are relatively small, they are not clinically significant at that moment, and it should be emphasized that they represent an early, potentially reversible change. The long-term effect of anticancer treatment may lead to more advanced stages of atherosclerosis, with future cardiovascular events, especially in the presence of other well known risk factors. The carotid IMT complex is an established, independent risk marker for cardiovascular disease. The usability of carotid IMT complex assessment in identification of subclinical atherosclerotic lesions, and in predicting the cardiovascular disease risk in patients with various types of malignancies treated with chemo- and radiotherapy, has already been well documented

in previous studies [24–26]. The diagnostic application of biomarkers needs to be further investigated in this particular population.

CONCLUSIONS

The presented study indicates that subjects treated in childhood for ALL may be at a higher risk of prematurely developing atherosclerosis. Therefore, the patients should be encouraged to lead a healthy lifestyle and undergo thorough, periodic follow-up cardiovascular evaluations.

Conflict of interests

The authors have no conflict of interests to declare and did not receive any funding from external sources.

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