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Plethysmographic and Biochemical Markers in the Diagnosis of Endothelial Dysfunction in Pediatric Acute Lymphoblastic Leukemia Survivors – New Applications

A. MASOPUSTOVÁ[1], P. JEHLIČKA[1], M. HUML[1], T. VOTAVA[1], L. TREFIL[2], M. KRESLOVÁ[1], J. SÝKORA[1]

[1]Department of Pediatrics, Faculty of Medicine in Pilsen, Faculty Hospital, Charles University in Prague, Pilsen, Czech Republic, [2]Department of Clinical Biochemistry and Hematology, Faculty of Medicine in Pilsen, Faculty Hospital, Charles University in Prague, Pilsen, Czech Republic

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Summary

Acute lymphoblastic leukemia (ALL) and its treatment are associated with endothelial dysfunction (ED) and increased cardiovascular risk in adulthood. There are no data on ED in children after successful treatment of ALL. We aimed to assess new ED in these children using the plethysmographic reactive hyperemia index (RHI) and biomarkers that are known to be related to ED. In all, 22 children (mean 15.6 years), after successful treatment of ALL, and 18 healthy subjects were included in this prospective study. RHI, plasma concentrations of asymmetric dimethyl arginine (ADMA), high-sensitive CRP (hsCRP) and E-selectin were measured in all children. RHI values were significantly lower in ALL patients when compared with healthy controls ($p<0.05$). hsCRP was significantly increased in ALL patients compared with the control group ($p<0.001$). E-selectin plasma levels were higher in ALL patients as compared to healthy controls ($p=0.05$). This is the first study that combines both plethysmographic and biochemical methods to assess ED in ALL survivors. Significantly decreased RHI with elevated plasma concentrations of biochemical markers imply a possible association with premature ED in ALL patients. The combined diagnostic approach seems to be a valuable tool for more accurate detection of ED and preventive cardiovascular management in these patients.

Key words

Endothelial dysfunction • Reactive hyperemia index • E-selectin • Acute lymphoblastic leukemia • Children

Corresponding author

A. Masopustová, Department of Pediatrics, Faculty of Medicine in Pilsen, Faculty Hospital, Charles University in Prague, Alej Svobody 80, 304 00, Pilsen, Czech Republic. Fax: +420 377104694. E-mail: masopustovaa@fnplzen.cz

Introduction

Acute lymphoblastic leukemia represents one fourth of all childhood malignancies (Gurney *et al.* 1995). The continuously improving cure rates of ALL exceed 85 % nowadays (Horner *et al.* 2009). However, this success come hand-in-hand with increasing numbers of late adverse effects (Silverman 2014, Oeffinger *et al.* 2006). There is a growing population of pediatric ALL survivors at risk for developing late complications of their chemotherapy and radiotherapy given years or decades earlier (Bottomley and Kassner 2003, De Caro *et al.* 2011, Leger *et al.* 2015, Mody *et al.* 2008, Kada-Lottick *et al.* 2010, Bhatia 2003). Accordingly, extended life-long medical care for all survivors of ALL is warranted (Nathan *et al.* 2005, Essig *et al.* 2014). Anhracycline-induced cardiotoxicity may lead to heart failure and dilated cardiomyopathy in children (Bryant *et al.* 2007). In this regard, anthracyclines may also induce changes in the vascular endothelium and could be a causative agent related to the pathogenesis of ED and premature atherosclerosis in young cancer survivors (Woo *et al.* 2013, Jarvela *et al.* 2013, Barry *et al.* 2007).

ED might be regarded as an integrated index of all atherogenic and atheroprotective factors present in an individual, including known, as well as yet-unknown, variables and genetic predispositions (Bonneti *et al.* 2003, Morisson *et al.* 2010). Altered endothelial cell biology is thought to be part of the early pathogenesis of atherosclerosis, although the precise mechanism is yet to be discovered. The decreased bioactivity of nitric oxide (NO), leading to the promotion of vasoconstriction, inflammation and thrombosis, plays a crucial role in the pathogenesis of ED. From a clinical point of view, ED is characterized by reduced vasodilative responses to an appropriate ischemic stimulus (Hamburg *et al.* 2008).

The reversibility of ED may be an attractive primary target in the effort to optimize therapeutic strategies and to decrease cardiovascular risk in pediatric ALL survivors. Early detection of this process may have therapeutic and prognostic implications (Widlanski *et al.* 2003). Unfortunately, ED has received little attention from researchers and studies on ED incidence in comparable pediatric ALL populations are lacking. Expanding on this, novel non-invasive diagnostic tools ensuring the capture of ED are needed (Bonneti *et al.* 2003). Based on these premises, the current study was conducted with the following specific aims: 1) to determine whether a significant difference in reactive hyperemia index (RHI) is found in pediatric ALL survivors as compared to controls, 2) to discern if the association between RHI and specific biochemical markers in ALL survivors exists, and 3) to demonstrate whether the combination of RHI and biochemical parameters can be used for the detection of ED in pediatric ALL survivors.

Materials and Methods

The ALL group included 22 ALL survivors at least two years after successful cytostatic therapy. All of these subjects were treated according to either BFM ALL 95 or ALL IC-BFM 2002 protocols. Neither patients with relapsed ALL nor with secondary ALL were enrolled in the study. All subjects had undergone a standard cardiology examination and all subjects had normal both systolic and diastolic heart function throughout the follow-up. Treatment protocols involved a combination of cytostatic drugs, including anthracycline chemotherapy agents (daunorubicine, doxorubicine) and cyclophosphamide. Cumulative doses of anthracyclines and cyclophosphamide were 240-360 mg/m^2 and 3,000 mg/m^2, respectively.

In the control group, 18 healthy subjects without a history of inflammatory, metabolic or neoplastic diseases were enrolled. None of them reported a history of heart disease, treatment with antibiotics, anti-inflammatory drugs or medication with known adverse effects on the endothelial function. Demographic characteristics of the study sample are listed in Table 1. Exclusion criteria for both groups were dyslipidemia, positive family history of premature cardiovascular events, abnormal left-ventricular function, and smoking.

Table 1. Demographic and laboratory characteristics of the study sample.

Data	ALL	Controls	P value
Subjects	22	18	
Gender (boys/girls)	15/7	5/13	
Age (years)	15.6 (12.72; 17.95)	16.1 (12.91; 17.33)	NS
BMI (kg/m^2)	21.1 (19.3; 25.13)	19.97 (18.79; 22.85)	NS
Arterial blood pressure (mm Hg)	116 (105; 121)/64 (60; 70)	114.5 (110;119)/62.5 (59; 69.5)	NS
Reactive hyperemia index (RHI)	1.5 (1.3; 2.0)	1.80 (1.59; 2.46)	p<0.05
E-selectin (µg/l)	76 (58.32; 108.98)	62.5 (31.66; 70.99)	p=0.05
ADMA (µmol/l)	0.6 (0.53; 0.66)	0.58 (0.49; 0.61)	NS
hsCRP (mg/l)	1.1 (0.71; 2.29)	0.19 (0.18; 0.45)	p<0.001
VCAM (µg/l)	941.7 (818.66; 1074.0)	918.4 (793.08; 1017.90)	NS
Total cholesterol (mmol/l)	4.2 (3.77; 4.67)	4.32 (3.93; 4.77)	NS

Values are expressed as median with inter-quartile range. ADMA – asymmetric dimethyl arginine, VCAM – vascular cell adhesive molecule, hsCRP – high sensitive C-reactive protein, BMI – body mass index, ALL – acute lymphoblastic leukemia, NS – non significant.

Reactive hyperemia index measurement

An EndoPAT® recorder (Itamar Caesarea®, Israel) was used for the determination of the RHI by measuring post-occlusive endothelium-dependent changes in vascular tone (PAT) in the subject´s fingertips. Initially, body mass index (BMI) and blood pressure in the non-dominant arm were measured. The subject was asked to rest in the supine position for 15 min in a temperature-controlled, quiet room with the unique biosensors placed on the fingertips. Then followed a five-minute brachial occlusion (60 mm Hg above the systolic pressure, at least 200 mm Hg). The post-occlusive dilatation with a reactive hyperemia was captured as an increase of the PAT signal amplitude. The RHI was calculated automatically thereafter. The contralateral arm was measured simultaneously to eliminate endothelium-independent changes in vascular tone.

Laboratory measurements

We obtained blood samples from all participating children in standard clinical settings after an overnight fasting. Specific biochemical markers of ED were: asymmetric dimethyl arginine (ADMA) as a competitive NO-synthase inhibitor, E-selectin and vascular cell adhesive molecules (VCAM) measured with Elisa (OLD Diagnostica®, BioVendor®), high-sensitive CRP (hsCRP) measured by a particle-enhanced immuno-turbidimetric assay technique (Orion Diagnostica®). All samples were collected by a qualified person and the tests were performed in a blinded fashion.

Statistics

Values obtained from individual measurements are expressed using descriptive statistics (mean, standard deviation, median, inter-quartile range). Statistical differences between healthy controls and patients with ALL were calculated using a sum rank test (Mann-Whitney test). All statistical analyses were performed using Software SAS 9.4 (Cary®, NC, USA). For all analyses, a p-value of 0.05 or less was accepted as being statistically significant.

Ethical considerations

Our study was conducted in accordance with the principles of the Declaration of Helsinki. The local Ethics Committee approved the study protocol. An informed consent was obtained from the parents or legal guardians of all enrolled participant. The research was not sponsored by any company.

Results

Demographics of the study sample

A total of 40 children were eligible for inclusion. Refer to Table 1 for an overview of the baseline demographic and clinical characteristics of both the ALL survivors and the healthy controls (HC). Probands were of Caucasian ethnicity, comparable in regard to number ratio. There were no differences in lipid profile between the study groups.

Reactive hyperemia index and biochemical parameters

RHI was significantly lower in the ALL patients as compared to HC: 1.5 (1.3; 2.0), 1.8 (1.59; 2.46), median and interquartile range, respectively; $p<0.05$ (Fig. 1).

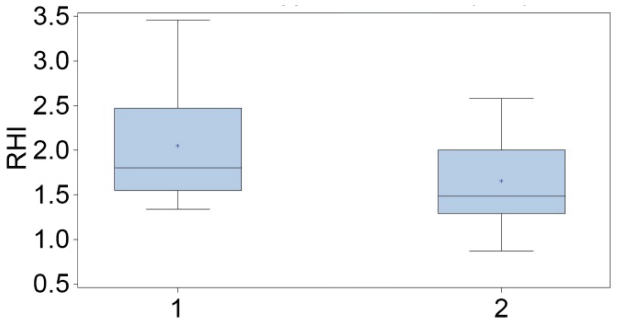

Fig. 1. RHI in healthy controls and ALL. Boxes indicate the inter-quartile range. Horizontal lines within boxes indicate medians. Whiskers extend to the highest or lowest values. 1 – healthy controls, 2 – acute lymphoblastic leukemia.

hsCRP plasma levels were significantly higher in ALL patients as compared to HC: 1.1 (0.71; 2.29) mg/l; 0.19 (0.18; 0.45) mg/l; respectively; $p<0.001$ (Fig. 2).

E-selectin levels of the children with ALL were higher when compared to HC: 76.0 (58.32; 108.98) µg/l, 62.5 (31.66; 70.99) µg/l; respectively; $p=0.05$ (Fig. 2).

No significant difference in ADMA levels was observed between ALL patients and the HC 0.60 (0.53; 0.66) µmol/l; 0.58 (0.49; 0.61) µmol/l, respectively (Fig. 2).

No significant difference in VCAM plasma levels was observed between ALL patients and the HC: 941.7 (818.65; 1074.0) µg/l, 918.4 (793.08; 1017.90) µg/l; respectively (Fig. 2).

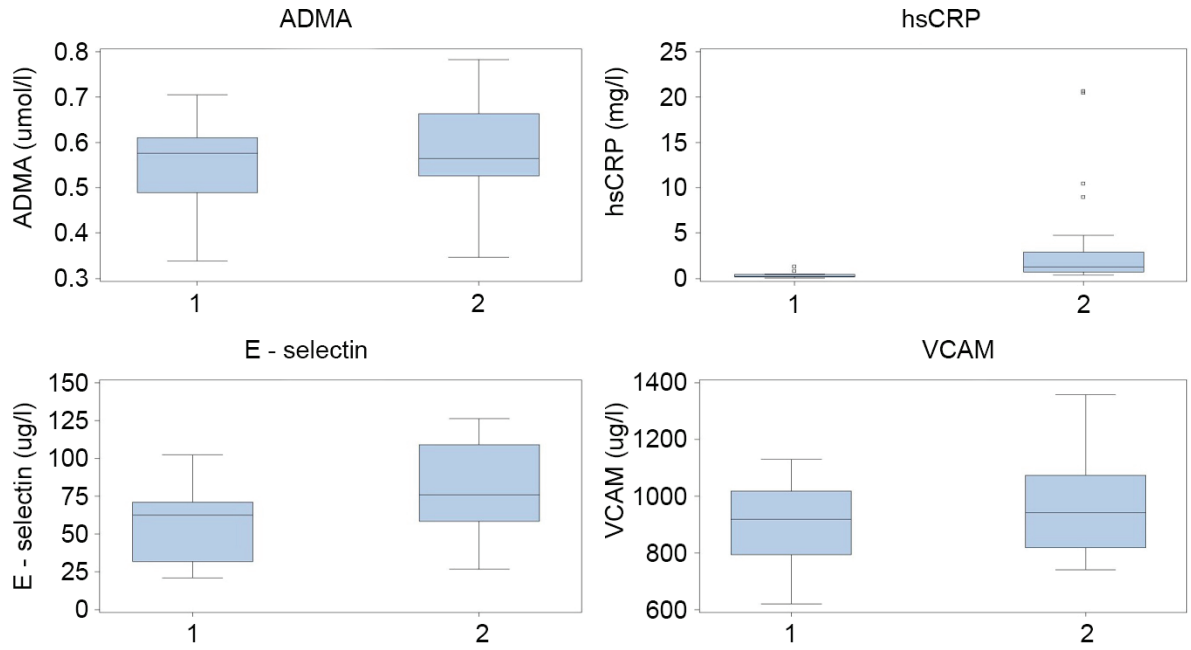

Fig. 2. Biochemical markers in healthy controls and ALL. Boxes indicate the inter-quartile range. Horizontal lines within boxes indicate medians. Whiskers extend to the highest or lowest values. 1 – healthy controls, 2 – acute lymphoblastic leukemia.

Discussion

Our study is the first to comprehensively evaluate ED in pediatric ALL survivors with respect to biochemical markers and RHI. Results of the study indicate that this combined approach may be used for the detection of ED in pediatric ALL survivors. There are several innovative findings in this study: 1) significantly reduced RHI and 2) elevated plasma levels of biochemical markers of ED when compared to healthy controls.

Several studies confirmed an increased cardiovascular risk in adult ALL survivors. The sequelae include predominantly well-documented components of metabolic syndrome with obesity, hypertension, insulin resistance and diabetes that increase cardiovascular risk (Alberti *et al.* 2006). Reported experience regarding ED and risk of cardiovascular diseases in young ALL survivors is limited. Only two studies implicated ED as marker of early development of atherosclerosis in ALL survivors in childhood (Woo *et al.* 2013, Jarvela *et al.* 2013). Probands in our study have a normal lipid profile and absence of metabolic syndrome (Table 1). Based on this fact, the association between treatment-related changes and ED seems to be plausible. Significantly elevated hsCRP level in children with ALL is in accordance with the proven relationship of hsCRP to atherogenic processes. Its elevated level is a predictive factor of morbidity and mortality from cardiovascular events in apparently healthy individuals, regardless of conventional risk factors (Wilson *et al.* 2008). One should respect that high plasmatic levels of hsCRP may be associated with acute inflammatory changes instead ED. Elevated E-selectin level reflects endothelial activation. ADMA as a competitive NO-synthase inhibitor is related to increased oxidative stress and post-occlusive vasodilation. The association of significantly elevated ADMA levels and ED was confirmed in our previous studies involving children with type 1 diabetes mellitus, familial hypercholesterolemia and Crohn's disease as well (Jehlicka *et al.* 2014, Jehlicka *et al.* 2009). This relationship is supported by experimental studies confirming an intimate link among biochemical and functional endothelial changes (Mori-Kawabe *et al.* 2015). Surprisingly, we observed only slightly elevated ADMA plasma levels in ALL patients compared to healthy controls.

In respect to cardiovascular prevention, most of the published studies focused on cardiotoxicity of anthracyclines and related cardioprotection. Dexrazoxan, carvedilol or various alimentary supplements, such as L-carnitine, coenzyme Q and others, were assessed in many studies that ultimately had ambiguous results (Jarvela *et al.* 2013, Van Dalen *et al.* 2011). No valuable information regarding the effect of cardioprotective agents to endothelial functions in ALL survivors has been

published to date. Only one study confirmed the positive effect of home-based exercise on endothelial function in children ALL survivors (Jarvela *et al.* 2013). From this point of view, further studies are highly needed.

From a technical aspect, many studies focused on ED used ultrasound methods, such as flow mediated dilatation (FMD) or intima media thickness (IMT). Reproducibility and accuracy of FMD are still matters of debate in children, mostly due to high dependency on operator‘s experience and demanding technical requirements (Al-Qaisi *et al.* 2008). IMT gradually increases during childhood, thus the precise cut-off values remain difficult to set in this population (Touboul *et al.* 2012). RHI, as a plethysmographic method, is technically easy to perform. Automatic analysis substantially reduces operator dependency and simultaneous measurement of the contralateral non-occluded arm allows for the elimination of unintended exogenous changes during examination. The close relationship between decreased RHI and coronary dysfunction was confirmed *via* invasive methods (Selamet *et al.* 2009). Osika *et al.* (2011) demonstrated a satisfactory variation coefficient of RHI and no sex differences in RHI score in a study including 248 school children.

Several limitations of the study merit consideration. One weakness of this study is the lack of a precise cut-off limit for RHI in children. Another limitation is that the sensors are unified and the minimum finger thickness is not defined. To avoid inaccurate results caused by measuring younger children with fingers too small for sensors, we only selected participants of age 12 and higher. Kelly *et al.* (2014) demonstrated that younger age is associated with lower RHI but not lower FMD among children and adolescents and results of their study suggest that age is associated with RHI. Therefore, we selected the control group with age comparable to ALL survivors. Hamburg *et al.* (2008) demonstrated increased baseline pulse amplitude (PAT) in obese persons with metabolic syndrome and an inverse relationship between baseline and response PAT to hyperemia. Some studies did not find a correlation between FMD and RHI with respect to ED (Allan *et al.* 2013). This controversial finding might partly be explained by different properties of large conductive brachial arteries and the peripheral resistive artery bed. The precise underlying condition is still unclear and a definitive explanation still remains an area of research. There were published conflicting results regarding the ability of RHI to detect acute alterations of vasodilatation following smoking in a small study by Moerland *et al.* (2012). However, smokers were not enrolled in the current sample.

Conclusions

Plethysmographic RHI and biochemical markers were used to assess ED in young ALL survivors in this study. Significantly decreased RHI, elevated plasma levels of hsCRP and E-selectin support a hypothesis of increased risk of premature ED in these patients. The combined approach seems to be a promising method for the assessment of endothelial function. Further clinical ED investigations providing data for diagnostic and therapeutic applications are needed. To what extent it will influence the late cardiovascular risk remains to be answered by long-term studies.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

ADMA – asymmetric dimethyl arginine, ALL – acute lymphoblastic leukemia, ED – endothelial dysfunction, FMD – flow-mediated dilatation, hsCRP – high-sensitive C-reactive protein, IMT – intima-media thickness, NO – nitric oxide, PAT– peripheral arterial tone, RHI – reactive hyperemia index, VCAM – vascular cell adhesive molecule.

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