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Markers of inflammation and endothelial dysfunction in young survivors from acute lymphoblastic leukemia.

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Abstract:	Purpose: To assess subclinical markers of endothelial inflammation in young survivors from acute lymphoblastic leukemia (ALL) treated with chemotherapy without cranial irradiation. Methods: Anthropometric parameters [height (H), body mass index (BMI), waist circumference (WC), hip circumference (HC), WC/H and WC/HC ratio], blood pressure, lipid profile, serum markers of inflammation and endothelial dysfunction [Interleukin 6 (IL-6), Vascular Cell Adhesion Molecule (VCAM), Intercellular Adhesion Molecule (ICAM), Tumor Necrosis Factor-alfa (TNF-α), Endogenous secretory Receptor for Advanced Glycation Endproducts (Es-RAGE)] and carotid intima–media thickness (c-IMT) were assessed in a group of young ALL survivors and

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in matched controls.

Results: 28 ALL survivors (71% male, 18% prepubertal, aged 15.98±4.41 years, mean follow-up 8.57±3.14 years) exhibited lower levels of Es-RAGE than controls (0.18±0.07 vs. 0.27±0.08 ng/ml, p<0.001). Among survivors, Es-RAGE values significantly correlated with BMI-SD off-therapy (R^2 -0.42), WC/H ratio (R^2 -0.41), WC/HC ratio (R^2 -0.38) and with low-density-lipoprotein cholesterol (LDL-C; R^2 -0.43). Most of ALL survivors (78%) presented c-IMT above the 95th centile if compared with gender- and age-standard. Mean c-IMT value correlated with blood pressure (R^2 0.56) and with LDL-C levels (R^2 0.56). Metabolic syndrome (MetS) was fully detected only in one ALL survivor. Nevertheless, 18% ALL survivors presented more than one MetS diagnostic criteria: 14% insulin-resistance, 25% dyslipidemia and 17.8% hypertension.

Conclusions: We demonstrated an initial functional vascular alteration in young ALL survivors even when treated with standard risk protocols. Our data already supports the activation at endothelial level of glycosylation and oxidation processes persistent long after the end of the treatment.

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EXACT TITLE:

Markers of inflammation and endothelial dysfunction in young survivors from acute lymphoblastic leukemia.

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ABSTRACT:

Purpose: To assess subclinical markers of endothelial inflammation in young survivors from acute lymphoblastic leukemia (ALL) treated with chemotherapy without cranial irradiation.

Methods: Anthropometric parameters [height (H), body mass index (BMI), waist circumference (WC), hip circumference (HC), WC/H and WC/HC ratio], blood pressure, lipid profile, serum markers of inflammation and endothelial dysfunction [Interleukin 6 (IL-6), Vascular Cell Adhesion Molecule (VCAM), Intercellular Adhesion Molecule (ICAM), Tumor Necrosis Factor-alfa (TNF- α), Endogenous secretory Receptor for Advanced Glycation Endproducts (Es-RAGE)] and carotid intima–media thickness (c-IMT) were assessed in a group of young ALL survivors and in matched controls.

Results: 28 ALL survivors (71% male, 18% prepubertal, aged 15.98±4.41 years, mean follow-up 8.57±3.14 years) exhibited lower levels of Es-RAGE than controls (0.18±0.07 vs. 0.27±0.08 ng/ml, p<0.001). Among survivors, Es-RAGE values significantly correlated with BMI-SD off-therapy (R^2 -0.42), WC/H ratio (R^2 -0.41), WC/HC ratio (R^2 -0.38) and with low-density-lipoprotein cholesterol (LDL-C; R^2 -0.43). Most of ALL survivors (78%) presented c-IMT above the 95t[h] centile if compared with gender- and age-standard. Mean c-IMT value correlated with blood pressure (R^2 0.56) and with LDL-C levels (R^2 0.56). Metabolic syndrome (MetS) was fully detected only in one ALL survivor. Nevertheless, 18% ALL survivors presented more than one MetS diagnostic criteria: 14% insulin-resistance, 25% dyslipidemia and 17.8% hypertension.

Conclusions: We demonstrated an initial functional vascular alteration in young ALL survivors even when treated with standard risk protocols. Our data already supports the activation at endothelial level of glycosylation and oxidation processes persistent long after the end of the treatment.

BACKGROUND

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer with a 5-year survival rate of 90% in high-income countries.[1] Nowadays, therapeutic progresses in childhood and adolescence bring to high vulnerability later on in life and survival is still tempered by a wide variety of sequelae. An increase in the prevalence of metabolic syndrome (MetS) and of cardiovascular risk (CVR) over time is already well documented in survivors treated according to high-risk protocols including hematopoietic stem-cell transplantation (HSCT) conditioned with total-body irradiation (TBI) and/or cranial and/or abdominal radiotherapy (RT), regardless of body mass index (BMI).[2] Nevertheless, no homogeneous data are yet available on cardiovascular health and its risk of worsening in young ALL survivors treated with standard risk protocols without RT.[3-4] Moreover, little is known about the molecular mechanisms leading to endothelial dysfunction and increase of CVR in this group of patients: cardiotoxicity could directly derived from the exposure to anthracyclines and local RT and, in addition, other endocrine dysfunctions could contribute to the development of MetS.

Several studies have already shown that early atherosclerotic lesions develop from morphological damages of the endothelium.[5] When the normal physiology of the endothelium is altered, its permeability rises, its anti-thrombotic capacity reduces, the adhesion of leukocyte increases due to high levels of Vascular Cell Adhesion Molecule (VCAM) and Intercellular Adhesion Molecule (ICAM) and, at the same time, its tone augmented due to the reduced production of nitric oxide and the proliferation of vascular muscle cells.[6] Interleukin 6 (IL-6) and Tumor Necrosis Factor-alfa (TNF-α) are primary inflammatory cytokines. IL-6 stimulates the production of acute phase proteins, amplifying the mechanisms of innate immunity and tissue remodelling.[7] TNF-α influences each phase of the atherogenetic process: in fact, it promotes the

recruitment of monocytes inducing their adhesion to the endothelium and it could contribute to the transformation and activation of macrophages.[8] Advanced glycation end products (AGEs) are complex and heterogeneous molecules derived *in vivo* from glycosylation and oxidation processes. In addition to the membrane receptor, there is a soluble receptor, which is formed either as a result of proteolytic cleavage of the membrane receptor (sRAGE) or by alternative splicing of the mRNA that codes for RAGES (EsRAGE or endogenous secretory RAGE). In particular conditions such as hyperglycemia, oxidative stress and inflammation, an increase of AGEs and a concomitant reduction of EsRAGE have already been demonstrated.[9] Previously, only few studies measured these endothelium-derived blood vasoactive factors in similar cohorts of childhood ALL survivors.[10-11]

The evaluation of carotid intima-media thickness (c-IMT) is nowadays recommended by the American Heart Association (AHA) as a non-invasive ultrasound parameter that identifies the early stages of intimal wall alterations and, therefore, it represents an excellent indicator of the pre-clinical stage of atherosclerosis also in childhood and adolescence .[12]

In the present study, we assess these subclinical markers of inflammation and endothelial dysfunction in a uniform cohort of young survivors from ALL who underwent standard risk therapeutic protocols without RT.

METHOD AND MATERIALS

Patients

Twenty-height adolescents and young adults (93% Caucasian and 7% from South America) survivors from childhood ALL treated locally (Oncology and Hematology Pediatric Unit, Department of Medical and Surgical Sciences for Mothers, Children and Adults, University of Modena and Reggio Emilia, Modena, Italy) according to ALL AIEOP 2000 and ALL AIEOP

2009 standard risk protocols[13] (see Appendix A) and successfully off-therapy from at least 5 years were enrolled into the study (GROUP ALL, group A). Exclusion criteria counted the exposure to RT, HSCT and/or TBI, the presence of pre-existent conditions increasing CVR (eg. previous CV diseases, type 1 diabetes, familial hypercholesterolemia, genetic syndromes, smoking habit, family history of precocious cardiovascular disease in parents and grandparents…), the presence of one or more endocrine deficiencies and the use of other medications with potential effects on endothelial and CV functions (eg. growth hormone, inhaled corticosteroids, cholesterol lowering medications...).

Additionally, twenty-two age- and gender-matched healthy adolescents who met the same exclusion criteria worked as a control group (GROUP CONTROLS, group B). A written informed consent was obtained from all of them.

The study was approved by the Local Ethics Committee (code 56/13, approved on 24th June 2013).

Study design

This is an observational, transversal and case-control study.

The collection of anamnestic/therapeutic data together with an accurate anthropometric evaluation was performed in the group A during their follow up visit and in the group B.

Anthropometrical parameters [Height (H), body weight, body mass index (BMI), waist circumference (WC), hip circumference (HC), pubertal development] and blood pressure (BP) were evaluated according to standard procedures and expressed as mean ± standard deviation (SD) with respect to chronological age and using national growth charts.[14-17] Metabolic syndrome (MetS) comprises a cluster of CVR factors (hypertension, altered glucose metabolism,

dyslipidemia and abdominal obesity). In our cohort, MetS was defined according to Weiss'
criteria.[18]

Blood samples were drawn early in the morning after overnight fasting. Moreover, group A
underwent an evaluation of endothelial function through c-IMT assessment.

Laboratory methods

Plasma concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C),
triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), fasting glucose and insulin
were measured enzymatically using commercial kits (Cholesterol CHOD-PAD, HDL plus,
Triglycerides GPO-PAP, Hitachi, Roche Diagnostic; Gluco-QuantR, Roche Diagnostic;
Immulite 2000 system, Siemens Healthcare; respectively).

ICAM-1 and VCAM-1 levels were determined using OriGene company's ELISA kit (Human
ICAM-1 and VCAM-1 ELISA kit). The lower limit of sensitivity was 10 pg/ml, with the
reference range 156-10000 pg/ml. The levels of EsRAGE were determined through ELISA kit
(B-Bridge International Company); normal values were 0.25-1.84 ng/ml. IL-6 and TNF- α
concentrations were determined using ELISA kit (Diaclone Company). The lower limit of
sensitivity was 2 pg/ml (reference range 6.25-200 pg/ml) and 10 pg/ml (reference range 25-800
pg/ml), respectively.

Assessment of c-IMT

A single experienced sonographer performed all the measurements after a fasting night, in a
quiet and temperature-controlled room. An ultrasound system (ESAOTE, MyLab70XVision)
equipped with vascular software for two-dimensional (2D) imaging, color Doppler and high-
frequency vascular transducer (8-15 MHz) was used. This performance followed standardized
protocols and results were interpreted according to pediatric standard.[19]

Statistical Analysis

All variables were expressed as mean ± SD. The comparisons among clinical and biochemical data between groups were performed using *U* Mann-Whitney test (STATISTICA, StatSoft Inc., Tulsa, OK, USA). Univariate analysis was performed to correlate variables and Pearson's *r* coefficient was calculated. Statistical significance was determined at a p value of < 0.05.

RESULTS

Clinical and metabolic features

Table 1 shows the main clinical characteristics of all enrolled subjects.

Groups A and B did not differ in age, anthropometrical parameters and rates of distribution of gender, pubertal status and ethnicity, as shown in Table 1.

In Group A, BMI SD significantly increased from diagnosis of ALL to study time (off-therapy). Nevertheless, fasting glycemic metabolism [fasting glucose/insulin ratio 13.03 ± 6.71 (range 2.23 – 28.51); HOMA index 1.96 ± 1.54 (range 0.5 – 8.47)] and lipid parameters [TC 155.41 ±24.84 mg/dl (range 99-193); LDL-C 90.28 ± 21.76 mg/dl (range 47-134); HDL-C 55.96 ± 14.21 (range 37 – 89); TG 70.77 ± 39.74 mg/dl (range 32-189)] were within the normal range.

According to Weiss' definition[18], metabolic syndrome (MetS) was fully detected only in one ALL survivor. Nevertheless, 18% ALL survivors presented more than one MetS diagnostic criteria: 14% (4/28) showed insulin-resistance, 25% dyslipidemia (7/28), 17.8% hypertension (5/28) and 42% (12/28) abdominal obesity.

Inflammation and vascular parameters

Group A exhibited lower levels of Es-RAGE than group B (Table 2). No other differences in inflammation and vascular markers were detected between groups (Table 2). Among survivors, Es-RAGE values inversely correlated with BMI-SD off-therapy (R^2 -0.42), WC/H ratio (R^2 -

0.41), WC/HC ratio (R^2 -0.38) and with LDL-C values (R^2 -0.43) (Table 3). IL-6 levels directly correlated with WC/H ratio (R^2 0.41), WC/HC ratio (R^2 0.51) and TG values (R^2 0.40) while TNF-α values correlated with diastolic blood pressure (DBP; R^2 0.50) (Table 3).

Ultrasound parameter – c IMT

In group A, mean c-IMT is located above the 95th age-standard centile (mean value 0.55±0.14 mm, range 0.4-0.85).[19] Even when single data were analyzed, in 70% of males and in all females, c-IMT was above the 95th centile if compared to gender- and age-standard.[19] In group A, mean c-IMT correlated with both systolic (R^2 0.56) and diastolic blood pressure (R^2 0.66) and LDL-C levels (R^2 0.56)(Table 3).

DISCUSSION

Clinical and metabolic features

Treatment-related risk factors for obesity and MetS in ALL survivors have been previously reviewed in literature[20] and extensively by our group.[21] In 2014, we have already demonstrated that, among 107 Caucasian ALL patients treated on AIEOP protocols without radiotherapy between 1989 and 2000, BMI-SD increased persistently from the start of chemotherapy until the achievement of final height.[22] As current data shown, we confirmed the increase of BMI-SD from ALL diagnosis through a long-term follow-up. Despite BMI-SD trend, in group A, at study time, mean values of BMI-SD, WC/H and WH/HC ratios, glucose and lipid profile were within the normal age-standard (table 1). Nevertheless, our population is not considerable as "metabolic healthy": the 3.5% (1/28) showed MetS, 14% (4/28) insulin-resistance, 25% dyslipidemia (7/28), 17.8% hypertension (5/28) and 42% (12/28) abdominal obesity. Similar findings are presented in other studies of adult survivors of childhood ALL even if prevalence rates for components of

MetS vary widely in literature, due to heterogeneity in diagnostic criteria and study reporting methods.[23-24]

Inflammation and vascular parameters

Our data show that, after a long follow-up period since the end of standard chemotherapy, even without previous exposition to high-risk therapies (as TBI and/or HSCT), childhood ALL survivors showed a pathological c-IMT and a marked increase in Es-RAGE consumption in comparison to age-matched healthy controls. In line with our results, Felicetti F et al. recently reported increased plasmatic levels of AGEs in a cohort of 18 adult survivors of childhood ALL treated with HSCT conditioned with TBI.[2] As already known, AGEs may contribute to the beginning and the development of vascular lesions, attracting monocytes to bind to the vessel surface, followed by their migration into the vessel wall and subsequent activation of endothelial cells and production of other mediators. In Felicetti's study, survivors showed also higher levels of C-reactive protein (CRP), IL-1□, and IL-17 than controls, suggesting the presence of chronic inflammatory state, in agreement with other study.[25] In our study only esRAGE but no other inflammatory molecules differed between ALL survivors and controls. Considering the central role of AGEs in glycoxidation of lipoproteins and foam cells formation, we can speculate that glycosylation and oxidation processes are more expressed in this category of patients at that age (adolescence) than inflammation itself. The latter could be more pronounce later on in life (adulthood). An unbalanced oxidative stress could be not only the trigger but also a persistent and silent disorder leading to inflammation and endothelium dysfunction: this is the so called "inflammaging" process already described in cancer survivors.[26] In addition to a well-known radiation-induced damage, also chemotherapy agents alone, such as anthracyclines, cause an immediate increase in the production of reactive oxygen species (ROS), generating a persistent

oxidative stress[27] and a direct apoptosis of vascular endothelial cells[28] and high dose of corticosteroids could increase visceral adiposity and reduce insulin-sensitivity.[10] Our patients were exposed to a cumulative mean dose of anthracyclines of 244 ± 3.14 mg/m^2 and prednisone of 926 ± 42 mg/m^2 (*data not shown*). In the study performed by Sadurska et al in 2018 among an heterogeneous group of 64 childhood ALL survivors (70.3% treated with only chemotherapy, 70% exposed to a cumulative dose of anthracyclines less than 240 mg/m^2), blood concentration of sICAM, IL-6 and high-sensitivity C-reactive protein as well as cIMT values were higher in ALL group than controls.[11] We can only speculate that dissimilarities between our and these results derived from differences in chemotherapeutic protocols and from the exposition of about 30% of survivors to high-risk therapy, such as RT, in Sadurska's cohort. Nevertheless, endothelial damage appears to be persistent.

In summary, ALL survivors, even if treated only with chemotherapy, have to be considered as unhealthy metabolic subjects similar to their obese and overweight peers.

Ultrasound parameter – c IMT

Among ALL survivors, we demonstrated a morphological alteration of the vascular wall.

Our results seem far from a recent study performed among 81 childhood ALL survivors aged 5-25 years (mean follow-up length 5.7 ± 3.9 years).[29] In this cohort, mean cIMT value was not statistically different between childhood ALL survivors and controls (0.43 ± 0.03 vs 0.42 ± 0.03, respectively).[29] Discrepancies could be justified by the age of survivors (older in our study) and by the length of follow-up after the end of chemotherapy (longer in our study). Moreover, in this recent publication, no details about ALL protocols were provided and thus, any speculation in comparing our and their results seems to be inconsistent.[29] In 2016, Giordano et al demonstrated that childhood ALL survivors had lower FMD of the brachial artery but not pathological cIMT

when compared to healthy controls.[4] As reported in literature in other categories of patients with an high CV risk as children and adolescents with heterozygous familial hypercholesterolemia, alterations in FMD are considered earlier indicators of endothelial dysfunction than cIMT.[30] In line with this finding, when our and Giordano's data are compared, our population presented as older (15.9±4.4 vs. 9.5±4.1 years old, respectively) and the follow-up as longer (average 8.5±3.1 vs. 1.17 years, respectively) than Giordano's ones.[4] Moreover, their study population comprises 52 childhood ALL survivors treated with different risk protocols (6 high-risk, 29 intermediate risk, 17 standard risk; 8 patients underwent cranial RT).[4] The importance of the timing (age) when cIMT is performed, is confirmed by Sadurska and colleagues: in their study, ALL survivors, similar to our cohort in terms of age (15.5±5.5 years old) and length of follow-up (9.2 ± 4.4 years), presented an increased cIMT than controls.[11]

In the already mentioned Ociepa's study, a significant correlation between 24-hour systolic BP SD and cIMT-SD in ALL survivors was documented.[29] This seems to be in line with our results demonstrating a direct correlation between mean c-IMT and both systolic and diastolic blood pressure and LDL-C levels (Table 3). Other study are in line with our results.[31] Moreover, in long-term ALL survivors, endothelial dysfunction, measured by both FMD and cIMT, seems to be reversible and counteracted with physical activity as a simple home-based exercise program[32-36] and some therapies, like vitamin D replacement, may be CV protective in this category.[37]

As happened in many other medical fields, there is a well-known gender dimorphism in pediatric oncologic research: in fact, female ALL survivors seem to be more affected than males by low cardiorespiratory fitness. They are less active than boys and they maybe face more social and environmental barriers as well as greater overprotection by parents. From a biological point of view, females show a genetic predisposition and a more sensible cardiac autonomic nervous

system leading them to 4 times greater risk of developing anthracycline-induced clinical heart failure than male cancer survivors.[33] In our study, 70% of male and all female ALL survivors presented a pathological c-IMT and we did not observe sex differences in BMI values, blood pressure, lipid profile and serum markers of inflammation and endothelial dysfunction (*data not shown*). Considering the exiguity of our population as the main study's limitation, the influence of gender needs to be analyzed in larger cohorts.

CONCLUSION

Our study demonstrated that even standard risk childhood ALL survivors, treated on modern protocols, presented precocious functional and structural endothelial alterations and we support the use of cIMT in screening such complications at least in adolescence. **We hope to shortly reinforce present data measuring cIMT also in healthy controls: this improvement is now limited by the current Covid19 emergency.**

In our opinion, considering the state-of-art, the measurement of serum inflammation and vaso-active molecules (e.g. Es-RAGE, IL-6, VCAM, ICAM and TNF- α) and their interpretation seems more challenging in the clinical practice: their meanings and sensitivity could be influenced by many biochemical factors that still have to be identified leading to a clarification of CV etio-pathogenetic mechanisms.

Currently, our data support the inclusion in the long-term survivor systematic follow-up programs of an early and routinely evaluation of both standard metabolic parameters (e.g. BMI, WC/H ratio, WC/HC ratio, BP, glucose and lipid profile) and endothelial function through non-invasive useful tools (FMD and c-IMT). This addition to current practice seems fundamental for providing early detection and treatment of potentially serious late-onset CV complications, which can be ameliorated by lifestyle modifications[34]. Therefore, the counseling and promotion

of healthy lifestyles are important aspects in the long-term follow-up care and must be encourage

soon after the end of treatment.[35] Screening and prevention could, therefore, partially reverse the

course.

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TABLES:

Table 1. Clinical features of total study population (Group A and B).

Data are presented as mean value ± SD (range)

Legend: ALL, Acute Lymphoblastic Leukemia; BMI, Body Mass Index; N.A., Not Applicable; SD, Standard Deviation; TH, Target Height; WC/H, Waist circumference/Height; WC/HC, Waist circumference/Hip circumference.

* $p<0.05$ at diagnosis ALL vs. off-therapy

Parameters	Group A (n.28)	Group B (n.22)	p-value
Age (years)	15.98 ± 4.41 (9.18 – 24.88)	16.59 ± 5.60 (6.00 – 27.13)	0.48
Male (%)	71	64	0.45
Prepubertal (%)	18	25	0.35
Caucasian (%)	93	92	0.60
Follow-up (years)	8.57 ± 3.14 (4.05 – 15.69)	N.A.	N.A.
Height SD at diagnosis ALL	0.15 ± 1.27 (-2.66 – 2.86)	N.A.	N.A.
Height SD off-therapy	0.01 ± 1.05 (-1.82 – 2.19)	0.09 ± 0.98 (-1.45 – 1.88)	0.09
Height SD off-therapy TH-adjusted	0.26 ± 0.84 (-1.80 – 1.65)	N.A.	N.A.
BMI SD at diagnosis ALL	-0.34 ± 1.42 (-3.35 - 2.80)	N.A.	N.A.
BMI SD off-therapy	0.55 ± 1.30 (-1.25 - 3.13)*	0.42 ± 1.01 (-1.33 – 2.34)	0.37
WC/H ratio off-therapy	0.48 ± 0.08 (0.39 – 0.71)	N.A.	N.A.
WC/HC ratio off-therapy	0.91 ± 0.07 (0.73 – 1.06)	N.A.	N.A.

Table 2. Serum endothelial biomarkers in both groups.

Data are presented as mean value ± SD.

Legend: Es-RAGE, Endogenous secretory Receptor for Advanced Glycation Endproducts; ICAM, Intercellular Adhesion Molecule; IL-6, Interleukin 6; SD, Standard Deviation; TNF-α, Tumor Necrosis Factor-alfa; VCAM, Vascular Cell Adhesion Molecule.

Parameters	Group A	Group B	p- value
IL-6 (pg/ml)	1.39 ± 0.74	1.70 ± 1.24	0.67
VCAM (pg/ml)	1488.92 ± 534.53	1614.31 ± 725.11	0.65
ICAM (pg/ml)	109.96 ± 55.3	104.30 ± 55.63	0.46
TNF-α (pg/ml)	8.15 ± 7.39	7.78 ± 5.15	0.75
Es-RAGE (ng/ml)	0.18 ± 0.07	0.27 ± 0.08	< 0.001

Table 3. Correlation analysis (R^2) between cardiovascular risk markers and clinical and biochemical parameters in Group A (*$p<0.05$).

Legend: BMI, Body Mass Index; c-IMT, carotid-Intima Media Thickness; DBP, Diastolic Blood Pressure; Es-RAGE, Endogenous secretory Receptor for Advanced Glycation Endproducts; IL-6, Interleukin 6; LDL-C, Low-density Lipoprotein- Cholesterol; SBP, Systolic Blood Pressure; SD, Standard Deviation; TG, Triglycerides; TNF-α, Tumor Necrosis Factor-alfa; WC/H, Waist circumference/Height; WC/HC, Waist circumference/Hip circumference.

Parameters	Es-Rage	IL-6	TNF-α	c-IMT
BMI-SDS off-therapy	-0.42*	+0.32	+0.02	+0.40
WC/H ratio	-0.41*	+0.41*	+0.12	+0.36
WC/HC ratio	-0.38*	+0.51*	+0.20	-0.03
LDL-C (mg/dl)	-0.43*	+0.08	-0.21	+0.56*
TG (mg/dl)	-0.33	+0.40*	+0.04	+0.39
SBP (mmHg)	-0.16	-0.02	+0.04	+0.56*
DBP (mmHg)	+0.13	+0.11	+0.50*	+0.66*

Appendix A.
AIEOP ALL 2000 and 2009 standard risk protocol (cumulative doses expressed in mg/m^2).

	Patients Group A N (%)	Chemotherapy: Phases		
		Induction	Consolidation	Reinduction
AIEOP ALL 2000	23 (82%)	**63 days** PDN (1680 + tapering) VCR (6) DNR (120) L-ASP (40000) CPM (2000) ARA-C (1200) 6MP (1680)	**56 days** MTX (8000) 6MP (1400)	**49 days** DXM (210 + tapering) VCR (6) ADM (120) L-ASP (40000) CPM (1000) ARA-C (600) 6TG (840)
AIEOP ALL 2009	5 (18%)	**63 days** PDN (1680 + tapering) VCR (6) DNR (120) PEG-ASP (5000) CPM (2000) ARA-C (1200) 6MP (1680)	**56 days** MTX (20000) 6MP (1400)	**49 days** DXM (210 + tapering) VCR (6) ADM (120) PEG-ASP (2500) CPM (1000) ARA-C (600) 6TG (840)

Legend: PDN, Prednisone; DXM, dexamethasone; VCR, vincristine; DNR, daunorubicin; ADM, Adriamycin; CPM, cyclophosphamide; ARA-C, cytarabine; L-ASP, l-asparaginase (cumulative dose expressed in UI/m^2); PEG-ASP, pegylated asparaginase; 6MP, mercaptopurine; 6TG, thioguanine; MTX, methotrexate

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