# Serum level of salusin β as an indicator of metabolic disorders in Acute Lymphoblastic Leucaemia and Wilms Tumour survivors

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# Abstract

Background Cardiovascular diseases (CVD) are one of long-term side effects of the childhood cancers treatment. Salusin β is an indicator of developing atherosclerosis.

Aim To assess the prevalence of established risk factors for CVD and the assessment of new indicator for CVD risk - salusin β in long-term acute lymphoblastic leukemia (ALLs) and Wilms tumour (WTs) survivors.

Methods 37 ALLs and 11 WTs at least 5 years after the end of oncological treatment undergone physical examination and laboratory tests after an overnight fast. Laboratory tests included lipid profile, serum level of glucose, renal parameters and salusin β.

Results The both groups didn't vary in age, time from the end of the treatment, number of obese persons, BP, lipid profile, serum creatinine and glucose level. ALLs had higher weight and greater waist circumference. Serum cystatin was higher and cystatin-based eGFR lower among WTs. Salusin β was higher in group WTs, but the result was not statistically significant.

Conclusions ALLs and WTs differ in types of long-term side effects. ALLs present rather metabolic problems, WTs - lower eGFR. Salusin β seems to implicate development of hypertension rather than metabolic disorders like obesity. Further investigations are necessary to confirm this statement.


# Background

In recent years, the number of childhood cancer survivors (CCS) has been constantly and significantly increasing. However, this effect was not achieved without a price. Oncological treatment is associated with long-term morbidity and mortality. The cardiovascular sequelae of cancer treatment are one of the most serious complications. These effects are largely caused by the direct toxic effects of radio- and chemotherapy. In addition, the prevalence of other risk factors for cardiovascular diseases (CVDs), such as obesity, hypertension and hypercholesterolemia, are also increased in CCS. These facts have been confirmed by numerous studies in older populations of survivors(1)(2)(3)(4).

Parallelly, CVDs are a great concern of researchers, as one of the main causes of death in the world. Scientists are looking for substances that could diagnose developing CVDs early on. Salusin β is considered a potential biomarker of atherosclerosis. Salusin β is a peptide that contributes to endothelial injury (5)(6). The concentration of salusin β has a positive correlation with blood pressure (BP) and triglyceride levels and is elevated in conditions that lead to cardiovascular complications, such as diabetes mellitus or polycystic ovary syndrome (7)(8)(9).

The aim of this study was to assess the prevalence of established risk factors for CVDs and to assess new indicators for CVD risk, such as salusin β in long-term acute lymphoblastic leukaemia survivors (ALLs) and Wilms tumour survivors (WTs).

# Patients And Methods

## Patients

This study enrolled 37 ALL and 11 WT survivors who were treated between 2000 and 2013 at the Department of Paediatrics, Haematology and Oncology of the Medical University of Gdansk. The patients, from 7 to 18 years of age, were examined at least 5 years after the end of oncological treatment. The clinical study was performed during routine follow-ups. The study consisted of a patient history and a physical examination, including anthropometric measurements, triple BP measurements, and blood and urine sample collection.

## Methods

The height, weight, and waist circumference were measured using standard techniques (Mensor WE 150, 2014).

During laboratory testing, we evaluated the morphology, serum creatinine, cystatin C, glucose, lipid profile and salusin β after an overnight fast.

Serum creatinine concentration was assayed using an enzymatic method (Alinityc Creatinine Reagent Kit Abbott). Serum cystatin C levels were detected by immunonephelometry (N Latex Cystatine C Siemens). The estimated glomerular filtration rate (eGFR) was calculated based on both the creatinine and cystatin C serum levels.

EGFR was measured indirectly using the original Schwartz, creatinine and BUN-based equation, and Filler formulas.

The Schwartz formula is defined as follows: GFR in mL/min/1.73 sq m = k x height of child in cm/serum creatinine concentration in mg/dL, where the constant k was defined using the published literature values of k=0.413 for children (10). Creatinine and BUN-based eGFR was calculated according to equation - 40.7(height/SCr)0.64(30/BUN)0.202 (11).

Additionally, the serum concentration of cystatin C was evaluated, and GFR was calculated according to the Filler formula: logGFR=1.962+[1.123xlog(1/cystatin C)] (12).

The plasma lipid profile was determined with electrophoresis (Hydragel 15 Lipo + Lp(a) Sebia). The concentration of salusin β was determined by an immunoenzymatic method using an Elisa set for salusin β (produced by Cloud-Clone Corp. 2018).

The International Diabetes Federation criteria were used to identify metabolic syndrome and central obesity (13).

## Blood Pressure

Blood pressure (BP) was measured in every child in the study by an oscillometric method using a standard clinical sphygmomanometer (professional blood pressure monitor HBP-1100-E, OMRON HEALTHCARE Co., Ltd. Kyoto, Japan, 2014) according to guidelines and recommendation of the Polish Pediatric Nephrology Society (14). BP was measured three times in each patient. Mean values of the systolic and diastolic pressure were determined. The results were then compared to the reference values matched according to gender, age and height.

### Statistical methods

The data are expressed as the mean, median and SDS values, and were compared with statistical tests, such as analysis of variance (ANOVA), the Mann-Whitney U test, the Kruskal-Wallis test with ranking and the chi-square test with the Yates correction. Analysis of the correlation between analysed parameters was evaluated using Spearman's rank correlation coefficient.

$P<0.05$ was considered statistically significant.

The standard deviation score value was evaluated using the following formula:

SDS value = (observed value – mean value in referenced population)/SDS value in reference population.

For the reference population, we used the results of the OLAF research, which was performed among children from the Polish population aged from 7 to 18 years (15)(16).

Statistical analysis was performed using EPIINFO Ver. 7.1.1.14 software.

## Results

All of the patients with ALL undergone standard chemoterapii, 9 of them received cranial radiotherapy additionally.

Nine patients with WT undergone total nephrectomy, 3 of them received abdominal radiotherapy in addition. Two patients with WT who sufferd from bilateral toumor undergone partial nefrectomy. Accept from surgery, all the WT patients were treated with chemotherapy.

The two examined groups of patients did not vary significantly in the time from the end of their treatment (ALLs vs WTs (8 (25Q-75Q: 6-9) vs 10 years (25Q-75Q: 6-13), p=0.188). The detailed characteristics of the studied groups are shown in table 1.

There were 12 persons with central obesity in the ALLs group and 2 in the WTs group, which was not a significant difference. However, in ALLs, the weight and waist circumference were significantly higher than those in the WTs (table 2). Central obesity was revealed in 3 out of 9 patients with ALL and cranial radiotherapy. No patients met the criteria of metabolic syndrome.

We found no significant differences in both the systolic and diastolic BP, as well as in the serum levels of total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides, between the two groups (tables 3 and 4).

The median serum salusin β concentration was higher in the WTs group than the ALLs group, but the difference was not statistically significant. The serum level of cystatin C was significantly higher, and the cystatin-based eGFR was significantly lower, in the WTs than in the ALLs. This type of a difference was not observed for creatinine (table 4).

# Discussion

Recent studies of Polish child and adolescent cancer survivors revealed a high incidence of cardiovascular problems. The assessment of the health status of Polish children and adolescents after cancer treatment has shown that circulatory problems were observed in 31.7% of the whole group, and obesity or short stature were present in 21.4% of all survivors. A higher frequency of circulatory system problems was observed in males than in females ($p = 0.029$), in children diagnosed between the ages of 1–4 years, 5–9 years, 10–14 years, and > 15 years, than in children who were infants when they were diagnosed ($p < 0.0001$), and in the groups of patients 5–10 and 11–15 years after treatment completion, than in children with time of follow-up < 2 years ($p < 0.0001$). Thirty-eight percent of patients who underwent treatment for ALL presented symptoms or complaints that suggested circulatory system problems, in contrast to 26.6% of patients after WT treatment. Symptoms such as short stature and obesity were present in 23.7% and in 13% of ALL and WT survivors, respectively (17).

The research by Ociepa et al. reported a prevalence of hypertension among ALL survivors of 37% (18).

These facts have been confirmed by numerous other national studies among older populations of survivors (1)(2). The results of these studies justify the search for new indicators of cardiovascular diseases.

Salusins have recently been identified as endogenous bioactive peptides that have hypotensive and bradycardiac impacts. They are synthesized and present in many tissues of the human body. Salusin α seems to suppress the formation of macrophage foam cells and atherosclerosis. The concentration of salusin α is decreased in conditions leading to atherosclerosis compared to that in healthy patients. Salusin β influences BP and heart rate through parasympathetic stimulation and negative inotropism. The central action of salusin β is regulating fluid balance. The peripheral effect is potentially atherosclerotic. Serum level increases of salusin β have been observed in patients with coronary artery disease and diseases that lead to cardiovascular disorders (8)(19). Elevated serum salusin β was observed in children with primary hypertension and was positively correlated with the serum triglyceride level and triglyceride/HDL-cholesterol ratio (20). Thus, salusin β seems to be a useful parameter for developing CVD.

In our study, the median serum salusin β concentration was higher in the WTs group, but the difference was not statistically significant. Although ALLs seem to have a higher risk of developing CVD and, in our study, had significantly higher weight and waist circumference, they had lower serum levels of salusin β than those in WTs. The explanation for this may be associated with the worse renal function expressed by the higher levels of cystatin c and lower cystatin-based eGFR in the WTs than in the ALLs. Poor renal function in WTs was also observed in our previous studies (21)(22). Research performed by Kołakowska et al. revealed a higher level of serum salusin β among patients with hypertension than in the reference group (the subjects diagnosed with white-coat hypertension). This finding may confirm the important role of salusin β in the pathogenesis of hypertension (23).

The lack of significance in the levels of salusin β in both groups might have been influenced by the low number of enrolled patients, which was not high enough to reach definite conclusions. In particular, the group of WT survivors was markedly small. The young age, relatively short time from the end of the treatment and variety of the undergone trement could also play an important role. The fact that the patients were not diagnosed with metabolic syndrome may also be relevant. Atherosclerosis develops gradually and is exacerbated in middle age. Obesity, hyperlipidaemia, hypertension and insulin resistance significantly accelerate the development of CVD. Thus, further studies need to be performed to determine whether the concentration of salusin β correlates with the development of endothelial injury and atherosclerosis in survivors of childhood cancers.

# Conclusions

Many types of long-term side effects are observed among survivors of paediatric cancers. Patients treated for ALL and WT differ in type of side effects that they experience. ALL survivors more often develop obesity and metabolic problems, whereas WT survivors tend to develop renal disorders.

Salusin β seemed to predict the development of hypertension rather than metabolic disorders such as obesity, but the results were not statistically significant. Further investigations are necessary to confirm this result.

It is necessary to continue follow-up among adults who were treated for childhood cancers to reveal long-term side effects such as cardiovascular disorders.

# Availability Of Data And Materials

Not applicable.

# Abbreviations

ALL - acute lymphoblastic leukaemia

ALLs - acute lymphoblastic leukaemia survivors

BP - blood pressure

CCS - childhood cancer survivors

CVDs - cardiovascular diseases

eGFR – estimated glomerular filtration rate WT - Wilms' tumour

WTs - Wilms' tumour survivors

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# Declarations

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### Contributions

AJ and JS took part in the study design, literature research, assessment of research, data analysis and manuscript preparation. AO took part in study design, literarure research and assessments of research. EAD was the guarantor of integrity of entire stydy and let the study design. All authors read and approved the final manuscript.

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### Ethics Declarations

### Ethics approval and consent to participate

This study was approved by the Independent Bioethical Committee of Scientific Researchers at the Medical University of Gdansk. Written informed consent was obtained from the legal guardians of the children.

**Consent for publication**Not applicable.

### Competing interest

The authors declare that they have no competing interests.

# Tables

Table 1. The characteristics of ALLs[1] and WTs[2]

| | ALLs[1] (n=37) | WTs[2] (n=11) | P |
|---|---|---|---|
| Sex (F/M) | 14/23 | 6/5 | 0.324 |
| Age at diagnosis [years] | 3 | 2 | 0.081 |
| Age at the time of the study [years] | 14 | 14 | 0.753 |
| Time from the end of treatment [years]<br><br>(median, 25Q-75Q) | 8 (6-9) | 10 (6-13) | 0.188 |

[1]ALLs - acute lymphoblastic leukaemia survivors

[2]WTs - Wilms' tumour survivors

Table 2. The comparison of established risk factors for CVD in ALLs[1] and WTs[2] (median, 25Q-75Q)

| | ALLs[1] (n=37) | WTs[2] (n=11) | P |
|---|---|---|---|
| Central obesity | 12 | 2 | 0.361 |
| Weight [kg] | 64.6 (52.5÷69.5) | 48.2 (40.7÷62.7) | 0.024* |
| SDS[3] of the weight | 0.464 (0.088÷1.286) | 0.149 (-0.479÷1.168) | 0.263 |
| Height [cm] | 166 (154÷177) | 160 (154÷169) | 0.243 |
| SDS[3] of the height | 0.626 (-0.287÷1.420) | 0.711 (0.153÷1.738) | 0.847 |
| BMI [kg/m$^2$][5] | 21.7 (18.7÷24.0) | 19.0 (17.0÷21.8) | 0.065 |
| SDS[3] of the BMI[4] | 0.607 (-0.206÷1.298) | -0.173 (-0.672÷1.350) | 0.123 |
| Waist circumference [cm] | 74.0 (67.0÷81.0) | 67.0 (64.0÷71.5) | 0.019* |

[1]ALLs – acute lymphoblastic leukaemia survivors

[2]WTs - Wilms' tumour survivors

[3]SDS - standard deviation score

[4]BMI – body-mass index

*statistically significant difference

Table 3. The comparison of blood pressure (BP) in ALLs[1] and WTs[2] (median, 25Q-75Q)

| | ALLs[1] (n=37) | WTs[2] (n=11) | P |
|---|---|---|---|
| Systolic BP[3] [mm Hg] | 117 (113÷122) | 111 (111-124) | 0.263 |
| Pc of the systolic BP[3] | 71 (41÷81) | 56 (43÷88) | 0.981 |
| Diastolic BP[3] [mm Hg] | 75 (72÷78) | 75 (74÷81) | 0.594 |
| Pc of the diastolic BP[3] | 94 (85÷97) | 96 (89÷99) | 0.285 |

[1]ALLs - acute lymphoblastic leukaemia survivors

[2]WTs - Wilms' tumour survivors

[3]BP – blood pressure

Table 4. Comparison of the biochemical parameters in ALLs[4] and WTs[5] (median, 25Q-75Q)

| | ALLs[4] (n=37) | WTs[5] (n=11) | P |
|---|---|---|---|
| Salusin β | 94.3 (56.0÷188.3) | 133.8 (72.7÷193.1) | 0.513 |
| Total cholesterol [mg/dl] | 154 (137÷177) | 151 (132÷167) | 0.722 |
| LDL [mg/dl] | 90 (74÷108) | 83 (64÷97) | 0.426 |
| HDL [mg/dl] | 50 (44÷58) | 56 (51÷58) | 0.373 |
| TG [mg/dl] | 58 (48÷85) | 67 (60÷83) | 0.320 |
| eGFR[1] [ml/min/1.73 $m^2$] | 111 (98÷129) | 106 (94÷114) | 0.164 |
| eGFR[2] [ml/min/1.73 $m^2$] | 92 (85÷100) | 89 (85÷89) | 0.114 |
| Cystatin C [mg/dl] | 0.77 (0.7÷0.81) | 0.85 (0.73÷1.11) | 0.042* |
| Cystatin-based eGFR[3] [ml/min/1.73 $m^2$] | 123 (116÷137) | 110 (82÷131) | 0.042* |

* statistically significant differences

[1] eGFR calculated from revised Schwartz equation

[2] eGFR calculated from creatinine and BUN-based equation

[3]eGFR calculated from Filler equation

[4]ALLs – acute lymphoblastic leukaemia survivors

[5]WTs - Wilms’ tumour survivors