

ORIGINAL RESEARCH

# Promotion of Arterial Stiffness by Childhood Cancer and Its Characteristics in Adult Long-Term Survivors

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**BACKGROUND:** Vascular alterations induced by antineoplastic treatment might be considered as a possible underlying mechanism of increased cardiovascular sequelae in childhood cancer survivors (CCSs). We aimed to evaluate arterial stiffness among long-term CCSs and to compare the data against a population-based sample.

**METHODS AND RESULTS:** Arterial stiffness was assessed by digital photoplethysmography (stiffness index; m/s) among 1002 participants of the CVSS (Cardiac and Vascular Late Sequelae in Long-Term Survivors of Childhood Cancer) study, diagnosed with neoplasia (1980–1990) before an age of 15 years. A population-based sample from the GHS (Gutenberg Health Study) (n=5252) was investigated for comparison. All subjects underwent a comprehensive, standardized clinical examination in the same study center. CCSs had higher stiffness index ( $\beta=0.66$  m/s; 95% CI, 0.51–0.80 m/s) in multivariable linear regression analysis after adjustment for cardiovascular risk factors compared with the population sample of comparable age range. Stiffer vessels were found among CCSs also in absence of arterial hypertension ( $\beta=0.66$ ; 95% CI, 0.50–0.81) or history of chemotherapy/radiotherapy ( $\beta=0.56$ ; 95% CI, 0.16–0.96) in fully adjusted models. Moreover, stiffness index differed by tumor entity, with highest values in bone and renal tumors. Almost 5.2-fold higher prevalence of stiffness index values exceeding age-specific, population-based reference limits was observed among CCSs compared with GHS participants.

**CONCLUSIONS:** This is the first study demonstrating increased arterial stiffness among long-term CCSs. The data suggest that vascular compliance might differ in survivors of childhood cancer from the established development concept for arterial stiffness in the population; cancer growth and antineoplastic treatment might be relevant determinants of the pathobiological features.

**REGISTRATION:** URL: <https://www.clinicaltrials.gov>; Unique identifier: NCT02181049.

**Key Words:** arterial stiffness ■ childhood cancer survivors ■ general population

**R**emarkable progress in cancer treatment during the past 40 years resulted in improved survival rates for most pediatric malignancies.<sup>1,2</sup> However, childhood cancer survivors (CCSs) are still at high risk for long-term cardiovascular morbidity

and mortality, considered to be late effects of cancer therapy.<sup>2</sup> Although earlier investigations in the field of cardio-oncology have been mostly dedicated to cardiotoxicity of antineoplastic therapy,<sup>3</sup> recent research rather focused on vasculotoxic effects.<sup>4</sup> Indeed,

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## CLINICAL PERSPECTIVE

### What Is New?

- Among long-term childhood cancer survivors, arterial stiffness was markedly higher than in a population sample of comparable age, even after controlling for the cardiovascular risk profile.
- This difference was also present among individuals without arterial hypertension or former chemotherapy or radiotherapy; arterial stiffness varied substantially by tumor entity, with highest values for a history of bone or renal tumors.
- The data suggest that promotion of arterial stiffness in childhood cancer survivors might differ from the established development concept for arterial stiffness in the population.

### What Are the Clinical Implications?

- These vascular alterations might indicate a possible underlying mechanism for premature arterial hypertension and overall cardiovascular sequelae among these high-risk individuals, such as long-term childhood cancer survivors.
- These findings contribute to the growing body of evidence supporting an early and regular evaluation of cardiovascular health in this patient group.
- Nonetheless, additional research is needed to understand whether arterial stiffness, measured at the asymptomatic, subclinical stage, represents a reliable prognostic marker for the cardiovascular outcome and whether it supports the risk stratification for prevention and treatment of cardiovascular sequelae.

## Nonstandard Abbreviations and Acronyms

<b>CCS</b>	childhood cancer survivor
<b>CVSS</b>	Cardiac and Vascular Late Sequelae in Long-Term Survivors of Childhood Cancer
<b>GHS</b>	Gutenberg Health Study
<b>SI</b>	stiffness index

arterial hypertension has been indicated as one of the most frequent complications following malignancy-related treatment.<sup>5</sup> It has been further shown, also by previous data from our group,<sup>6</sup> that arterial hypertension develops in cancer survivors earlier than in the general population.<sup>2,6</sup> The underlying pathophysiological processes of this association are still not completely elucidated. Several lines of evidence

suggested that a decreased compliance of the arterial wall, clinically measured as arterial stiffness, might be a major determinant and rather precursor of arterial hypertension.<sup>7</sup> In the general population, a 1-m/s increase in pulse wave velocity, a most studied marker of arterial stiffness,<sup>7</sup> was related to  $\approx 15\%$  increase in future cardiovascular events.<sup>8</sup> Furthermore, data from short-term cancer treatment with low to moderate doses of anthracycline-based chemotherapy revealed an increase in pulse wave velocity by  $\approx 3$  m/s in 6 months.<sup>9</sup> Hence, arterial stiffness might be considered as a potent predictor of future cardiovascular events in CCSs. However, only a limited number of sizeable studies investigated arterial stiffness in long-term CCSs.<sup>10–12</sup>

The aim of the present analysis was to evaluate differences in arterial stiffness among CCSs compared with the general population, using data from the CVSS (Cardiac and Vascular Late Sequelae in Long-Term Survivors of Childhood Cancer) study and the population-based GHS (Gutenberg Health Study).

## METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request after institutional approval and following institutional process.

### CVSS Study Design

The CVSS study represents an observational cohort study, aiming to investigate cardiovascular sequelae of pediatric cancer. The CVSS study source population was identified using the data from the nationwide GCCR (German Childhood Cancer Registry), which has systematically collected sociodemographic and malignant disease information of patients with pediatric cancer since 1980.<sup>13</sup> Inclusion criteria were as follows: (1) neoplasia, diagnosed in accordance to the *International Classification of Childhood Cancer* between 1980 and 1990 before 15 years of age, (2) at least  $>5$  years' survival after initial cancer diagnosis, and (3) previous antineoplastic therapy (chemotherapy, radiotherapy, or surgery). Survivors of Hodgkin lymphoma and a negligible proportion of subjects with nephroblastoma diagnosed in 1990 were not included in the study, because they participated in other clinical investigations. Only CCSs from former treating medical centers located at acceptable travel distance to the study center at University Medical Center Mainz (radius of  $\approx 300$  km;  $n=34$  of 82 participating centers) were eligible for participation.

Because the CVSS study aimed to examine  $\approx 1000$  long-term survivors, a sample of 2894 subjects of 4320

potentially eligible CCSs (nonrandomized recruitment) from all 34 participating centers were invited by mail via the GCCR. Of invited survivors, 39% consented to participate (n=1140). In total, 1002 individuals (aged 23–48 years at enrollment) have been examined between October 2013 and February 2016. All participants gave informed written consent on entry into the study. Among them, subjects with subsequent neoplasia (n=51) were later excluded from the analysis. Therefore, the final study sample includes 951 subjects. The study protocol and study documents had been approved by the responsible ethics committee (medical association of the federal state Rhineland-Palatinate, Germany) and the local data safety commissioner. The study was performed according to the principles of Good Clinical Practice and the Declaration of Helsinki.

### Population-Based Sample as Reference Group

For the present analysis, a subsample from the population-based GHS (n=5252) with comparable age range (aged 35–≤50 years) was chosen as comparison group. The GHS is a population-based, observational cohort study, including residents of the city of Mainz and the county of Mainz-Bingen located in Western Germany. A representative population sample of 15 010 individuals, aged 35 to 74 years, with stratification for age, sex, and residence was drawn randomly from the local governmental registry. Details of the study design have been reported elsewhere.<sup>14</sup>

### Data on Cancer Diagnosis and Therapy

Information on primary cancer disease was abstracted from the GCCR in Mainz. Treatment-related data were collected from primary health records of former treating medical centers and/or the centrally documented individual therapy data of the respective study centers of the Society for Pediatric Oncology and Hematology.

### Data Collection and Assessment of Cardiovascular Risk Profile and Comorbidities

The study participants of the CVSS study and GHS underwent an identical, highly standardized cardiovascular examination program of 5.5 hours in the same study center. Smoking was dichotomized into non-smokers (never/former) and smokers (occasional/daily). Body mass index  $\geq 30$  kg/m<sup>2</sup> was used as an indicator of obesity. Diabetes mellitus, dyslipidemia, and arterial hypertension were diagnosed by a physician and/or by using appropriate medication (oral blood glucose-lowering therapy or insulin substitution, lipid-lowering medication, or antihypertensive drugs, respectively). Additional diagnostic criteria were a blood glucose level

$\geq 126$  mg/dL after an overnight fast (at least 8 hours), a blood glucose level of  $\geq 200$  mg/dL after a fasting period  $< 8$  hours, or hemoglobin A1c concentration  $\geq 6.5\%$  for diabetes mellitus; and a low-density lipoprotein/high-density lipoprotein ratio of  $> 3.5$  for dyslipidemia and a mean systolic blood pressure  $\geq 140$  mm Hg and/or a mean diastolic blood pressure  $\geq 90$  mm Hg on examination (averaging the second and third standardized measurements after 8 and 11 minutes of rest) for arterial hypertension. A positive family history of myocardial infarction or stroke was defined in first-degree relatives aged  $< 65$  years in women or aged  $< 60$  years in men.

For detailed information on data collection and definition of traditional cardiovascular risk factors as well as comorbidities, please see the supplementary description of methods within Data S1.

### Assessment of Arterial Stiffness

Stiffness index (SI) was assessed by digital photoplethysmography (PCA2 device; Carefusion) and used as measure for systemic arterial stiffness. Detailed description of this method has been provided recently.<sup>14</sup> Briefly, a volume pulse waveform with an early systolic and a second diastolic/reflected peak was recorded by transmission of an infrared light through the finger pulp. SI was calculated as the subject's height (in meters) divided by time difference between these 2 peaks (so-called "peak-to-peak time") (in seconds). If no discrimination between systolic and diastolic peaks could be made on the digital pulse signal (class 4 waveform, according to Dawber et al<sup>15</sup>), vasculature was characterized as "very stiff" (Figure S1). All measurements were done in accordance to standard operating procedures with device calibration and subsequent quality control. The intraclass correlation coefficient for assessment of peak-to-peak time and SI in healthy subjects (n=9) showed a strong agreement of 2 successive measurements in 1-week intervals (peak-to-peak time, 0.92 [95% CI, 0.69–0.98]; and SI, 0.91 [95% CI, 0.65–0.98]).

### Statistical Analysis

Descriptive variables were presented as relative frequencies for binary variables and means $\pm$ SDs or medians with interquartile ranges for continuous traits. SI was categorized into tertiles. Sex- and age-adjusted mean values for SI were presented according to *International Classification of Childhood Cancer* classes. A subsample of the GHS (n=5252) with a comparable age range (35–50 years) was used as reference group, with age used as confounder in regression analysis. In sensitivity analysis, a subsample of the CVSS study (n=383) was compared with 1149 age- and sex-matched GHS participants (1:3 case/control ratio with 2-year tolerance for age). To visualize

the difference in SI according to age between cancer survivors and the population, a conditional density plot was generated and nonparametric bootstrap samples ( $n=1000$ ) were run to estimate 95% CIs. Linear regression models were calculated with SI as dependent variable to assess the differences between cohorts. Models were adjusted for age and sex and additionally for traditional cardiovascular risk factors (ie, arterial hypertension, diabetes mellitus, dyslipidemia, family history for myocardial infarction or stroke, obesity, and smoking).

To estimate prevalence ratios for dichotomous outcomes, the Poisson regression technique with robust variance estimator was used as replacement for less stable binomial regression models to overcome convergence problems. Regression coefficient with 95% CI values were determined for tumor entities, for treatment status (with or without chemotherapy/radiotherapy), as well as in the subgroup of subjects without prevalent arterial hypertension by regression analysis. Finally, the impact of the variables “age at cancer diagnosis” and “time since diagnosis” on arterial stiffness was analyzed by linear regression analysis in the large subsample of leukemia survivors ( $n=368$ ), with both variables used simultaneously in the same model.

Because of the explorative character of the analysis, a significance threshold was not defined for  $P$  values.  $P$  values should be interpreted as a continuous measure of statistical evidence. The statistical analysis

was performed with R software, version 3.14.2 (<http://www.r-project.org>).

## RESULTS

In total, 1002 individuals have been examined between October 2013 and February 2016. Among them, subjects with subsequent neoplasia ( $n=51$ ) were later excluded from the analysis. Therefore, the final study sample includes 951 subjects. SI could not be measured in 88 subjects because of technical and logistical constraints; 8 additional participants demonstrated “very stiff” arteries (according to Dawber et al<sup>15</sup>) with no computable SI. This resulted in an analysis sample of 855 subjects, of whom 72 did not receive any chemotherapy or radiotherapy (please see Figure S2 for the study flowchart).

### Sample Characteristics, According to the Distribution of Arterial Stiffness

Characteristics of the CVSS study participants, according to tertiles of SI, with the cut points 5.67 and 6.81 m/s, are summarized in Table 1. Age at examination increases with higher tertiles of SI. Time since diagnosis also was larger in individuals with higher SI values, whereas age at diagnosis of the malignancy was lower in those with lower SI. The frequency of female participants decreases with higher tertiles of SI. Distinct changes were seen for systolic blood pressure, where the difference between

**Table 1. Characteristics of CCSs From the CVSS Study, According to Tertiles of SI (n=855)**

Characteristics	Tertiles of SI		
	Tertile 1: $\leq 5.67$ m/s (n=284)	Tertile 2: $> 5.67/\leq 6.81$ m/s (n=282)	Tertile 3: $> 6.81$ m/s (n=289)
Sex (women), %	71.8	31.2	21.8
Age, y	32.3 $\pm$ 5.0	33.7 $\pm$ 5.3	36.1 $\pm$ 5.5
BMI, kg/m <sup>2</sup>	24.0 (21.8/27.3)	24.9 (22.5/27.5)	26.1 (23.3/29.6)
Heart rate, bpm	63.0 $\pm$ 9.9	66.2 $\pm$ 11.1	65.8 $\pm$ 9.7
Systolic blood pressure, mm Hg	118.2 $\pm$ 12.3	124.2 $\pm$ 11.8	127.3 $\pm$ 12.8
Diastolic blood pressure, mm Hg	77.0 $\pm$ 8.5	79.9 $\pm$ 8.7	83.0 $\pm$ 8.9
Arterial hypertension, %	15.1	21.6	31.9
Diabetes mellitus, %	1.4	4.7	0.7
Smoking, %	18.0	22.1	26.0
Obesity, %	16.5	13.8	22.8
Dyslipidemia, %	16.5	32.3	39.8
Family history of MI/stroke, %	12.0	13.5	17.3
History of radiotherapy, %	52.6	49.8	57.0
History of chemotherapy, %	84.6	89.7	88.8
Age at diagnosis, y	5.02 $\pm$ 3.95	5.94 $\pm$ 4.06	7.58 $\pm$ 4.50
Time since diagnosis, y	27.7 $\pm$ 3.1	28.3 $\pm$ 3.1	29.1 $\pm$ 3.0
Reflection index, %	51.4 $\pm$ 14.0	60.6 $\pm$ 15.8	70.8 $\pm$ 13.3

Data are expressed as relative frequencies for binary variables or mean $\pm$ SD/median (first/third quartile) for continuous traits. BMI indicates body mass index; bpm, beats per minute; CCS, childhood cancer survivor; CVSS, Cardiac and Vascular Late Sequelae in Long-Term Survivors of Childhood Cancer; MI, myocardial infarction; and SI, stiffness index.

the outer tertiles was 9.1 mm Hg. This was also reflected by the prevalence of arterial hypertension across SI tertiles, which almost doubled from the lowest to the highest tertile. No differences were seen across SI tertiles, according to treatment status or prevalence of established cardiovascular disease (data not shown). The overall characteristics of the GHS subsample have been provided in Table S1. In addition, demographics and clinical characteristics of both study groups were compared according to tertiles of SI, using the same cutoff values (Table S2). Although CVSS study subjects were slightly younger and a comparable increase in systolic blood pressure was observed with increasing SI tertiles in both samples, the prevalence of several traditional risk factors, such as arterial hypertension, diabetes mellitus, and dyslipidemia, in the lowest SI tertile was found to be higher among subjects from CCSs compared with subjects from the general population.

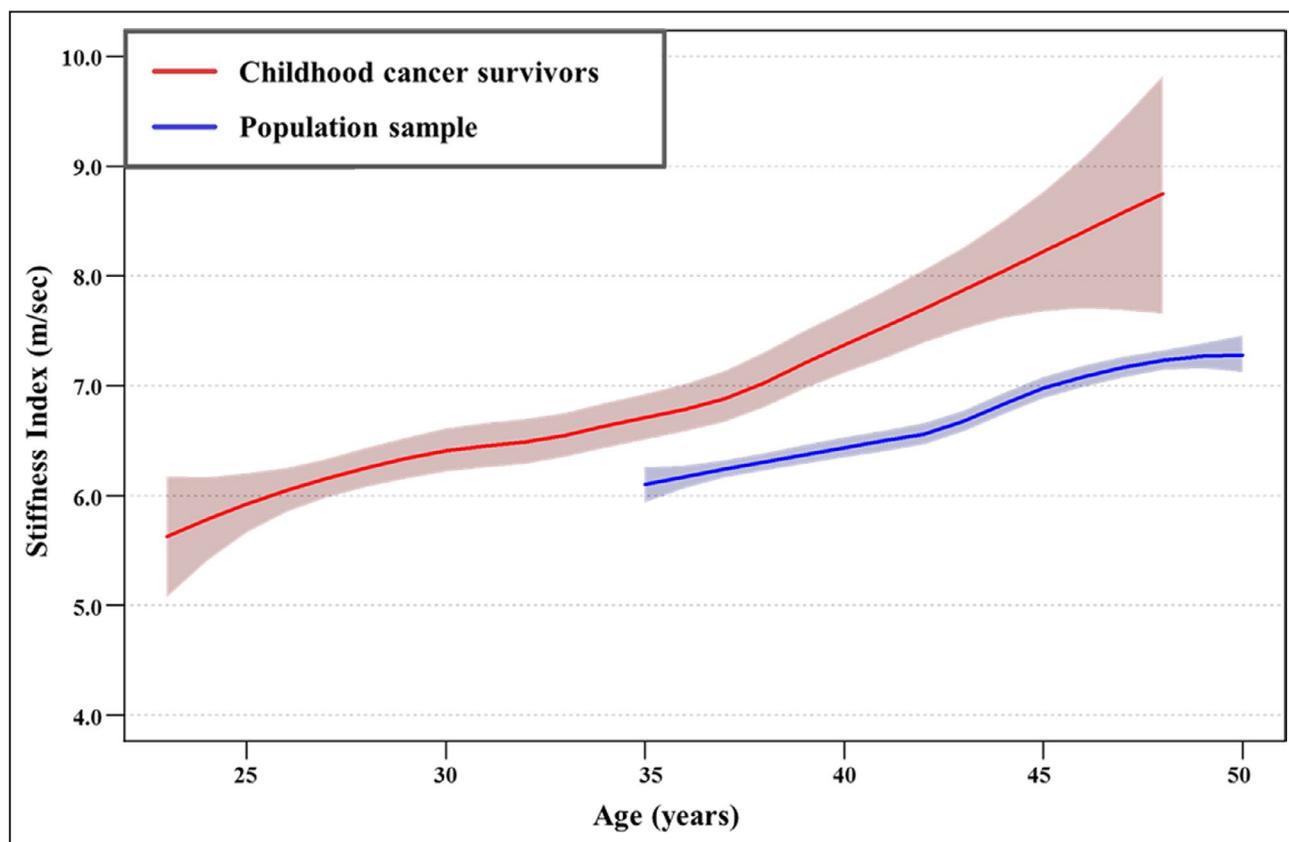
### Arterial Stiffness in CCSs and in Comparison to the Population

Among CCSs, mean SI was  $6.77 \pm 1.91$  m/s. Further stratification for the treatment status (after exclusion

of 64 subjects with no available information on treatment status) revealed an SI of  $6.75 \pm 1.87$  m/s for those with previous antineoplastic therapy, whereas the SI was lower among 72 participants without any chemotherapy or radiotherapy ( $6.42 \pm 1.87$  m/s; mean SI in this subgroup,  $6.72 \pm 1.87$  m/s). The difference in SI over age between both CCSs and the population is illustrated by a conditional density plot in Figure 1.

Linear regression analysis was applied to further investigate whether CCSs have increased arterial stiffness compared with subjects from the population. In an age- and sex-adjusted model, SI was 0.7 m/s higher in CCSs compared with the GHS population. Adjustment for traditional cardiovascular risk factors, including arterial hypertension, did not change this result ( $\beta = 0.66$  m/s; 95% CI, 0.51–0.80 m/s) (Table 2). Similar estimates were obtained for the subgroup of CCSs with history of chemotherapy or radiotherapy. CCSs without any chemotherapy or radiotherapy, however, also had stiffer vessels than individuals from the population. This association was independent from potential confounders ( $\beta = 0.56$  m/s; 95% CI, 0.16–0.96) (Table 2).

Interestingly, CCSs had a 5.2-fold higher prevalence of SI values, exceeding the age-related reference



**Figure 1. Age-related increase of stiffness index in childhood cancer survivors and in the population.**

Conditional density plots were generated and nonparametric bootstrap samples ( $n=1000$ ) were run to estimate 95% CIs. Data were calculated on the basis of a CVSS (Cardiac and Vascular Late Sequelae in Long-Term Survivors of Childhood Cancer) study sample, aged 30 to 45 years, and a GHS (Gutenberg Health Study) sample, aged 35 to 50 years.

**Table 2. Arterial Stiffness Among CCSs (n=855) Compared With the Population (n=5252)**

Variable	Differences in Stiffness Index Between CCSs and General Population			
	Adjusted for Age and Sex		Adjusted for Age, Sex, and Cardiovascular Risk Factors*	
	$\beta$ Estimate (95% CI)	P Value	$\beta$ Estimate (95% CI)	P Value
CCSs vs population (reference)	0.70 (0.56–0.85)	<0.0001	0.66 (0.51–0.80)	<0.0001
CCSs with chemotherapy/radiotherapy vs reference	0.68 (0.52–0.84)	<0.0001	0.63 (0.48–0.79)	<0.0001
CCSs without chemotherapy/radiotherapy vs reference	0.59 (0.18–1.00)	0.0049	0.56 (0.16–0.96)	0.0066

CCSs without chemotherapy/radiotherapy, n=72. CCS indicates childhood cancer survivor.

\*Cardiovascular risk factors included diabetes mellitus, arterial hypertension, smoking status, obesity, dyslipidemia, and family history of myocardial infarction/stroke.

limit compared with the population (prevalence ratio, 5.18 [95% CI, 3.88–6.93] [ $P<0.0001$ ] after adjustment for age and sex; and prevalence ratio, 5.18 [95% CI, 3.86–6.96] [ $P<0.0001$ ] in a fully adjusted model).

Sensitivity analysis with stricter age (2-year tolerance for age) and sex matching, comparing 383 CVSS study participants with 1149 age- and sex-matched GHS individuals (1:3 case/control ratio) confirmed the results above (for details, see Table S3).

### Change in Arterial Stiffness, According to Tumor Entity

SI in CCSs compared with the population was evaluated for different types of tumor. Age- and sex-adjusted mean values and 95% CIs for SI, according to the cancer diagnosis based on *International Classification of Childhood Cancer* classification, are presented in Figure 2. Values of SI were varying from a minimum of 6.63 m/s in those with previously diagnosed leukemia to a maximum of 7.07 m/s in survivors of bone tumors.

Overall, the results of linear regression analysis revealed higher values of SI in CCSs compared with the population among all types of cancer. These differences were only negligibly smaller after controlling for traditional cardiovascular risk factors as confounders (Table S4). Again, these differences were varying with tumor entity, being less pronounced in hematopoietic malignancies (lymphoma or leukemia) and more prominent in solid tumors, such as bone and renal cancer (Figure 3). SI values above the reference limits were seen more frequently among all tumors, with the highest prevalence among renal tumor survivors (multivariable-adjusted prevalence ratio, 9.81; 95% CI, 4.36–22.09; Table S5).

### Determinants of SI in CCSs

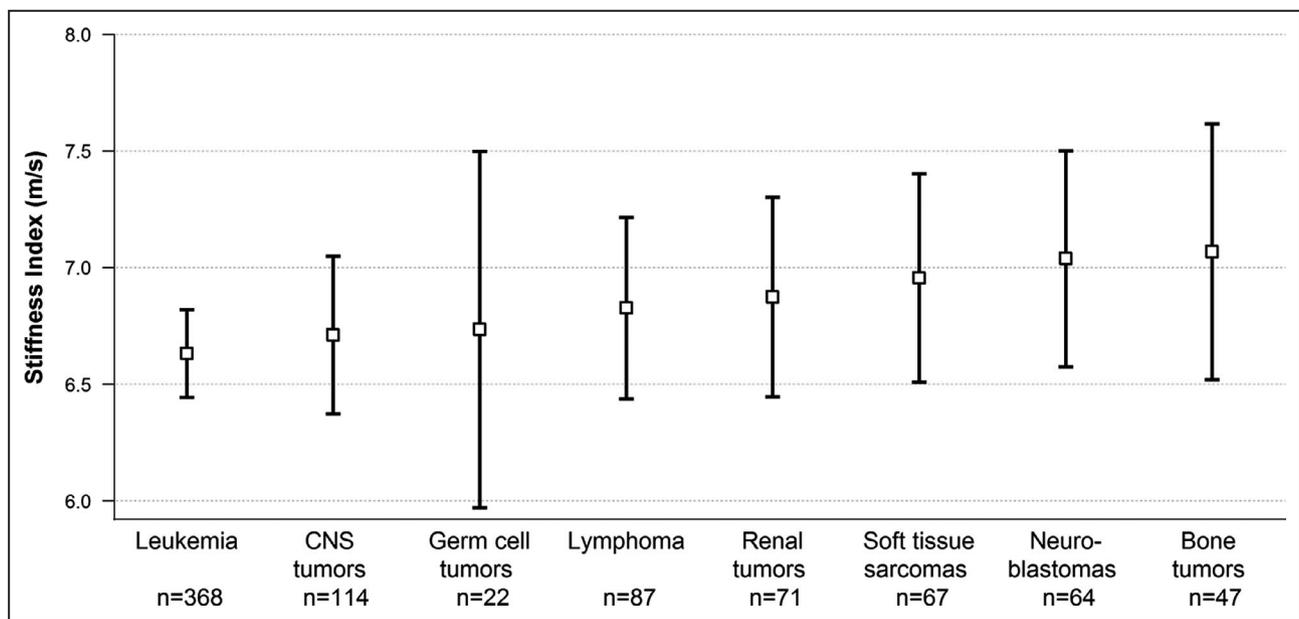
As present cardiovascular risk factors had only a minimal influence on the differences in arterial stiffness observed between CCSs and the population, data were explored for possible other factors.

Interestingly, in a subgroup analysis on subjects without prevalent arterial hypertension as major causal factor related to arterial stiffness across both cohorts (CCSs, n=662; GHS population, n=3852), SI was also markedly higher in CCSs than in the population ( $\beta=0.66$  m/s; 95% CI, 0.50–0.81 m/s; fully adjusted model; Table 3).

In a subgroup analysis of patients with history of leukemia (n=368) as largest group with homogeneous type of cancer, time since diagnosis from cancer to the current examination and age at diagnosis were explored as putative determinants of SI. Multivariable linear regressions were calculated by replacing “age” with these variables. After adjustment for sex, there was a positive association with “time since diagnosis.” Also, a positive and pronounced association with age at diagnosis was found, indicating that younger age at time of cancer diagnosis is associated with lower SI at the time of the follow-up examination (Table 4). Additional adjustment for traditional cardiovascular risk factors resulted in a weakening of the association between SI and “time since cancer diagnosis,” but a strong association with “age at diagnosis” remained ( $\beta$  per 1 year of age, 0.13 m/s; 95% CI, 0.09–0.17 m/s).

## DISCUSSION

Within the present analysis, arterial stiffness has been investigated among 855 long-term CCSs and has been compared against 5252 subjects from the general population. Remarkably higher arterial stiffness was observed among survivors of childhood cancer, which varied with the tumor entity. Moreover, the higher arterial stiffness observed among CCSs was widely independent from the presence of cardiovascular risk factors and was also detected in individuals without arterial hypertension. Intriguingly, increased arterial stiffness was not only found among CCSs with history of antineoplastic treatment, but



**Figure 2. Age- and sex-adjusted arterial stiffness, according to tumor entity.**

Data presented as age- and sex-adjusted means for stiffness index, measured by digital photoplethysmography, with their 95% CIs. CNS indicates central nervous system.

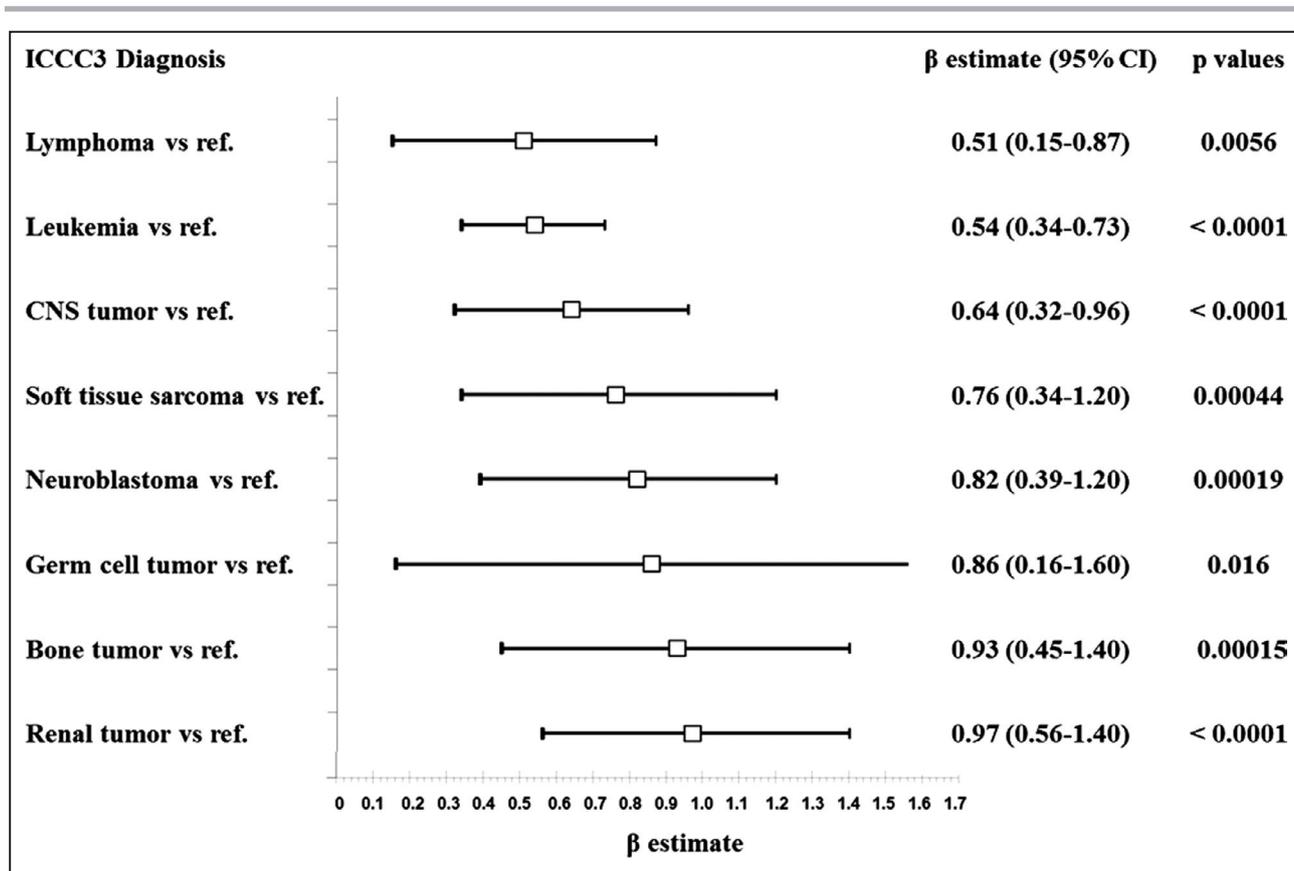
also in individuals without chemotherapy or radiotherapy, although to a slightly lower extent. Finally, in the subgroup of individuals with history of leukemia, age at time of cancer diagnosis and therapy seemed to be important for arterial stiffness progression. Thus, the findings of the present investigation suggest that other pathophysiological mechanisms than well-established factors (eg, arterial hypertension) promote a stiffer vasculature in this high-risk group for cardiovascular disease.

Arterial hypertension represents the most powerful predictor of arterial stiffness in the population, and a long-term increase in blood pressure is usually associated with changes in arterial stiffness.<sup>7</sup> Within the present analysis, increase in SI among CCSs compared with the population was, however, independent of arterial hypertension. An alternative, putative mechanism for stiffness promotion among CCSs might be a vasculotoxic effect mediated by prior chemotherapy and radiotherapy. Functional and structural changes in the vasculature, induced by neoplasm-associated treatment, are well-known.<sup>5</sup> They include beyond others an induction of endothelial injury by chemotherapeutic agents (eg, anthracyclines<sup>9,16</sup>) with subsequent impairment of vasodilatory properties of vessels and initiation of potent inflammatory and hemostatic responses.<sup>2,4</sup> Also, radiation exposure might lead to induction of chronic oxidative stress,<sup>17</sup> with further upregulation of numerous signaling pathways, like nuclear factor- $\kappa$ B,<sup>18</sup> as well as initiation of subendothelial fibrosis,<sup>19</sup> potentially leading to reduced vascular compliance. Cranial

radiotherapy-driven changes in hypothalamic-pituitary axis with subsequent hormonal imbalance might be another underlying mechanism for vascular toxicity.<sup>19</sup>

Within the present study, however, also CCSs without history of anticancer treatment demonstrated increased arterial stiffness. Interestingly, Lipshultz et al<sup>20</sup> also reported abnormalities in left ventricular structure and function, traditional cardiovascular risk factors, and low-grade systemic inflammation among CCSs, who were not exposed to cardiotoxic therapy (eg, anthracyclines and cardiac irradiation). One might speculate that cancer per se might contribute to the stiffening of vessels, because of the proinflammatory and hypercoagulable milieu, which might be long-term persisting after survival of cancer<sup>21</sup> and involved in its pathomechanism. Indeed, it was recently demonstrated that a strong association between SI and numerous inflammatory and hemostatic markers exists, which was more prominent in individuals with higher cardiovascular risk profile.<sup>14</sup> Moreover, presence of other determinants for arterial stiffness, such as poor physical activity<sup>22</sup> or sedentary lifestyle,<sup>23</sup> and subsequent metabolic abnormalities,<sup>24</sup> which are more common among CCSs than in the population, might have further impact on development of arterial stiffness, even in absence of antineoplastic therapy.

Although increased arterial stiffness was observed among all types of cancer in the cohort, its extent varied with tumor entity. The largest changes in SI among CCSs compared with individuals from the population were found in individuals with former renal tumors,



**Figure 3.** Difference in stiffness index in childhood cancer survivors compared with a population sample, according to tumor entity.

Results from multivariable linear regression analysis with stiffness index as dependent variable and adjustment for age, sex, diabetes mellitus, arterial hypertension, smoking, obesity, dyslipidemia, and family history of myocardial infarction/stroke. Data presented as  $\beta$  estimates with their 95% CIs. CNS indicates central nervous system; *ICCC3*, *International Classification of Childhood Cancer*; and ref., reference.

and prevalence of values exceeding the reference limit among these subjects was almost 10-fold higher than in the population sample. In contrast, the estimate for increase in arterial stiffness in survivors with malignant blood disorders had approximately half the size. Differences in SI are likely to be attributed to the varying therapy regimens for tumor entities, with more aggressive strategies and higher cumulative doses of applied chemotherapeutics and radiation for solid tumors. On

the other hand, more information on the entity-specific effect on arterial stiffness, independent of the treatment, as reported above, is of interest. This aspect could not be further elucidated within the present analysis and remains an important task for further investigations.

Also, synergistic effects of impaired renal function, which is a well-known determinant of arterial stiffness,<sup>7</sup> promoting vascular calcification or renin-angiotensin-aldosterone system dysregulation,<sup>25</sup> and frequently

**Table 3.** Arterial Stiffness Among CCSs Without Prevalent Hypertension Compared With Hypertension-Free Subjects From the General Population

Variable	Differences in Stiffness Index Between CCSs and General Population			
	Adjusted for Age and Sex		Adjusted for Age, Sex, and Cardiovascular Risk Factors*	
	$\beta$ Estimate (95% CI)	P Value	$\beta$ Estimate (95% CI)	P Value
CCSs vs population (reference)	0.65 (0.50–0.81)	<0.0001	0.66 (0.50–0.81)	<0.0001

CVSS (Cardiac and Vascular Late Sequelae in Long-Term Survivors of Childhood Cancer) study (n=662) vs GHS (Gutenberg Health Study) (n=3852) participants without prevalent arterial hypertension. CCS indicates childhood cancer survivor.

\*Cardiovascular risk factors included diabetes mellitus, smoking status, obesity, dyslipidemia, and family history of myocardial infarction/stroke.

**Table 4. Age at Cancer Diagnosis and Arterial Stiffness in the Subsample With Survivors With Leukemia (n=365)**

Variable	Differences in Stiffness Index			
	Adjusted for Age and Sex		Adjusted for Age, Sex, and Cardiovascular Risk Factors*	
	$\beta$ Estimate (95% CI)	P Value	$\beta$ Estimate (95% CI)	P Value
Time since diagnosis (per 1 y)	0.06 (0.007–0.11)	0.025	0.05 (–0.003 to 0.10)	0.067
Age at cancer diagnosis (per 1 y)	0.14 (0.09–0.18)	<0.0001	0.13 (0.09–0.17)	<0.0001

\*Cardiovascular risk factors included diabetes mellitus, arterial hypertension, smoking status, obesity, dyslipidemia, and family history of myocardial infarction/stroke.

observed in survivors of renal cancer, might be a possible and relevant component. However, renal function in the present cohort was predominantly in normal range, based on estimated glomerular filtration rate, although one has to keep in mind that established clinical routine markers increase only in case of substantial impairment of kidney function and subclinical changes might already contribute to the process.

Higher age at diagnosis of malignancy was unexpectedly associated with higher arterial stiffness in patients with leukemia in the study, although a priori an inverse association with SI (ie, earlier age of diagnosis was associated with higher SI) was hypothesized because of the earlier initiation of antineoplastic therapy and herewith associated earlier exposure to the treatment-related vascular toxicity. Recent investigations have already shown that diagnosis of leukemia in later childhood seems to be associated with worse survival compared with younger ages.<sup>26</sup> The higher frequency of the more unfavorable T-cell immunophenotype and/or lower proportion of favorable biological features, such as hyperploidy (DNA index  $\geq 1.16$ ) seen in older pediatric patients with leukemia,<sup>27</sup> might also represent possible mechanisms involved in the stronger progression of arterial stiffness.

Despite the intriguing results, several limitations of the current study merit consideration. No individuals with Hodgkin lymphoma, which predisposes to cardiovascular sequelae, have been included in the study. Because the recruitment of CVSS study participants was not completely performed at random, a possible selection bias, resulting in different proportions of leukemia and retinoblastoma compared with the eligible source population (A. Schneider, S. Eckerle, H. Merzenich, M. Blettner, P. Kaatsch, C. Jünger, J. Prochaska, A. Wingerther, M. Neu, J. Faber, P. Wild, C. Spix, unpublished data, 2019), might affect the findings. However, the analyses of the external validity (A. Schneider, S. Eckerle, H. Merzenich, M. Blettner, P. Kaatsch, C. Jünger, J. Prochaska, A. Wingerther, M. Neu, J. Faber, P. Wild, C. Spix, unpublished data, 2019) showed that the CVSS study contributes a representative sample to address cardiovascular long-term effects among CCSs. The influence of antineoplastic therapy on the vasculature (ie, its dose dependency or

modality specificity) was not investigated in more detail, because treatment-related data were not available at this time. Furthermore, digital photoplethysmography is a not widely spread method for assessment of arterial stiffness, with far less epidemiological evidence than carotid-femoral pulse wave velocity.

In summary, this is the first study demonstrating remarkably increased arterial stiffness among long-term CCSs compared with the population, which complementarily fits to the findings of a higher prevalence of arterial hypertension and cardiovascular disease found in these individuals. The present data intriguingly suggest that promotion of arterial stiffness in CCSs differs from the established concept: Increased arterial stiffness was only marginally explained by traditional cardiovascular disease risk factors and hypertension in particular and was also present in absence of arterial hypertension. Increased stiffness was varying with tumor entities, but also present in cancer survivors without chemotherapy and radiation, partially refuting its explanation by therapy-induced vasculotoxicity. Last, in the largest patient subgroup with history of leukemia, the age at diagnosis of cancer seems to be relevant for the development of structural vascular changes. The findings suggest arterial stiffness is a consequence of a complex process, which urgently requires a better understanding for potential prevention and treatment of cardiovascular sequelae in CCSs.

## ARTICLE INFORMATION

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### Supplementary Material

#### Data S1

#### Tables S1–S5

#### Figures S1–S2

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# **SUPPLEMENTAL MATERIAL**

## Data S1.

### Supplemental Methods

#### Data Collection and Definition of Cardiovascular Risk Factors and Diseases

All subjects participated in a 5.5-hour baseline-examination at the study center, which was performed according to standard operating procedures by certified medical technical assistants. They also underwent a standardized computer-assisted personal interview carried out by a specially trained team of interviewers. Cardiovascular (CV) risk factors were defined as follows: smoking was dichotomized into non-smokers (never and former smokers) and smokers (occasional and daily smokers). Body mass index was calculated as weight in kilograms divided by height in meters squared (both measured) and used as a marker of obesity with a cut-point of  $\geq 30 \text{ kg/m}^2$  for dichotomized analyses. Diabetes mellitus was defined as a diagnosis of diabetes by a physician or a blood glucose level of  $\geq 126 \text{ mg/dl}$  at the baseline examination after an overnight fast of at least 8 hours, a blood glucose level of  $\geq 200 \text{ mg/dl}$  in the baseline examination after a fasting period  $< 8$  hours or HbA1c concentration  $\geq 6.5 \%$ . In addition, those who were on oral blood glucose-lowering therapy or on insulin substitution were also classified as diabetics. Subjects with a LDL/HDL-ratio of  $> 3.5$ , with diagnosis of dyslipidemia by general practitioners or on lipid-lowering medication were classified as having dyslipidemia. Hypertension was diagnosed, if antihypertensive drugs were taken, or a mean examination systolic blood pressure of  $\geq 140 \text{ mmHg}$  or a mean diastolic blood pressure of  $\geq 90 \text{ mmHg}$  (averaging the 2<sup>nd</sup> and 3<sup>rd</sup> standardized measurements after 8 and 11 minutes of rest). A positive family history of myocardial infarction or stroke was defined as history of myocardial infarction in female first-degree relatives  $< 65$  years or in male first-degree relatives  $< 60$  years.

Cardiovascular diseases were documented in a computer assisted personal interview by specifically trained and certified interviewers. Participants were asked to bring their medical records and reports to the interview. A disease was recorded as present, if a physician had diagnosed the disease.

**Table S1. Overall Characteristics of a Subsample of the Gutenberg Health Study (n=5,252\*).**

	<b>GHS</b>
Sex (Women), % (n)	50.4 (2,649)
Age, years	43.0±4.5
BMI, kg/m <sup>2</sup>	25.6 (23.0/28.8)
Heart rate, bpm	69.3±10.5
Systolic blood pressure, mmHg	123.8±14.1
Diastolic blood pressure, mmHg	81.5±9.4
Arterial hypertension, % (n)	26.6 (1,397)
Diabetes mellitus, % (n)	2.6 (134)
Smoking, % (n)	25.8% (1,352)
Obesity, % (n)	19.2% (1,011)
Dyslipidemia, % (n)	28.6 (1,500)
Family history of MI/stroke, % (n)	20.6 (1,083)

\*For comparison with the GHS cohort, only the overlapping age range between samples (35-50 years) was analyzed. Data are expressed as relative frequencies for binary variables or mean ± standard deviation (SD)/medians (with 1<sup>st</sup>/3<sup>rd</sup> quartile) for continuous traits.

BMI stands for body mass index; bpm for beats per minute; MI for myocardial infarction.

**Table S2. Characteristics of Childhood Cancer Survivors from the CVSS Study and GHS participants according to Tertiles of Stiffness Index derived from the CVSS sample.**

	Tertiles of Stiffness Index					
	CVSS			GHS		
	T1 ≤6.0 m/s n=126	T2 >6.0/≤7.6 m/s n=126	T3 >7.6 m/s n=126	T1 ≤6.0 m/s n=2,202	T2 >6.0/≤7.6 m/s n=1,191	T3 >7.6 m/s n=1,089
Sex (Women), %	57.9	33.3	18.3	65.4	42.7	26.5
Age, years	38.4±3.3	39.0±3.2	40.1±3.8	41.2±3.9	42.0±3.9	43.1±3.7
Heart rate, bpm	64.5±11.3	67.6±10.4	64.9±9.0	68.4±10.1	70.8±10.7	69.6±10.2
Systolic BP, mmHg	120.1±12.8	124.0±14.1	128.0±11.8	119.5±12.5	124.8±13.3	128.7±14.5
Diastolic BP, mmHg	78.0±9.2	80.8±9.8	84.4±8.1	78.7±8.5	82.1±8.7	85.1±9.6
Arterial hypertension, %	23.0	28.0	38.1	16.1	27.2	37.1
Diabetes mellitus, %	4.0	1.6	0.8	1.1	3.1	4.0
Smoking, %	12.7	19.0	23.8	21.2	26.2	34.1
Obesity, %	15.1	15.9	23.8	14.0	22.1	25.2
Dyslipidemia, %	24.6	35.7	44.4	17.4	31.9	45.0

Family history of MI/stroke, %	13.5	19.0	16.7	18.4	20.9	23.2
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Data are expressed as relative frequencies for binary variables or mean  $\pm$  standard deviation (SD). CVSS stands for *Cardiac and Vascular Late Sequelae in Long-term Survivors of Childhood Cancer*; GHS for *Gutenberg Health Study*; T for *Tertile*; bpm for *beats per minute*; BP for *blood pressure*; MI for *myocardial infarction*.

**Table S3. Stiffness Index in Childhood Cancer Survivors Compared to an Age-and Sex-matched\* Population Sample.**

	<b>CVSS-Study</b>	<b>Gutenberg Health Study</b>	<b>p value</b>
	<b>(n=383)</b>	<b>(n=1,149)</b>	
Sex (Women), %	36.8	36.8	1.00
Age, years	39.1±3.5	39.1±3.5	1.00
Stiffness index, m/s	7.31±2.15	6.54±1.78	< 0.0001
SI above population-based reference limit, %	15.6	3.3	< 0.0001

\*Matching in 1:3-case/control ratio with a 2 year tolerance for age matching.

Data are expressed as relative frequencies for binary variables or means ± standard deviations (SD).

CVSS stands for Cardiac and vascular late sequelae in long-term survivors of childhood cancer; SI for stiffness index

**Table S4. Arterial Stiffness in Childhood Cancer Survivors Compared to a Population Sample according to Tumor Entity.**

ICCC3 Diagnosis	Differences in Stiffness Index (m/s)			
	Adjustment for age and sex		Adjustment for age, sex and CVRFs*	
	$\beta$ Estimate (95% CI)	p value	$\beta$ Estimate (95% CI)	p value
Lymphoma vs ref.	0.58 (0.22-0.95)	0.0019	0.51 (0.15-0.87)	0.0056
Leukaemia vs ref.	0.58 (0.38-0.78)	< 0.0001	0.54 (0.34-0.73)	< 0.0001
CNS tumour vs ref.	0.67 (0.35-1.00)	< 0.0001	0.64 (0.32-0.96)	< 0.0001
Soft tissue sarcoma vs ref.	0.83 (0.40-1.30)	0.00014	0.76 (0.34-1.20)	0.00044
Neuroblastoma vs ref.	0.94 (0.51-1.40)	< 0.0001	0.82 (0.39-1.20)	0.00019
Germ cell tumour vs ref.	0.83 (0.11-1.50)	0.024	0.86 (0.16-1.60)	0.016
Bone tumour vs ref.	1.00 (0.51-1.50)	< 0.0001	0.93 (0.45-1.40)	0.00015
Renal tumour vs ref.	1.00 (0.59-1.40)	< 0.0001	0.97 (0.56-1.40)	< 0.0001

Data presented as  $\beta$ -estimates with their 95% CI.

\*Diabetes mellitus, arterial hypertension, smoking, obesity, dyslipidemia, family history of myocardial infarction/stroke.

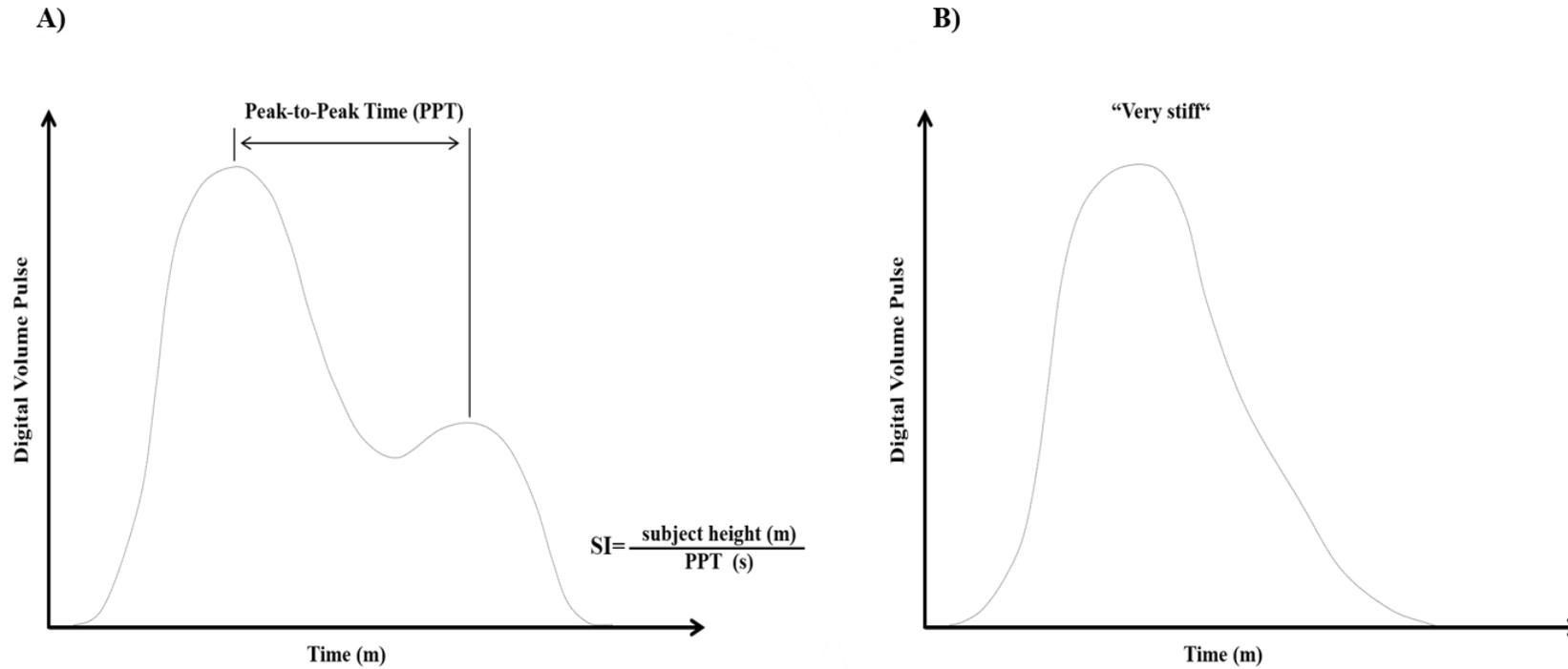
CVRFs stands for cardiovascular risk factors; ICC3 for international classification of childhood cancer 3; CI for confidence interval; Ref for reference.

**Table S5. Prevalence of SI values Above the Age-related, Population-based Reference Range Among Childhood Cancer Survivors Compared to Subjects from the General Population.**

ICCC3 Diagnosis	Prevalence of Values of SI, Exceeding the Population-based Reference Limit	
	Adjustment for age and sex	Adjustment for age, sex and CVRFs*
	PR (95% CI)	PR (95% CI)
Bone tumor vs ref.	4.13 (1.97-8.67)	4.00 (1.90-8.42)
Leukemia vs ref.	4.37 (2.77-6.89)	4.50 (2.85-7.11)
Lymphoma vs ref.	6.30 (3.54-11.19)	5.81 (3.28-10.28)
CNS tumor vs ref.	5.64 (3.10-10.26)	5.95 (3.26-10.84)
Soft tissue sarcoma vs ref.	6.60 (3.25-13.42)	6.22 (3.13-12.38)
Germ cell tumor vs ref.	5.73 (1.59-20.59)	6.36 (1.52-26.59)
Renal tumor vs ref.	9.24 (3.86-22.09)	9.81 (4.36-22.09)

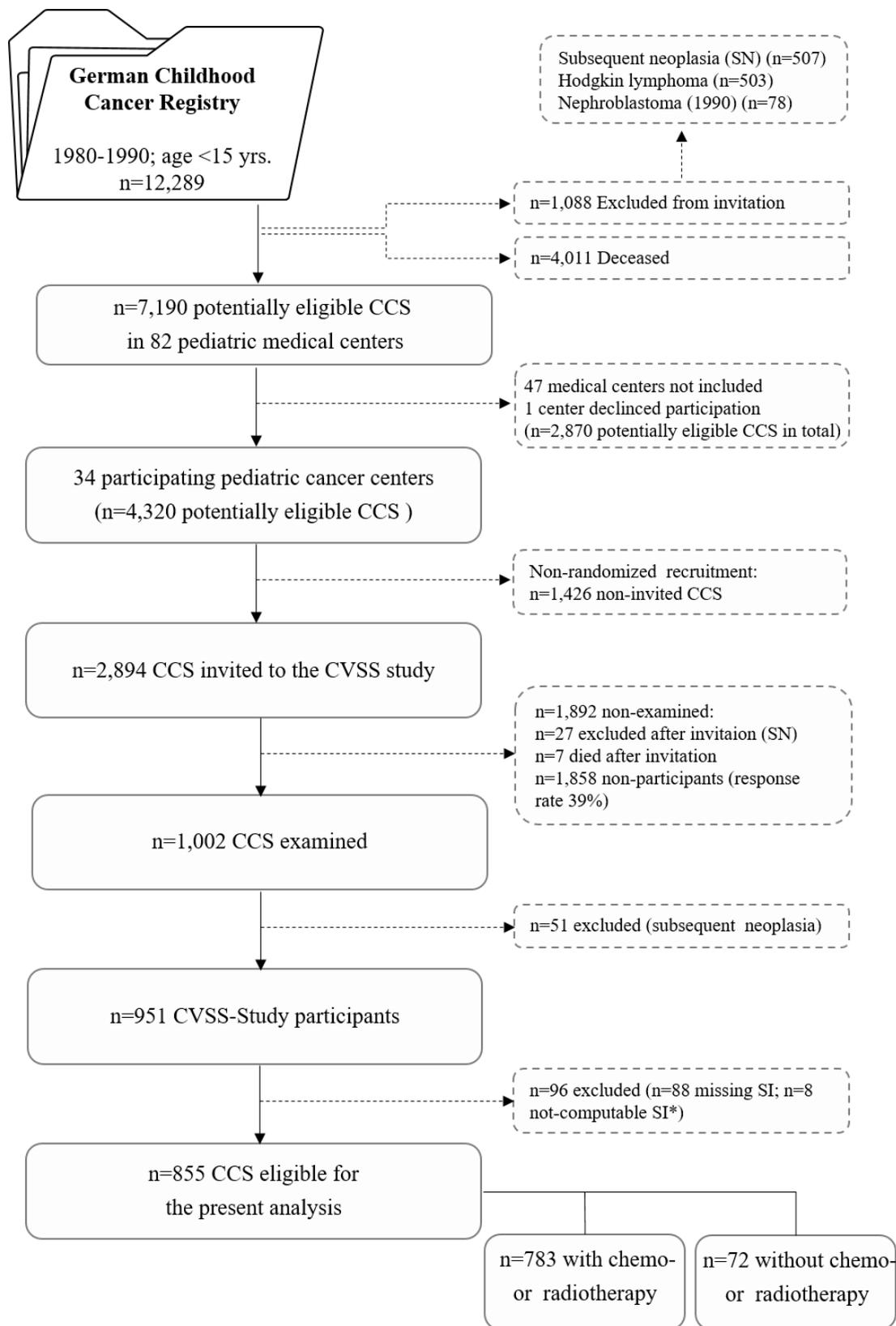
Data presented as PR with their 95% CI. \*Diabetes mellitus, arterial hypertension, smoking, obesity, dyslipidemia, family history of myocardial infarction/stroke. SI stands for stiffness index; CVRFs for cardiovascular risk factors; ICC3 for international classification of childhood cancer 3; PR for prevalence ratio; CI for confidence interval; ref for reference.

**Figure S1. Assessment of Stiffness Index by Digital Photoplethysmography.**



Panel A: Curve of digital volume pulse with measurable peak-to-peak time (PPT); Panel B: Curve of digital volume pulse, indicating „very stiff“ vessels according to Dawber et al.<sup>15</sup> SI denotes stiffness index.

**Figure S2. Flowchart of the Present Analysis on Arterial Stiffness in the Cardiac and Vascular Late Sequelae in Long-term Survivors of Childhood Cancer (CVSS) Study.**



SI denotes stiffness index; CCS childhood cancer survivors; SN subsequent neoplasia. \* „very stiff“ vessels according to Dawber et al.<sup>15</sup>