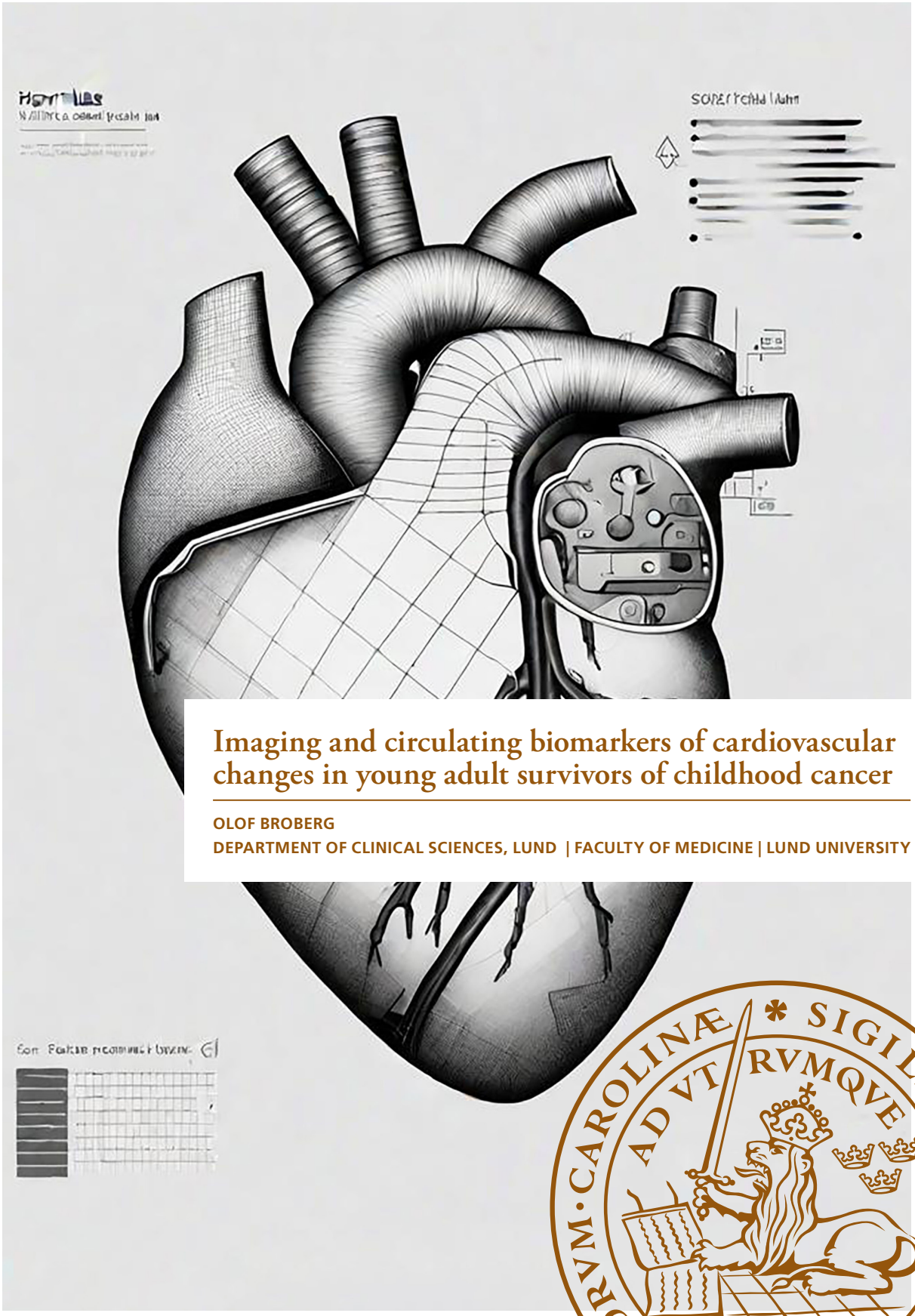
Imaging and circulating biomarkers of cardiovascular changes in young adult survivors of childhood cancer

Broberg, Olof

2024

Document Version:
Publisher's PDF, also known as Version of record



Imaging and circulating biomarkers of cardiovascular changes in young adult survivors of childhood cancer

OLOF BROBERG
DEPARTMENT OF CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

Imaging and circulating biomarkers of cardiovascular changes in young adult survivors of childhood cancer

Imaging and circulating biomarkers of cardiovascular changes in young adult survivors of childhood cancer

Olof Broberg

LUNDS UNIVERSITET

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Pediatrics at Lund University to be publicly defended on 31st of January 2024 at 09:00 in Belfragesalen, Biomedical Centre, Lund, Sweden

Faculty Opponent

Professor Eero Jokinen, Pediatric Cardiology, University of Helsinki and Finnish Foundation for Cardiovascular Research, Helsinki, Finland

Organization: LUND UNIVERSITY, Faculty of medicine, Clinical Sciences, Pediatrics

Document name: Doctoral thesis **Date of issue:** 2024-01-31

Author: Olof Broberg **Sponsoring organization:** None

Title and subtitle: Imaging and circulating biomarkers of cardiovascular changes in young adult survivors of childhood cancer

Abstract:

Childhood cancer survivors (CCS) are prone to so called “late-effects” to the cardiovascular system. Some anti-cancer treatments, in particular anthracyclines and mediastinal radiotherapy, are cardio- and vasculotoxic. They cause damage to the cardiovascular tissues and increase the risk for early cardiovascular diseases, such as heart failure, myocardial infarction and stroke.

It has recently been approximated that one out of ten of CCS treated with cardiotoxic treatments will have a diagnosis of heart failure within 40 years after treatment. Thus, there is a need to find those CCS at risk for developing cardiovascular diseases and to increase the knowledge of the cardiovascular health in CCS.

This thesis includes four papers in a cohort of CCS of different childhood cancer diagnoses. In Paper I we investigated cardiac and arterial function with ultrasound, as well as circulating lipid and apolipoprotein biomarkers. We found cardiac, vascular, lipid, and apolipoprotein changes that could account for the increased risk for CVD later in life among CCS. In Paper II, we studied the cardiac reserve function, with dobutamine stress echocardiography, using both conventional left ventricular ejection fraction (LVEF) and strain echocardiography. We found that CCS had impaired cardiac reserve as measured by strain echocardiography but not with LVEF, suggesting that strain echocardiography is a sensitive method to quantify cardiac reserve in asymptomatic CCS.

In Paper III we did an explorative study by a proteomic approach. We measured 92 different circulating cardiovascular proteins in CCS and controls. Of the cardiovascular proteins, leptin was significantly higher, after correction for multiple testing in CCS compared with controls. Leptin was correlated with arterial stiffness and could potentially be a protein that drives cardiovascular risk in CCS. In Paper IV, we investigated in the same cohorts cardiotoxic ceramides associated with cardiovascular risk and a ceramide-based cardiovascular risk score (Coronary Event Risk Test 2, CERT2), which has earlier been shown to independly predict the risk for major adverse events in adult patients. According to this score, we found that 35% of CCS studied, had a high risk for cardiovascular disease compared with 9% of the controls. Further, all cardiotoxic ceramides were higher in CCS compared with controls.

In conclusion, the results of this thesis suggest several important lines of future research aimed at lowering cardiovascular risk in CCS. Some of the results could be implemented in the current screening for early asymptomatic cardiovascular disease.

Keywords:

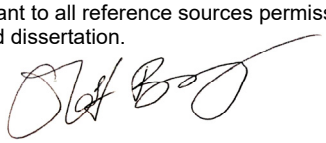
Classification system and/or index terms (if any) Supplementary bibliographical information

Language: English **ISSN and key title**: 1652-8220

ISBN: 978-91-8021-508-4

Recipient’s notes **Number of pages**: 85

Price Security classification

Signature  Date 2023-12-18

Imaging and circulating biomarkers of cardiovascular changes in young adult survivors of childhood cancer

Olof Broberg

LUNDS
UNIVERSITET

Supervisor

Petru Liuba, Associate Professor, University Lecturer

Co-supervisors

Ingrid Øra, Associate Professor

Constance Weismann, Associate Professor

Omslagsbild: The heart

Copyright: Olof Broberg

Fakultet: Medicinska Fakulteten, Lunds Universitet

Institution: Pediatrik

ISBN 978-91-8021-508-4

ISSN 1652-8220

Tryckt i Sverige av Media-Tryck, Lunds universitet

Lund 2024

For Bruno, Aron, Lo, Jasmin, Karin, Anna, Lovisa, Mom, and Dad

Table of contents

Populärvetenskaplig sammanfattning

En stor framgång inom barnonkologin är förbättrade överlevnadstal för olika barncancerdiagnoser vilket är resultatet av bättre behandlingsprotokoll från mitten på 1970-talet. Snittöverlevanden för olika diagnoser ökade från under 50 % till numera över 85 % i Sverige för alla barncancerdiagnoser kombinerat.
Detta möjliggjordes genom en ökad förståelse för cancerbiologin, individuella förutsättningar hos patienten samt bättre cellgifter och mer precis cancerkirurgi och strålbehandling. Därför finns det nu en växande skara av barncanceröverlevare i samhället. När dessa barncanceröverlevare växer upp och går in i vuxenlivet kan unika problem för barncanceröverlevare uppstå – så kallade sen-effekter. Dessa är sent uppträdande negativa effekter av de kraftfulla behandlingarna de genomgått och den svåra sjukdomen de drabbades av under barnaåren.
Kardiovaskulära sjukdomar (sjukdomar i hjärta och kärl) är inte ovanliga sen-effekter och är den näst vanligaste orsaken till en för tidig död hos barncanceröverlevare. Problem med ämnesomsättningen (övervikt, fetma, diabetes) är inte ovanliga hos barncanceröverlevare och kan i sin tur förvärra sen-effekterna i det kardiovaskulära systemet.
Forskningen som presenteras i detta arbete omfattar studier av förändringar i hjärta, kärl och fetter samt potentiella nya blodmarkörer för kardiovaskulär sjukdom hos en grupp unga, 20 – 30 år gamla, friska barncanceröverlevare. Detta har genomförts genom insamling av forskningsdata med blodprover, ultraljud av kärl och av hjärtat i vila och med stimulering med medicin som gör att hjärtat pumpar bättre, mätning av blodkärlens funktion, blodprovsinsamling och genom jämförelser med en grupp friska unga vuxna individer i samma ålder.
Det första av de fyra arbetena studerade hjärt- och kärlfunktion samt blodfetter hos symtomfria barncanceröverlevare. Resultaten visade att det redan i ung ålder hos barncanceröverlevarna fanns försämrad hjärt- och kärlfunktion samt att de hade sämre nivåer av de dåliga blodfetterna. Det andra arbetet studerade hjärtats reservkapacitet, vilken var försämrad hos barncanceröverlevare och kan vara en tidig markör för senare sjukdom. Det tredje arbetet undersökte en panel med 92 kardiovaskulära proteiner och visade att hormonet leptin var högre hos barncanceröverlevare. Det fjärde arbetet undersökte hjärtskadande typer av fetterna ceramider och visade att barncanceröverlevarna hade högre nivåer av dessa ceramider jämfört med kontroller – vilket är förknippat med en ökad risk för att drabbas av hjärtkärlsjukdom. Sammantaget har vi visat med olika tekniker i en grupp av unga vuxna och hjärtfriska barncanceröverlevare negativa förändringar i hjärta, kärl och i ämnesomsättningen, förändringar som i den allmänna befolkningen kan förutspå hjärtkärlsjukdom. För att se om dessa fynd kan förutspå eller förbättra den kardiovaskulära hälsan i uppföljningen utav barncanceröverlevare behövs uppföljande studier.

List of papers

Paper I
Broberg O, Øra I, Wiebe T, Weismann CG, Liuba P. *Characterization of Cardiac, Vascular, and Metabolic Changes in Young Childhood Cancer Survivors*. Front Pediatr. 2021 Dec 8;9:764679. doi: 10.3389/fped.2021.764679. PMID: 34956978; PMCID: PMC8692667.

Paper II
Broberg O, Øra I, Weismann CG, Wiebe T, Liuba P. *Childhood Cancer Survivors Have Impaired Strain-Derived Myocardial Contractile Reserve by Dobutamine Stress Echocardiography.* J Clin Med. 2023 Apr 9;12(8):2782. doi: 10.3390/jcm12082782. PMID: 37109119; PMCID: PMC10145059.

Paper III
Broberg O, Feldreich T, Weissman C, Øra I, Wiebe T, Ärnlöv J, Liuba P. *Circulating Leptin is Associated with Adverse Vascular Changes in Young Adult Survivors of Childhood Cancer.* Under revision in – Cardiol Young (submitted 28th oct 2023).

Paper IV
Broberg O, Øra I, Weismann CG, Wiebe T, Laaksonen R, Liuba P. *Ceramides - a Potential Cardiovascular Biomarker in Young Adult Childhood Cancer Survivors?* (submitted to Eur Heart J Open 20th dec 2023)

Papers by the author not included in the thesis:

Mohlkert LA, Hallberg J, **Broberg O**, Hellström M, Pegelow Halvorsen C, Sjöberg G, Edstedt Bonamy AK, Liuba P, Fellman V, Domellöf M, Norman M. *Preterm arteries in childhood: dimensions, intima-media thickness, and elasticity of the aorta, coronaries, and carotids in 6-y-old children born extremely preterm.* Pediatr Res. 2017. 2017 Feb;81(2):299-306.

Mohlkert LA, Hallberg J, **Broberg O**, Rydberg A, Halvorsen CP, Liuba P, Fellman V, Domellöf M, Sjöberg G, Norman M. *The Preterm Heart in Childhood: Left Ventricular Structure, Geometry, and Function Assessed by Echocardiography in 6-Year-Old Survivors of Periviable Births.* J Am Heart Assoc. 2018 Jan 20;7(2).

Mohlkert LA, Hallberg J, **Broberg O**, Sjöberg G, Rydberg A, Liuba P, Fellman V, Domellöf M, Norman M, Halvorsen CP. *Right Heart Structure, Geometry and Function Assessed by Echocardiography in 6-Year-Old Children Born Extremely Preterm-A Population-Based Cohort Study*. J Clin Med. 2020 Dec 31;10(1):122.

Abbreviations

AC	Anthracycline
Ai75	Augmentation index normalized to a heart rate of 75
Apo	Apolipoprotein
CAD	Coronary artery disease
CCA	Common carotid artery
CCS	Childhood cancer survivors
CER	Ceramide
CERT2	Coronary event risk test 2
CRF	Cardiac reserve function
CIMT	Carotid intima media thickness
cMRI	Cardiac magnetic resonance imaging
CRF	Cardiac reserve function
CVD	Cardiovascular disease
DI	Distensibility index
DSE	Dobutamine stress echocardiography
LVEDV	Left ventricular end-diastolic volume
LVESV	Left ventricular end-systolic volume
GEDSR	Global early diastolic strain rate
GLS	Global longitudinal strain
GSR	Global systolic strain rate
HDL	High density lipoprotein
LDL	Low density lipoprotein
MACE	Major adverse cardiovascular events
LVEF	Left ventricular ejection fraction
NPX	Normalized protein expression
RHI	Reactive hyperemia index
RT	Radiotherapy
SI	Stiffness index
TAPSE	Tricuspid annular plane systolic excursion
TDI	Tissue Doppler Imaging
TG	Triglycerides

Introduction

Childhood cancer survival, in many cases, used to be fatal because there were no effective treatments available[1]. Over the past 50 years, important improvements in pharmacology, diagnostics, treatment combinations and techniques have led to substantially higher survival from childhood cancer and declining mortality rates[2]. Since the 2000s, the 5-year survival rates for all childhood cancers combined is now over 80 % in developed countries[3]. Due to this success, the population of childhood cancer survivors (CCS) is continuously growing. In Europe there are currently approximately 500 000 CCS and in Sweden 10 000 – 11 000 CCS[4,5].

Effective treatment for cancer comes with a cost – so called "*late effects"*. These late effects are diseases that occur years after the cancer disease and are due to unwanted damage to healthy organs[6]. Second to recurrence of the patient's initial cancer or development of a secondary cancer, multiple studies have demonstrated in the long-term, that cardiovascular disease (CVD) is the leading cause of morbidity and mortality in CCS[7,8].

The risk for CVD is attributed to cardiotoxic treatments with radiotherapy (RT) and the commonly used chemotherapeutic anthracycline (AC)[9]. In more recent treatment periods, the mortality from cardiovascular late effects have decreased due to better treatment options for severe CVDs. However, the incidence of severe or life-threatening heart failure has increased and it has been estimated that 10% of CCS that were exposed to cardiotoxic treatments will have heart failure within 40 years after the treatment ended[10,11]. Compared with healthy persons of the same age, CCS are at an 8-fold risk of death by CVD such as heart failure, myocardial infarction, and stroke, [12].

Thus, for the vulnerable growing population of CCS there is a pressing need to find early signs of asymptomatic cardiovascular disease, as early discovery might better the chances of recovery and enables primary prevention strategies [13].

This thesis aims to identify and evaluate the cardiovascular status in CCS by combining different early imaging and circulating biomarkers for cardiotoxicity in a young adult cohort of healthy CCS.

Cardiotoxicity in Childhood Cancer Survivors

Cardiotoxicity from cancer treatment manifests as heart failure, coronary artery disease, arrhythmias, stroke, and pericardial disease. Heart failure is the most common manifestation[11].

Based on the time to onset of cardiac symptoms[14-16], clinical cardiotoxicity can be categorized as:

- *Acute*: Cardiotoxicity that presents within seven days of given therapy. Possible manifestations are acute congestive heart failure, pericarditis, and arrhythmias. Acute cardiotoxicity is uncommon and seen in less than 1% of patients. The acute form is usually reversible with cessation of treatment.
- *Early*: Cardiotoxicity that presents < one year after therapy completion. It presents with heart failure due to restrictive or dilated cardiomyopathy and can be progressive.
- *Late*: Cardiotoxicity that presents > one year after ending therapy. This type can be progressive and symptomatic disease can occur up to 30 – 40 years, or even later, after treatment but much earlier than in the general population.

The following text focuses on late cardiotoxicity.

Heart Failure

Heart failure is the most common cardiovascular late effect after treatment for childhood cancer[17]. The latency between exposure to cardiotoxic treatments and clinically evident disease can be very long, up to 30 – 40 years[18-20]. Of the different chemotherapeutic drugs used, one group of drugs is particularly associated with heart failure due to cardiotoxicity – AC[9]. This group includes the drugs doxorubicin, epirubicin, daunorubicin and mitoxantrone. Initially ACs were used as antibiotics, but due to the serious side effects this usage was stopped. For cancer treatment ACs were discovered to be highly effective[21]. ACs have been, and are still extensively used to treat leukaemia, lymphoma, and sarcoma. Approximately 50 – 60 % of childhood cancer patients are treated with AC[22]. Radiotherapy (RT) directed to the chest is also strongly associated with heart failure[23].
Non-AC chemotherapeutic agents such as alkylating agents (cyclophosphamide and ifosfamid) and platinum agents (cisplatin and carboplatin) can also cause both acute and late-occurring heart failure[14].
The incidence of symptomatic heart failure from AC is proportional to the cumulative dose (mg/m^2) of the drug given during cancer treatment[14]. The increased risk for cardiac impairment following cumulative doses > 250 – 300 mg/m2 is well

established whereas doses < 100 mg/m2 are considered relatively safe[24,25]. However, no AC dose is without risk and cardiotoxicity has been demonstrated with very low doses of AC[18,26].

Two large studies of CCS, from 2013 and 2019, studied late occurring cancer treatment related heart failure and found similar results: Irrespective of exposure to cardiotoxic treatments the cumulative incidence at an acquired age of 45 and 40 years after cancer treatment the cumulative incidence was 4.8% and 4.4% respectively[10,27]. In the study from 2019 by Feijen *et al*., the incidence was low in CCS not exposed to cardiotoxic treatments (0.3%) and high in those exposed (10.6%). Importantly, in both studies, only heart failure with symptoms at minimal exertion to fatal heart failure were included, meaning that 1/10 of CCS that were exposed to cardiotoxic treatments will need treatment for heart failure or die from it at a much earlier age than people in the general population.

CCS requiring heart transplantation for heart failure after cardiotoxic cancer treatment have been shown to be younger than other patients[28]. The prognosis of symptomatic heart failure after childhood cancer treatment has been shown to be worse than for other heart failure etiologies[29,30].

Coronary Artery Disease

Coronary artery disease (CAD) risk is higher in CCS and linked to RT directed at the chest. Asymptomatic CAD in CCS has been detected by electrocardiogram, echocardiography, and patient history in 3.8 % of CCS at follow-up 20 years after childhood cancer treatment[31]. The presence of traditional cardiovascular risk factors (hypertension, obesity, diabetes, smoking) in CCS increase this elevated risk even further[32]. As with AC and risk of heart failure, the risk for CAD after RT is dose dependent with a cumulative incidence of symptomatic CAD as high as 20 % in survivors exposed to > 35 Gray of ionizing radiation to the chest[33]. A study of Hodgkin lymphoma survivors with computed tomography (CT) showed that coronary artery stenoses were proximally located after chest RT, and thus, more severe[34]. However, anatomic screening for coronary lesions with angiography or computed tomography in CCS is not used in the clinical setting[35].

Stroke

Stroke is particularly associated with higher doses of cranial RT[36]. Stroke rate has been reported to be 77 cases per 100 000 person years in CCS compared 9.3 in siblings at 23 years follow up regardless of exposure to RT treatment or not[36]. Also, risk for a recurrent stroke is higher in CCS compared to the general population in the following 10 years after the first stroke (10 % vs 21 %)[37]. RT to the circle of Willis appears to be an important risk factor for stroke in CCS but RT to the heart and neck also increase the risk for stroke[38].

The etiology of stroke in CCS treated with cranial RT can be due to vasculitis induced by vascular damage from ionizing radiation, which can lead to large or small vessel infarction[39]. Another suggested mechanism in non-RT treated CCS is that AC causes endothelial dysfunction that accelerates atherosclerosis leading to stroke[40]. Further, conventional risk factors for stroke such as hypertension are also more common among CCS[36].

Valvar Disease and Pericardial Disease

Clinically significant valvar disease after treatment for childhood cancer is uncommon (< 1%)[41]. Valvar sequelae likely have a longer latency before symptoms develop than other CVDs from treatment for childhood cancer and the incidence might therefore be underestimated[42].

As with CAD, higher doses of ionizing radiation to the chest increase the risk for valvar disease[43]. High doses of chest RT cause scar formation with valvar collagen deposits and fibrosis followed by thickening and calcification with progressive valve dysfunction[42,44].

Constrictive pericarditis, due to fibrotic remodelling of the pericardium, is a feared complication after RT to the chest with low survival even after surgical treatment[45,46]. The disease is very uncommon in the general population but in CCS the risk is 10-fold higher[47].

Arrhythmia

A prolonged, and arrhythmogenic, corrected QT interval (QTc) on electrocardiography has been documented in CCS after AC treatment[48]. Ectopic beats and atrioventricular blocks have been reported in CCS irrespective of cardiotoxic treatment[49]. Incidence of symptomatic arrhythmias have been reported to be low (<1%) in middle aged CCS[27]. Symptomatic arrhythmias have been associated with higher cumulative doses of AC and RT[47].

Mechanisms and Risk Factors for Cardiotoxicity

Anthracyclines

The anti-cancer action of AC on rapidly proliferating cancer cells is by transport of AC into the cell nucleus, where it interchelates between DNA base pairs. The interchelation leads to inhibition of DNA replication and RNA transcription and arrests cell growth. Further, AC form a complex with the DNA and the enzyme Topoisomerase-2α, which inhibits the function of Topoisomerase-2α in repairing DNA and eventually the cancer cell dies[50].

By similar mechanisms to kill cancer cells, AC exert its cardiotoxic effect (**Figure 1**)[50]. The enzyme Topoisomerase-2α is only expressed in dividing cells (such as cancer cells) but in the non-dividing cardiomyocyte, the iso-form Topoisomerase-2β is present and responsible for DNA replication and transcription[51]. By a similar mechanism as in cancer cells, the AC molecule is transported into the cardiomyocyte nucleus causing and forms a complex with Topoisomerase-2β causing DNA breaks[52]. Such DNA breaks activates apoptosis (programmed cell death) pathways[53]. As support for the role of Topoisomerase-2β in anthracycline cardiotoxicity, an animal study of Topoisomerase-2β knockout mice did not observe cardiotoxicity as measured by cardiac function[52].

Other important mechanisms for cardiotoxicity by AC need mentioning: AC is also retained within the mitochondria of cardiomyocytes by forming complexes with mitochondrial surface membrane proteins. This disturbs the mitochondrial electron transport chain and reactive oxygen species such as superoxide (O_2^-) are formed. AC also interact with the enzyme endothelial nitric oxide synthase (eNOS). This enzyme produces nitric oxide (NO) but in the presence of AC it shifts to formation of O_2^- [54].

Further, AC induce an iron-mediated increase of reactive oxygen species inside cardiomyocyte mitochondria[55]. Iron overload has been suggested as a risk for developing more severe cardiotoxicity and carriers of mutations in the HFE-gene for hemochromatosis have been shown to be at an increased risk for AC-mediated cardiotoxicity[56].

The resulting increase of reactive oxygen species from the over-production by these mentioned mechanisms activates cardiomyocyte apoptosis[57]. Still, the full spectrum of mechanisms responsible for AC-mediated cardiotoxicity is not yet fully understood. There is most likely an interplay between DNA damage, oxidative stress, and iron overload with a unified result of cardiotoxicity[58]. These mechanisms all promote decreased cardiomyocyte function and apoptosis, explaining the elevated risk for heart failure in CCS[56,59].

Besides cardiotoxicity, vascular changes due to the toxic effects of AC on endothelial cells have recently become more recognized[60]. It has been proposed that AC induce dysfunction and apoptosis of endothelial cells by similar mechanisms as

in cardiomyocytes, with formation of reactive oxygen species and DNA damage[61]. Apoptosis of endothelial cells cause a decrease of the density of the endothelium and extra incorporation of collagen causing less elasticity (stiffer) arteries that are more prone to atherosclerosis[62]. Stiffer peripheral arteries might cause indirect cardiotoxicity by increasing left ventricular afterload and wall stress[61,63].

Radiotherapy

RT is an important adjuvant therapy to chemotherapy or surgery for different types of childhood cancer[64]. Treatment with RT of lymphoma, a cancer that often is located on the neck or the area between the lungs and behind the breastbone (the mediastinum), may expose the heart and neck vessels to the negative effects of ionizing radiation[65]. Similar to AC – the cumulative dose of chest RT has a linear relationship with cardiotoxicity[66]. Concomitant use of AC and RT multiplies the risk for cardiotoxicity even further[11,65].

Highly differentiated cardiomyocytes are relatively resistant to ionizing radiation[67]. Damage to cardiac and vascular endothelial cells is likely important for RT induced cardiovascular late effects[68,69]. Still, the cardiomyocyte can undergo functional changes, apoptosis, and also differentiate to myofibroblasts due to damage from ionizing radiation causing interstitial fibrosis[23].

Ionizing radiation cause fibrosis in cardiovascular tissues through a stepwise process[70]. In the acute phase, radiotherapy causes DNA damage and formation of reactive oxygen species to the endothelial cells lining the heart[69]. The endothelial cells, as a response, enter senescence (stops dividing) or undergo apoptosis. Senescent endothelial cells secrete pro-inflammatory substances[71]. This inflammatory response attracts inflammatory cells, which secrete molecules that further promote inflammation and fibrosis. Fibroblasts are recruited and as a result extracellular matrix proteins such as collagen are deposited in the heart and blood vessels[67,69].

Also, capillaries, and arteries, such as coronary or carotid arteries, can be affected with resulting endothelial dysfunction and narrowing and stiffening of arteries, ultimately causing stroke and coronary artery disease[67].

Individual Risk Factors for Cardiotoxicity

Besides cumulative doses of AC and chest RT, there are several patient-specific risk factors that are associated with higher risk for cardiotoxicity: female sex, Down syndrome, young age at diagnosis (< 4 years), and pre-existing cardiac disease[15,72]. Also, pharmacogenetic studies have identified genes that increase the risk for cardiotoxicity in some CCS[73].

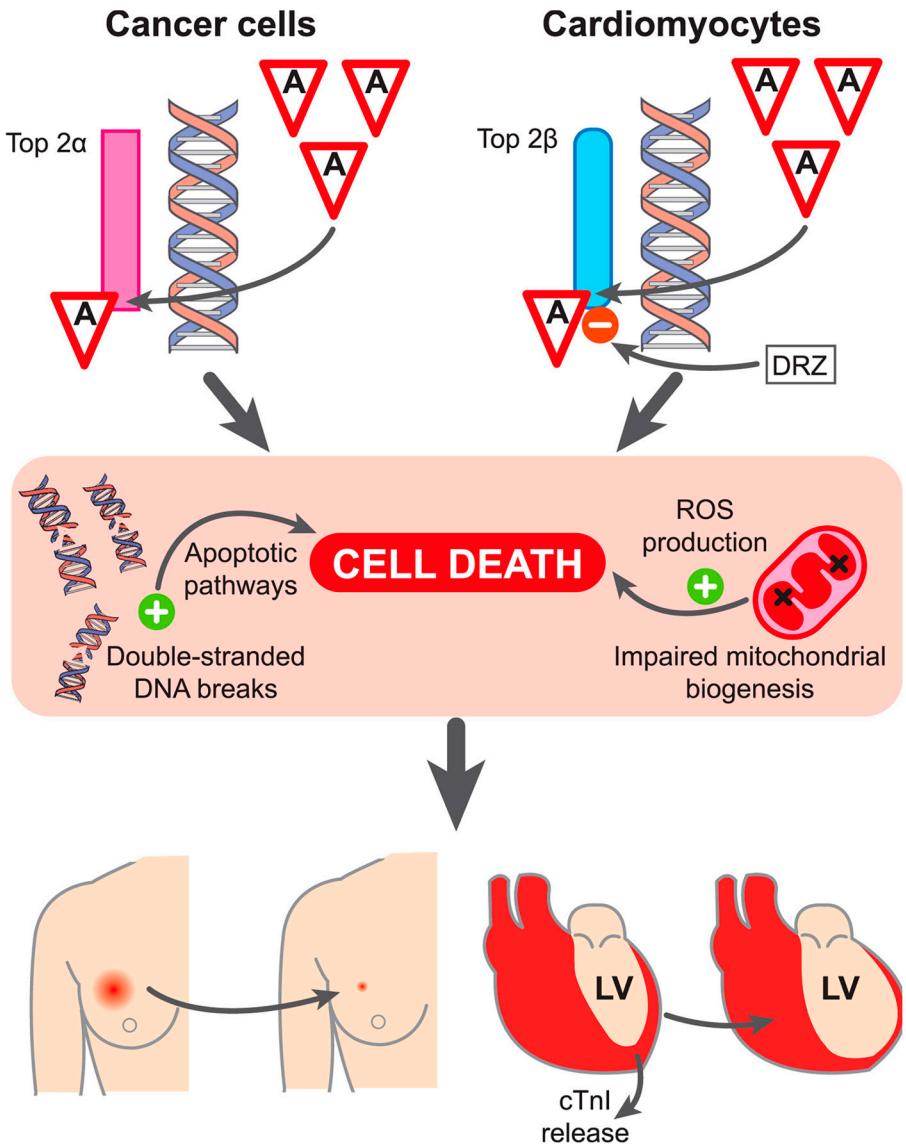


Figure 1. Anthracycline (AC)-mediated treatment mechanism against cancers cells and the mechanism for AC (A) mediated cardiotoxicity against cardiomyocytes. Rapidly dividing cancer cells contain Topoisomerase-2α which AC binds to and inhibits and thereby cause DNA breaks, inhibition of DNA repair and accumulation of reactive oxygen species leading to cancer apoptosis. In cardiomyocytes, Topoisomerase 2β (Top-2β) is present. AC inhibits Top-2β and by similar mechanisms cause apoptosis as in cancer cells. Cardiac damage can be detected during AC treatment with cardiac biomarkers such as cardiac troponin I (cTnI). With time there are fewer cardiomyocytes and there is fibrosis leading to a thinner walled heart (LV – left ventricle) and may lead to heart failure. The cardioprotectant Dexrazoxane (DRZ) inhibits the binding of AC to Top-2β, and is used as a cardioprotective drug in some countries in selected patients during AC treatment**. Reproduced with permission from Peter A Henriksen, Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention, Heart, 2018**[50]**.**

Modifiable Cardiovascular Risk Factors

Cardiovascular risk factors relevant to the general population, such as hypertension, diabetes, obesity, and dyslipidaemia are also important determinants of the increased cardiovascular risk in CCS[32]. Specific cancer treatments might predispose to such cardiovascular risk factors[74].

The metabolic syndrome is defined as the presence of a cluster of these risk factors and the incidence of the metabolic syndrome was shown to be between 9 – 32% in two different cohorts of CCS aged 20 – 30 years[75,76]. Patients treated with cranial RT (brain tumor patients and some leukemia patients) are particularly prone to obesity and the metabolic syndrome[77,78].

Hypertension is one of the most common cardiovascular risk factors in CCS, with a cumulative incidence between 14 – 40%, in middle-aged CCS (40 years)[27,79]. Hypertension, in adult CCS, has shown to be associated with major adverse cardiovascular events (MACE) and death[27,80]. Causes for hypertension in CCS can be due to treatment factors such as abdominal RT with subsequent kidney damage[81].

The prevalence of obesity in CCS, as defined by body mass index (BMI) > 30, has been shown to be the same as in the general population[82]. However, sarcopenic obesity, as defined by a normal BMI with high body fat mass, is more prevalent in CCS[82]. Sarcopenic obesity might be associated with higher risk for CVD than non-sarcopenic obesity[83]. Possible causes of obesity in CCS are cranial RT, chronic inflammation, an unhealthy diet, and physical inactivity[84,85].

Dyslipidemia is prevalent in CCS[86]. Presence of dyslipidemia is associated with CVD in CCS[87]. Dyslipidemia is frequently underdiagnosed in CCS possibly due to the increased prevalence of sarcopenic obesity[82,88].

Diabetes is more common in CCS than in the general population[89,90]. CCS treated cardiotoxic treatments who later develop diabetes have a very high risk for CVD[80]. The sometimes-observed increased visceral obesity in CCS is associated with insulin resistance, inflammation and is likely contributing to the risk of developing diabetes in CCS[91,92]. Possible mechanisms for the increased risk for diabetes in CCS are abdominal RT and treatment with corticosteroids[93,94].

In summary, CCS are prone to develop several cardiovascular risk factors causing indirect cardiotoxicity[32,95,96]. CCS also have higher incidence of psychiatric diseases, negative stress, and negative lifestyle measures[97]. With these risk factors, taken together with an earlier exposure to cardiotoxic anti-cancer treatments, the etiology of CVD in CCS is complex and multi-factorial (**Figure 2**).

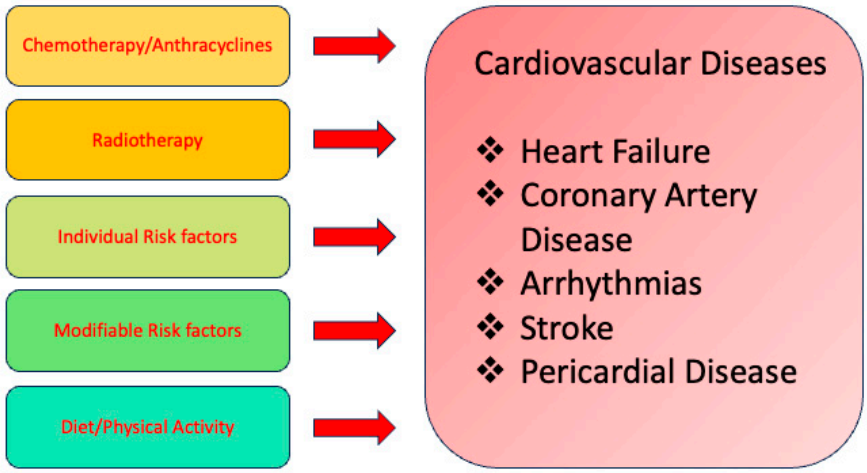


Figure 2. The many components to be considered in the development of cardiovascular diseases (CVD) in childhood cancer survivors. 50 – 60% of survivors undergo treatments with cardiotoxic anti-cancer treatments such as anthracyclines and radiotherapy to the cranium or chest. Young age (<4 years), female sex, Down syndrome and genetic predisposition increase cardiac damage from these treatments. Modifiable cardiovascular risk factors such as hypertension, dyslipidemia, diabetes and obesity further drive the development of cardiovascular diseases. An unhealthy diet and low physical activity add to the burden of CVD risk.

Detection of Cardiotoxicity

Different methods such as imaging by echocardiography, cardiac magnetic resonance imaging (cMRI), electrocardiography, and circulating biomarkers in the blood are used to diagnose cardiac damage after cardiotoxic treatments[11,14,98]. Early detection of chemotherapy- and RT-induced cardiotoxicity enables prompt preventive pharmacological or life-style measures[21]. Therefore, research has been focused on finding adverse cardiovascular changes in CCS early – before symptoms occur because of the benefits of early interventions[29,99].

The development of symptomatic CVD after cardiotoxic anti-cancer treatment is a continuous process as shown in **Figure 3**[100]. Newer methods utilizing advanced echocardiography can diagnose subclinical cardiovascular functional changes in CCS in the early stages[98]. One of current challenges is to find such early markers for subclinical cardiotoxicity that can predict future symptomatic cardiovascular late effects[25,101].

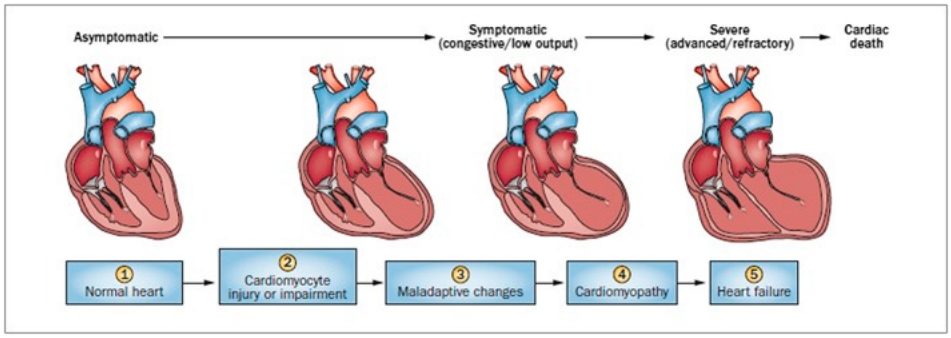


Figure 3. Possible progression of heart failure in childhood cancer survivors (CCS) exposed to cardiotoxic treatments – from subclinical cardiomyopathy to severe disease. (1-2) In a CCS with a normal heart but exposed to cardiotoxic treatments, primary preventive measures are possible. (3) Cardiac remodeling is detectable by advanced imaging and possibly by circulating cardiovascular biomarkers. Function is preserved, and prevention might be possible. (4) Diastolic and/or systolic functional impairment are evident by standard echocardiography and can progress to (5) heart failure and prevention is no longer possible and in severe cases radical treatments such as ventricular assist device or cardiac transplantation may be needed. Adopted with permission from the authors, Hutchins KK, et al. **Prevention of cardiotoxicity among survivors of childhood cancer.** British Journal of Clinical Pharmacology. 2017[100].

In Sweden, six late effects clinics in collaboration with the cardiology outpatient clinics are responsible for cardiac follow-up in CCS. The late effects clinics are situated at the six hospitals also specialized for treatment of childhood cancer.
The current Swedish protocol for cardiac cardiotoxicity monitoring is based on the exposure to cardiotoxic treatments with AC and/or chest RT (**Table 1**)[102]. International follow-up protocols are similar[25,101]. If abnormalities are found, or the CCS has cardiovascular symptoms, a referral to the cardiology clinic is recommended for further evaluation and treatment. Heart failure in CCS is treated according to standard guidelines for heart failure treatment with drugs such as angiotensin converting enzyme inhibitors (ACEi), beta-blockers, and diuretics[9]. A current dilemma is if asymptomatic, subclinical, abnormalities in cardiac systolic and diastolic function in CCS should be treated or not[103].

Table 1. Cardiac follow-up after childhood cancer in Sweden.

Cardiotoxicity Risk group	
1. Cumulative AC-dose < 250 mg/m^2, no chest RT	• TTE within 6 months of completion of treatment • TTE at puberty and at age 18 • TTE is not recommended during adulthood. • Cardiac evaluation if competitive sports/pregnancy
2. Cumulative AC-dose > 250 mg/m^2, no chest RT	• TTE within 6 months of completion of treatment • TTE after 5 years, at puberty and at age 18 • TTE every 5th year during adulthood • Cardiac evaluation if competitive sports/pregnancy
3. AC-treatment and chest RT > 15 GY or TBI irrespective of GY	• TTE within 6 months of completion of treatment • TTE after 5 years, at puberty and at age 18 • TTE and exercise-test every 5th year during adulthood • Cardiac evaluation if competitive sports/pregnancy
4. Chest RT and no AC-treatment	• If Chest RT dose > 15 GY – TTE and exercise test every 5th year. • Cardiac evaluation if competitive sports/pregnancy

Recommended cardiac follow-up in Sweden for childhood cancer survivors exposed to cardiotoxic anthracycline (AC) and/or chest RT or total body irradiation (TBI). TTE – transthoracic echocardiography.[102]

Echocardiography

Echocardiography (cardiac ultrasound) is considered the modality of choice for detection of cardiotoxicity in CCS, because it is non-invasive, widely available, and without radiation[9,11,98,104,105].

With echocardiography, cardiac functional, morphological and hemodynamical measures can be assessed[106]. A standard 2D-echocardiographic analysis before cardiotoxic treatment in childhood cancer patients should include evaluation of left- and right ventricular systolic and diastolic function, pulmonary artery pressure, valvular functions, and signs of pericardial disease[98,101,107].

In the long-term follow-up of CCS, systolic (pumping) function, as measured with left ventricular ejection fraction (LVEF) and shortening fraction (FS) by 2D-echocardiography and M-mode echocardiography respectively, are the most used measures to monitor cardiotoxicity (**Figures 4 and 5**)[25]. The normal value for LVEF in females is ≥54% and in males ≥52%[105]. Reductions in LVEF and FS can be detected via the routine screening in CCS. An important limitation for LVEF and FS is that they detect cardiotoxicity at a late stage when primary prevention is no longer possible.

The 2D-echocardiographic and M-mode assessments of LVEF and FS have inherent limitations because they make geometric assumptions of the left ventricle and reproducibility is low. With three-dimensional (3D) echocardiography these assumptions are not needed[21]. LVEF assessed by 3D echocardiography has better reproducibility and less intra- and interobserver variability due to the semi-automatic acquisition and is now the recommended modality when available[108]. However, 3D assessment of LVEF requires an operator with experience with the modality and is dependent on superior image quality compared to 2D LVEF.
A high reproducibility of cardiac measurements is important in the aspect of CCS with a long-term follow-up with serial measurements[98]. It is important to note that a decrease in LVEF (both 2D and 3D acquired) and FS often are a late finding, and a normal result does not exclude cardiotoxicity[109].

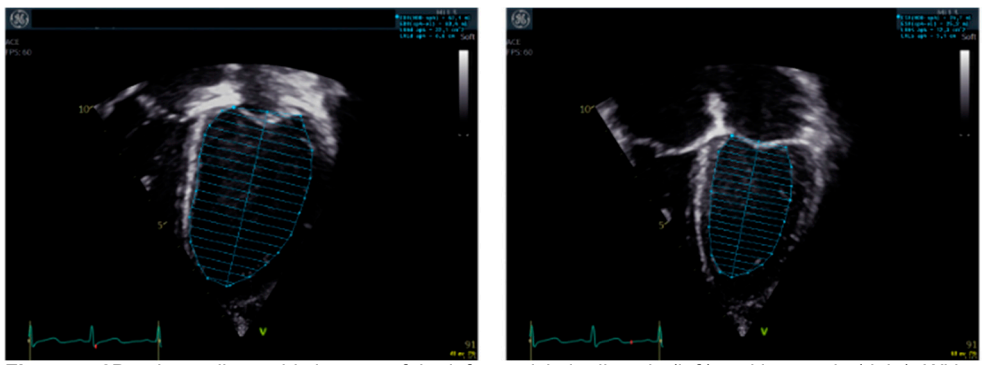


Figure 4. 2D-echocardiographic images of the left ventricle in diastole (left) and in systole (right). With calculation of end diastolic- (LVEDV, left) and end systolic volumes (LVESV, right). left ventricular ejection fraction (LVEF) can be calculated: $LVEF = (LVEDV - LVESV) / EDV * 100\,\%$. This measure is used in the follow up in childhood cancer survivors after exposure to cardiotoxic anti-cancer treatments.

Cardiac structural changes also occur in CCS after cardiotoxic treatments with AC and RT, and cardiac remodeling can be seen by reductions in the thickness of the walls of the left ventricle[72](**Figure 5**). Because of the wall thinning, the stress on the cardiac walls increases. This can lead to an increase in the diameter of the left ventricle and the cardiac morphology after cardiotoxic treatment can have similarities to dilated cardiomyopathy[110]. Sometimes, in patients exposed to high doses of chest RT and high cumulative doses of AC a small thin-walled heart with a restrictive physiology, due to interstitial fibrosis, is observed ("Grinch syndrome")[111].
Diastolic dysfunction, meaning a defective cardiac filling, is a predictor for heart failure in the general population and can be detected with echocardiography. This is sometimes seen in CCS[112]. In a recent study, diastolic dysfunction was diagnosed in 15% of CCS, being mostly found in combination with systolic dysfunction[113]. Diastolic dysfunction is diagnosed by specific mitral inflow Doppler flow patterns and tissue Doppler imaging (TDI) (**Figure 6**)[106,114].

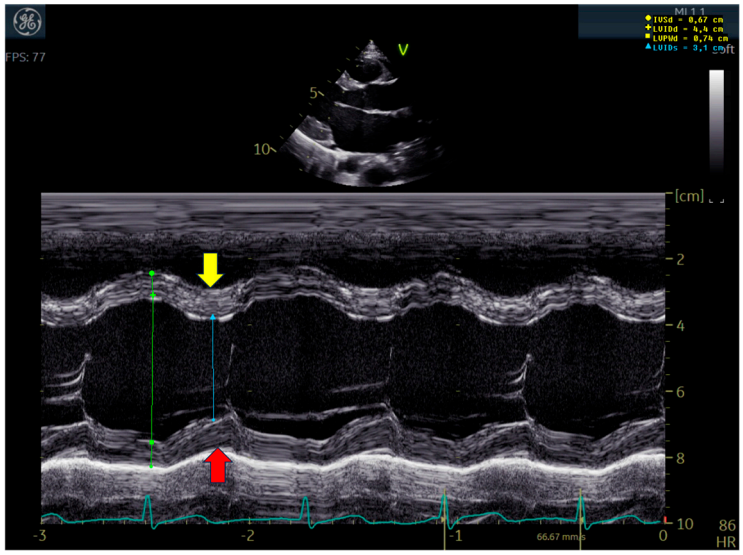


Figure 5. M-mode assessment in the parasternal long-axis view with measurements of the dimensions of the left ventricle in systole and diastole, enabling the calculation of shortening fraction (FS), a marker for systolic function used in the cardiac follow-up of childhood cancer survivors (CCS). Image also depicts measurements of the ventricular septum (yellow arrow) and the posterior wall (red arrow). These structures can become thinner in CCS after anthracycline treatment.

Strain (Speckle Tracking) Echocardiography

Strain is a novel measure of the relative shortening of a cardiac segment in systole. As with LVEF, moving images (loops) are acquired with echocardiography for strain analysis. A special software identifies patterns of gray values in the myocardium called "speckle patterns". These speckle patterns are unique for each myocardial segment. The software measures the movement of speckle patterns and calculates the shortening during systole for a particular cardiac segment. Global longitudinal strain (GLS) corresponds to a mean of the sum of such shortening of the different segments of the whole left ventricle[101]. LVEF and GLS are both measures of systolic function with the difference that LVEF corresponds to the changes in left ventricular volumes during the heart cycle and GLS measures the cardiomyocyte movement directly in systole and is a more sensitive measure of systolic function[105].

Since decreases in LVEF and FS reflect later stages of cardiotoxicity in CCS, advanced imaging techniques are of importance to detect early remodelling. GLS, has been shown to detect cardiotoxicity at an early stage in CCS (**Figure 7**)[9,11,13,14,115].

In survivors of adult cancer, GLS can predict a future decline in LVEF[116]. Several studies in CCS have shown a decreased GLS in both patients with a preserved and impaired LVEF[109,114,115,117]. The frequency of impaired GLS in CCS has been shown

to be as high as 28 %, at 10 – 48 years from cancer diagnosis, and is associated with higher cumulative doses of AC and chest RT[118]. Importantly the incidence of abnormal strain is dependent of what GLS values is defined as abnormal.
GLS values differ somewhat depending on both the manufacturer of the ultrasound machine and the brand of the software that is used to analyze GLS, and defining normal values is a challenge[119]. In recent studies -16% has been defined as the upper limit for normal GLS (lower, more negative, global longitudinal strain values are better) [120,121].
GLS is currently used as a validated marker and predictor in several CVDs not related to exposure to cardiotoxic anti-cancer treatments[122,123]. Despite being a proven sensitive measure for cardiotoxicity in the follow-up of CCS, reductions in GLS have not yet been shown to be predictive for symptomatic heart failure in this population[11].

Stress Echocardiography

By stressing the heart, by physical activity (e.g., treadmill or cycling) or pharmacologically during echocardiography, the cardiac reserve function (CRF) can be measured. Cardiac function, in the normally functioning heart, is then expected to increase. Thus, CRF is the difference in LVEF or GLS (or other cardiac functional measures) between rest and during physical or pharmacological stress (CRF = heart function during stress - heart function at rest)[124]. A persistent decrease in CRF precedes clinical heart failure (**Figure 8**)[125].
Impairments in CRF have also been shown in CCS but predictive abilities for heart failure are not established[126-128]. Another benefit of stress echocardiography is the ability to screen for coronary artery disease in CCS treated with chest RT[35].

Cardiac Magnetic Resonance Imaging

Cardiac magnetic resonance imaging (cMRI) can be used in the follow up of CCS if echocardiography is inconclusive or if echocardiography is not feasible[25]. cMRI renders well defined and reproducible images of the moving heart and enables accurate assessments of functional measures, such as LVEF and GLS and cardiac morphology irrespective of acoustic windows[129].
A major advantage with cMRI is that cardiac fibrosis is detectable, which is particularly important in patients exposed to chest RT[130]. Disadvantages with cMRI are the high cost, the long examination time, and the limited availability[98]. cMRI imaging could be more sensitive for the detection of cardiotoxicity[130-132]. Results in studies of the use of cMRI in CCS have been conflicting regarding benefit over echocardiography[114,133].

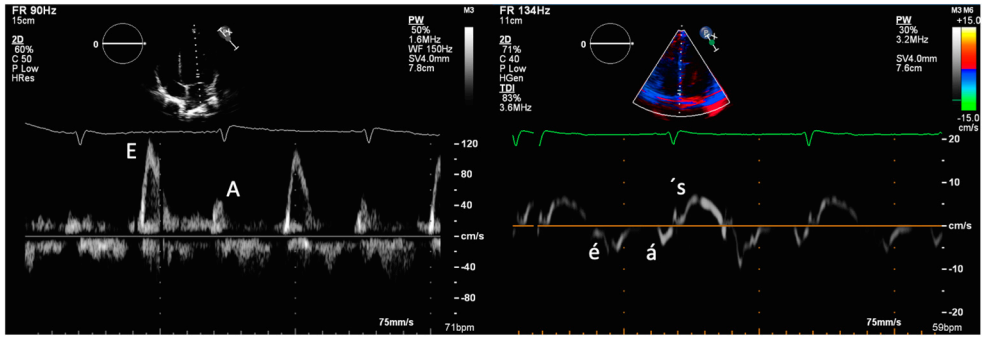


Figure 6. Image depicts mitral inflow Doppler (left) and tissue Doppler imaging (TDI, right) analysis of the septal mitral point for evaluation of diastolic function in a childhood cancer survivor (CCS) treated with anthracyclines (AC). Results show diastolic (filling) dysfunction with high E/A ratio (2.5) indicating restrictive filling. E/é is abnormal at 12-15, further indicating diastolic dysfunction. The E wave is the early mitral flow and A wave is the late mitral flow. TDI é wave is the early diastolic movement and the á-wave is the late diastolic movement. The positive TDI reflection is the ´s-wave – reflecting systolic function. The TDI ´s-wave is also abnormal (5.5 cm/s) indicating abnormal systolic function as well in this patient. Importantly this CCS was treated with 217 mg/m^2 AC and no radiotherapy wich corresponds to the low risk cardiotoxicity cathegory with no further cardiac screening during adulthood according to the Swedish guidelines (see Table 1).

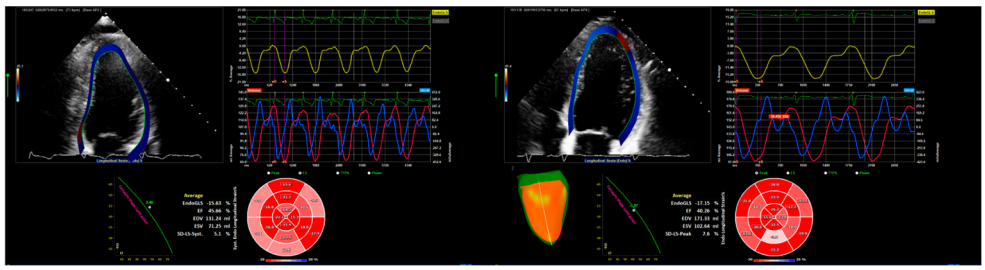


Figure 7. Measurement of global longitudinal strain (GLS) in two childhood cancer survivors (CCS) treated with a cumulative anthracycline dose of 446 mg/m^2 (left) and 150 mg/m^2 (right). In the CCS treated with 446 mg/m^2 GLS was abnormal at -15.6%, and in the other CCS GLS was normal at -17.8%. Both of these CCS had normal LVEF (>55 %). As seen in the images, GLS values are calculated semi-automatically by the software from 3 – 8 consecutive heart cycles.

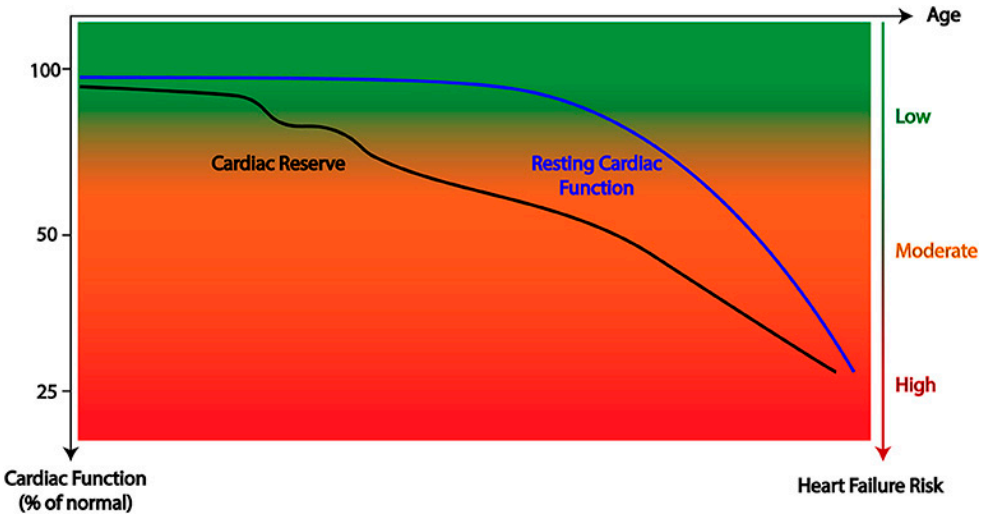


Figure 8. Possible benefit of stress echocardiography in detecting a declining cardiac reserve function that might precede a decline in resting cardiac function after exposure to cardiotoxic anti-cancer treatments. Adopted from with permission: Foulkes S., et al. **The Utility of Cardiac Reserve for the Early Detection of Cancer Treatment-Related Cardiac Dysfunction: A Comprehensive Overview.** Frontiers Cardiovascular Medicine (2020)[124]. Licensed by http://creativecommons.org/licenses/by/4.0/.

Vasculotoxicity

Measures of vasculotoxicity (endothelial dysfunction, arterial stiffness, intima media thickness) are not currently used in the clinical context of cardiotoxicity detection in CCS[134].

Endothelial dysfunction is the impairment of endothelial cells to balance the secretion of vasodilating and vasoconstrictive substances such as NO and O_2^-. Arterial stiffness is the consequence of a decrease in elastic fibres of the arterial wall. Intima-media thickness is the measured thickness of the inner vascular layers – the intima and the media[135]. The intima-media thickness increases with fat accumulation.

Endothelial dysfunction, arterial stiffness and intima-media thickness are all early subclinical manifestations of vascular disease[136]. They are intimately associated with each other and relate to CVD risk[135]. AC and platinum agents are examples of chemotherapeutic compounds that cause vascular dysfunction seen in CCS[135,137-139]. However, premature vascular disease has been demonstrated by increases in arterial stiffness in both CCS exposed and unexposed to cardiotoxic treatments[60,140,141]. Vasculotoxicity is also important in the understanding of cerebrovascular disease, coronary artery disease and the development of heart failure in CCS[60,134].

Vasculotoxicity is not routinely measured in clinical settings but is investigated in research[135,140].

Circulating Biomarkers

Conventional circulating blood biomarkers in cardiac disease are N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) and cardiac troponins. These have been demonstrated to be higher in some CCS exposed to cardiotoxic anticancer treatments[142]. However, these biomarkers have shown limited diagnostic value for cardiotoxicity in CCS[143]. The predictive values of these biomarkers are also limited[144].

The current Swedish guidelines and international guidelines for late cardiac effects after childhood cancer does not recommend testing CCS with either NT-pro-BNP or troponins but these circulating biomarkers are of course used in symptomatic patients[101,102].

Current Problems in the Follow-up of CCS

With the growing numbers of CCS reaching adulthood, the prevalence of CCS with CVD will continue to rise. CCS presenting at a young age with CVD are probably just the tip of the iceberg. In most CCS treated with cardiotoxic treatments, lifelong cardiovascular follow-up is warranted because the latency from treatment to clinical onset can be very long. Improved detection of early signs of subclinical CVD in this population is thus crucial to decrease the rate of major adverse cardiovascular events later in life.

Cost-effectiveness and proper resource utilization are also important aspects that need to be considered. Since late effects clinics are centralized to larger hospitals with access to specialized cardiac care, the related costs and workforce are expected to further increase, thereby posing an additional burden on a Western healthcare system that is already affected by increasing expenditure and staffing shortage.

Therefore, there are several areas that need to be addressed to better the lifespan and life-quality for CCS and to also improve the accessibility and effectiveness of highly specialized services in cardiac care. Some of these areas are:

- Better characterization of cardiovascular health in CCS before reaching the ages when CVDs become apparent.
- Better screening tools to detect subclinical CVD early in CCS.
- Easy to measure, early CVD-specific, inexpensive biomarkers such as circulating biomarkers that can be used both in an outpatient setting as well as in highly specialized clinical settings.

In the papers of this thesis, we addressed these areas of interest in CCS.

Aims

The overall purpose of this thesis was to identify and evaluate different early imaging and circulating biomarkers for cardiotoxicity in CCS.

Paper I

The aim of this study was to evaluate different cardiac, vascular, lipid, and apolipoprotein measures for early CVD risk in young adult CCS and in healthy controls.

Paper II

The aim of this study was to evaluate left ventricular contractile reserve function (CRF) by dobutamine stress echocardiography (DSE) combined with measures of myocardial strain in CCS previously treated with AC and in healthy controls.

Paper III

The aim of this study was to investigate circulating cardiovascular proteins in young adult CCS and healthy controls and their relationship to subclinical CVD.

Paper IV

The aim of this study was to investigate circulating ceramides specific for cardiovascular disease in young adult CCS and their correlations to previously reported adverse cardiovascular changes in this cohort.

Funding

The work in this thesis was supported by the Swedish Childhood Cancer Foundation, the Swedish Heart Lung Foundation, the Lund University and the Skåne University Hospital in Lund, Sweden.

Study Cohorts and Methods

Paper I

Paper I was a single-centre cross-sectional study of lipid and apolipoprotein profile, carotid artery, and peripheral endothelial function as well as cardiac size and function using standard echocardiography in CCS exposed to cardiotoxic anticancer treatment (AC with or without RT). We also studied the correlations between these indices and treatment with AC and RT.

All CCS were identified from the Registry for Childhood Malignancies in Southern Sweden (BarnOnkologiskt Register I Södra Sjukvårdsregionen, BORISS)[145]. Inclusion criteria were cancer diagnosis under the age of 18, survival more than 5 years after the disease remission, and age between 20–30 years. Exclusion criteria were previous cardiovascular disease (CVD), CVD medication or any cardiovascular complication during cancer treatment, any current chronic disease or syndrome, and pregnancy. Healthy controls with similar sex and age between 20-30 years were recruited via written announcements at the Skåne University Hospital area in Lund, Sweden.

The cumulative dose of different ACs was converted into doxorubicin equivalents using conversion factors (**Table 2**)[146].

Table 2. Anthracycline Conversion into Doxorubicin Equivalents

Type of Anthracycline	Conversion factor
Doxorubicin	1.0
Idarubicin	5.0
Mitoxantrone	4.0
Daunorubicin	0.83
Epirubicin	0.67

Doxorubicin isotoxic equivalents of cardiotoxic potential of different types of anthracyclines.

Systolic and diastolic brachial blood pressure (SBP and DBP) were measured in the supine position after 15 min of rest in the right arm. Weight and height were measured, and body mass index (BMI) was calculated.
Fasting blood samples were collected for lipid and apolipoprotein cardiovascular biomarkers (HDL, LDL, total cholesterol, TG, Apo-A and Apo-B). The ratio of Apo-B to Apo-A (Apo-B/Apo-A-ratio) was calculated.

Echocardiography and Vascular Ultrasound

Standard echocardiography was performed according to current guidelines for 2D-echocardiography, Doppler and TDI measurements of systolic and diastolic function, and cardiac morphology[105]. Systolic function was measured with LVEF, FS and TDI ´s-wave velocity. Diastolic function was measured with TDI é-wave velocities. Cardiac left ventricular morphology was assessed by measurements of left ventricular dimensions of septal and posterior wall thickness (LVPW) and left ventricular diameters in systole and diastole.
Carotid ultrasound was performed according to current guidelines[147]. For common carotid artery (CCA) stiffness, the stiffness index (SI) and distensibility index (DI) were calculated from blood pressure measurements and CCA systolic and diastolic dimensions[148]. SI is the log-transformed ratio of systolic/diastolic blood pressure to the relative change in the CCA diameter during the cardiac cycle and DI is the relative change in the CCA lumen diameter during systole in percent (%) for every 10 mmHg of pressure change (**Figure 9**). Carotid intima media thickness (CIMT) was measured by a semi-automated algorithm (**Figure 10**).

Endothelial Function

Endothelial function was measured with reactive hyperemia index (RHI) using the EndoPat-2000 (EndoPAT-2000, Itamar Medical, Caesarea, Israel) (**Figure 11**)[149]. This method utilizes fingertip pneumatic probes that record pulsatile changes at the right and left index fingers before and after blood flow occlusion with a cuff on the non-dominant arm. Once the cuff is released, blood flow is restored causing the release of dilating compounds by the endothelium. Using a specific algorithm, the pulsatile changes are analyzed, and the RHI is calculated.
The EndoPat-2000 also calculates augmentation index, a measure of arterial stiffness, during the baseline period and because this measure is inversely correlated with heart rate the value is normalized to 75 beats per minute (Ai75). Lower values, more negative results, indicate better arterial elasticity.

Statistical analysis

Data of measurements of vascular and cardiac function, lipid and apolipoprotein biomarkers and patient characteristics were entered into Excel spreadsheets, which were exported to the statistical software (SPSS version 27, IBM, Chicago). Student´s t-test and analysis of variance (ANOVA) were used to assess the group differences. Analysis of co-variance (ANCOVA) was used to adjust for unbalanced baseline variables. For multiple group (3 groups) comparisons, Bonferroni post-hoc test was used. Partial correlations adjusted for age and sex were performed within the CCS group.

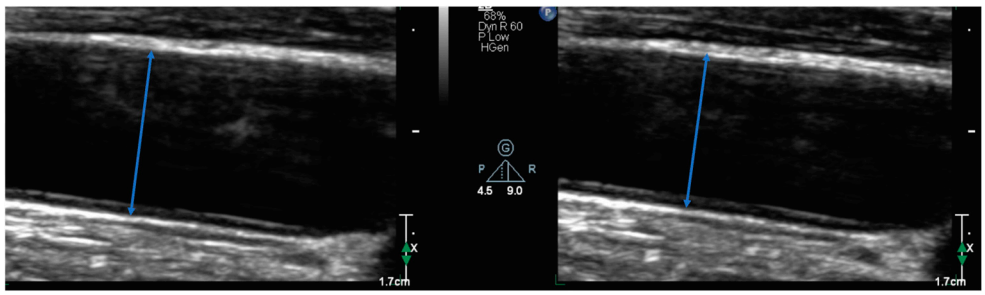


Figure 9. Image depicts the common carotid artery. Images were acquired using a linear vascular probe for vascular ultrasound. Measurements were done at systole and diastole to obtain the DI and the SI. The formulas used were: **Distensibility index, *DI*** $(\%\ diameter\ change\ /\ 10mmHg) = (Ds - Dd)\ /\ Dd * [SBP - DBP] * 1000$, and **Stiffness index, *SI*** $(no\ unit) = ln(SBP/DBP)/([Ds - Dd]\ Dd)$. Ds – diameter in systole, Dd – diameter in diastole, SBP – systolic blood pressure, DBP – diastolic blood pressure.

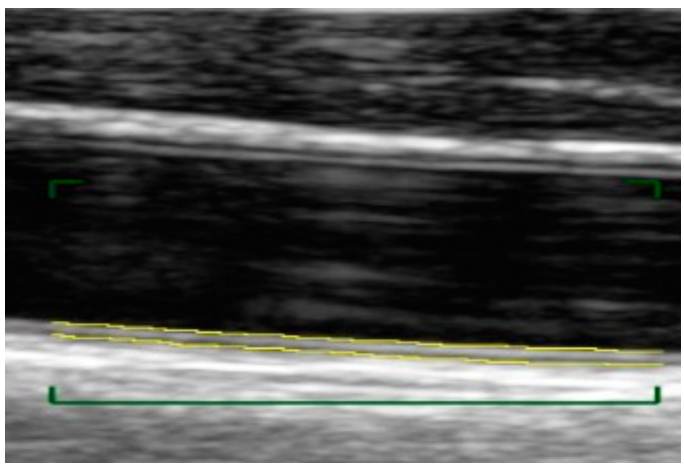


Figure 10. Image depicts measurement of carotid intima media thickness (CIMT). The far wall of a 1-cm long segment of the proximal common carotid artery was used to measure the CIMT in diastole with a semiautomated tracing algorithm (QLAB, Philips Healthcare Netherlands). The CIMT is the combined dimension of the tunica intima and the tunica media. The tunica intima consists of endothelial cells and the tunica media consists of smooth muscle cells, and elastic and collagen fibres.

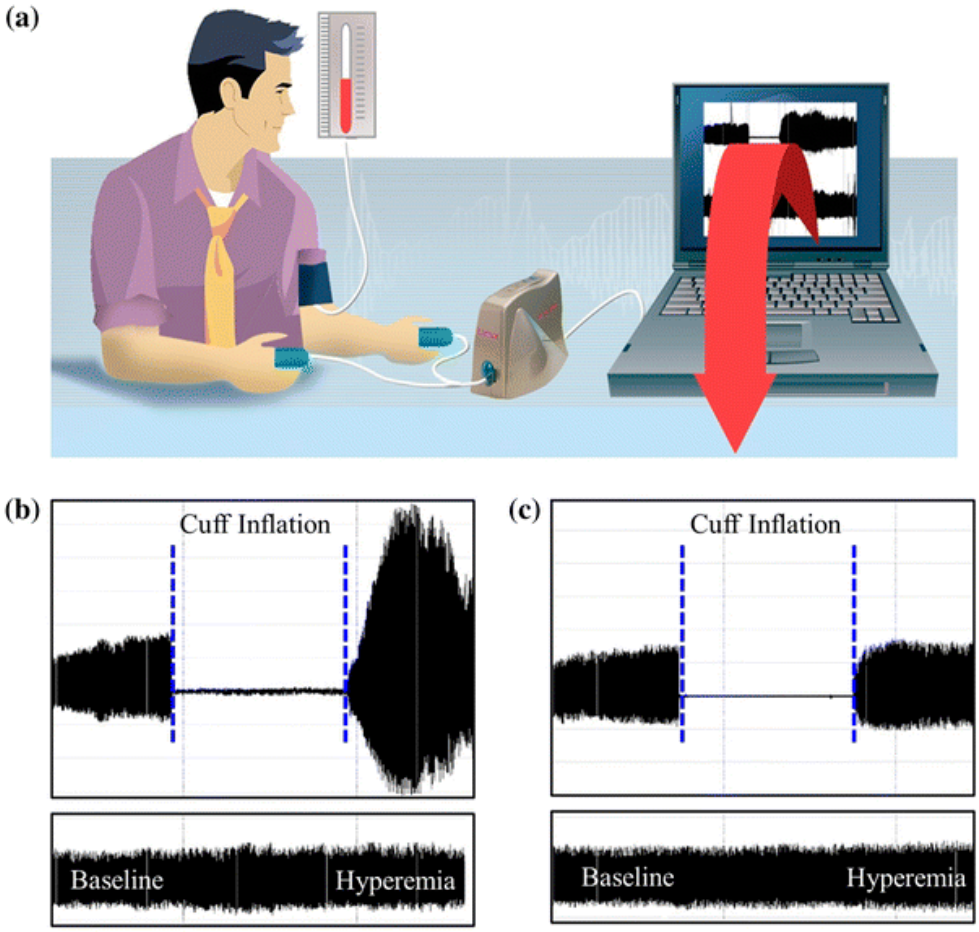


Figure 11. (a) Schematic presentation of the measurement of reactive hyperemia index (RHI), a measure of endothelial function, with EndoPAT2000. (b) depicts a normal RHI. (c) depicts abnormal RHI. With permission from Kang J. et al. **Endothelial function estimated by digital reactive hyperemia in patients with atherosclerotic risk factors or coronary artery disease**. Heart Vessels. 2018[150].

Paper II

Paper II was a study of cardiac reserve function (CRF) by dobutamine stress echocardiography (DSE) in CCS. The CCS group studied in Paper I underwent DSE via a standardized protocol of dobutamine infusion used clinically in adults at the Skåne University Hospital **(Figure 12)**.
As seen in **Figure 12**, echocardiography was performed at *rest* and after 3 min of 5µg/kg/min dobutamine infusion (*low phase*). The dobutamine infusion was then increased gradually to 10, 20, 30 and 40 µg/kg/min to achieve the target maximum heart rate, $max\ phase$ $(220 - age * 0.85)$. If this was not achieved with only dobutamine, atropine was added.
DSE was interrupted if there was 1) significant arrhythmia, 2) severe hypertension or 3) significant discomfort such as anxiety or chest pain. During the procedure there was continuous monitoring with electrocardiography and blood pressure measurements.

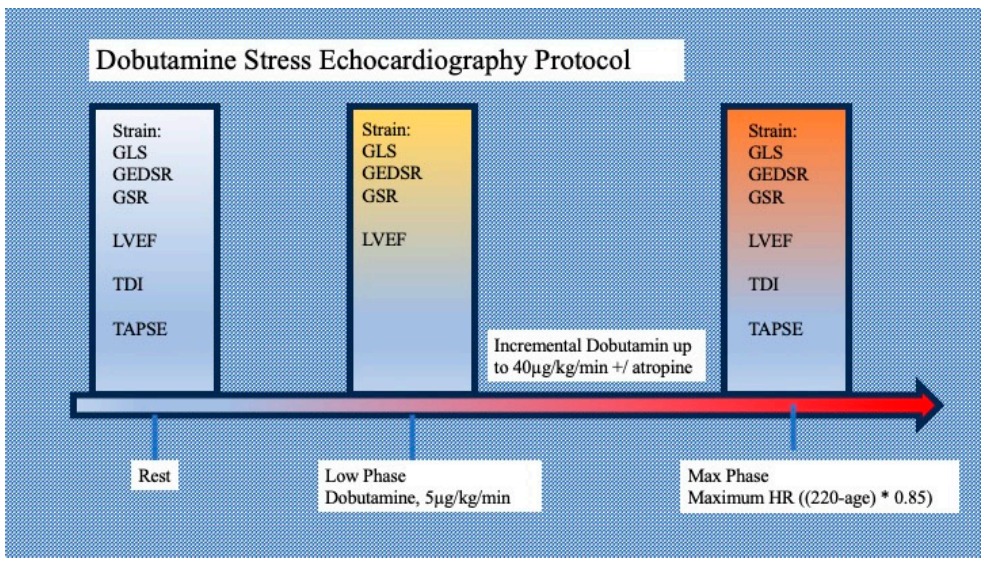


Figure 12. The dobutamine stress echocardiography protocol used in Paper II. Participants were monitored with blood pressure measurements and continuous ECG. At rest, images for off-line measurements global longitudinal strain (GLS), global early-diastolic strain rate (GEDSR), global longitudinal strain rate (GSR), left ventricular ejection fraction (LVEF), tissue Doppler imaging measures (TDI) and tricuspid annular plane systolic excursion (TAPSE) were taken. At *low phase*, only strain and LVEF measurements were performed. At max phase (as defined by reaching the calculated maximum heart rate of 220-age * 0.85) the same measurements carried out at rest were redone.

Off-line analysis of LVEF and global longitudinal strain (GLS), strain rate (GSR) and early diastolic strain rate (GEDSR) from apical 2-, 3-, and 4-chamber views were performed using TOMTEC 2D Cardiac Performance Analysis software (version 1.3.0.147, TOMTEC imaging systems, Unterschleissheim, Germany) for semi-automated speckle tracking strain analysis (**Figure 13**). TDI left ventricular mitral plane diastolic é and systolic ´s waves were also measured. Tricuspid annular plane systolic excursion (TAPSE) was measured as a measure of right ventricular function.
The CRF was measured using LVEF, GLS GSR, GEDSR, TDI measures and TAPSE as the difference between 1) rest and the *low phase* as well as the difference between 2) rest and the *max phase*.

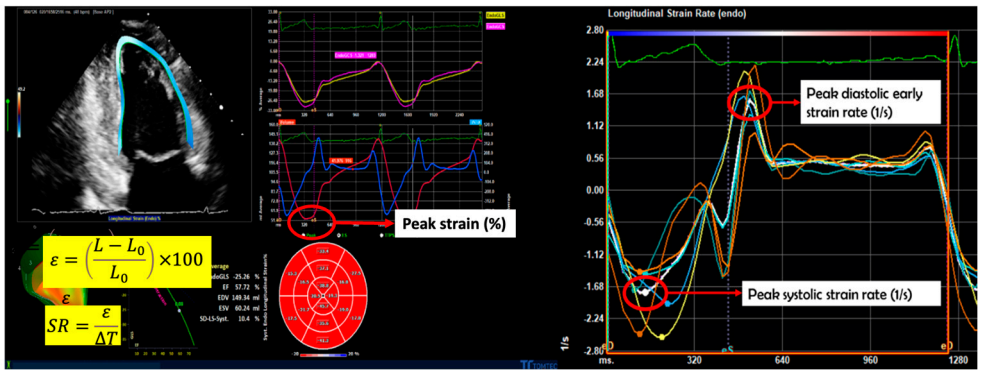


Figure 13. Measurements of strain, strain-rate and early diastolic strain rate using TOMTEC, a semi-automated software. The mean longitudinal values from 18 segments (six segments per each chamber view) for strain (S), strain rate (SR), and early diastolic strain rate (EDSR) were calculated and expressed as global longitudinal strain (GLS), global strain rate (GSR), and global early diastolic strain rate (GEDSR).

Statistical analysis

Repeated measures ANCOVA was performed to analyze differences in outcome variables between groups at the three phases (*rest*, *low phase,* and *max phase*), adjusting for age and sex. ANCOVA with age and sex as covariates was used to calculate differences between CCS and control subjects for numerical changes of these variables between phases. Means (SD) were presented. Simple linear regression was used to investigate relationships between cardiotoxic risk factors and CRF in CCS. Fisher's exact test was used for dichotomous variables. *p*-values < 0.05 were considered statistically significant.

Paper III

In paper III we studied, with a proteomic approach, a panel of 92 circulating cardiovascular proteins in CCS and in controls. We investigated if there were differences in these proteins between these groups. We also investigated possible correlations of proteins with different measures of vascular function that were previously reported in **Paper I**. This paper was a collaboration with Professor Johan Ärnlöv (Karolinska University), and Senior Lecturer Tobias Feldreich (the School of Health and Social Studies, Dalarna University), both experts in proteomics.

Proteomic Assay

Blood samples from CCS and controls were sent to Olink in Uppsala, Sweden, for analysis of the cardiovascular panel (Cardiovascular Panel II - https://olink.com/products-services/target/cardiometabolic-panel/). The analysis was performed using antibodies for these specific proteins linked to DNA. When two antibodies bind to a protein, it enables the DNA to bind to each other and amplify in a polymerase chain reaction (PCR), whereby the proteins can be quantified. The results of the analyses were given as NPX – Normalized Protein eXpression – an arbitrary unit which is in Log2 scale. Because of the Log2 scale, a one-unit difference means a doubling of protein concentration (**Figure 14**).
As a quality control, proteins with >25% missing values (below the limit of detection) were excluded. The limit of detection was calculated separately for each Olink sample plate and was based on the background signal, estimated from negative controls included on every plate. Missing values for biomarkers between 0 and 25% missingness were imputed by the lower limit of detection threshold divided by the square root of two. After carrying out the quality control, one protein was excluded (B-type natriuretic protein, BNP), leaving 91 proteins included in the analysis.

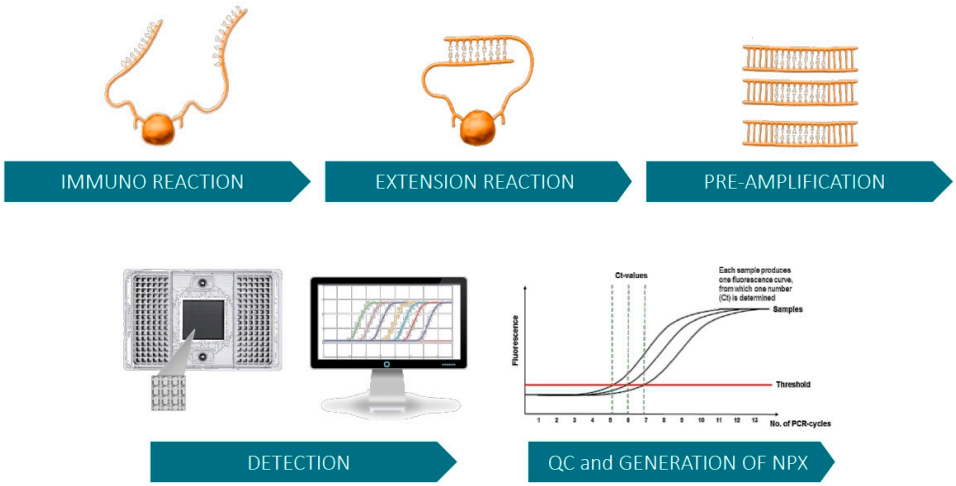


Figure 14. Analysis of the cardiovascular protein panel. By immune reaction the circulating proteins are bound to antibodies that carry complementary DNA. Using polymerase chain reaction, the DNA is amplified and detected by fluorescence intensity. This result is then converted to a Normalized Protein eXpression (NPX) value.

Statistical analysis

In the primary analysis, we assessed using linear regression the differences between CCS and controls for the 91 cardiovascular proteins adjusted for age and sex and corrected for multiple testing. Associations that were nominally statistically significant ($p<0.05$) were also addressed.

In the second analysis, we assessed among CCS potential associations of proteins different in the primary analysis with cardiotoxic treatments with AC or RT. In the third analysis, we investigated correlations of proteins different in the first analysis with cancer diagnosis lipid and apolipoprotein biomarkers, renal biomarkers, and vascular measures (CCA DI and CCA SI, Ai75 and RHI – measured in **Paper I**). This was performed using partial correlations adjusted for (A) sex, age, body mass index and (B) cumulative AC dose and radiotherapy.

Paper IV

In this paper, we investigated in CCS and in controls several circulating ceramides known to be associated with CVD. Further, we evaluated possible associations of these ceramides to clinical and cardiovascular measures previously reported in **Papers I and II**. The study was done in collaboration with Professor Reijo Laaksonen (University of Tampere, Finland), an expert of ceramides in CVD.
Investigated ceramides species were:

- N-palmitoyl-D-erythro-sphingosine (C16:0)
- N-stearoyl-D-erythro-sphingosine (C18:0)
- N-lignoceroyl-D-erythro-sphingosine (C24:0)
- N-nervonoyl-D-erythro-sphingosine (C24:1)

The coronary event risk test 2 (CERT2), which is based on ratios of these ceramides and two phosphatidylcholine species[151], was also calculated. CERT2 can be used in adults with and without CVD to estimate the 10-year relative risk for both fatal and non-fatal CVD (**Figure 15**).

Ceramide Assay

Ceramide analysis was done at Zora Biosciences laboratory in Esbo, Finland. Plasma samples from CCS and controls were analyzed using mass spectrometry. Quantification was done with calibration samples constructed with known amounts of synthetic ceramides and corresponding standards. Peak area ratios of analyzed ceramides, to its corresponding deuterated form, were calculated and plotted against the known added synthetic ceramide concentrations and finally linear regression analysis was performed. Plasma ceramide concentrations were then derived from the obtained individual regression equations.

Statistical analysis

Previously reported outcome cardiac, vascular, lipid and apolipoprotein measures known to be different between the CCS cohort and controls were used as possible markers for ceramide induced cardiac remodeling.
Differences in mean values of different ceramides (C16:0, C18:0, C24:0 and C24:1) and CERT2 score between CCS and controls were assessed by ANCOVA, adjusting for sex, BMI, and age. Based on CERT2 score, CCSs were further divided into 2 groups: low to moderate score (0-6), and high to very-high (7-12) CERT2 score[152]. Differences between these two groups for outcome variables were assessed by ANCOVA adjusted for cumulative AC dose, and cranial- and mediastinal RT. Lastly, in the CCS cohort, we performed partial correlations of analyzed ceramides to the above-mentioned cardiovascular measures as well as the cumulative AC dose,

exposure to cranial and mediastinal RT, BMI, heart rate, CRP, and NT-pro-BNP, adjusting for sex, BMI, SBP, DBP, and age.

RELATIVE RISK									
Smoker	SysBp	No diabetes				Diabetes			
Yes	160	5	8	12	20	13	23	32	47
	140	3	5	7	11	8	13	19	30
	120	2	3	4	6	4	8	11	18
No	160	3	6	8	13	9	16	23	34
	140	2	3	5	8	5	9	13	21
	120	1	2	3	4	3	5	8	12
	CERT2	0-3	4-6	7-8	9-12	0-3	4-6	7-8	9-12

Figure 15. Coronary event risk test 2 (CERT2) score chart for 10-year relative risk for fatal and non-fatal cardiovascular disease (CVD) comparing between low (0-3), medium (4-6), high(7-8) and very high (9-12) CERT2 score. This score is validated for adults with and without CVD > 40 years of age. With permission from Hilvo et al. **Absolute and relative risk prediction in cardiovascular primary prevention with a modified SCORE chart incorporating ceramide-phospholipid risk score and diabetes mellitus,** Eur Heart J 2021[152].

Results and Discussion

Clinical characteristics in CCS and controls and CCS treatments

The clinical characteristics of the CCS group and the control group are shown in **Table 3**. For Papers I and II, the CCS cohort consisted of 53 individuals. In Papers III and IV, additional 5 CCS with Wilms tumor, who had not been exposed to AC, were included.
None of the participants (CCS and controls) had any previous or known current chronic disease or used any cardiovascular medications and none of the female participants were pregnant. The CCS cohort was shorter and had a higher resting heart rate compared to controls. The cohort in Papers III and IV had a higher mean age compared to controls (one year, p=0.04).
Treatment characteristics are shown in supplement 1 (**S1**)

Table 3. Clinical Characteristics of CCS and Controls

	Cohort paper I+II (n=53)	Cohort paper III+IV (n=58)	Controls (n=53)
Age (years)	25.3 (2.5)	25.4 (2.5)*	24.4 (2.4)
Sex (males,%)	32 (60.4%)	35 (60.3%)	35 (66.0%)
Weight (kg)	74.8 (14.1)	74.1 (13.2)	79.2 (15.3)
Height (cm)	174.8 (10.4)*	174.7 (10.1)*	179.1 (8.7)
BMI (kg/m^2)	24.4 (3.5)	24.2 (3.5)	24.6 (3.8)
SBP (mmHg)	118.6 (11.3)	118.8 (11.1)	118.8 (11.6)
DBP (mmHg)	75.9 (8.5)	75.7 (8.2)	73.4 (5.9)
Tobacco use (y, %)	12 (22.6 %)	12 (20.7 %)	9 (17.0 %)
Exercise (hours/week)	4.4 (5.6)	4.6 (6.1)	4.4 (2.9)
Resting HR	74.1 (11.0)*	72.1 (11.2) *	66.2 (11.3)

Main clinical characteristics. Differences were assessed by Student`s t-test. *Denotes p value <0.05 vs controls. **Abbreviations:** BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, HR –heart rate. Tobacco use includes smokeless tobacco, casual smoking, and regular smoking. Exercise level is expressed as number of hours with pulse-increasing exercise per week.

Paper I

The aim was to determine and evaluate different measures of lipid, apolipoprotein, carotid and cardiac measures associated with cardiovascular function and disease in young adult CCS and in controls.

Lipids and Apolipoproteins

The analysis of fasting circulating lipids and apolipoproteins revealed significantly higher levels of several biomarkers associated with higher cardiovascular risk[153,154] in CCS compared with controls (**Table 5**). The largest difference between CCS and controls were for triglycerides (50 % higher) followed by LDL (26 % higher). High-density lipoprotein (HDL) and Apolipoprotein-A1 (Apo-A), associated with a decrease in cardiovascular risk[155], however, were not different between CCS and controls.

Table 5. Lipid and apolipoprotein biomarkers in CCS and controls

	CCS (n=53)	LAD (n=26)	HAD (n=27)	Control (n=53)
HDL	1.33 (0.33)	1.36 (0.30)	1.29 (0.34)	1.40 (0.32)
LDL	2.81 (0.74)**	2.64 (0.69)*	3.06 (0.77)**	2.22 (0.77)
Total Cholesterol	4.57 (0.86)**	4.32 (0.76)*	4.82 (0.88)**,a	3.84 (0.82)
Triglycerides	1.19 (0.78)**	1.01 (0.58)*	1.37 (0.93) **	0.79 (0.35)
Apo-A1	1.46 (0.26)	1.57 (0.40)	1.44 (0.27)	1.42 (0.22)
Apo-B	0.86 (0.21)**	0.80 (0.19)	0.92 (0.21)**,a	0.72 (0.20)
Apo-B/Apo-A1	0.61 (0.17)*	0.56 (0.16)	0.65 (0.19) **	0.52 (0.16)

Different lipid and apolipoprotein circulating markers for cardiovascular disease, compared between childhood cancer survivors (CCS) and controls. CCS were divided into two subgroups by the median cumulative anthracycline (AC) dose: low- (LAD) and high- (HAD) cumulative AC dose. Analysis by ANCOVA adjusted for height and heart rate. **Abbreviations:** HDL – high density lipoprotein, LDL – low density lipoprotein, Apo-A – apolipoprotein A1, Apo-B – apolipoprotein B. * - $p < 0.05$ vs control group, ** - $p < 0.005$ vs control group, a – $p < 0.05$ HAD vs LAD group.

These findings are in agreement with previous studies of cardiovascular lipid biomarkers in CCS. In a recent large study by Goldberg et al.[156] on 4115 CCS with a mean age of 35 years, lipid biomarkers were abnormal in up to 30 % of survivors. In this study, the lipid abnormalities were associated with significantly increased risk of myocardial infarction and heart failure, and with increased overall cardiovascular mortality by 40 %[156].

Using the same cut-offs for abnormal lipid biomarkers as in the study by Goldberg et al, 27 % of CCS in Paper I had dyslipidemia. Although no follow-up data for cardiovascular events is available in our study, it is reasonable to believe that the

findings in Paper I could highlight a potential mechanism for increased cardiovascular risk in this subgroup.
In Paper I, we also found higher Apo-B and higher Apo-B/Apo-A1 ratio in CCS compared to controls. The Apo-B/Apo-A1 ratio was abnormal in 30 % of the CCS with a high anthracycline dose. Prospective multicentre studies in adults without CVD have shown a positive linear association between risk of any cardiovascular event and an increasing Apo-B/Apo-A1 ratio[157,158].
Studies on apolipoproteins in CCS are sparse but our findings correspond well to a previous study of an adult cohort recruited via BORISS[159]. In this study by *Link et al.* (2004), the cohort included only acute lymphoblastic leukemia survivors (ALL) that had been treated with cranial radiotherapy, with 90 % of the cohort being growth hormone deficient. In this study, CCS had significantly higher body mass index compared to controls and more fat mass. In our study (Paper 1), there was no difference in body mass index between CCS and controls. When looking at only normal-weight (BMI < 25 kg/m^2) CCS and controls, CCS still had significantly higher Apo-B and Apo-B/Apo-A1 ratio. Thus, body mass index is a poor marker for obesity in CCS.
The reason for higher lipid and apolipoprotein biomarkers CCS is probably multifactorial. As shown in Paper I, CCS with a higher cumulative AC dose had the worst lipid and apolipoprotein values compared to controls. With higher doses of AC, overall fitness appears to be impaired, contributing to an unhealthy lifestyle[160]. Poor dietary habits including increased intake of high-energy, high-density food are also more common in CCS[161]. Further, a relevant number of CCS had received treatments with corticosteroids and dexamethasone that has been shown to be linked to future metabolic complications in survivors[162].

Carotid Stiffness measures

Common carotid artery (CCA) distensibility index (DI) was lower and CCA stiffness index (SI) was higher in CCS compared to controls in Paper I (**Table 6**). In a subgroup analysis of low (LAD) and high (HAD) cumulative dose of AC, the HAD group had more impaired carotid distensibility and stiffness compared to both the LAD group and controls. Endothelial function measured with reactive hyperemia index (RHI) was lower in CCS. There was no difference in carotid intima media thickness (CIMT) between CCS and controls.

Table 6. Measures of Arterial Stiffness and RHI in CCS and controls

	CCS (n=53)	LAD (n=26)	HAD (n=27)	Control (n=53)
CCA DI (%/10 mmHg)	2.64 (0.62)**	2.94 (0.60)**	2.40 (0.63)**,a	3.47 (0.89)
CCA SI (no unit)	4.55 (1.17)**	3.93 (0.76)**	4.94 (1.30) **,a	3.31 (0.78)
RHI (no unit)	1.92 (0.60)*	1.86 (0.69)*	2.02 (0.61)	2.22 (0.61)
Ai75 (no unit)	-11.30 (10.19)*	-12.84 (1.76)	-8.81 (1.84)*	-15.16 (9.77)
CIMT (mm)	0.45 (0.05)	0.45 (0.04)	0.46 (0.07)	0.44 (0.05)

Vascular outcomes in childhood cancer survivors (CCS) compared to controls. Subgroups of CCS divided by the median anthracycline dose into low-, and high cumulative anthracycline dose (LAD and HAD group). Analysis by ANCOVA adjusted for height and heart rate. **Abbreviations:** CCA DI – common carotid artery distensibility index (mean left and right), CCA SI – common carotid artery stiffness index (mean left and right), RHI – reactive hyperemia index, Ai75 – augmentation index normalized to a heart rate of 75, CIMT – carotid intima media thickness. *, p < 0.05, **, p < 0.005, a, p< 0.05 HAD vs LAD group.

DI and SI correlated with cranial RT among CCS (r=-0.31, p=0.034) and was worse in the HAD group, suggesting synergistic effects of cranial RT and AC in promoting impaired arterial stiffness. Further, Apo-A1 and the Apo-B/Apo-A1 ratio were correlated with higher and lower CCA DI respectively (**Figure 16**). These findings highlight the complex interplay of cardio- and vasculotoxic treatments, lipid derangements and adverse cardiovascular remodeling in CCS.

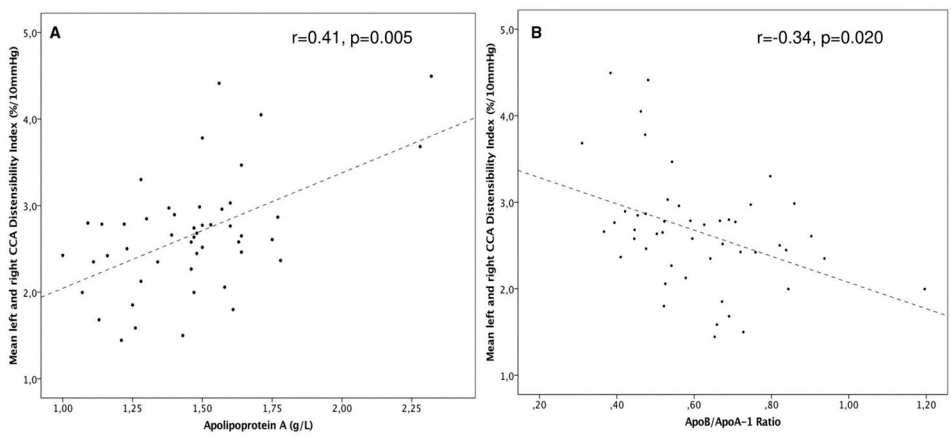


Figure 16. Scatter plots of common carotid distensibility index and Apolipoprotein A1 and the Apo-B/Apo-A1 ratio in childhood cancer survivors. Partial correlation coefficients are adjusted for age and sex.

It has been reported that AC exposure leads to decreased vascular elastin and increased collagen content in rodents, suggesting a possible structural derangement caused by ACs[62]. RT induces oxidative stress leading to inflammation and fibrosis that acts locally in the irradiated area[23], but also nearby or systemically through the so-called "non-targeted effects" which could in part explain why cranial RT in the current study correlated with CCA DI[163].
Impaired carotid artery DI has been described as an independent marker for cardiovascular disease and mortality[164]. Further, in older CCS, CCA stiffness measures have been suggested as surrogate markers for stroke[165].

Cardiac measures

We found several subclinical impairments in both systolic and diastolic function in the HAD group (**Table 7**). Both shortening fraction (SF) and left ventricular ejection fraction (LVEF) were lower in the HAD group most probably due to the cumulative, dose-dependent, effect of AC[9,11,14]. This was shown by the correlation of LVEF with the cumulative AC dose (mg/m^2) (**Figure 17A)**.
LVEF was moderately correlated with the follow-up time, i.e., the time after cancer treatment (**Figure 17B)**. A longer follow-up time is associated with an increased risk for cardiotoxicity[9,11,14]. This is expected since adverse cardiac remodeling is an ongoing process after exposure to cardiotoxic treatments[19]. Further, the young heart might be particularly vulnerable because younger children have a higher percentage of body fat which might result in higher AC levels in blood and non-adipose tissues[166].
In Paper I, the subclinical changes in cardiac function and in CCA DI/SI showed no correlation with each other in the CCS group. Theoretically, the increased afterload caused by impaired carotid elasticity could affect left ventricular diastolic parameters. Such correlations between arterial stiffness and cardiac diastolic function have been observed in hypertensive patients[167]. CCS are different from hypertensive patients, as they do not have left ventricular hypertrophy but instead thinner cardiac walls and decreased left ventricular mass due to cardiomyocyte apoptosis[111].

Table 7. Cardiac measures in CCS and in controls

	CCS (n=53)	LAD (n=26)	HAD (n=27)	Control (n=53)
Systolic Function				
LVEF (%)	58.90 (4.02)**	60.34 (3.00)	57.50 (5.03)**,a	62.21 (3.84)
FS (%)	36.22 (6.01)**	38.23 (5.73)	34.29 (6.34)**,a	39.98 (6.21)
Mean ´s (cm/s)	8.42 (1.32) *	8.57 (1.41)*	8.27 (1.28) **	9.34 (1.15)
Diastolic function				
Mean é (cm/s)	12.80 (1.84)**	13.20 (2.01)**	12.41 (1.52)**	15.30 (2.35)
LV dimensions and mass				
LVIDd/BSA (mm/m2)	27.24 (3.65)	26.67 (2.90)	27.78 (4.42)	26.65 (3.35)
LVIDs/BSA (mm/m2)	17.38 (3.01)*	16.47 (2.29)	18.25 (3.44)*	16.24 (2.49)
LVPWd/BSA (mm/m2)	5.87 (1.19)*	5.81 (0.98)*	5.94 (1.41)**,a	6.97 (1.28)
LV-mass (g/m2)	64.67 (14.23)*	66.15 (13.29)	63.65 (15.38)*	73.94 (18.87)

Systolic, diastolic and morphologic measures of cardiac function. Statistical analysis by ANCOVA adjusted for height and heart rate. **Abbreviations:** LVEF – left ventricular ejection fraction, FS – shortening fraction, mean ´s – tissue Doppler systolic ´s, mean septal and mitral point of measurement ´s-wave, Mean é – mean septal and mitral point diastolic é-wave, LV – left ventricular, LVIDd – left ventricular diastolic inner diameter, LVIDs – left ventricular systolic inner diameter, LVPWd – left ventricular posterior wall diastolic diameter, BSA – body surface area. * - p<0.05 and ** - p< 0.005, [a] – p>0.05 vs the LAD group.

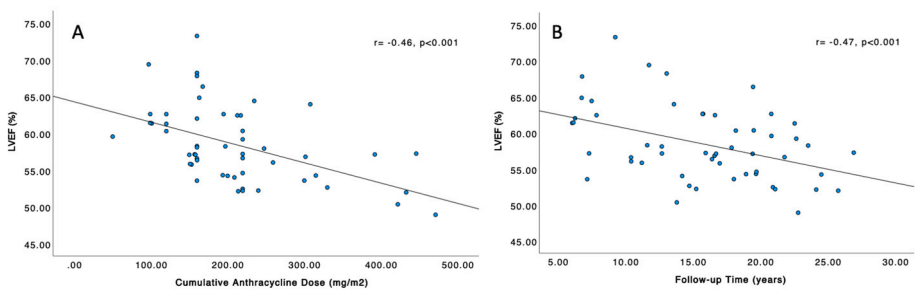


Figure 17. Figure depicts scatter plots of left ventricular ejection fraction and (A) correlation to the cumulative anthracycline dose and (B) the follow-up time. Correlation coefficients are adjusted for sex and age.

Endothelial Function

The reactive hyperemia index (RHI) measured with the EndoPat-2000 (**Figure 8**) is the only FDA approved non-invasive clinical test for endothelial dysfunction. RHI by this method has been shown to predict future cardiovascular events[150]. In Paper I, we found lower RHI in CCS compared to controls. Further, there were more

survivors with an abnormal test (RHI < 1.67) compared to controls (19 vs 9, p=0.046).

The reason for this finding could be similar to the finding of increased arterial stiffness – both AC and RT exhibit systemic vasculotoxic effects and this is reflected in a blunted hyperaemic response in CCS exposed to such therapies[135,137].

While the literature on reactive hyperemia index measured with EndoPat-2000 is extensive in other patient populations, there is very limited data on reactive hyperemia index in CCS[138]. The usefulness in young adults might be limited since it has been reported that in younger individuals (<30 years) the RHI response might be blunted and show "pseudo endothelial dysfunction"[168]. This might explain why RHI did not show any correlation to any cardiac or arterial stiffness parameters analyzed.

The phenomenon of "pseudo endothelial dysfunction" in young adults further questions the usefulness of EndoPat-2000 derived RHI in our study.

We also assessed the peripheral augmentation index normalized to a heart rate of 75 (Ai75) with the EndoPat-2000 (not presented in Paper I). This measure reflects arterial stiffness by the augmentation of the pulse wave in the artery. If the augmented peak is higher than the first peak there is less elasticity. This measure was moderately correlated with carotid distensibility (and stiffness) as shown in **Figure 18**, indicating that arterial stiffness measured with Ai75/EndoPat-2000 could potentially be a useful measure.

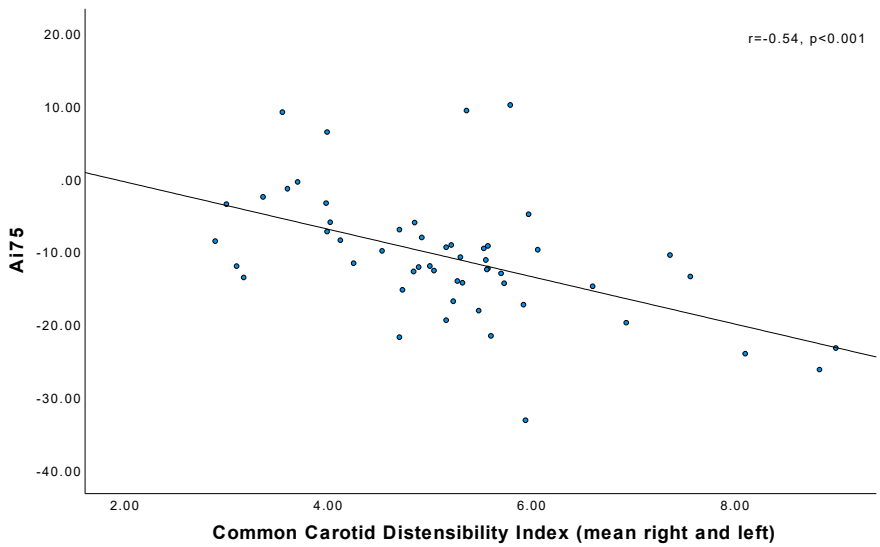


Figure 18. Augmentation index normalized to 75 beats (Ai75) was moderately correlated with common carotic artery (CCA) distensibility index (DI) in the childhood cancer survivor group (r=-0.54, p<0.001). In the control group (not shown) the correlation was similar between Ai75 and CCA DI (r=-0.51, p<0.001).

Limitations

Because of the known associations of lipid biomarkers to amount of fat tissue, it would have been valuable to account for such measures. Also, more information on diet would have been informative and useful to better interpret the data. Additional biomarkers for the metabolic syndrome such as blood glucose and HOMA-IR would have better profiled the CCS cohort. CCA DI and SI reflect local arterial stiffness. The same measurement could also have been done in other arteries.

Paper II

The aim of paper II was to investigate and evaluate CRF with dobutamine stress echocardiography (DSE) by cardiac functional measures in CCS compared to controls.

Dobutamine Stress echocardiography

At *baseline* and at *low phase* (5µg/kg/min dobutamine infusion), 2D image loops of the left ventricular 2-, 3- and 4-chamber views were acquired in all CCS and controls for strain analysis. At *max phase*, despite extensive efforts to record image loops with sufficient quality for strain analysis, the images were inadequate in 3 CCS and 2 controls. Factors that influenced the image quality were breathing-related, artifacts and poor echo windows.

In clinical setting, DSE is used for individuals unable to perform exercise[169]. In Paper II we used DSE to achieve better imaging. Some previous studies in CCS with exercise stress echocardiography (ESE) have shown that strain imaging is less feasible with exercise stress echocardiography (ESE), with dropout of up to 30 % of patients due to suboptimal imaging[170,171]. To overcome this, a simplified global longitudinal strain calculation in only 4-chamber view has been used with ESE[127]. Because we used DSE, we were able to perform a thorough strain analysis in multiple (2-, 3- and 4 chamber) views with little dropout to obtain GLS, GSR and GEDSR.

In two CCS the *max phase* was interrupted: in one CCS because of irregular heart rate and in one CCS due to anxiety. The irregular heart rate disappeared shortly after cessation of dobutamine infusion. Although DSE is considered to be very safe with a risk for life-threatening complications being reported in less than 0.2 % in non-selected populations[172], in a substantial proportion of participants in both CCS and control group there was a noticeable discomfort at *max phase* due to sweating, heavy breathing, and nausea. Data from 48 (90.5%) CCS and 50 (94.0%) controls were included in the ANOVA repeated measures analysis.

Reliable strain imaging (GLS, GSR, GEDSR) is dependent on corresponding number of frames (images) during every heart cycle. At least 25 – 30 frames per heart cycle have been proposed as the lower limit of a correct strain analysis[173]. In our study, the frame rates in the recorded image loops at baseline and at low phase were 58 – 86 and 60 – 89 frames per second respectively. However, at max phase there was under-sampling with frames per second of 55 – 89 and mean heart rates of 160 in CCS. This corresponds to less than 30 frames per heart cycle, which could possibly explain why the peak strains at max phase were lower than at low phase.

A lower increase at *max-phase* (40 µg/kg/min) compared to the *low-phase* (5 µg/kg/min) of both LVEF and GLS from baseline was observed. This was probably due to lower preload because of unphysiologically low systemic venous return,

causing lower stroke volumes and increased cardiac output by the increased heart rate[174]. This is a drawback of DSE compared to exercise stress echocardiography[174]. The overnight fasting (due to blood sampling) with a relative hypovolemia due to this might have influenced the LVEF and strain measures that were acquired to some degree at the higher heart rates.

Physiological Adaption to Dobutamine

Heart rate and blood pressure were recorded (**Table 8**). Heart rate was higher at *baseline* in CCS compared to controls (63.7 vs 59.3 beats per minute (bpm), p=0.014). At *low-phase,* CCS increased their heart rate by 17 % and controls by 10 % from baseline (74.3 vs 66.3 bpm, p=0.003). This difference in response to dobutamine might be attributed to a baseline increase in β_1-receptor tone in CCS due to increased catecholamines[175]. A higher resting heart rate might also indicate a lower fitness in the CCS[176].
All CCS and controls needed 40µg/kg/min dobutamine infusion to reach the target heart rate. Further, most participants also needed 0.25 – 1 mg of atropine in addition to the maximum dobutamine dose. At *max-phase*, heart rate was higher in survivors compared to controls (160.5 vs 156.4 bpm, p=0.007).
Diastolic blood pressure was higher in survivors (76.9 vs 73.4 mmHg, p=0.048) at rest. At *low*- and *max-phase,* there were no differences in blood pressure.

Table 8. Heart rate and Blood Pressure during DSE

	CCS (n=53)	Controls (n=53)
Baseline		
HR	59.3 (8.4)	63.7 (8.5)*
SBP	117.0 (14.3)	118.7 (11.2)
DBP	73.4 (5.6)	76.9 (10.8)*
Low-Dose DSE		
HR	66.3 (10.4)	74.3 (15.1)*
SBP	127.6 (17.1)	126.3 (12.0)
DBP	84.17 (12.5)	86.6 (11.2)
Max-phase DSE		
HR	156.4 (8.1)	160.5 (8.8)*
SBP	168.5 (26.0)	163.3 (26.2)
DBP	91.92 (15.9)	90.68 (14.7)

Heart rate and blood pressure during DSE in childhood cancer survivors (CCS) and controls. **Abbreviations:** HR – heart rate, SBP – systolic blood pressure, DBP – diastolic blood pressure, DSE – dobutamine stress echocardiography. * - p<0.05.

Resting Strain Measurements

CCS had lower strain measures (GLS and GSR) at rest than controls (mean (SD) CCS -18.8% (2.2), controls -20.4% (2.0), p=0.012). Four out of 53 CCS (7.5%) had clinically abnormal GLS values (>-16%). None of the controls had abnormal GLS. Although LVEF was lower in CCS (mean (SD) CCS 58.1% (5.2), controls 60.8% (5.1), p=0.035) than in controls, LVEF was within normal range in all CCS suggesting that GLS is a more sensitive measure for cardiotoxicity.

These data in Paper II, are in line with previously published studies of long-term follow-up in CCS with strain (speckle tracking) echocardiography[114,115,118]. In adult cancer survivors, studies have aimed to use GLS before and after cancer treatment to predict later cardiotoxicity with some success[177]. In adult cancer patients, relative reductions in GLS of > 15% from baseline during cancer treatment has been shown to be associated with later occurrence of significant cardiotoxicity including decline in LVEF and heart failure[177]. The usefulness of GLS is still hampered by clear cut-offs and GLS is currently only useful for serial follow up with evaluations of changes from baseline in adults[104].

According to the latest European guidelines for adult cardio-oncology from 2022[104], LVEF is the only clinically accepted functional measure used in guiding treatment in adult cancer survivors. Even in the updated international guidelines for cardiotoxicity surveillance released in 2023, the use of GLS is still not recommended because of the non-proven usefulness[101].

Difficulties with normal values for GLS and CRF

The definition of abnormal GLS remains debatable due to the significant range of GLS values between vendors[119,121]. In some studies of CCS, GLS values beyond 2 SD from the mean has been used as cut-off, while in others a pre-defined cut-off value was selected based on meta-analysis in different healthy populations[120,178].

In our study, we decided to use -16% as the upper value for normal GLS. Had we used -2SD from the mean of the control group as the upper normal value for GLS it would also have been -16%. For CRF we chose to use values >2 SD from the mean.

Cardiac Reserve Function

Compared to controls, both LVEF and GLS were lower in CCS at both *low*- and *max-phase* ($p<0.018$ for LVEF and $p<0.001$ for GLS).

CRF measured with ΔLVEF (i.e., change in LVEF from baseline to either *low*- or *max-phase*), was not different between CCS and controls (**Figure 19**). CRF measured with ΔGLS was significantly higher in controls compared to CCS at both *low*- and *max-phase* ($p<0.048$) (**Figure 20**).

GSR was higher (lower values are better) in CCS compared to controls at both *low*- and *max-phase* ($p<0.024$). GEDSR was lower (higher values are better) in CCS compared to controls at both *low*- and *max phase* ($p<0.009$). Both ΔGSR and ΔGEDSR were less augmented in CCS compared to controls at both *low*- and *max phase* ($p<0.040$ for both).

In summary, CRF measured with ΔGLS, ΔGSR and ΔGEDSR but not with LVEF was impaired in CCS compared to controls. These strain-derived measures for CFR might thus be more valuable than LVEF to detect cardiotoxicity in CCS. The underlying mechanism is probably related to cardiomyocyte apoptosis or cardiomyocyte dysfunction[23,51].

Previous studies of CRF with strain measures in CCS [126,127,170,171] have yielded conflicting results. These studies utilized exercise stress echocardiography (ESE) instead of DSE and different, simplified imaging for strain analysis.

As already mentioned above, the drawback with ESE is that the image acquisition is harder. Strain analysis is sensitive to image artifacts and requires high quality image loops of the cardiac movement with adequate frame rates and well-defined cardiac walls[119,179].

Cifra *et al.* (2018) used a simplified protocol for the evaluation of cardiotoxicity in young CCS nearly 10 years after AC chemotherapy, and strain measures were calculated only in 4-chamber view, excluding thus two thirds of the left ventricular segments[127]. Although strain could be measured in all participants at peak exercise, there were no differences in CFR between CCS and controls. In another study by Von Scheidt et al. (2022), with a fairly similar follow-up time as in the study by Cifra et al., there was a trend to abnormal CRF with increasing exercise in CCS[126]. In our study, the follow-up time was longer (15.8 years), suggesting that follow-up time is important, and that cardiotoxicity is an ongoing phenomenon [**Figure 8**].

Indeed, in our study, GLS at low phase was correlated with follow-up time ($p=0.004$) and to the cumulative AC dose ($p<0.001$). The correlation to the cumulative AC dose was much weaker at max-phase and follow-up time was not significantly correlated at max-phase to GLS, seemingly due to technical limitations with less reliable strain measurements at high heart rates as discussed previously.

Importantly, we found differences at *low-phase*, and we had no incremental information at *max phase*. This suggests that low-dose DSE (5 μg/kg/min) is an alternative to ESE to obtain imaging of good quality suitable for strain analysis and to detect early cardiotoxicity in young CCS.

Using TDI **(Figure 21),** we found that septal ´s-wave velocity was lower in CCS compared to controls at baseline ($p<0.009$) and that both lateral and septal velocities were lower in CCS compared to controls at *max phase* ($p<0.003$ for both). CCS also had a lower increase in lateral and septal ´s-wave (Δ´s) velocities from baseline to *max phase* ($p<0.019$ for both).

The diastolic function measured with TDI (septal and lateral é-waves velocities) in CCS showed differences at *baseline* but not at *max phase* compared to controls (**Figure 22**). The reason for the discrepancy in the findings regarding the impaired

CRF measured with GEDSR and the comparable TDI é-wave velocities might be due to the fact that é-wave velocity is measured close to the mitral hinge points whereas GEDSR is a measure of the diastolic function of the whole left ventricle. Different cardiac segments could be more affected after exposure to cardiotoxic therapies and locally measured TDI does not take this into account.
TDI is a fast method to measure either systolic and a diastolic function that does not require superior image quality and is not affected by heart rate to frame rate mismatch at high heart rates[106]. Normal values are established for resting TDI values making TDI useful in a clinical setting[180]. Unfortunately, we did not measure TDI with low-dose DSE, which would have enabled a comparison with strain measures. Tricuspid annular plane systolic excursion (TAPSE), a measure of right ventricular systolic function, was lower at baseline and at *max phase* and the increase between these two phases was lower in CCS compared to controls (**Figure 19**). This suggests that the right ventricular CRF is also impaired in CCS and that cardiotoxicity is not limited to the left ventricle.

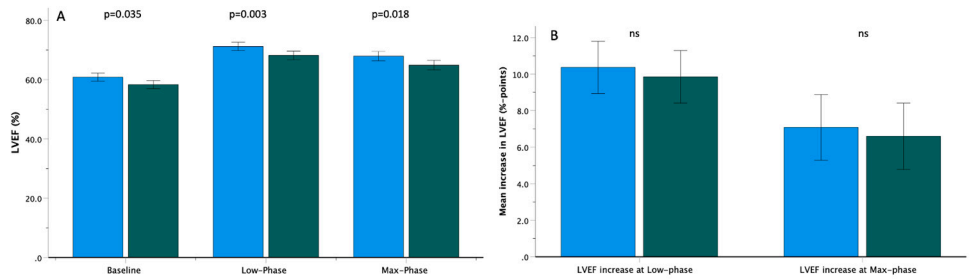


Figure 19. (A) Left ventricular ejection fraction (LVEF) at baseline, low phase and at max phase dobutamine stress echocardiography (DSE). LVEF was lower in childhood cancer survivors (CCS) at all phases. (B) The increase in LVEF was not different between CCS compared to controls. Statistical analysis was done with analysis of co-variance (ANCOVA) repeated measures.

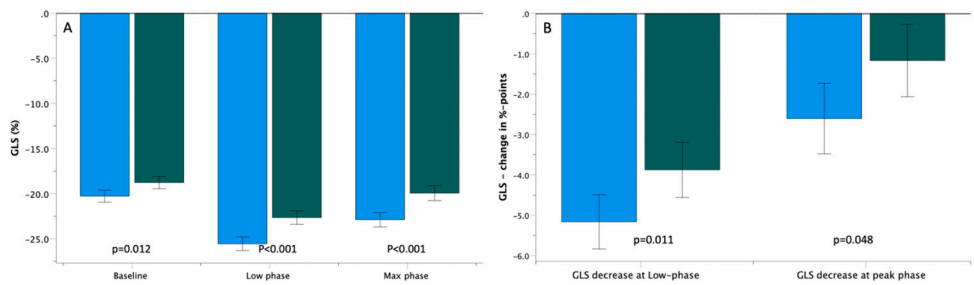


Figure 20. (A) Global longitudinal strain (GLS) at baseline, low phase and at max phase dobutamine stress echocardiography (DSE). GLS was higher (lower, more negative values are better) in childhood cancer survivors (CCS) at all phases. (B) The improvement in GLS was worse during DSE in CCS compared to controls. Statistical analysis with analysis of co-variance (ANCOVA) repeated measures.

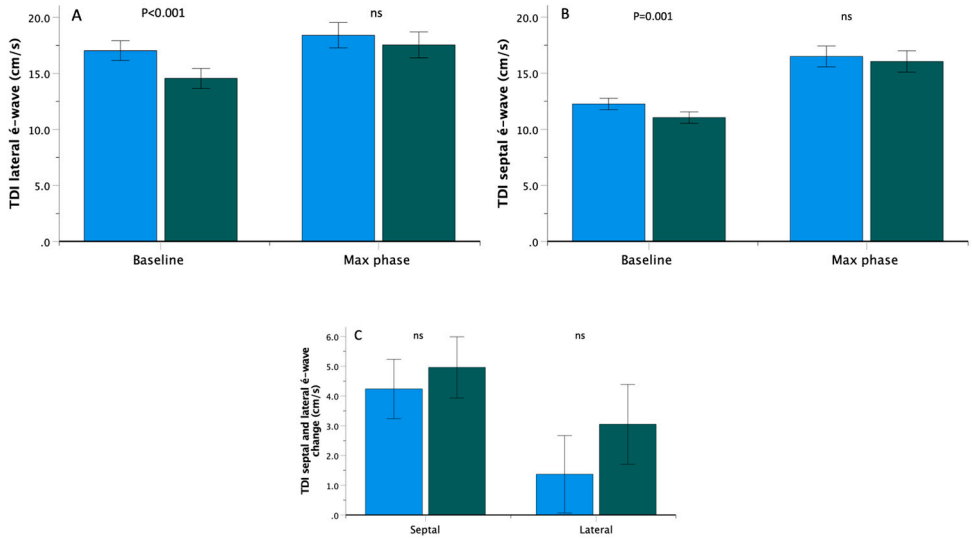


Figure 21. (A) Lateral tissue Doppler imaging (TDI) é wave was lower at baseline in childhood cancer survivors (CCS) compared to controls but there was no difference at max phase. (B) Septal TDI é wave was also lower at baseline compared to control with no difference at max phase. (C) The magnitude of TDI é-wave velocity increase between baseline and max phase was not different between CCS and controls.

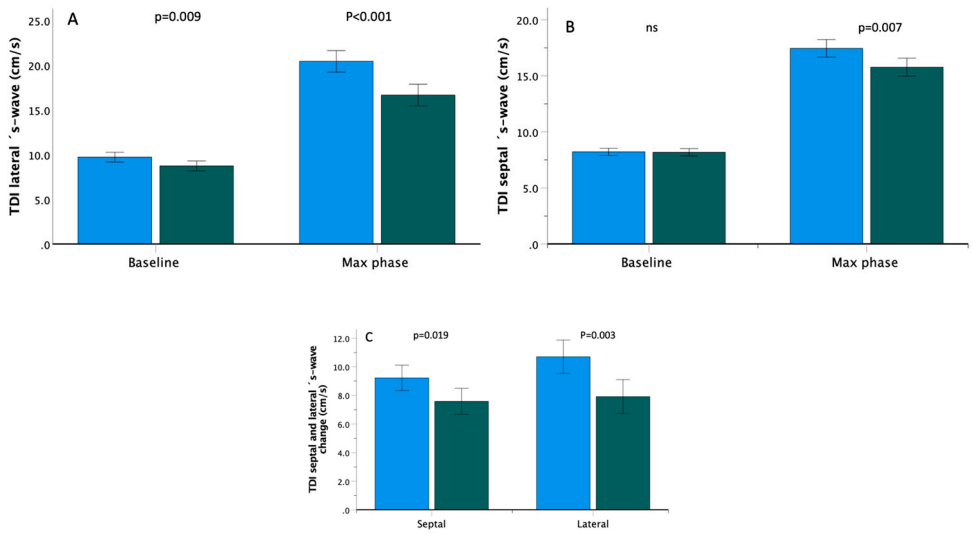


Figure 22. (A) Lateral tissue Doppler imaging (TDI)´s wave was lower at baseline in childhood cancer survivors (CCS) compared to controls and max phase this difference was more pronounced. (B) Septal TDI ´s wave was not different at baseline in CCS compared to controls but at max phase ´s-wave velocity weas lower in CCS (C) The magnitude of TDI ´s wave velocity increase between baseline and max phase was different between CCS and controls and more pronounced in the lateral wall.

Limitations

DSE has the advantage of better image acquisition compared to ESE, since ESE causes breathing and movement artifacts. However, image acquisition during high-dose DSE was not easily accomplished in several participants because high doses of dobutamine caused discomfort in some patients who needed to move or take deep breaths, making image acquisition harder and more time-consuming. Another limitation of this study is that we did not measure other markers for decreased cardiac reserve such as VO_2 uptake, exercise capacity, and pulmonary function, which would have helped to better characterize LVCR. The number of CCS included in this study was 53, so a type 2 error may have occurred.

Paper III

Proteomic Analysis

One protein (brain natriuretic peptide, BNP) was excluded from the analysis because of low level of detection. Of the remaining 91 analyzed proteins, only leptin was different between CCS and controls ($p<0.000001$) after adjusting for sex and age and after correction for multiple testing (**Figure 23**). Because leptin is known to be associated with body fat, we also adjusted for BMI with the same result, with higher leptin in CCS ($p<0.0000001$).

Leptin

The observed association between leptin and vascular dysfunction (**Figure 24A**) has not previously been reported in CCS. However, it has been documented in healthy young people, in the elderly, and in relation to obesity[181-183]. In these earlier studies[181-183], leptin was associated with arterial stiffness also after adjusting for fat mass, suggesting a direct link between these two.

Hyperleptinemia has been suggested to indirectly promote arterial stiffness via immune and inflammatory mechanisms[181]. CCS are, however, a unique population, because of their exposure to cardio- and vasculotoxic treatments, such as AC and RT, as well as their burden of hypertension and dyslipidemia[140]. Our results raise an important question whether CCS carry a risk for CVD due to increased circulating leptin.

In our study, leptin was associated with lower CCA DI adjusted for BMI and exposure to cardiotoxic treatments ($r=0.35$, $p=0.011$) suggesting that leptin alone has a negative effect on vascular function in CCS. Leptin was also associated with higher BMI (**Figure 24B**) and with exposure to higher cumulative AC doses ($r=0.46$, $p<0.001$). BMI and possibly AC are important determinants of leptin levels in CCS. Taken together our results point to a negative impact of leptin on vascular function in CCS. The relationship of leptin to vascular dysfunction and cardiotoxic treatment in CCS needs to be studied further.

Our finding of the association of leptin with BMI is also in concordance with previous metabolic studies in CCS where leptin has been correlated with obesity, central fat mass and other components of the metabolic syndrome[86,184]. In the current study, when comparing CCS and controls with normal BMI (<25 kg/m^2), CCS still had higher leptin. CCS might have more fat mass than muscle mass compared to controls. They can thus have a relative obesity with a normal BMI. This condition, named sarcopenic obesity, has been described in CCS[92]. Besides sarcopenic obesity, CCS have been suggested to have abnormal fat deposition and leptin has been proposed as a biomarker to replace the waist-to-hip ratio in order to better identify metabolic syndrome in CCS[185].

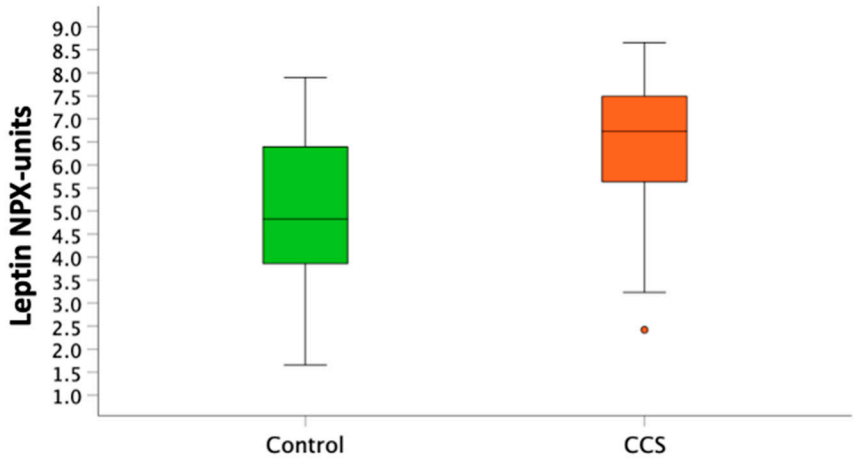


Figure 23. Leptin was higher in childhood cancer survivors compared to controls, adjusted for sex and age (p=0.0000082). Leptin is expressed as NPX – Normalized Protein eXpression units.

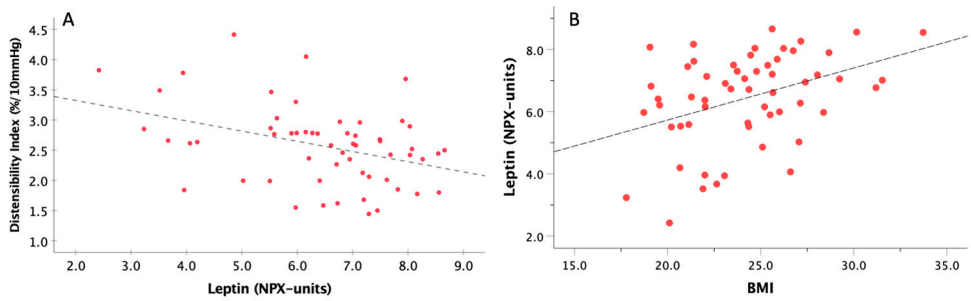


Figure 24. (**A**) Scatterplot shows that leptin negatively correlated with common carotid artery (CCA) distensibility index (DI), r=-0.44, p<0.001, using partial correlations adjusted for age and sex and BMI. (**B**) Scatterplot of the correlation of leptin to BMI, r=0.43, p<0.001, adjusted for age and sex.

Other Proteins

Besides leptin, six other proteins were different in CCS compared to controls, but not after correction for multiple testing: Kidney injury molecule 1 (KIM1), alpha-1-microglobulin/bikunin precursor (AMBP), decorin (DCN), MER proto-oncogene tyrosine kinase (MERTK), pentraxin 3 (PTX3), and selectin P ligand (PSGL1).

KIM1 and AMBP

Two markers for kidney injury, kidney injury marker 1 (KIM1) and alpha-1-microglobulin/bikunin precursor (AMBP) were elevated in CCS compared to controls (**Figure 25**). Both KIM1 and AMBP in CCS were correlated with

endothelial dysfunction as measured with RHI (r=-0.38 and r=-0.37 respectively; p<0.05 for both) (**Figure 26A**). We also found that KIM1 and AMBP were correlated with lower kidney function in CCS (**Figure 26B**). Both KIM1 and AMBP have been suggested as prognostic markers for developing chronic kidney disease (CKD)[186,187].

CKD has been estimated to occur in as many as 7.4 % of CCS at a studied mean age of 32 years[188]. In CKD, uremic toxins cause endothelial dysfunction and accelerate atherosclerosis formation[189]. Subclinical CKD as well as clinical CKD are independent risk factors for CVD[190]. Our observation of correlations of these two proteins with both endothelial dysfunction and reduced kidney function suggests that renal damage after cancer treatment contributes to the future increased risk of CVD in CCS.

Also, Wilms tumor (nephroblastoma – a pediatric renal tumor) was associated with higher levels of these proteins suggesting that KIM1 and AMBP might be useful markers in the follow-up of Wilms patients[191]. Taken together, KIM1 and AMBP could be interesting markers in the follow-up of CCS in future studies, for kidney disease as well as for vascular dysfunction.

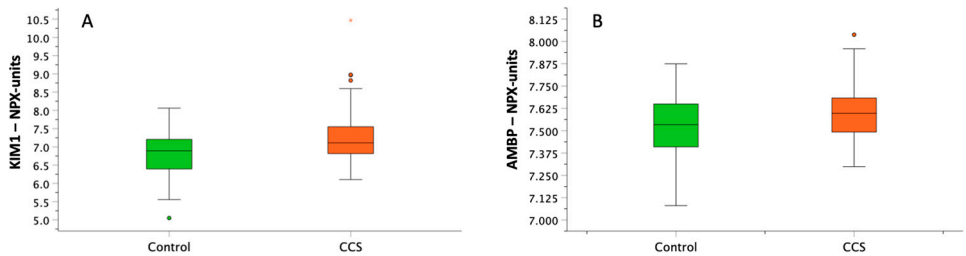


Figure 25. (**A**) Boxplot showing higher levels of kidney injury molecule 1 (KIM1) in childhood cancer survivors (CCS) compared to controls (p=0.0013) adjusted for age and sex. (**B**) Boxplot showing higher levels of alpha-1-microglobulin/bikunin precursor in CCS compared to controls adjusted for sex and age (p=0.042).

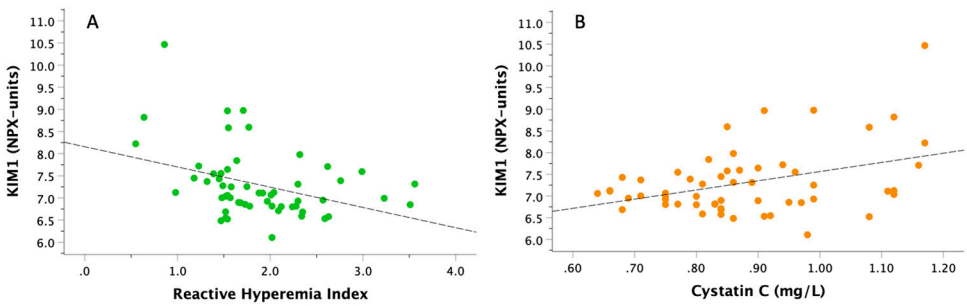


Figure 26. (**A**) Scatterplot shows that KIM1 was correlated with reactive hyperemia index (RHI) in childhood cancer survivors (CCS), r=-0.38, p=0.005 using partial correlations adjusting for age and sex. (**B**) Scatterplot shows that KIM1 was correlated with cystatin C in CCS, r=0.45, p<0.001.

Other proteins different in CCS

The other proteins different in CCS are related to the extracellular matrix (DCN) and inflammation (MERTK, PTX3 and PSGL1).

DCN, a proteoglycan found in the extracellular matrix[192], was lower in CCS than in controls. In CCS, DCN was correlated with better CCA SI ($r=-0.44$, $p<0.001$) and better RHI ($r=0.38$, $p=0.005$). This protein has been shown to correlate with better cardiopulmonary function (as measured by a 6 min walk test) and overall survival in cancer patients[193]. DCN has been suggested to protect against cardiac disease by inhibiting cardiac fibrosis[192]. The role of the observed decreased DCN levels in our CCS cohort is unclear, and whether downregulation of DCN is important for CVD in CCS should be investigated further.

Some previous studies in CCS have found increased levels of high sensitivity CRP, which is a known marker of chronic low-grade inflammation[194]. The dyslipidemia in CCS could contribute to a pro-inflammatory milieu, which could also explain the observed adverse changes in carotid elasticity and endothelial function.

PTX3, an anti-inflammatory protein that is thought to be protective in CVD[195], was lower in CCS than in controls, suggesting some dysregulation of inflammation-related mechanisms. Also, PSGL1, a cellular adhesion molecule responsible for the leukocyte-endothelial cell interplay and implicated in inflammation and atherosclerosis formation[196], was higher in CCS than in controls in our study.

Limitations

Only leptin was different between CCS and controls. The cardiovascular protein panel used in our study has previously been shown to be related to cardiac remodeling[197,198]. The lack of difference in other proteins between the groups could be due to the relatively young age and the absence of concomitant chronic diseases.

False positivity cannot be excluded since we also included proteins that were different without correction for multiple testing. In addition, we performed several post-hoc statistical tests in our secondary analyses of associations between proteins and cardiovascular risk factors and vascular properties without taking the multiple testing into account. Therefore, these associations should be interpreted with caution. A stricter approach could have excluded important observations since several of the included proteins were inter-correlated ($r>0.5 – 0.7$).

Paper IV

Ceramides

Cardiotoxic ceramides (C16:0, C18:0, C24:0, and C24:1) were higher in CCS compared to controls, after adjustments for sex, age, and BMI (**Figure 27**). To our knowledge, this is the first study of ceramides known to be strongly associated to CVD risk in CCS.

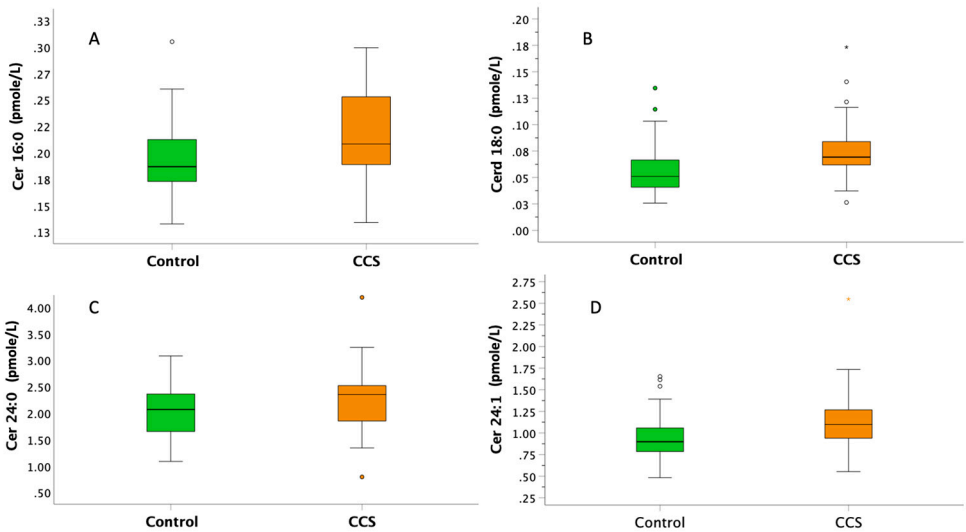


Figure 27. Boxplots for cardiotoxic ceramides in childhood cancer survivors (CCS) and controls. All ceramide species were significantly higher in CCS compared to controls. The largest difference was seen for C18:0 (**A**) which was 33 % higher in CCS compared to controls ($p<0.000002$). The smallest difference was observed for C24:0 (**C**) that was 13 % higher in CCS compared to controls ($p=0.012$)

Ceramides are linked to the pathophysiology of CVD, because accumulation in cardiovascular tissues leads to an increase in lipid uptake, mitochondrial dysfunction, and inhibition of glucose utilization[199]. Knocking out ceramide production in rodents have showed regression of atherosclerosis and improvements in cardiac function[200,201]. In humans, certain types of ceramides (C16:0, C18:0 and C24:1) were elevated in patients with heart failure. These elevations in ceramides were reversed after treatment with mechanical assist device suggesting that ceramides are accumulated in the failing heart[202].

Of the studied ceramides in Paper IV, the largest difference (33% higher in the CCS compared to controls) was found for C18:0 (**Figure 23A**). C18:0, C16:0 and C24:1 have all been demonstrated to be predictors for CVDs and major cardiovascular

events (MACE) in older patients with and without known CVD, independently of other cardiovascular risk factors[203,204].
BMI was correlated with C18:0, suggesting adiposity as possible cause for higher C18:0 in CCS. Leptin, investigated in Paper III and known to be associated with adiposity, showed however no correlation with any of the ceramides (data not shown). Other indices for adiposity, such as waist-hip ratio or fat mass, not measured in our study, may have helped to further assess the putative association of adiposity with ceramides in CCS. Increased ceramides are associated with poor diet and a sedentary lifestyle and BMI-lowering interventions might reverse ceramide levels in CCS[205,206].

Cardiac Remodeling

Better understanding of the role of cardiotoxic ceramides in cardiac remodeling is important. In a study of 2700 adults with mean age of 66 years, there were several interesting correlations of ceramides with cardiac functional measures such as LVEF and global circumferential strain (GCS)[207]. However, to our knowledge this is the only study on cardiotoxic ceramides and cardiac remodeling in humans and further studies are needed.
In Paper IV, both C18:0 and C24:1 were associated with a thinner left ventricular posterior wall. This finding contrasts with a previous study in rodents demonstrating a causal relationship between accumulation of ceramides and cardiac hypertrophy[208]. Because > 90% of the CCS in our study were exposed to cardiotoxic treatments, such associations between ceramides and cardiac hypertrophy might not be found in CCS[111].
In our study, there was a weak correlation of C18:0 with tricuspid annular plane systolic excursion (TAPSE) and of C16:0 with CCA DI, but not with the other markers of cardiac function. The young age of the CCS in our study might preclude additional associations since accumulation of cardiotoxic ceramides in the heart likely increases with age.

Treatment Exposure

Since cranial RT, but not mediastinal RT or AC, correlated with elevated levels of circulating ceramides, we investigated if growth hormone (GH) correlated with ceramides. GH deficiency can be caused by RT exposure to the pituitary gland[184] and is known to cause cardiometabolic complications. In our study, GH concentrations were not lower in CCS compared to controls, but GH in CCS was significantly correlated with all ceramides except C:18:0.
We also assessed differences in ceramides between CCS and controls after removing patients treated with cranial RT. All studied ceramides were still elevated (**Figure 28**) and GH was still a significant covariate. GH deficiency might therefore

be important also in CCS not treated with cranial RT[209]. Increased GH testing and perhaps more liberal GH substitution strategies in CCS might perhaps help to reduce the risk for CVD in CCS.

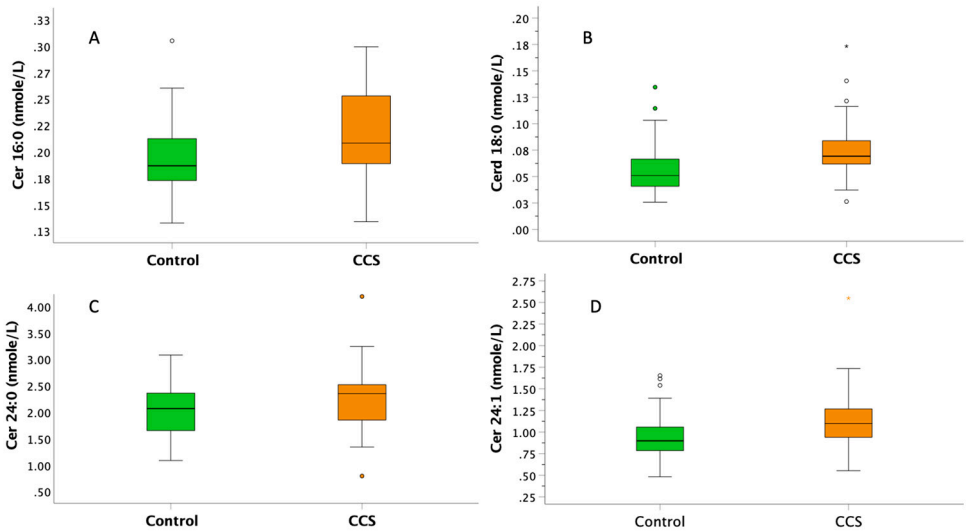


Figure 28. Differences in cardiotoxic ceramides between childhood cancer survivors (CCS) and controls after removing CCS treated with cranial radiotherapy, adjusted for sex, age, and BMI (ANCOVA). All ceramides were significantly higher in CCS: (A) p=0.002, (B) p<0.001, (C) p=0.032 and (D) p<0.001).

CERT2-score

Broad ceramide panels are difficult to use in clinical practice to predict cardiovascular risk. Instead, some studies have used ratios of ceramides rather than individual ceramides to predict cardiovascular risk[210,211]. ,Therefore, ceramide risk tests using combination of multiple ceramide ratios have been developed. CERT2 is based on ratios of C16:0, C18:0, C24:0 and C24:1 and two phosphatidylcholines and yields a score between 0 (best) and 12 (worst)[151]. A score of 0 – 3 is considered low, 4 – 6 moderate, 7 – 8 high, and 9 – 12 very high[151]. CERT2 has been validated for adults aged above 40 years. It independently predicts 10-year absolute and relative risk for fatal and non-fatal CVD and is currently being used clinically in some centres in patients with and without known CAD[152,212,213].

In our study, the CERT2 score was higher in CCS compared to controls. After categorizing CCS and controls based on CERT2 score, we found that 35 % of CCS versus 9 % of controls had a high – very high CERT2 (chi-square, p=0.001) (**Figure 25**).

In our data, the CERT2 score was higher in CCS compared to controls. After dividing CCS and controls into groups with a CERT2 score ≤ 6 points (low – moderate) and one group with ≥ 7 points (high – very high), it was shown that 35 % of CCS had high – very high CERT2 score compared to 9 % among the controls (chi-square, p=0.001) (**Figure 29**).

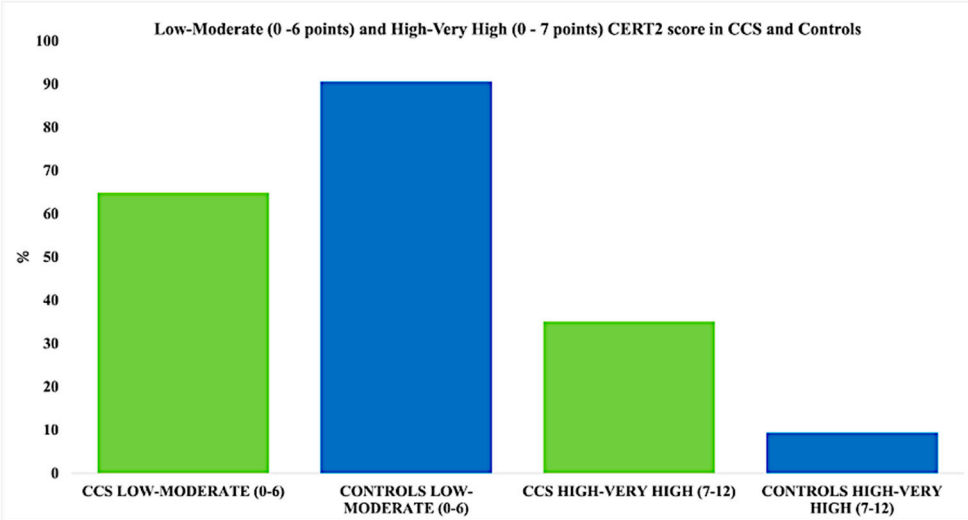


Figure 29. Coronary event risk test 2 (CERT2) score in childhood cancer survivors (CCS) (green) and controls (blue). The CERT2 score was higher in CCS compared to controls (mean (SD), CCS 5.7 (2.2) vs controls 4.0 (2.0), p<0.001). The distribution of CERT2 scores showed that 35 % of CCS versus 9 % of controls had a high – very high CERT2 score (chi-square, p=0.001).

We further compared clinical, cardiac, and vascular characteristics of CCS with a low – moderate CERT2 score to CCS with a high – very high CERT2-score, with adjustment for cardiotoxic treatments (RT and AC). We found that systolic blood pressure (SBP), heart rate (HR), Apo-B, and the Apo-B/Apo-A ratio were higher whereas the CCA DI was lower in the high CERT2 score group (**Table 9**). In CCS, SBP correlated with CERT2 score (r=0.41, p=0.002) (**Figure 30**). C16:0 correlated with lower CCA DI (r=-0.38, p=0.007) and with higher resting heart rate (r=0.36, p=0.008). There was no difference in cardiac measures by echocardiography between CERT2 score groups.

Table 9. Clinical, lipid and cardiovascular characteristics in CCS according to CERT2 score.

	CERT2: 0–6	CERT2: 7–12	P-value
n, %	37 (65.00%)	20 (35.00%)	
SBP (mmHg)	115.22 (8.54)	125.40 (12.73)	<0.001
DBP (mmHg)	74.65 (7.42)	78.35 (8.65)	ns
HR (bpm)	69.03 (11.24)	76.60 (9.41)	0.002
Apo-B	0.80 (0.17)	0.95 (0.25)	0.010
Apo-B/Apo-A1	0.56 (0.14)	0.68 (0.20)	0.011
CCA DI (%/10mmHg)	5.56 (1.52)	4.67 (0.83)	0.022
LVPWd/BSA (mm/m^2)	3.73 (0.90)	3.68 (1.07)	ns
LVEF (%)	57.92 (7.38)	58.76 (6.44)	ns
TDI é (cm/s)	12.48 (2.57)	12.01 (2.53)	ns
GLS (%)	-19.43 (3.65)	-18.91 (3.25)	ns
TAPSE (mm)	24.00 (7.75)	23.50 (4.75)	ns

Differences between chilhood cancer survivors (CCS) with a low- (≤ 6 points) or high- (≥ 7 points) coronary event risk test 2 (CERT2) score for different lipid and cardiovascular measures analyzed with ANCOVA, adjusting for radiotherapy and anthracycline exposure. SBP – systolic blood pressure, DBP – diastolic blood pressure, HR – heart rate, Apo – apolipoprotein, CCA DI – common carotid artery distensibility index, LVPWd – left ventricular posterior wall thickness in diastole, LVEF, left ventricular ejection fraction, TDI é– tissue Doppler imaging diastolic é-wave, GLS – global longitudinal strain, TAPSE – tricuspid annular plane systolic excursion.

Taken together, these findings suggest a role of these ceramides in the early stages of vascular remodeling in CCS. Ceramides have been implicated as key drivers of atherosclerosis via their uptake into the endothelial cell mitochondria membranes with subsequent cytochrome c release and induction of apoptosis[214]. Further, ceramides cause endothelial dysfunction by decreasing NO synthesis and by increasing NO breakdown via reactive oxygen species[215]. Our findings suggest that cardiotoxic ceramides, perhaps driven by C:16, might cause an increase in vascular tone coincident with increased arterial stiffness.

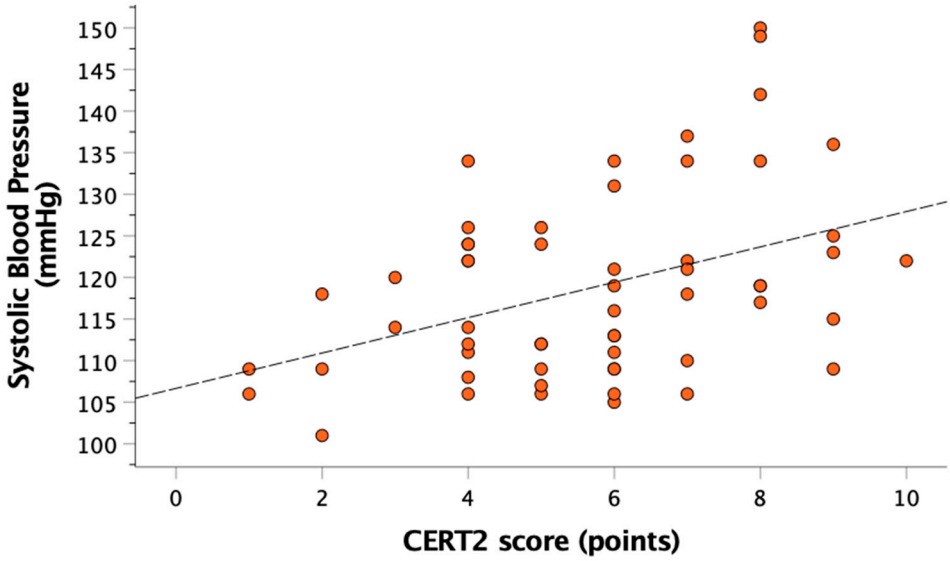


Figure 30. Scatterplot showing the correlation between the CERT2 score and systolic blood pressure (r=0.41, p=0.002, adjusted for sex age and BMI) in childhood cancer survivors.

Limitations

The cross-sectional design precludes conclusions regarding the predictive role of the studied ceramides and elevated CERT2 scores in clinical CVD. Other measures of the metabolic syndrome would have given a better insight into the pathophysiology of cardiotoxic ceramides in CCS. The large number of performed statistical tests increase the risk for type I errors.

Conclusions

This thesis provides new knowledge regarding cardiotoxicity in young adult CCS by combining measurements of cardiac and vascular impairments, endothelial dysfunction, and dyslipidemia with analysis of cardiovascular-related proteins and cardiotoxic ceramides.

CCS without overt CVD had changes in the cardiovascular system, lipid, and apolipoprotein profiles with potential implications for their CVD risk later in life. (*Paper I*)

CRF measured with GLS, compared to LVEF, was a sensitive measure for cardiotoxicity in CCS. Low-dose DSE combined with GLS should be further evaluated as a possible investigation of routine echocardiographic follow-up of CCS in young adulthood for better risk stratification of CCS, at least in CCS exposed to higher doses of cardiotoxic treatments.
(*Paper II*)

With a proteomic approach, we found that leptin was increased in childhood cancer survivors compared to controls. Higher leptin was associated with cardiotoxic treatment and lower carotid distensibility in CCS. Our findings indicate a role for leptin in driving the cardiovascular disease burden in this CCS. Leptin might be a promising marker in the cardiometabolic follow up in childhood cancer survivors.
(*Paper III*)

We showed markedly elevated serum concentrations of cardiotoxic ceramides in CCS compared to controls. Ceramides might be important biomarkers in understanding the pathophysiology of CVD in CCS. Ceramides and the CERT2 score could be important for CVD risk assessment in CCS beyond the exposure to cardiotoxic treatments.
(*Paper IV*)

In summary, in this thesis, we identified a phenotype of subclinical adverse cardiac and vascular remodeling and dyslipidemia in young adult asymptomatic CCS. We identified low-dose DSE and different circulating biomarkers that can be used in a clinical setting to detect and evaluate subclinical CVD in CCS.
(*Paper I-IV*)

Future perspectives

The papers included in this thesis could provide important incentives for:

- Large prospective follow-up studies to assess changes in dyslipidemia and other components of the metabolic syndrome as well as changes in cardiac and vascular dysfunction in CCS from treatment to late survivorship based on the findings in papers I, III, and IV.

- Studies on monitoring and interventions in diet and physical activity in CCS – factors that are likely to improve the future cardiovascular risk in CCS. These are based on the findings in papers I – IV.

- Studies in pediatric heart failure patients of cardiotoxic ceramides to assess lipotoxicity as a possible mechanism based on the findings in paper IV.

- Studies on the predictive value of low-dose DSE in different adult and pediatric heart failure cohorts based on the findings in paper II.

- Studies using novel advanced ultrasonographic techniques such as blood speckle echocardiography and ultra-high frequency vascular ultrasound to better characterize cardiotoxicity and vascular wall pathology based on the findings in paper I.

Acknowledgements

Petru for the invaluable support and his encouragement and knowledge pushing for science in the everyday clinics and in the cath-lab. Saving lives globally and doing science simultaneously – one of a kind.

Ingrid for her invaluable knowledge of pediatric oncology, her swift answers, and data from the BORISS registry. Always busy but still with a precise answer from Lund to Amsterdam.

Constance for her great knowledge of echocardiography, vascular function and in statistics – all things invaluable for my thesis. A great colleague with great shoes.

Thomas for invaluable insights and his enormous knowledge of pediatrics and pediatric oncology and for data from the BORISS registry. A pioneer in saving kids with cancer and one of the greats.

Annica for her invaluable help with putting the logistics and data collection together. We miss you.

Elin for enormous help with blood samples and great laughs. We are so blessed to have you.

Johan and *Tobias* for their patience and enormous help with paper III. Great talks from Tel-Aviv to Dalarna.

Reijo Laaksonen for his knowledge of cardiotoxic ceramides in paper IV.

Philippe for his enormous help with statistics. One of the smoothest guitar players. A dear friend.

Amra Jujic and *Morten Kraen* for their help with DSE.

Michal and *Zahraa* for great discussions and friendship at work. Never a boring day.

Peter my first teacher in echocardiography and pediatric cardiology. Great instructor and cardiologist.

Judith for her invaluable help with looking at echoes for inter-variability analysis.

Karin, for being the best sister and professor and her great support and proof reading.

Bruno, *Aron,* and *Lo* – the best sons to wish for – strong, smart, and witty, I love you guys.

Jasmin for being the best possible partner, I love you so much – no more words needed.

Anna – for being a great sister, proof reading, staying sharp and curious – a true inspiration.

Lovisa – the family law expert, constantly working for the greater good, and for being my great sister

Mom for being the best mom/grandmom and for teaching me and helping med with so many things. I love you.

Jan-Axel – the rock – thank you for everything, you are one of a kind.

Dad for being the best dad/grandad/professor and a great inspiration – so sad you left us too soon. I hope you get to visit Birdland in heaven.

Finally – all my fantastic colleagues at *Barnhjärtcentrum – Katarina* you are a fantastic clinician and a great inspiration to all of us.

References

1 Erdmann, F. *et al.* Childhood cancer: Survival, treatment modalities, late effects and improvements over time. *Cancer Epidemiol* **71**, 101733, doi:https://doi.org/10.1016/j.canep.2020.101733 (2021).

2 Pritchard-Jones, K. *et al.* Sustaining innovation and improvement in the treatment of childhood cancer: lessons from high-income countries. *Lancet Oncol* **14**, e95-e103, doi:10.1016/s1470-2045(13)70010-x (2013).

3 Pui, C. H. & Evans, W. E. A 50-year journey to cure childhood acute lymphoblastic leukemia. *Semin Hematol* **50**, 185-196, doi:10.1053/j.seminhematol.2013.06.007 (2013).

4 Barncancerfonden. *Barncancerrapporten 2019*, https://www.barncancerfonden.se/barncancerrapporter/barncancerrapporten-2019/ (accessed on 20231018)

5 Winther, J. F. *et al.* Childhood cancer survivor cohorts in Europe. *Acta Oncologica* **54**, 655-668, doi:10.3109/0284186X.2015.1008648 (2015).

6 Sørensen, G. V. *et al.* Long-Term Risk of Hospitalization for Somatic Diseases Among Survivors of Childhood Acute Lymphoblastic Leukemia. *JNCI Cancer Spectr* **6**, doi:10.1093/jncics/pkac029 (2022).

7 Pedersen, C. *et al.* Somatic Disease in Survivors of Childhood Malignant Bone Tumors in the Nordic Countries. *Cancers (Basel)* **13**, doi:10.3390/cancers13184505 (2021).

8 Armenian, S. H. *et al.* Cardiovascular Disease in Survivors of Childhood Cancer: Insights Into Epidemiology, Pathophysiology, and Prevention. *J Clin Oncol* **36**, 2135-2144, doi:10.1200/jco.2017.76.3920 (2018).

9 Tolani, D., Wilcox, J., Shyam, S. & Bansal, N. Cardio-oncology for Pediatric and Adolescent/Young Adult Patients. *Curr Treat Options Oncol* **24**, 1052-1070, doi:10.1007/s11864-023-01100-4 (2023).

10 Feijen, E. *et al.* Risk and Temporal Changes of Heart Failure Among 5-Year Childhood Cancer Survivors: a DCOG-LATER Study. *J Am Heart Assoc* **8**, e009122, doi:10.1161/jaha.118.009122 (2019).

11 Leerink, J. M. *et al.* Cardiac Disease in Childhood Cancer Survivors: Risk Prediction, Prevention, and Surveillance: JACC CardioOncology State-of-the-Art Review. *JACC CardioOncol* **2**, 363-378, doi:10.1016/j.jaccao.2020.08.006 (2020).

12 Armstrong, G. T. *et al.* Aging and risk of severe, disabling, life-threatening, and fatal events in the childhood cancer survivor study. *J Clin Oncol* **32**, 1218-1227, doi:10.1200/jco.2013.51.1055 (2014).

13 Kubota, S., Hara, H. & Hiroi, Y. Current status and future perspectives of onco-cardiology: Importance of early detection and intervention for cardiotoxicity, and cardiovascular complication of novel cancer treatment. *Glob Health Med* **3**, 214-225, doi:10.35772/ghm.2021.01024 (2021).

14 Bennati, E. *et al.* Cardio-Oncology in Childhood: State of the Art. *Curr Oncol Rep* **24**, 1765-1777, doi:10.1007/s11912-022-01329-6 (2022).

15 Krischer, J. P. *et al.* Clinical cardiotoxicity following anthracycline treatment for childhood cancer: the Pediatric Oncology Group experience. *J Clin Oncol* **15**, 1544-1552, doi:10.1200/jco.1997.15.4.1544 (1997).

16 Bansal, N., Amdani, S. M., Hutchins, K. K. & Lipshultz, S. E. Cardiovascular disease in survivors of childhood cancer. *Curr Opin Pediatr* **30**, 628-638, doi:10.1097/mop.0000000000000675 (2018).

17 Lipshultz, S. E., Franco, V. I., Miller, T. L., Colan, S. D. & Sallan, S. E. Cardiovascular disease in adult survivors of childhood cancer. *Annu Rev Med* **66**, 161-176, doi:10.1146/annurev-med-070213-054849 (2015).

18 Lipshultz, S. E. *et al.* Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. *J Clin Oncol* **23**, 2629-2636, doi:10.1200/jco.2005.12.121 (2005).

19 Lipshultz, S. E. *et al.* Long-term cardiovascular toxicity in children, adolescents, and young adults who receive cancer therapy: pathophysiology, course, monitoring, management, prevention, and research directions: a scientific statement from the American Heart Association. *Circulation* **128**, 1927-1995, doi:10.1161/CIR.0b013e3182a88099 (2013).

20 Hegazy, M., Ghaleb, S. & Das, B. B. Diagnosis and Management of Cancer Treatment-Related Cardiac Dysfunction and Heart Failure in Children. *Children (Basel)* **10**, doi:10.3390/children10010149 (2023).

21 Loar, R. W., Noel, C. V., Tunuguntla, H., Colquitt, J. L. & Pignatelli, R. H. State of the art review: Chemotherapy-induced cardiotoxicity in children. *Congenit Heart Dis* **13**, 5-15, doi:10.1111/chd.12564 (2018).

22 Saleh, Y., Abdelkarim, O., Herzallah, K. & Abela, G. S. Anthracycline-induced cardiotoxicity: mechanisms of action, incidence, risk factors, prevention, and treatment. *Heart Fail Rev* **26**, 1159-1173, doi:10.1007/s10741-020-09968-2 (2021).

23 Siaravas, K. C., Katsouras, C. S. & Sioka, C. Radiation Treatment Mechanisms of Cardiotoxicity: A Systematic Review. *Int J Mol Sci* **24**, doi:10.3390/ijms24076272 (2023).

24 Nysom, K. *et al.* Relationship between cumulative anthracycline dose and late cardiotoxicity in childhood acute lymphoblastic leukemia. *J Clin Oncol* **16**, 545-550, doi:10.1200/jco.1998.16.2.545 (1998).

25 Armenian, S. H. *et al.* Recommendations for cardiomyopathy surveillance for survivors of childhood cancer: a report from the International Late Effects of Childhood Cancer Guideline Harmonization Group. *Lancet Oncol* **16**, e123-136, doi:10.1016/s1470-2045(14)70409-7 (2015).

26 Leger, K. *et al.* Subclinical cardiotoxicity in childhood cancer survivors exposed to very low dose anthracycline therapy. *Pediatr Blood Cancer* **62**, 123-127, doi:10.1002/pbc.25206 (2015).

27 Armstrong, G. T. *et al.* Modifiable risk factors and major cardiac events among adult survivors of childhood cancer. *J Clin Oncol* **31**, 3673-3680, doi:10.1200/jco.2013.49.3205 (2013).

28 Oliveira, G. H. *et al.* Characteristics and survival of patients with chemotherapy-induced cardiomyopathy undergoing heart transplantation. *J Heart Lung Transplant* **31**, 805-810, doi:10.1016/j.healun.2012.03.018 (2012).

29 Nadruz, W., Jr. *et al.* Cardiovascular phenotype and prognosis of patients with heart failure induced by cancer therapy. *Heart* **105**, 34-41, doi:10.1136/heartjnl-2018-313234 (2019).

30 Felker, G. M. *et al.* Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. *N Engl J Med* **342**, 1077-1084, doi:10.1056/nejm200004133421502 (2000).

31 Mulrooney, D. A. *et al.* Cardiac Outcomes in Adult Survivors of Childhood Cancer Exposed to Cardiotoxic Therapy: A Cross-sectional Study. *Ann Intern Med* **164**, 93-101, doi:10.7326/m15-0424 (2016).

32 Lipshultz, E. R. *et al.* Cardiometabolic Risk in Childhood Cancer Survivors: A Report from the Children's Oncology Group. *Cancer Epidemiol Biomarkers Prev* **31**, 536-542, doi:10.1158/1055-9965.Epi-21-0360 (2022).

33 Chow, E. J. *et al.* Prediction of Ischemic Heart Disease and Stroke in Survivors of Childhood Cancer. *J Clin Oncol* **36**, 44-52, doi:10.1200/jco.2017.74.8673 (2018).

34 Mulrooney, D. A. *et al.* Coronary artery disease detected by coronary computed tomography angiography in adult survivors of childhood Hodgkin lymphoma. *Cancer* **120**, 3536-3544, doi:10.1002/cncr.28925 (2014).

35 Velusamy, R., Nolan, M., Murphy, A., Thavendiranathan, P. & Marwick, T. H. Screening for Coronary Artery Disease in Cancer Survivors: JACC: CardioOncology State-of-the-Art Review. *JACC CardioOncol* **5**, 22-38, doi:10.1016/j.jaccao.2022.12.007 (2023).

36 Mueller, S. *et al.* Radiation, atherosclerotic risk factors, and stroke risk in survivors of pediatric cancer: a report from the Childhood Cancer Survivor Study. *Int J Radiat Oncol Biol Phys* **86**, 649-655, doi:10.1016/j.ijrobp.2013.03.034 (2013).

37 Fullerton, H. J. *et al.* Recurrent stroke in childhood cancer survivors. *Neurology* **85**, 1056-1064, doi:10.1212/wnl.0000000000001951 (2015).

38 El-Fayech, C. *et al.* Cerebrovascular Diseases in Childhood Cancer Survivors: Role of the Radiation Dose to Willis Circle Arteries. *Int J Radiat Oncol Biol Phys* **97**, 278-286, doi:10.1016/j.ijrobp.2016.10.015 (2017).

39 Chow, E. J. *et al.* Individual prediction of heart failure among childhood cancer survivors. *J Clin Oncol* **33**, 394-402, doi:10.1200/jco.2014.56.1373 (2015).

40 Zito, C., Manganaro, R., Carerj, S., Antonini-Canterin, F. & Benedetto, F. Peripheral Artery Disease and Stroke. *J Cardiovasc Echogr* **30**, S17-s25, doi:10.4103/jcecho.jcecho_4_19 (2020).

41 van der Pal, H. J. *et al.* Valvular Abnormalities Detected by Echocardiography in 5-Year Survivors of Childhood Cancer: A Long-Term Follow-Up Study. *International Journal of Radiation Oncology*Biology*Physics* **91**, 213-222, doi:https://doi.org/10.1016/j.ijrobp.2014.09.010 (2015).

42 Desai, M. Y. *et al.* Increased aorto-mitral curtain thickness independently predicts mortality in patients with radiation-associated cardiac disease undergoing cardiac surgery. *Ann Thorac Surg* **97**, 1348-1355, doi:10.1016/j.athoracsur.2013.12.029 (2014).

43 Christiansen, J. R. *et al.* Left Ventricular Function in Long-Term Survivors of Childhood Lymphoma. *The American Journal of Cardiology* **114**, 483-490, doi:https://doi.org/10.1016/j.amjcard.2014.04.055 (2014).

44 van Rijswijk, J. W. *et al.* Fibrotic aortic valve disease after radiotherapy: an immunohistochemical study in breast cancer and lymphoma patients. *Cardiovascular Pathology* **45**, 107176, doi:https://doi.org/10.1016/j.carpath.2019.107176 (2020).

45 Szpakowski, N. & Desai, M. Y. Radiation-Associated Pericardial Disease. *Curr Cardiol Rep* **21**, 97, doi:10.1007/s11886-019-1192-y (2019).

46 Belzile-Dugas, E. & Eisenberg, M. J. Radiation-Induced Cardiovascular Disease: Review of an Underrecognized Pathology. *J Am Heart Assoc* **10**, e021686, doi:10.1161/jaha.121.021686 (2021).

47 Mulrooney, D. A. *et al.* Major cardiac events for adult survivors of childhood cancer diagnosed between 1970 and 1999: report from the Childhood Cancer Survivor Study cohort. *Bmj* **368**, l6794, doi:10.1136/bmj.l6794 (2020).

48 de Baat, E. C. *et al.* Electrocardiographic abnormalities in childhood cancer survivors treated with cardiotoxic therapy: a systematic review. *Pediatr Blood Cancer* **69**, e29720, doi:10.1002/pbc.29720 (2022).

49 Larsen, R. L. *et al.* Electrocardiographic changes and arrhythmias after cancer therapy in children and young adults. *Am J Cardiol* **70**, 73-77, doi:10.1016/0002-9149(92)91393-i (1992).

50 Henriksen, P. A. Anthracycline cardiotoxicity: an update on mechanisms, monitoring and prevention. *Heart* **104**, 971-977, doi:10.1136/heartjnl-2017-312103 (2018).

51 Pantazi, D. & Tselepis, A. D. Cardiovascular toxic effects of antitumor agents: Pathogenetic mechanisms. *Thrombosis Research* **213**, S95-S102, doi:https://doi.org/10.1016/j.thromres.2021.12.017 (2022).

52 Zhang, S. *et al.* Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med* **18**, 1639-1642, doi:10.1038/nm.2919 (2012).

53 Menna, P. & Salvatorelli, E. Primary Prevention Strategies for Anthracycline Cardiotoxicity: A Brief Overview. *Chemotherapy* **62**, 159-168, doi:10.1159/000455823 (2017).

54 Octavia, Y. *et al.* Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. *J Mol Cell Cardiol* **52**, 1213-1225, doi:10.1016/j.yjmcc.2012.03.006 (2012).

55 Ichikawa, Y. *et al.* Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. *J Clin Invest* **124**, 617-630, doi:10.1172/jci72931 (2014).

56 Miranda, C. J. *et al.* Hfe deficiency increases susceptibility to cardiotoxicity and exacerbates changes in iron metabolism induced by doxorubicin. *Blood* **102**, 2574-2580, doi:10.1182/blood-2003-03-0869 (2003).

57 Kim, S. C. *et al.* Extracellular heat shock protein 60, cardiac myocytes, and apoptosis. *Circ Res* **105**, 1186-1195, doi:10.1161/circresaha.109.209643 (2009).

58 Dempke, W. C. M. *et al.* Anthracycline-induced cardiotoxicity - are we about to clear this hurdle? *Eur J Cancer* **185**, 94-104, doi:10.1016/j.ejca.2023.02.019 (2023).

59 Lipshultz, S. E. *et al.* Cardiotoxicity and cardioprotection in childhood cancer. *Acta Haematol* **132**, 391-399, doi:10.1159/000360238 (2014).

60 Schneider, C. *et al.* Time-Dependent Effect of Anthracycline-Based Chemotherapy on Central Arterial Stiffness: A Systematic Review and Meta-Analysis. *Front Cardiovasc Med* **9**, 873898, doi:10.3389/fcvm.2022.873898 (2022).

61 Terwoord, J. D., Beyer, A. M. & Gutterman, D. D. Endothelial dysfunction as a complication of anti-cancer therapy. *Pharmacol Ther* **237**, 108116, doi:10.1016/j.pharmthera.2022.108116 (2022).

62 Uhm, J. S., Chung, W. B., Yoon, J. S., Oh, Y. S. & Youn, H. J. Effects of adriamycin and candesartan on the collagen and elastin of the aorta in rats. *Clin Hypertens* **20**, 8, doi:10.1186/2056-5909-1-2 (2014).

63 Soultati, A. *et al.* Endothelial vascular toxicity from chemotherapeutic agents: Preclinical evidence and clinical implications. *Cancer Treatment Reviews* **38**, 473-483, doi:https://doi.org/10.1016/j.ctrv.2011.09.002 (2012).

64 Ehrhardt, M. J., Fulbright, J. M. & Armenian, S. H. Cardiomyopathy in Childhood Cancer Survivors: Lessons from the Past and Challenges for the Future. *Curr Oncol Rep* **18**, 22, doi:10.1007/s11912-016-0510-4 (2016).

65 Mrotzek, S. M., Rassaf, T. & Totzeck, M. Cardiovascular Damage Associated With Chest Irradiation. *Front Cardiovasc Med* **7**, 41, doi:10.3389/fcvm.2020.00041 (2020).

66 Chen, D. H. *et al.* Cardiovascular Disease Amongst Women Treated for Breast Cancer: Traditional Cytotoxic Chemotherapy, Targeted Therapy, and Radiation Therapy. *Current Cardiology Reports* **23**, 16, doi:10.1007/s11886-021-01446-x (2021).

67 Spetz, J., Moslehi, J. & Sarosiek, K. Radiation-Induced Cardiovascular Toxicity: Mechanisms, Prevention, and Treatment. *Curr Treat Options Cardiovasc Med* **20**, 31, doi:10.1007/s11936-018-0627-x (2018).

68 Saiki, H. *et al.* Experimental cardiac radiation exposure induces ventricular diastolic dysfunction with preserved ejection fraction. *Am J Physiol Heart Circ Physiol* **313**, H392-h407, doi:10.1152/ajpheart.00124.2017 (2017).

69 Venkatesulu, B. P. *et al.* Radiation-Induced Endothelial Vascular Injury: A Review of Possible Mechanisms. *JACC Basic Transl Sci* **3**, 563-572, doi:10.1016/j.jacbts.2018.01.014 (2018).

70 Koutroumpakis, E. *et al.* Modern Radiotherapy and Risk of Cardiotoxicity. *Chemotherapy* **65**, 65-76, doi:10.1159/000510573 (2020).

71 Dong, X. *et al.* NEMO modulates radiation-induced endothelial senescence of human umbilical veins through NF-κB signal pathway. *Radiat Res* **183**, 82-93, doi:10.1667/rr13682.1 (2015).

72 Lipshultz, S. E. *et al.* Anthracycline-related cardiotoxicity in childhood cancer survivors. *Curr Opin Cardiol* **29**, 103-112, doi:10.1097/hco.0000000000000034 (2014).

73 Aminkeng, F. *et al.* Recommendations for genetic testing to reduce the incidence of anthracycline-induced cardiotoxicity. *Br J Clin Pharmacol* **82**, 683-695, doi:10.1111/bcp.13008 (2016).

74 Chueh, H. W. & Yoo, J. H. Metabolic syndrome induced by anticancer treatment in childhood cancer survivors. *Ann Pediatr Endocrinol Metab* **22**, 82-89, doi:10.6065/apem.2017.22.2.82 (2017).

75 Oudin, C. *et al.* Prevalence and risk factors of the metabolic syndrome in adult survivors of childhood leukemia. *Blood* **117**, 4442-4448, doi:https://doi.org/10.1182/blood-2010-09-304899 (2011).

76 Smith, W. A. *et al.* Lifestyle and metabolic syndrome in adult survivors of childhood cancer: a report from the St. Jude Lifetime Cohort Study. *Cancer* **120**, 2742-2750, doi:10.1002/cncr.28670 (2014).

77 Pluimakers, V. G. *et al.* Metabolic syndrome detection with biomarkers in childhood cancer survivors. *Endocr Connect* **9**, 676-686, doi:10.1530/ec-20-0144 (2020).

78 van Waas, M., Neggers, S. J. C. M. M., Pieters, R. & van den Heuvel-Eibrink, M. M. Components of the metabolic syndrome in 500 adult long-term survivors of childhood cancer. *Annals of Oncology* **21**, 1121-1126, doi:https://doi.org/10.1093/annonc/mdp414 (2010).

79 Noyd, D. H. *et al.* Cardiovascular Risk Factor Disparities in Adult Survivors of Childhood Cancer Compared With the General Population. *JACC CardioOncol* **5**, 489-500, doi:10.1016/j.jaccao.2023.01.011 (2023).

80 Dixon, S. B. *et al.* Specific causes of excess late mortality and association with modifiable risk factors among survivors of childhood cancer: a report from the Childhood Cancer Survivor Study cohort. *Lancet* **401**, 1447-1457, doi:10.1016/s0140-6736(22)02471-0 (2023).

81 Sapkota, Y. *et al.* Contribution of Polygenic Risk to Hypertension Among Long-Term Survivors of Childhood Cancer. *JACC CardioOncol* **3**, 76-84, doi:10.1016/j.jaccao.2021.01.007 (2021).

82 Pluimakers, V. G. *et al.* Prevalence, risk factors and optimal way to determine overweight, obesity and morbid obesity, in the first Dutch cohort of 2,338 long-term survivors of childhood cancer: a DCCSS-LATER study. *Eur J Endocrinol*, doi:10.1093/ejendo/lvad139 (2023).

83 Kim, J. H., Cho, J. J. & Park, Y. S. Relationship between sarcopenic obesity and cardiovascular disease risk as estimated by the Framingham risk score. *J Korean Med Sci* **30**, 264-271, doi:10.3346/jkms.2015.30.3.264 (2015).

84 Casirati, A. *et al.* Fat-to-lean mass ratio as a tool to detect the dysmetabolic profile in childhood cancer survivors. *Nutrition* **113**, 112129, doi:10.1016/j.nut.2023.112129 (2023).

85 Delacourt, L. *et al.* Risk factors for obesity in adulthood among survivors of childhood cancer. *Obesity (Silver Spring)* **31**, 1942-1952, doi:10.1002/oby.23784 (2023).

86 Morel, S. *et al.* Biomarkers of cardiometabolic complications in survivors of childhood acute lymphoblastic leukemia. *Sci Rep* **10**, 21507, doi:10.1038/s41598-020-78493-x (2020).

87 Chen, Y. *et al.* Traditional Cardiovascular Risk Factors and Individual Prediction of Cardiovascular Events in Childhood Cancer Survivors. *J Natl Cancer Inst* **112**, 256-265, doi:10.1093/jnci/djz108 (2020).

88 Chow, E. J. *et al.* Underdiagnosis and Undertreatment of Modifiable Cardiovascular Risk Factors Among Survivors of Childhood Cancer. *J Am Heart Assoc* **11**, e024735, doi:10.1161/jaha.121.024735 (2022).

89 Holmqvist, A. S. *et al.* Adult life after childhood cancer in Scandinavia: diabetes mellitus following treatment for cancer in childhood. *Eur J Cancer* **50**, 1169-1175, doi:10.1016/j.ejca.2014.01.014 (2014).

90 Lega, I. C. *et al.* Diabetes Risk in Childhood Cancer Survivors: A Population-Based Study. *Can J Diabetes* **42**, 533-539, doi:10.1016/j.jcjd.2018.01.004 (2018).

91 Bhandari, R., Armenian, S. H., McCormack, S., Natarajan, R. & Mostoufi-Moab, S. Diabetes in childhood cancer survivors: emerging concepts in pathophysiology and future directions. *Front Med (Lausanne)* **10**, 1206071, doi:10.3389/fmed.2023.1206071 (2023).

92 Mostoufi-Moab, S. *et al.* Adverse Fat Depots and Marrow Adiposity Are Associated With Skeletal Deficits and Insulin Resistance in Long-Term Survivors of Pediatric Hematopoietic Stem Cell Transplantation. *J Bone Miner Res* **30**, 1657-1666, doi:10.1002/jbmr.2512 (2015).

93 Meacham, L. R. *et al.* Diabetes mellitus in long-term survivors of childhood cancer. Increased risk associated with radiation therapy: a report for the childhood cancer survivor study. *Arch Intern Med* **169**, 1381-1388, doi:10.1001/archinternmed.2009.209 (2009).

94 Williams, H. E. *et al.* Diabetes mellitus among adult survivors of childhood acute lymphoblastic leukemia: A report from the St. Jude Lifetime Cohort Study. *Cancer* **126**, 870-878, doi:10.1002/cncr.32596 (2020).

95 Mainieri, F., Giannini, C. & Chiarelli, F. Cardiovascular Risk in Childhood Cancer Survivors. *Biomedicines* **10**, doi:10.3390/biomedicines10123098 (2022).

96 Pluimakers, V. G., van Waas, M., Neggers, S. & van den Heuvel-Eibrink, M. M. Metabolic syndrome as cardiovascular risk factor in childhood cancer survivors. *Crit Rev Oncol Hematol* **133**, 129-141, doi:10.1016/j.critrevonc.2018.10.010 (2019).

97 Lubas, M. M. *et al.* The Contribution of Stress and Distress to Cardiovascular Health in Adult Survivors of Childhood Cancer. *Cancer Epidemiol Biomarkers Prev* **30**, 286-294, doi:10.1158/1055-9965.Epi-20-1183 (2021).

98 Camilli, M. *et al.* Cardiac Imaging in Childhood Cancer Survivors: A State-of-the-Art Review. *Curr Probl Cardiol* **48**, 101544, doi:10.1016/j.cpcardiol.2022.101544 (2023).

99 Ehrhardt, M. J. *et al.* Cost-Effectiveness of the International Late Effects of Childhood Cancer Guideline Harmonization Group Screening Guidelines to Prevent Heart Failure in Survivors of Childhood Cancer. *J Clin Oncol* **38**, 3851-3862, doi:10.1200/jco.20.00418 (2020).

100 Hutchins, K. K., Siddeek, H., Franco, V. I. & Lipshultz, S. E. Prevention of cardiotoxicity among survivors of childhood cancer. *Br J Clin Pharmacol* **83**, 455-465, doi:10.1111/bcp.13120 (2017).

101 Ehrhardt, M. J. *et al.* Systematic review and updated recommendations for cardiomyopathy surveillance for survivors of childhood, adolescent, and young adult cancer from the International Late Effects of Childhood Cancer Guideline Harmonization Group. *Lancet Oncol* **24**, e108-e120, doi:10.1016/s1470-2045(23)00012-8 (2023).

102 Samverkan i Regionala Cancercentrum. *Långtidsuppföljning efter barncancer*, https://cancercentrum.se/globalassets/cancerdiagnoser/barn/vardprogram/vp-langtidsuppfoljning-barncancer.pdf (2022), accessed on 20231106.

103 Bansal, N. *et al.* Strategies to prevent anthracycline-induced cardiotoxicity in cancer survivors. *Cardiooncology* **5**, 18, doi:10.1186/s40959-019-0054-5 (2019).

104 Lyon, A. R. *et al.* 2022 ESC Guidelines on cardio-oncology developed in collaboration with the European Hematology Association (EHA), the European Society for Therapeutic Radiology and Oncology (ESTRO) and the International Cardio-Oncology Society (IC-OS). *Eur Heart J* **43**, 4229-4361, doi:10.1093/eurheartj/ehac244 (2022).

105 Lang, R. M. *et al.* Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* **28**, 1-39.e14, doi:10.1016/j.echo.2014.10.003 (2015).

106 Mitchell, C. *et al.* Guidelines for Performing a Comprehensive Transthoracic Echocardiographic Examination in Adults: Recommendations from the American Society of Echocardiography. *J Am Soc Echocardiogr* **32**, 1-64, doi:10.1016/j.echo.2018.06.004 (2019).

107 Dobson, R. *et al.* British Society for Echocardiography and British Cardio-Oncology Society guideline for transthoracic echocardiographic assessment of adult cancer patients receiving anthracyclines and/or trastuzumab. *Echo Res Pract* **8**, G1-g18, doi:10.1530/erp-21-0001 (2021).

108 Plana, J. C. *et al.* Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* **27**, 911-939, doi:10.1016/j.echo.2014.07.012 (2014).

109 Merkx, R. *et al.* Extensive Cardiac Function Analyses Using Contemporary Echocardiography in Childhood Cancer Survivors: A DCCSS LATER Study. *JACC CardioOncol* **5**, 472-485, doi:10.1016/j.jaccao.2023.06.003 (2023).

110 Sorensen, K., Levitt, G. A., Bull, C., Dorup, I. & Sullivan, I. D. Late anthracycline cardiotoxicity after childhood cancer: a prospective longitudinal study. *Cancer* **97**, 1991-1998, doi:10.1002/cncr.11274 (2003).

111 Lipshultz, S. E. *et al.* Hearts too small for body size after doxorubicin for childhood ALL: Grinch syndrome American Society of Clinical Oncology, 32:15_suppl, 10021 (2014).

112 Kosmala, W. & Marwick, T. H. Asymptomatic Left Ventricular Diastolic Dysfunction: Predicting Progression to Symptomatic Heart Failure. *JACC Cardiovasc Imaging* **13**, 215-227, doi:10.1016/j.jcmg.2018.10.039 (2020).

113 Palmer, C. *et al.* Prevalence of Diastolic Dysfunction in Adult Survivors of Childhood Cancer: A Report From SJLIFE Cohort. *JACC CardioOncol* **5**, 377-388, doi:10.1016/j.jaccao.2022.12.010 (2023).

114 Wolf, C. M. *et al.* Subclinical Cardiac Dysfunction in Childhood Cancer Survivors on 10-Years Follow-Up Correlates With Cumulative Anthracycline Dose and Is Best Detected by Cardiopulmonary Exercise Testing, Circulating Serum Biomarker, Speckle Tracking Echocardiography, and Tissue Doppler Imaging. *Front Pediatr* **8**, 123, doi:10.3389/fped.2020.00123 (2020).

115 Niemelä, J. *et al.* Cardiac Function After Cardiotoxic Treatments for Childhood Cancer—Left Ventricular Longitudinal Strain in Screening. *Frontiers in Cardiovascular Medicine* **8**, doi:10.3389/fcvm.2021.715953 (2021).

116 Sawaya, H. *et al.* Assessment of echocardiography and biomarkers for the extended prediction of cardiotoxicity in patients treated with anthracyclines, taxanes, and trastuzumab. *Circ Cardiovasc Imaging* **5**, 596-603, doi:10.1161/circimaging.112.973321 (2012).

117 Merkx, R. *et al.* Asymptomatic systolic dysfunction on contemporary echocardiography in anthracycline-treated long-term childhood cancer survivors: a systematic review. *J Cancer Surviv*, doi:10.1007/s11764-021-01028-4 (2021).

118 Armstrong, G. T. *et al.* Comprehensive Echocardiographic Detection of Treatment-Related Cardiac Dysfunction in Adult Survivors of Childhood Cancer: Results From the St. Jude Lifetime Cohort Study. *J Am Coll Cardiol* **65**, 2511-2522, doi:10.1016/j.jacc.2015.04.013 (2015).

119 Nagata, Y. *et al.* Intervendor variability of two-dimensional strain using vendor-specific and vendor-independent software. *J Am Soc Echocardiogr* **28**, 630-641, doi:10.1016/j.echo.2015.01.021 (2015).

120 D'Elia, N. *et al.* Normal Global Longitudinal Strain: An Individual Patient Meta-Analysis. *JACC Cardiovasc Imaging* **13**, 167-169, doi:10.1016/j.jcmg.2019.07.020 (2020).

121 Nyberg, J. *et al.* Echocardiographic Reference Ranges of Global Longitudinal Strain for All Cardiac Chambers Using Guideline-Directed Dedicated Views. *JACC Cardiovasc Imaging*, doi:10.1016/j.jcmg.2023.08.011 (2023).

122 Haji, K. *et al.* Incremental Value of Global Longitudinal Strain in the Long-Term Prediction of Heart Failure among Patients with Coronary Artery Disease. *J Am Soc Echocardiogr* **35**, 187-195, doi:10.1016/j.echo.2021.09.003 (2022).

123 Raafs, A. G. *et al.* Global Longitudinal Strain is Incremental to Left Ventricular Ejection Fraction for the Prediction of Outcome in Optimally Treated Dilated Cardiomyopathy Patients. *J Am Heart Assoc* **11**, e024505, doi:10.1161/jaha.121.024505 (2022).

124 Foulkes, S. *et al.* The Utility of Cardiac Reserve for the Early Detection of Cancer Treatment-Related Cardiac Dysfunction: A Comprehensive Overview. *Frontiers in Cardiovascular Medicine* **7**, doi:10.3389/fcvm.2020.00032 (2020).

125 Thein, P. M., Mirzaee, S., Cameron, J. D. & Nasis, A. Left ventricular contractile reserve as a determinant of adverse clinical outcomes: a systematic review. *Intern Med J*, doi:10.1111/imj.14995 (2022).

126 von Scheidt, F. *et al.* Left Ventricular Strain Analysis During Submaximal Semisupine Bicycle Exercise Stress Echocardiography in Childhood Cancer Survivors. *J Am Heart Assoc* **11**, e025324, doi:10.1161/jaha.122.025324 (2022).

127 Cifra, B. *et al.* Dynamic Myocardial Response to Exercise in Childhood Cancer Survivors Treated with Anthracyclines. *J Am Soc Echocardiogr* **31**, 933-942, doi:10.1016/j.echo.2018.02.003 (2018).

128 Sieswerda, E. *et al.* Exercise echocardiography in asymptomatic survivors of childhood cancer treated with anthracyclines: a prospective follow-up study. *Pediatr Blood Cancer* **54**, 579-584, doi:10.1002/pbc.22371 (2010).

129 Saunderson, C. E. D., Plein, S. & Manisty, C. H. Role of cardiovascular magnetic resonance imaging in cardio-oncology. *European Heart Journal - Cardiovascular Imaging* **22**, 383-396, doi:10.1093/ehjci/jeaa345 (2021).

130 van der Velde, N. *et al.* Detection of Subclinical Cardiovascular Disease by Cardiovascular Magnetic Resonance in Lymphoma Survivors. *JACC CardioOncol* **3**, 695-706, doi:10.1016/j.jaccao.2021.09.015 (2021).

131 Mawad, W. *et al.* Effect of anthracycline therapy on myocardial function and markers of fibrotic remodelling in childhood cancer survivors. *European Heart Journal - Cardiovascular Imaging* **22**, 435-442, doi:10.1093/ehjci/jeaa093 (2021).

132 Harries, I. *et al.* Cardiovascular magnetic resonance characterisation of anthracycline cardiotoxicity in adults with normal left ventricular ejection fraction. *International Journal of Cardiology* **343**, 180-186, doi:https://doi.org/10.1016/j.ijcard.2021.08.037 (2021).

133 Armstrong, G. T. *et al.* Screening adult survivors of childhood cancer for cardiomyopathy: comparison of echocardiography and cardiac magnetic resonance imaging. *J Clin Oncol* **30**, 2876-2884, doi:10.1200/jco.2011.40.3584 (2012).

134 Herrmann, J. Vascular toxic effects of cancer therapies. *Nature Reviews Cardiology* **17**, 503-522, doi:10.1038/s41569-020-0347-2 (2020).

135 Crocco, M. *et al.* Endothelial Dysfunction in Childhood Cancer Survivors: A Narrative Review. *Life (Basel)* **12**, doi:10.3390/life12010045 (2021).

136 Li, A. *et al.* Vascular Aging: Assessment and Intervention. *Clin Interv Aging* **18**, 1373-1395, doi:10.2147/cia.S423373 (2023).

137 Jenei, Z. *et al.* Anthracycline causes impaired vascular endothelial function and aortic stiffness in long term survivors of childhood cancer. *Pathol Oncol Res* **19**, 375-383, doi:10.1007/s12253-012-9589-6 (2013).

138 Blair, C. K. *et al.* Feasibility and preliminary efficacy of the effects of flavanoid-rich purple grape juice on the vascular health of childhood cancer survivors: a randomized, controlled crossover trial. *Pediatr Blood Cancer* **61**, 2290-2296, doi:10.1002/pbc.25202 (2014).

139 Dengel, D. R. *et al.* Signs of early sub-clinical atherosclerosis in childhood cancer survivors. *Pediatr Blood Cancer* **61**, 532-537, doi:10.1002/pbc.24829 (2014).

140 Arnold, N. *et al.* Promotion of Arterial Stiffness by Childhood Cancer and Its Characteristics in Adult Long-Term Survivors. *J Am Heart Assoc* **10**, e015609, doi:10.1161/jaha.119.015609 (2021).

141 Sadurska, E., Zaucha-Prażmo, A., Brodzisz, A., Kowalczyk, J. & Beń-Skowronek, I. Premature atherosclerosis after treatment for acute lymphoblastic leukemia in childhood. *Ann Agric Environ Med* **25**, 71-76, doi:10.5604/12321966.1230680 (2018).

142 Lipshultz, S. E. *et al.* Changes in cardiac biomarkers during doxorubicin treatment of pediatric patients with high-risk acute lymphoblastic leukemia: associations with long-term echocardiographic outcomes. *J Clin Oncol* **30**, 1042-1049, doi:10.1200/jco.2010.30.3404 (2012).

143 Leerink, J. M. *et al.* Biomarkers to diagnose ventricular dysfunction in childhood cancer survivors: a systematic review. *Heart* **105**, 210-216, doi:10.1136/heartjnl-2018-313634 (2019).

144 Moazeni, S., Cadeiras, M., Yang, E. H., Deng, M. C. & Nguyen, K.-L. Anthracycline induced cardiotoxicity: biomarkers and "Omics" technology in the era of patient specific care. *Clinical and Translational Medicine* **6**, 17, doi:10.1186/s40169-017-0148-3 (2017).

145 Wiebe, T., Hjorth, L., Marotta Kelly, M., Linge, H. M. & Garwicz, S. A population based pediatric oncology registry in Southern Sweden: the BORISS registry. *Eur J Epidemiol* **33**, 1125-1129, doi:10.1007/s10654-018-0437-1 (2018).

146 Group, C. O. Guidelines for Long Term Follow Up for Survivors of Childhood, Adolescent Young Adult Cancer Version 3. (2008).

147 Stein, J. H. *et al.* Use of carotid ultrasound to identify subclinical vascular disease and evaluate cardiovascular disease risk: a consensus statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force. Endorsed by the Society for Vascular Medicine. *J Am Soc Echocardiogr* **21**, 93-111; quiz 189-190, doi:10.1016/j.echo.2007.11.011 (2008).

148 Juonala, M. *et al.* Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study. *Circulation* **112**, 1486-1493, doi:10.1161/circulationaha.104.502161 (2005).

149 McCrea, C. E., Skulas-Ray, A. C., Chow, M. & West, S. G. Test-retest reliability of pulse amplitude tonometry measures of vascular endothelial function: implications for clinical trial design. *Vasc Med* **17**, 29-36, doi:10.1177/1358863x11433188 (2012).

150 Kang, J. *et al.* Endothelial function estimated by digital reactive hyperemia in patients with atherosclerotic risk factors or coronary artery disease. *Heart and Vessels* **33**, 706-712, doi:10.1007/s00380-018-1118-4 (2018).

151 Hilvo, M. *et al.* Development and validation of a ceramide- and phospholipid-based cardiovascular risk estimation score for coronary artery disease patients. *Eur Heart J* **41**, 371-380, doi:10.1093/eurheartj/ehz387 (2020).

152 Hilvo, M., Jylhä, A., Lääperi, M., Jousilahti, P. & Laaksonen, R. Absolute and relative risk prediction in cardiovascular primary prevention with a modified SCORE chart incorporating ceramide-phospholipid risk score and diabetes mellitus. *Eur Heart J Open* **1**, oeab010, doi:10.1093/ehjopen/oeab010 (2021).

153 Jung, E., Kong, S. Y., Ro, Y. S., Ryu, H. H. & Shin, S. D. Serum Cholesterol Levels and Risk of Cardiovascular Death: A Systematic Review and a Dose-Response Meta-Analysis of Prospective Cohort Studies. *Int J Environ Res Public Health* **19**, doi:10.3390/ijerph19148272 (2022).

154 Behbodikhah, J. *et al.* Apolipoprotein B and Cardiovascular Disease: Biomarker and Potential Therapeutic Target. *Metabolites* **11**, doi:10.3390/metabo11100690 (2021).

155 Boekholdt, S. M. *et al.* Levels and changes of HDL cholesterol and apolipoprotein A-I in relation to risk of cardiovascular events among statin-treated patients: a meta-analysis. *Circulation* **128**, 1504-1512, doi:10.1161/circulationaha.113.002670 (2013).

156 Goldberg, J. F. *et al.* Dyslipidemia and Cardiovascular Disease Among Childhood Cancer Survivors: A St. Jude Lifetime Cohort Report. *J Natl Cancer Inst*, doi:10.1093/jnci/djad222 (2023).

157 Tian, M. *et al.* Comparison of Apolipoprotein B/A1 ratio, Framingham risk score and TC/HDL-c for predicting clinical outcomes in patients undergoing percutaneous coronary intervention. *Lipids Health Dis* **18**, 202, doi:10.1186/s12944-019-1144-y (2019).

158 Holme, I., Aastveit, A. H., Jungner, I. & Walldius, G. Relationships between lipoprotein components and risk of myocardial infarction: age, gender and short versus longer follow-up periods in the Apolipoprotein MOrtality RISk study (AMORIS). *J Intern Med* **264**, 30-38, doi:10.1111/j.1365-2796.2008.01925.x (2008).

159 Link, K. *et al.* Growth hormone deficiency predicts cardiovascular risk in young adults treated for acute lymphoblastic leukemia in childhood. *J Clin Endocrinol Metab* **89**, 5003-5012, doi:10.1210/jc.2004-0126 (2004).

160 Florin, T. A. *et al.* Physical inactivity in adult survivors of childhood acute lymphoblastic leukemia: a report from the childhood cancer survivor study. *Cancer Epidemiol Biomarkers Prev* **16**, 1356-1363, doi:10.1158/1055-9965.Epi-07-0048 (2007).

161 Zhang, F. F. & Parsons, S. K. Obesity in Childhood Cancer Survivors: Call for Early Weight Management. *Adv Nutr* **6**, 611-619, doi:10.3945/an.115.008946 (2015).

162 Chow, E. J., Pihoker, C., Hunt, K., Wilkinson, K. & Friedman, D. L. Obesity and hypertension among children after treatment for acute lymphoblastic leukemia. *Cancer* **110**, 2313-2320, doi:10.1002/cncr.23050 (2007).

163 Russi, E. G., Raber-Durlacher, J. E. & Sonis, S. T. Local and systemic pathogenesis and consequences of regimen-induced inflammatory responses in patients with head and neck cancer receiving chemoradiation. *Mediators Inflamm* **2014**, 518261, doi:10.1155/2014/518261 (2014).

164 Yuan, C., Wang, J. & Ying, M. Predictive Value of Carotid Distensibility Coefficient for Cardiovascular Diseases and All-Cause Mortality: A Meta-Analysis. *PLoS One* **11**, e0152799, doi:10.1371/journal.pone.0152799 (2016).

165 Zaletel, L. Z., Popit, M. & Zaletel, M. Is Carotid Stiffness a Possible Surrogate for Stroke in Long-term Survivors of Childhood Cancer after Neck Radiotherapy? *Radiol Oncol* **52**, 136-142, doi:10.2478/raon-2018-0006 (2018).

166 Screever, E. M., Meijers, W. C. & Moslehi, J. J. Age-Related Considerations in Cardio-Oncology. *J Cardiovasc Pharmacol Ther* **26**, 103-113, doi:10.1177/1074248420968689 (2021).

167 Mottram, P. M. *et al.* Relation of arterial stiffness to diastolic dysfunction in hypertensive heart disease. *Heart* **91**, 1551-1556, doi:10.1136/hrt.2004.046805 (2005).

168 Jujic, A., Kennebäck, C., Johansson, M., Nilsson, P. M. & Holm, H. The impact of age on endothelial dysfunction measured by peripheral arterial tonometry in a healthy population-based cohort - the Malmö offspring study. *Blood Press* **32**, 2234059, doi:10.1080/08037051.2023.2234059 (2023).

169 Lee, C., Dow, S., Shah, K., Henkin, S. & Taub, C. Complications of exercise and pharmacologic stress echocardiography. *Front Cardiovasc Med* **10**, 1228613, doi:10.3389/fcvm.2023.1228613 (2023).

170 Kaneko, S. *et al.* Impaired Left Ventricular Reserve in Childhood Cancer Survivors Treated With Anthracycline Therapy. *Pediatr Blood Cancer* **63**, 1086-1090, doi:10.1002/pbc.25933 (2016).

171 Ryerson, A. B. *et al.* Assessing anthracycline-treated childhood cancer survivors with advanced stress echocardiography. *Pediatr Blood Cancer* **62**, 502-508, doi:10.1002/pbc.25328 (2015).

172 Varga, A., Garcia, M. A. & Picano, E. Safety of stress echocardiography (from the International Stress Echo Complication Registry). *Am J Cardiol* **98**, 541-543, doi:10.1016/j.amjcard.2006.02.064 (2006).

173 Rösner, A. *et al.* The influence of frame rate on two-dimensional speckle-tracking strain measurements: a study on silico-simulated models and images recorded in patients. *European Heart Journal - Cardiovascular Imaging* **16**, 1137-1147, doi:10.1093/ehjci/jev058 (2015).

174 Pellikka, P. A. *et al.* Normal stroke volume and cardiac output response during dobutamine stress echocardiography in subjects without left ventricular wall motion abnormalities. *Am J Cardiol* **76**, 881-886, doi:10.1016/s0002-9149(99)80254-9 (1995).

175 Fagundes, C. P. *et al.* Sympathetic and parasympathetic activity in cancer-related fatigue: more evidence for a physiological substrate in cancer survivors. *Psychoneuroendocrinology* **36**, 1137-1147, doi:10.1016/j.psyneuen.2011.02.005 (2011).

176 Webster, R. T. *et al.* Health behavior profiles in young survivors of childhood cancer: Findings from the St. Jude Lifetime Cohort Study. *Cancer* **129**, 2075-2083, doi:10.1002/cncr.34749 (2023).

177 Oikonomou, E. K. *et al.* Assessment of Prognostic Value of Left Ventricular Global Longitudinal Strain for Early Prediction of Chemotherapy-Induced Cardiotoxicity: A Systematic Review and Meta-analysis. *JAMA Cardiol* **4**, 1007-1018, doi:10.1001/jamacardio.2019.2952 (2019).

178 Wang, T. K. M. *et al.* Determining the thresholds for abnormal left ventricular strains in healthy subjects by echocardiography: a meta-analysis. *Cardiovasc Diagn Ther* **10**, 1858-1873, doi:10.21037/cdt-20-711 (2020).

179 Lancellotti, P. *et al.* The Clinical Use of Stress Echocardiography in Non-Ischaemic Heart Disease: Recommendations from the European Association of Cardiovascular Imaging and the American Society of Echocardiography. *J Am Soc Echocardiogr* **30**, 101-138, doi:10.1016/j.echo.2016.10.016 (2017).

180 Dalen, H., Thorstensen, A., Vatten, L. J., Aase, S. A. & Stoylen, A. Reference values and distribution of conventional echocardiographic Doppler measures and longitudinal tissue Doppler velocities in a population free from cardiovascular disease. *Circ Cardiovasc Imaging* **3**, 614-622, doi:10.1161/circimaging.109.926022 (2010).

181 Kang, K. W., Ok, M. & Lee, S. K. Leptin as a Key between Obesity and Cardiovascular Disease. *J Obes Metab Syndr* **29**, 248-259, doi:10.7570/jomes20120 (2020).

182 Singhal, A. *et al.* Influence of leptin on arterial distensibility: a novel link between obesity and cardiovascular disease? *Circulation* **106**, 1919-1924, doi:10.1161/01.cir.0000033219.24717.52 (2002).

183 Fantin, F. *et al.* Adipokines and Arterial Stiffness in the Elderly. *Vasc Health Risk Manag* **16**, 535-543, doi:10.2147/vhrm.S274861 (2020).

184 Follin, C. *et al.* Associations between Metabolic Risk Factors and the Hypothalamic Volume in Childhood Leukemia Survivors Treated with Cranial Radiotherapy. *PLoS One* **11**, e0147575, doi:10.1371/journal.pone.0147575 (2016).

185 Pluimakers, V. G. *et al.* Can biomarkers be used to improve diagnosis and prediction of metabolic syndrome in childhood cancer survivors? A systematic review. *Obes Rev* **22**, e13312, doi:10.1111/obr.13312 (2021).

186 Robles, N. R. *et al.* Alpha-1-microglobulin: Prognostic value in chronic kidney disease. *Med Clin (Barc)* **157**, 368-370, doi:10.1016/j.medcli.2020.06.061 (2021).

187 Liu, C. *et al.* Systematic Review and Meta-Analysis of Plasma and Urine Biomarkers for CKD Outcomes. *J Am Soc Nephrol* **33**, 1657-1672, doi:10.1681/asn.2022010098 (2022).

188 Green, D. M. *et al.* Kidney Function after Treatment for Childhood Cancer: A Report from the St. Jude Lifetime Cohort Study. *J Am Soc Nephrol* **32**, 983-993, doi:10.1681/asn.2020060849 (2021).

189 Harlacher, E., Wollenhaupt, J., Baaten, C. & Noels, H. Impact of Uremic Toxins on Endothelial Dysfunction in Chronic Kidney Disease: A Systematic Review. *Int J Mol Sci* **23**, doi:10.3390/ijms23010531 (2022).

190 Sarnak, M. J. *et al.* Chronic Kidney Disease and Coronary Artery Disease: JACC State-of-the-Art Review. *J Am Coll Cardiol* **74**, 1823-1838, doi:10.1016/j.jacc.2019.08.1017 (2019).

191 Chu, D. I. *et al.* Kidney Outcomes and Hypertension in Survivors of Wilms Tumor: A Prospective Cohort Study. *J Pediatr* **230**, 215-220.e211, doi:10.1016/j.jpeds.2020.12.005 (2021).

192 Berezin, A. E., Berezin, A. A. & Lichtenauer, M. Myokines and Heart Failure: Challenging Role in Adverse Cardiac Remodeling, Myopathy, and Clinical Outcomes. *Dis Markers* **2021**, 6644631, doi:10.1155/2021/6644631 (2021).

193 Kawaguchi, T. *et al.* Impact of Decorin on the Physical Function and Prognosis of Patients with Hepatocellular Carcinoma. *J Clin Med* **9**, doi:10.3390/jcm9040936 (2020).

194 Sulicka-Grodzicka, J. *et al.* Low-grade chronic inflammation and immune alterations in childhood and adolescent cancer survivors: A contribution to accelerated aging? *Cancer Med* **10**, 1772-1782, doi:10.1002/cam4.3788 (2021).

195 Doni, A., Mantovani, A., Bottazzi, B. & Russo, R. C. PTX3 Regulation of Inflammation, Hemostatic Response, Tissue Repair, and Resolution of Fibrosis Favors a Role in Limiting Idiopathic Pulmonary Fibrosis. *Front Immunol* **12**, 676702, doi:10.3389/fimmu.2021.676702 (2021).

196 Ye, Z. *et al.* GALNT4 primes monocytes adhesion and transmigration by regulating O-Glycosylation of PSGL-1 in atherosclerosis. *J Mol Cell Cardiol* **165**, 54-63, doi:10.1016/j.yjmcc.2021.12.012 (2022).

197 Weismann, C. G., Hlebowicz, J., Åkesson, A., Liuba, P. & Hanseus, K. Comprehensive Characterization of Arterial and Cardiac Function in Marfan Syndrome-Can Biomarkers Help Improve Outcome? *Front Physiol* **13**, 873373, doi:10.3389/fphys.2022.873373 (2022).

198 Pettersson-Pablo, P., Nilsson, T. K., Breimer, L. H. & Hurtig-Wennlöf, A. IGFBP-1 and IGFBP-2 are associated with a decreased pulse-wave velocity in young, healthy adults. *BMC Cardiovasc Disord* **21**, 131, doi:10.1186/s12872-021-01914-w (2021).

199 Chaurasia, B. & Summers, S. A. Ceramides in Metabolism: Key Lipotoxic Players. *Annu Rev Physiol* **83**, 303-330, doi:10.1146/annurev-physiol-031620-093815 (2021).

200 Chakraborty, M. *et al.* Myeloid cell-specific serine palmitoyltransferase subunit 2 haploinsufficiency reduces murine atherosclerosis. *J Clin Invest* **123**, 1784-1797, doi:10.1172/jci60415 (2013).

201 Park, T. S. *et al.* Ceramide is a cardiotoxin in lipotoxic cardiomyopathy. *J Lipid Res* **49**, 2101-2112, doi:10.1194/jlr.M800147-JLR200 (2008).

202 Ji, R. *et al.* Increased de novo ceramide synthesis and accumulation in failing myocardium. *JCI Insight* **2**, doi:10.1172/jci.insight.82922 (2017).

203 Havulinna, A. S. *et al.* Circulating Ceramides Predict Cardiovascular Outcomes in the Population-Based FINRISK 2002 Cohort. *Arterioscler Thromb Vasc Biol* **36**, 2424-2430, doi:10.1161/atvbaha.116.307497 (2016).

204 Di Pietro, P. *et al.* The Dark Side of Sphingolipids: Searching for Potential Cardiovascular Biomarkers. *Biomolecules* **13**, doi:10.3390/biom13010168 (2023).

205 Mathews, A. T. *et al.* Efficacy of nutritional interventions to lower circulating ceramides in young adults: FRUVEDomic pilot study. *Physiol Rep* **5**, doi:10.14814/phy2.13329 (2017).

206 Rigamonti, A. E. *et al.* Ceramide Risk Score in the Evaluation of Metabolic Syndrome: An Additional or Substitutive Biochemical Marker in the Clinical Practice? *Int J Mol Sci* **24**, doi:10.3390/ijms241512452 (2023).

207 Nwabuo, C. C. *et al.* Association of Circulating Ceramides With Cardiac Structure and Function in the Community: The Framingham Heart Study. *J Am Heart Assoc* **8**, e013050, doi:10.1161/jaha.119.013050 (2019).

208 Rodríguez-Calvo, R. *et al.* Peroxisome proliferator-activated receptor alpha down-regulation is associated with enhanced ceramide levels in age-associated cardiac hypertrophy. *J Gerontol A Biol Sci Med Sci* **62**, 1326-1336, doi:10.1093/gerona/62.12.1326 (2007).

209 Haddy, T. B., Mosher, R. B., Nunez, S. B. & Reaman, G. H. Growth hormone deficiency after chemotherapy for acute lymphoblastic leukemia in children who have not received cranial radiation. *Pediatr Blood Cancer* **46**, 258-261, doi:10.1002/pbc.20485 (2006).

210 Shu, H. *et al.* Emerging Roles of Ceramide in Cardiovascular Diseases. *Aging Dis* **13**, 232-245, doi:10.14336/ad.2021.0710 (2022).

211 Li, Q. *et al.* Associations between plasma ceramides and mortality in patients with coronary artery disease. *Atherosclerosis* **314**, 77-83, doi:10.1016/j.atherosclerosis.2020.09.004 (2020).

212 Gencer, B. *et al.* Plasma ceramide and phospholipid-based risk score and the risk of cardiovascular death in patients after acute coronary syndrome. *Eur J Prev Cardiol* **29**, 895-902, doi:10.1093/eurjpc/zwaa143 (2022).

213 Vasile, V. C. *et al.* Ceramide Scores Predict Cardiovascular Risk in the Community. *Arterioscler Thromb Vasc Biol* **41**, 1558-1569, doi:10.1161/atvbaha.120.315530 (2021).

214 Piccoli, M. *et al.* Sphingolipids and Atherosclerosis: The Dual Role of Ceramide and Sphingosine-1-Phosphate. *Antioxidants (Basel)* **12**, doi:10.3390/antiox12010143 (2023).

215 Symons, J. D. & Abel, E. D. Lipotoxicity contributes to endothelial dysfunction: a focus on the contribution from ceramide. *Rev Endocr Metab Disord* **14**, 59-68, doi:10.1007/s11154-012-9235-3 (2013).

Printed by Media-Tryck, Lund 2024 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Paediatrics
Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2024:15
ISBN 978-91-8021-508-4
ISSN 1652-8220