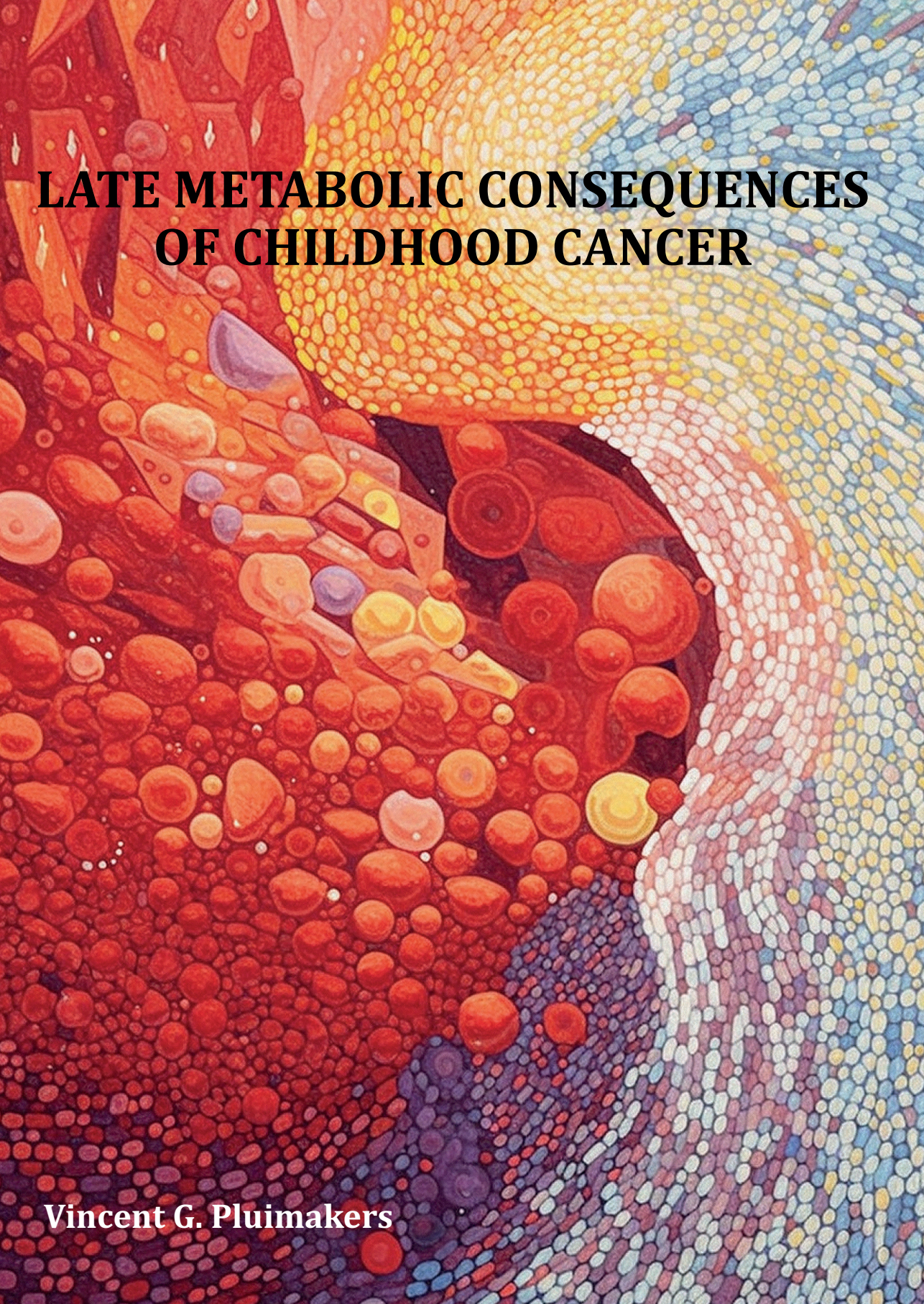

LATE METABOLIC CONSEQUENCES OF CHILDHOOD CANCER

Vincent G. Pluimakers

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INTRODUCTION

CHILDHOOD CANCER AND SURVIVAL

Annually, more than 400,000 children worldwide are diagnosed with cancer[1]. Forty to 45% of these diagnoses include hematologic malignancies (e.g., several types of leukemias and lymphomas), 30-35% are solid tumors (e.g., kidney, bone and muscle tumors) and 20-25% are brain and peripheral nervous system tumors[1]. Until the 1960's most childhood cancer diagnoses would almost certainly lead to death. The development of treatment strategies, including chemotherapy, radiotherapy, stem cell transplantation and, more recently, immunotherapy, made it possible to cure most childhood cancer patients. Advances in supportive care and better risk group stratification over time further increased survival rates for most of the pediatric cancer types. These treatments in young and developing children are often intensive and can take several years, but the results are impressive. To date, more than 80% of children with cancer in high-income countries survive[2, 3].

In The Netherlands, 600 childhood cancer patients are diagnosed annually[4]. Pediatric oncology centers started treating these children in the 1960s, and protocols were developed in clinical trials on a national level. From 1972, this started with protocols for leukemia treatment, coordinated by the Dutch Childhood Leukemia Group, and in 2002 this was expanded to brain and solid tumors by the Dutch Childhood Oncology Group. In 2014, centralization of pediatric oncology care on a national level started by centralizing care for children with solid tumors in the Princess Maxima Center for Pediatric Oncology, with the aim to further increase survival rates. In June 2018, all pediatric oncology care and research became centralized in this hospital. Rising survival rates have led to an increasing cohort of childhood cancer survivors. Currently, more than 15,000 childhood cancer survivors are alive in The Netherlands[4], and a dedicated outpatient clinic has been set up in the Princess Maxima Center.

LONG-TERM TOXICITY OF CHILDHOOD CANCER TREATMENT

These childhood cancer survival rates, however, reflect only the *five-year* survival. Recent studies showed that in the 25 years beyond that moment, an additional 20% of childhood cancer survivors die[5]. This indicates that one in five survivors will die at a substantially younger age than their peers in the general population. In the first years after the five-year survival moment, this is often caused by cancer relapse. Moreover, severe adverse effects of their cancer treatment are responsible for this excess mortality. The two most important late side-effects leading to early death are secondary tumors

in radiation exposed tissues and cardiovascular disease due to damage caused by chemo- and radiotherapy[6, 7].

Additionally, significant morbidity, that can affect all organ systems, occurs. This includes pulmonary disease, impaired motor performance, for instance after amputation or due to paralysis, ototoxicity including deafness and tinnitus, cardiotoxicity, and kidney failure. Among the most common late effects are several endocrine sequelae such as growth hormone deficiency, infertility, impaired bone health and body composition sequelae including sarcopenia and adiposity. More general consequences include fatigue, neuropsychological adverse effects, accelerated aging and frailty. Previous studies showed that 75% of survivors develops at least one adverse event, which was classified as severe in more than half of these, and 50% developed three or more adverse events[8, 9]. This seems to be a continuously increasing risk, as illustrated by a study from the United States which revealed that, by the age of 50 years, a survivor had on average *seventeen* chronic health conditions, of which *five severe*[10]. These numbers were twice as high as in the general population, clearly illustrating the excess morbidity among long-term childhood cancer survivors.

METABOLIC SYNDROME

One of the conditions that needs attention in childhood cancer survivors is metabolic syndrome. It is a cluster of conditions that occur together and aggravate each other: overweight, dyslipidemia, insulin resistance and hypertension.

Overweight is an unhealthy weight caused by the excess accumulation of fat. It is usually described as weight in relation to height (body mass index (BMI) in kg/m^2), with overweight defined as BMI $\geq 25 kg/m^2$ and obesity $\geq 30 kg/m^2$[11]. BMI can however underestimate the true adiposity status or overestimate overweight in muscular people. Therefore, another overweight measurement commonly used is waist circumference (WC), which is specifically directed at measuring abdominal, unhealthy fat[12]. An unhealthy WC is usually described per population, e.g., ≥94cm for Caucasian men and ≥80cm for Caucasian women[13], or as a single, higher threshold of ≥102cm in men and ≥88cm in women[14].

Dyslipidemia refers to unhealthy levels of one or more blood lipids. Routinely used parameters are high low-density-lipoprotein (LDL) cholesterol (≥4.1mmol/L), high triglycerides (≥1.7mmol/L), high total cholesterol (≥6.2mmol/L), and low high-density-lipoprotein (HDL) cholesterol (≤1.0mmol/L in men and ≤1.3mmol/L in women)[15, 16].

Insulin resistance is a condition in which muscles, fat and liver respond less to insulin. It causes the pancreas to produce more insulin to maintain glucose uptake in the cell. Insulin resistance is usually defined as a fasting plasma glucose between 5.6 and 7 mmol/L, indicating that the pancreas is unable to produce enough insulin to maintain healthy glucose levels[17]. This is also called prediabetes, whereas diabetes mellitus is defined as fasting glucose ≥7mmol/L[17].

In patients with arterial hypertension the systolic and/or diastolic blood pressure are consistently too high. Depending on location (office or ambulatory) and time (during the day or 24 hours) of measurement commonly used thresholds for hypertension are 130, 135 or 140mmHg systolic and 80, 85 or 90mmHg diastolic[18].

The central pathophysiologic mechanism of metabolic syndrome is a vicious circle of overweight leading to insulin resistance and vice versa, thereby also causing dyslipidemia and hypertension (Figure 1). Excess adipose tissue secretes an overabundance of free fatty acids, which increase gluconeogenesis in the liver and make muscles more resistant to insulin, both of which cause the pancreas to produce more insulin. Hyperinsulinemia and insulin resistance lead to lipogenesis, thus weight gain, as well as increased release of free fatty acids from this adipose tissue[19-21]. Additionally, dyslipidemia is caused by the accumulation of abundant free fatty acids in the liver, leading to steatosis hepatis, overproduction of very-low density lipoprotein cholesterol and consequent other lipid abnormalities including increased LDL cholesterol and triglycerides and decreased HDL cholesterol[22]. Also, hyperinsulinemia increases sodium reabsorption in the kidney and sympathetic nervous system activity, adipose tissue secretes more leptin which also activates the sympathetic nervous system, and adipocytes have an aldosterone releasing effect on the adrenal gland which also increases sodium reabsorption. All these mechanisms contribute to the development of hypertension. Overeating and a sedentary lifestyle have led to a drastic worldwide increase in the prevalence of metabolic syndrome. In a large study in The Netherlands ten years ago the prevalence was 25%[23], and this is also the expected current worldwide prevalence[24].

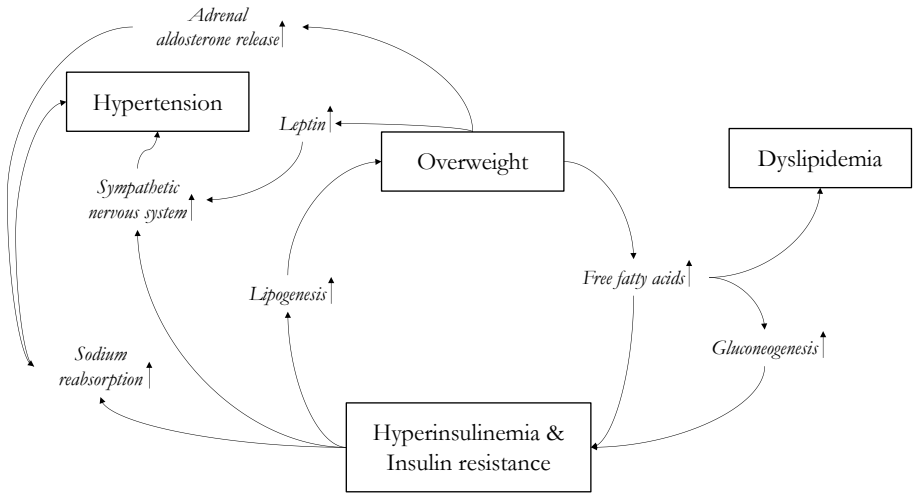

Figure 1. Pathophysiology of metabolic syndrome

Since its inception, multiple definitions have been used to classify the metabolic syndrome. These definitions used different methods and thresholds to measure the four components overweight, dyslipidemia, insulin resistance, and hypertension. Also, some definitions used overweight as an essential criterion, whereas others regarded all components equally important. One definition that is frequently used in clinical practice and research was released by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) in 2001 (Table 1)[16]. In 2009, in an attempt to align the different perspectives on metabolic syndrome classification, a collaboration between the International Diabetes Federation, the National Heart, Lung, and Blood Institute and a number of other health associations developed the Harmonized Metabolic Syndrome Definition, also known as Joint Interim Statement[24]. These two definitions, that require any three components for diagnosis, are currently most in use, and they only differ in overweight threshold. In addition to the components comprising the classifications, other components have been observed to be involved in metabolic syndrome pathophysiology, including hyperuricemia, inflammation, adipokine signaling and pro-thrombotic factors[21, 25].

Table 1. Metabolic syndrome definitions commonly used in clinical practice and research

	Joint Interim Statement*	NCEP ATP III
Required for diagnosis	3 or more criteria	
Overweight	Waist circumference with ethnicity specific thresholds	Waist circumference >102/88cm (men/women)
Insulin resistance	Fasting plasma glucose ≥5.6 mmol/L or treatment	
Dyslipidemia	Triglycerides ≥1.7mmol/L or treatment HDL cholesterol <1/1.3mmol/L (men/women) or treatment	
Hypertension	≥130/85mmHg or treatment	

* Involved organizations are the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity

Metabolic syndrome leads to an increased risk of diabetes and atherosclerotic disease and, hence, mortality. Previous studies have shown that in the general population metabolic syndrome is associated with a three-fold and five-fold increased risk of developing subsequent diabetes and atherosclerotic disease, respectively, that diabetes mellitus itself further increases atherosclerotic disease risk by three-fold, and that consequently patients with metabolic syndrome carry an up to doubled risk of dying from atherosclerotic disease[26-29].

METABOLIC SYNDROME IN CHILDHOOD CANCER SURVIVORS

Some previous studies have observed mechanisms that can induce metabolic syndrome and separate components on the long-term after childhood cancer treatment. One important mechanism is damage to the hypothalamus and pituitary gland, which leads to several hormonal deficiencies – growth hormone is the most important one, followed by sex hormone and thyroid hormone – that contribute to metabolic syndrome risk[30, 31]. This damage can be caused by brain tumors in this region, as well as local surgery and radiotherapy. Furthermore, it can be caused by cranial radiotherapy as administered in the first childhood leukemia treatment protocols and by total body irradiation as myeloablative conditioning regimen for stem cell transplantation. A second important mechanism for metabolic syndrome is primary hypogonadism caused by ovarian and testicular tumors, resections, abdominal and pelvic radiotherapy, and alkylating chemotherapy[31, 32]. Thirdly, abdominal radiotherapy can damage the pancreas, leading to reduced insulin-secretion and hence faster deterioration of insulin resistance into diabetes[33, 34]. Finally, nephrectomy and nephrotoxic chemotherapy have been shown to increase risk of hypertension[35, 36].

Altogether, these treatment-related risk factors, and additional comorbidities that can further aggravate the situation, lead to a higher metabolic syndrome risk in childhood cancer survivors, even at a very young age[37-40].

These insights have made clinicians aware of the importance to include metabolic syndrome screening in the follow-up of survivors. Metabolic syndrome is often a subclinical condition, i.e., it tends to develop without apparent symptoms for years. Hence, it is important to screen for the components in childhood cancer survivors, to be able to start early lifestyle and medical interventions, and thereby prevent further derangement into life-threatening diabetes and cardio- and cerebrovascular disease. However, there are challenges in identifying metabolic syndrome in survivors early and effectively.

First, there is a risk of underdiagnosis of metabolic syndrome with the standard criteria. After abdominal radiation the abdominal wall can be damaged, and therefore waist circumference may not reflect total body fat percentage. This leads to underestimation of overweight, thereby underdiagnosing metabolic syndrome[41, 42]. Diagnosis of metabolic syndrome could be improved by including other diagnostic markers of metabolic syndrome in surveillance. Potential alternatives include additional serum biomarkers, vascular ultrasonography, other anthropometric measurements, and more detailed body composition assessment with dual-energy X-ray absorptiometry. Although studies in the general population have indicated the potential value of these alternative methods, studies in survivors are scarce and adequate overweight and metabolic syndrome assessment remains challenging in subgroups of survivors.

Also, knowledge on national prevalence and risk factors of metabolic syndrome and its components remains incomplete. In addition to patient and treatment related risk factors, this also includes genetic risk, which is suspected because similarly treated survivors can have different occurrence of metabolic syndrome. This knowledge on prevalence and determinants is lacking because studies so far are either based on small and biased survivor cohorts, have insufficient treatment data, are questionnaire-based, or have short follow-up. Large cohort studies on metabolic syndrome that actively recruited childhood cancer survivors are scarce and for some subtopics unavailable. Therefore, there remain steps to be taken towards personalized metabolic syndrome risk assessment and effective follow-up.

AIMS OF THIS THESIS

This thesis attempts to contribute knowledge based on large, representative national cohorts of childhood cancer survivors. The aims of this thesis were to identify additional (bio)markers to improve metabolic syndrome diagnosis, and to describe prevalence as well as clinical and genetic determinants of metabolic syndrome, with a specific focus on the components overweight and dyslipidemia.

Chapter 1 describes a literature review on the occurrence of metabolic syndrome in childhood cancer survivors and established and possible essential links with treatment. In ***Chapter 2*** a single-center recruitment study among survivors of childhood nephroblastoma and neuroblastoma is described, that aimed to show the potential value of additional serum biomarkers and vascular ultrasonography for diagnosing metabolic syndrome. ***Chapter 3*** includes a systematic literature review and meta-analysis on the diagnostic and predictive value of nine novel metabolic syndrome related biomarkers that have been described in the normal population as well as in survivors. ***Chapter 4*** describes the methodology of our study on prevalence and determinants of metabolic syndrome in survivors based on the nationwide Dutch LATER cohort, using various assessment modalities. In ***Chapter 5*** the results on prevalence and determinants of overweight and obesity in this national cohort, and the optimal way to determine overweight, are described. ***Chapter 6*** reports the first genome-wide association study on the metabolic syndrome component dyslipidemia in childhood cancer survivors, in three large survivor cohorts in the United States. ***Chapter 7*** describes perspectives on optimal survivor follow-up from an international panel of healthcare workers who attended a meet-the-expert session on survivorship at the 2018 conference of the International Society of Pediatric Oncology. In ***Chapter 8*** the results of this thesis are further discussed including future perspectives.

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Chapter 1

Metabolic syndrome as cardiovascular risk factor in childhood cancer survivors

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ABSTRACT

Over the past decades, survival rates of childhood cancer have increased considerably from 5-30% in the early seventies to current rates exceeding 80%. This is due to the development of effective chemotherapy, surgery, radiotherapy and stem cell transplantation, combined with an optimized stratification of therapy and better supportive care regimens. As a consequence, active surveillance strategies of late sequelae have been developed to improve the quality of survival. Several epidemiological studies have reported an increased incidence of (components of) metabolic syndrome (MetS) and cardiovascular disease in childhood cancer survivors (CCS). Growth hormone deficiency (GHD) after cranial radiotherapy (CRT) has been previously described as an important cause of MetS. New insights suggest a role for abdominal radiotherapy as a determinant for MetS as well. The role of other risk factors, such as specific chemotherapeutic agents, steroids, gonadal impairment, thyroid morbidity and genetics, warrants further investigation. This knowledge is important to define subgroups of CCS that are at risk to develop (subclinical) MetS features. These survivors might benefit from standard surveillance and early interventions, for example lifestyle and diet advice and medical treatment, thereby preventing the development of cardiovascular disease.

INTRODUCTION

Over the past decades, survival rates of childhood cancer have increased considerably from 5-30% in the early seventies to current rates exceeding 80%[1]. This is due to the development of effective chemotherapy, surgery, radiotherapy and stem cell transplantation (SCT), combined with an optimized stratification of therapy and better supportive care regimens. These improved survival rates currently result in an ongoing increasing number of survivors[2], which in turn resulted in increased awareness of late side effects of treatment for childhood cancer, and research investigating these late sequelae.

Several epidemiological studies have reported an increased incidence of cardiovascular disease in survivors of childhood cancer (Supplemental table 1). Standardized mortality risk, e.g. due to stroke and coronary heart disease, ranges from 1.9 to 12.7, with higher risk for specific subgroups with regard to diagnosis, administered treatment and follow-up time[3-16].

The pathophysiology of the development of cardiovascular disease in childhood cancer survivors is a multifactorial process as in the normal population, but with additional treatment and disease specific modulators. Frequently reported risk factors for cardiovascular sequelae are adiposity, hypertension, diabetes mellitus and dyslipidemia, which cluster as the entity "metabolic syndrome"[17-19]. This narrative review summarizes existing literature on the frequency and determinants of metabolic syndrome and its components in childhood cancer survivors (CCS).

METHODS

We searched PubMed and Embase for the following terms and synonyms: "childhood cancer survivor", "metabolic syndrome", "obesity", "insulin resistance", "diabetes", "dyslipidemia" and "hypertension".

COMPONENTS OF THE METABOLIC SYNDROME IN CHILDHOOD CANCER SURVIVORS

Overweight and adiposity

Overweight and obesity are frequently described phenomena in CCS. Overweight is defined as body mass index (BMI) ≥25 and <30 kg/m2, obesity as BMI ≥30 kg/m2. Population based, the prevalence of overweight has increased enormously over the past decades, especially in developed countries. In 2014, an estimated 1.9 billion

adults (i.e. 39% of the adult population worldwide), suffered from overweight, of which a third was obese[20]. Overweight has a negative influence on blood pressure, lipid metabolism and insulin resistance. A five kg/m^2 BMI increase has been described to be associated with a 1.5- or 2-fold risk increase for coronary heart disease, and 4- or 8-fold for diabetes mellitus[21]. Also, overweight enhances the risk of stroke (1.3-fold[22]) and of several types of cancer, e.g. postmenopausal breast, colon, thyroid, renal, endometrium and esophageal, with a relative risk of 1.12-1.59 per 5 points BMI increase[23].

Adiposity is a broader term including more accurate measurements of adipose tissue accumulation, such as waist circumference, waist/hip ratio and sometimes fat percentage or body composition (assessed by Dual-energy X-ray Absorptiometry [DXA])[24-30]. There is increasing evidence that BMI values reflect underestimations of adiposity, and that the accumulation of visceral fat as well as body composition as measured by DXA are more reliable measures for overweight to predict the development of cardiovascular disease[30-36]. However, since DXA is a time consuming, financially less attractive diagnostic test which, in addition, requires low dose radiation in children who have often already been exposed to teratogenic treatments, BMI is the most commonly used tool to study overweight.

The first reports on obesity risk after childhood cancer were published in the eighties, initiated by the impression that many survivors of childhood leukemia were overweight or obese[37]. A correlation with CRT, often associated with growth hormone deficiency (GHD), was reported, which was confirmed in consecutive studies thereafter[24, 30, 38, 39]. Subsequently, further detailed studies pointed out that the risk of overweight was especially high among female survivors and survivors diagnosed at younger age and was radiation dose- and site-dependent[40-43]. On the other hand, a recent meta-analysis in 1742 ALL survivors reported a high prevalence of overweight – 80th BMI percentile –, independent of patient and treatment characteristics[44]. Nine recently published studies performed multivariable analysis to describe independent risk factors for overweight, six of which had a cross-sectional design, and three were retrospective studies[42, 43, 45-50]. The largest is a report from the Childhood Cancer Survivor Study (CCSS), comparing self-reported overweight between 13000 survivors, after median 24 years' follow-up, and 4000 siblings in 27 participating centers in the United States and Canada[42]. Overweight rate was the same in both study groups (RR 1.0, 95% CI 0.9-1.1). Among survivors, CRT >18Gy, total body irradiation (TBI) and abdominal radiotherapy were independent risk factors for overweight. After a follow up of 24.6 years, the St. Jude Lifetime cohort, consisting of ~2000 patients that underwent late effect surveillance in the After Completion of Therapy (ACT) Clinic, showed a prevalence of obesity of 36%, with a standardized morbidity ratio of 1.14

when compared to matched controls[43]. CRT (OR 1.66) and previous glucocorticoids treatment (OR 1.37) as well as older age at evaluation were independent risk factors of becoming obese, whereas previous chest/abdominal/pelvic radiation (OR 0.48) was associated with lower obesity prevalence among survivors. In the Swiss Childhood Cancer Survivor Study, the prevalence of self-reported overweight in 2400 CCS was similar to siblings and the general population, and CRT >20Gy was an independent risk factor for overweight among survivors[51]. The three other studies with a cross-sectional design comprised between 330 and 900 survivors, and reported the following independent risk factors: brain tumor, CRT, anthracyclines, high BMI at diagnosis and Hispanic race[45-47]. In summary, in studies of highest quality, CRT is the most frequently reported independent risk factor of overweight in CCS (Table 1a).

Insulin resistance and type II diabetes mellitus

Diabetes mellitus (DM) gives rise to the risk of micro- and macrovascular damage[52, 53] (Figure 1). Type II DM (DM2) is thought to be the result of insulin resistance (IR) and (visceral) adiposity-associated chronic inflammation and, ultimately, pancreatic β-cell dysfunction[54, 55]. It is estimated that worldwide 422 million people suffer from DM. As with obesity, the prevalence of DM – especially DM2 – in the general population has increased substantially over the past decades, from 4.7% in 1980 to 8.5% in 2014[56]. As adiposity is highly associated with the development of fatty liver disease, IR and DM2[57-60], it is anticipated that adipose survivors more frequently suffer from diabetes than non-adipose survivors. In addition, some studies suggest an increased prevalence of diabetes after adjusting for obesity, e.g. due to radiotherapy[27, 61-64].

Table 1a. Obesity in childhood cancer survivors

Author	Year	N	Population	Design	Follow-up	% Overweight	% Obese	MVA	Prognostic variable
Zhang	2014	1742	ALL	1. Meta-analysis of literature 20 studies	<10y	80th percentile	n.a.	Meta-analysis	None, high prevalence of obesity among survivors independent of patient- and treatment characteristics
Moustoufi	2016	13203	CCS	2. Cross-sectional	24y Mdn (5-39y)	RR 1.0 (95% CI 0.9-1.1)	n.a.	Yes	CRT>18Gy, TBI, Abd Rtx greater risk compared with other survivors; CRT<18Gy lower risk compared to siblings; No Abd Rtx or TBI greater risk than siblings
Wilson	2015	1996	CCS	2. Cross-sectional	24.6y Mdn (10.7-48.3y)	27.9	36.2	Yes	CRT, glucocorticoids, older age at evaluation; Abdominal, chest, pelvic radiation less obesity
Van Santen	2015	893	CCS	2. Cross-sectional	14.9y M (4.7-36.2y)	23.3	n.a.	Yes	CRT, younger age at diagnosis, high BMI at diagnosis
Brown	2016	406	ALL	2. Cross-sectional	11.39y M (±5.33y)	27.6	22.2	Yes	CRT, Hispanic
Felicetti	2015	330	CCS	2. Cross-sectional	16.1y Mdn (5.1-33.0y)	n.a.	8	Yes	Brain tumor (HR 10.0), anthracyclines (HR 1.3), age at diagnosis (HR 1.12)
Prasad	2015	648 (471 <18y)	CCS	3. Retrospective	6y Mdn (2-16y) (<18y); 11.5y Mdn (2-41y) (≥18y)	10.8 (<18y); 8.5 (≥18y)	2.7 / 0	Yes	ALL, brain tumor
Gunn	2016	276	CCS	3. Retrospective	n.a.	32.3	n.a.	Yes	CRT
Lindemulder	2015	269	ALL CRT-	3. Retrospective	9.1y Mdn (4.8-13.7y)	18.1	20.9	Yes	n.a.
Essig	2014	556	ALL	2. Cross-sectional	18.4y Mdn (0.0-33.0y)	n.a.	21	No	n.a.
Stolley	2015	452	CCS	2. Cross-sectional	18.4y (±9.3)/16.7y (±6.8)/20.2 (±7.9) M	n.a.	32 / 42 / 23	No	Hispanic, Afr-American
Berdan	2014	413	CCS	2. Cross-sectional	18.5y M (±8.1y)	28.9	32.4	No	Hispanic
Brouwer	2013	277	CCS	2. Cross-sectional	18y Mdn (5-31y)	33	n.a.	No	n.a.
Nayjager	2017	75	ALL	2. Cross-sectional	15.07y Mdn (10.22-26.30y)	25.3	8	No	n.a.
Latoch	2016	75	CCS	2. Cross-sectional	12.15y Mdn (1-23.5y)	29.3	n.a.	No	n.a.

Author	Year	N	Population	Design	Follow-up	% Overweight	% Obese	MVA	Prognostic variable
Siviero	2013	56	ALL	2. Cross-sectional	8.5y M (±3.9y)	n.a.	3.6	No	n.a.
Murphy	2015	53	CCS	2. Cross-sectional	Range 3.2-14.4y	0	n.a.	No	n.a.
Jahnukainen	2015	49	ALL male	2. Cross-sectional	20y Mdn (10-29y)	n.a.	15	No	Cranial/testicular radiation (NS)
Van Dorp	2013	191	CCS female	3. Retrospective	18.8y Mdn (2.3-48.8y)	13	10	No	n.a.
Shalitin	2014	139	Non-brain solid	3. Retrospective	9y Mdn (1.2-29.5y)	n.a.	1.4	No	n.a.
Harper	2013	27	ALL RTx-	3. Retrospective	6y	40.7	n.a.	No	n.a.

Highest quality evidence above dashed line, based on design (1 = meta-analysis, 2 = cross-sectional, 3 = retrospective) and multivariable analysis. CCS = (childhood) cancer survivors (heterogeneous group); ALL = acute lymphoblastic leukemia; RTx = radiotherapy; RR = relative risk; CRT = cranial irradiation

Table 1b provides an overview of recent literature on IR and DM in CCS. Twenty years ago, the first reports on an increased risk of DM after abdominal radiation in survivors of Wilms tumor were published[65, 66], suggesting a damaging effect of radiation to the pancreas. From a cross-sectional study in ~8600 survivors by Meacham, the prevalence of self-reported DM after 23.5 years of follow-up was 2.5% in survivors and 1.7% in siblings ($p<0.01$). Among survivors, this was explained in particular by TBI (OR 7.2), abdominal radiotherapy (OR 2.7), alkylating agents (OR 1.7) and younger age at diagnosis (OR 2.4). No association was found with CRT and corticosteroids[62]. Holmqvist retrospectively reported hospitalizations for DM in a large cohort of ~33000 survivors, ten years after diagnosis. The observed hospitalization rate was 1.6 times higher than expected and especially high in survivors treated with radiotherapy, i.e. Wilms tumor (OR 2.9), leukemia (2.0), CNS tumor (1.8), germ-cell tumor (1.7) and bone tumor (1.7)[67]. A large cross-sectional study in ~1000 adult survivors treated with HSCT also revealed TBI as an independent risk factor for DM (OR 3.42)[61]. A study in 750 pediatric HSCT treated survivors added asparaginase toxicity, defined as hyperglycemia and/or pancreatitis, as an independent risk factor[68], and a prospective study in 250 CCS reported TBI and hypogonadism as independent risk factors[27]. Chao found no significant increase in DM frequency in 650 survivors compared to 6520 non-cancer controls[69]. In summary, several studies investigated DM in large cohorts of cancer survivors, and radiotherapy – total body as well as abdominal – seems to be the most frequently reported independent risk factor.

The link with damage to the pancreas by radiotherapy was closely investigated by De Vathaire[63]. Radiation to the pancreatic tail, where the majority of insulin-secreting Langerhans islets is located, increased the risk of diabetes in a dose-dependent way (RR at 1 Gy 1.61), whereas the radiation dose to the head or body had no significant effect. A similar dose-dependent relation between radiation to the pancreatic tail and the occurrence of DM was found in adult Hodgkin lymphoma survivors[70]. In our study in nephro- and neuroblastoma survivors, radiotherapy to the whole pancreas increased the risk of IR, compared to controls and to radiation to parts of the pancreas[35]. A study in ALL survivors reported lower pancreatic volume and insulin secretion after TBI, suggesting a reduced beta cell reserve[71]. Apart from pancreatic radiation damage impairing insulin secretion, it might be that radiotherapy impairs fat cell expansion, which increases liver steatosis and circulation of free fatty acids (FFA), subsequently causing IR and DM. In mice, it has been shown that adipose tissue fibrosis restricts adipocyte enlargement and is associated with local inflammation and systemic IR[72, 73]. Whether these biological mechanisms determine the higher MetS risk in abdominally irradiated cancer survivors as well, needs to be investigated.

Table 1b. Insulin resistance and diabetes mellitus in childhood cancer survivors

Author	Year	N	Population	Design	Follow-up	% IR	% DM	MVA	Prognostic variable
Holmqvist	2014	32903	CCS	3. Retrospective	10y Mdn (0-42y)	n.a.	1.5, SHRR 1.6	Yes	Wilms', leukemia, CNS, germ-cell, bone, HL
Meacham	2009	8599	CCS	2. Cross-sectional	23.5y M (16.0-35.2y)	n.a.	2.5	Yes	TBI (OR 7.2), Abd Rtx (OR 2.7), alkylating agent (OR 1.7), young age at diagnosis (OR 2.4); no association: CRT, corticosteroids, asparaginase
Baker	2007	1089	HSCT (adult)	2. Cross-sectional	8.6y M (±5.1y)	n.a.	7.6	Yes	TBI (OR 3.42)
Hoffmeister	2004	748	HSCT	3. Retrospective	11y Mdn (2.0-30.0y)	n.a.	4.5	Yes	CML (HR 85.0), AML/ALL (HR 13.0), asparaginase toxicity
Chao	2016	652	CCS	2. Cross-sectional	6.2y M (±4.1y)	n.a.	1.1 vs 0.05; 1.8/1000p-yr; adjusted IRR 1.6 (diabetes) (NS)	Yes	n.a.
Neville	2006	248	CCS	1. Prospective	12.9y Mdn (2.3-33.6y)	6.9 (IGT)	9.7	Yes	TBI, hypogonadism
Latoch	2016	75	CCS	2. Cross-sectional	11.8y M (±5.2y)	1.33	n.a.	Yes	(BMI)
Moustoufi	2016	14290	CCS	2. Cross-sectional	24y Mdn (5-39y)	n.a.	RR 1.8 (95% CI 1.4-2.3)	No	TBI, CRT
Kero	2016	2530	CCS	3. Retrospective	10.4y Mdn (0-18y)	n.a.	HR 3.0	No	ALL, AML, CNS
De Vathaire	2012	2520	CCS	3. Retrospective	?	n.a.	2.6	No	Pancreatic tail radiation
Van Waas	2013	532	CCS	3. Retrospective	17.9y median (5.0-48.8y)	n.a.	0.9	No	Wilms', Abd Rtx
Felicetti	2015	330	CCS	2. Cross-sectional	16.1y Mdn (5.1-33.0y)	n.a.	1.5	No	n.a.
Wilhelmsson	2014	204	HSCT	3. Retrospective	12y Mdn (4-28y)	n.a.	9	No	n.a.

Table 1b. Continued

Author	Year	N	Population	Design	Follow-up	% IR	% DM	MVA	Prognostic variable
Shalitin	2014	139	Non-brain solid	3. Retrospective	9y Mdn (1.2-29.5y)	1.4 (IGT)	n.a.	No	n.a.
Gunn	2016	62	CCS	3. Retrospective	18.0y M (6.8-37.9y)	32.3	n.a.	No	n.a.
Wei	2016	35	A(L/M)L + HSCT	2. Cross-sectional	12.5y Mdn (3.2-18.2y)	34.2 (IGT)	17.1	No	n.a.
Cohen	2014	24	HR NBL + SCT	3. Retrospective	6.1y Mdn (1.0-15.2y)	45.9 (HbA1c)	4.2	No	n.a.

Highest quality evidence above dashed line, based on multivariable analysis, patient number and design (1 = prospective, 2 = cross-sectional, 3 = retrospective). (H)SCT = (hematologic) stem cell transplantation; A(L/M)L = acute lymphoblastic/myeloid leukemia; NBL = neuroblastoma; IGT = impaired glucose tolerance; HR = hazard ratio; TBI = total body irradiation; HL = Hodgkin lymphoma; Abd Rtx = abdominal radiotherapy.

Dyslipidemia

Classic parameters of dyslipidemia include elevated fasting levels of total cholesterol and low-density lipoprotein cholesterol and triglycerides, and low levels of high-density lipoprotein cholesterol. These alterations in lipid metabolism are associated with cardiovascular disease[74-76]. Adipose tissue plays an important causal role in the occurrence of dyslipidemia through the release of FFA, which leads to increased triglyceride and very low-density lipoprotein cholesterol production in the liver[77]. Hence, cancer survivors with an increased risk of overweight carry an increased risk of dyslipidemia as well. Hypogonadism following cancer therapy can cause dyslipidemia directly as well; this was observed in survivors of adult testicular cancer[78, 79], breast cancer treated with aromatase inhibitors[80] and prostate cancer treated with LHRH-agonists[81].

As depicted in Table 1c, the rate of dyslipidemia in CCS varied greatly and different outcome measures are reported. Only one study reported independent risk factors for dyslipidemia in CCS. In 330 survivors, after 16.1 years of follow-up, age at diagnosis (HR 1.1), TBI (2.7), GHD (2.3) and autologous SCT (3.2) were independent risk factors for hypercholesterolemia, and TBI (6.5) and GHD (7.2) were also independent risk factors for hypertriglyceridemia[47]. Chao studied dyslipidemia in 650 survivors and reported a higher risk (incidence rate ratio 1.9) compared to controls, but no specific prognostic variables were identified in multivariable analysis[69]. In the CCSS the incidence of dyslipidemia was 8.9%, compared to 6.0% in siblings; this increased to a significant difference at age 50 (23.0 vs 13.6%), whereas the obesity rate at older age in this cohort was significantly higher among siblings[7]. In a large Finnish cohort of ~2500 survivors, the rate of dyslipidemia, defined as the purchase of lipid-lowering drugs, was 4.3 times higher than in siblings[82].

Table 1c. Dyslipidemia in childhood cancer survivors

Author	Year	N	Population	Design	Follow-up	% Dyslipidemia	MVA	Prognostic variable
Chao	2016	652	CCS	2. Cross-sectional	6.2y M (±4.1y)	1.8 vs 0.8; 3/1000 p-yr; adjusted IRR 1.9	Yes	n.a.
Felicetti	2015	330	CCS	2. Cross-sectional	16.1y Mdn (5.1-33.0y)	20% hyperchol., 6% hypertrigl.	Yes	Hyperchol: age at diagnosis (HR 1.1), TBI (HR 2.7), GHD (HR 2.3), auto-HSCT (HR 3.2); hypertrigl: TBI (HR 6.5), GHD (HR 7.2)
Armstrong	2013	10724	CCS	3. Retrospective	25.6y Mdn (7.4-39.3y)	8.9	No	n.a.
Kero	2016	2530	CCS	3. Retrospective	10.4y Mdn (0-18y)	HR 4.3	No	n.a.
Brouwer	2013	277	CCS	2. Cross-sectional	18y Mdn (5-31y)	33 (hyperchol.)	No	n.a.
Wilhelmsson	2014	204	HSCT	3. Retrospective	12y Mdn (4-28y)	7	No	n.a.
Staba	2013	168	CCS	2. Cross-sectional	11.4y M (±6.3y)	16	No	n.a.
Shalitin	2014	139	Non-brain solid	3. Retrospective	9y Mdn (1.2-29.5y)	10.9	No	n.a.
Morel	2017	80	ALL	2. Cross-sectional	12.4y M (±0.71)	50	No	n.a.
Gunn	2016	72	CCS	3. Retrospective	18.0y M (6.8-37.9y)	50	No	n.a.
Wei	2016	35	A(L/M)L + HSCT	2. Cross-sectional	12.5y Mdn (3.2-18.2y)	62.9 (highTRI), 34.3 (lowHDL)	No	n.a.

Highest quality evidence iabove dashed line, based on design (2 = cross-sectional, 3 = retrospective) and multivariable analysis. GHD = growth hormone deficiency.

Hypertension

Arterial hypertension is a condition in which blood pressure is persistently raised, defined as ≥140mmHg systolic or ≥90 diastolic. Globally, the overall prevalence of hypertension in the general population aged 25 and over has been reported to be around 40%[83]. The availability of low-cost medication has significantly decreased the occurrence of hypertension to e.g. 18% in the USA[83, 84]. Hypertension is a major risk factor for coronary heart disease and ischemic as well as hemorrhagic stroke, being responsible for ~50% of deaths due to these diseases[85]. In addition, blood pressure level as continuous variable has been shown to be related to the risk of stroke, coronary heart disease, heart failure, peripheral vascular disease, renal impairment and retinal hemorrhage[86-90].

Already in 1989, Kantor described hypertension in 20% of long-term survivors of childhood renal cancer[91]. According to a Cochrane review by Knijnenburg, prevalence of hypertension in childhood cancer survivors ranges from 0% to 18.2%[92]. Three reports thereafter showed even higher prevalence, and one of these observed a sharp increase with age, exceeding 70% by age 50[50, 93, 94] (Table 1d). In most case control studies, survivors reveal relatively high hypertension rates[7, 10, 82, 94]. A study in ~650 survivors found no significant difference between survivors and controls, but this was a study with a rather short follow-up time of 6 years[69]. In the CCSS, the presence of hypertension significantly increased the risk of major cardiac events and cardiac-specific mortality[7]. The aforementioned Cochrane review included 24 studies with ~4000 survivors in total, and a high BMI was the only consistent independent risk factor for hypertension reported in multiple studies. Other reported independent risk factors are the use of total body or abdominal irradiation, nephrectomy, acute kidney injury, SCT, growth hormone therapy, older age at screening and male sex[50, 92, 94].

Hypertension in CCS may be caused by direct kidney damage through irradiation[95]. Unilateral nephrectomy is known to induce hyperfiltration in the remaining kidney, which may give rise to hypertension[96]. Ifosfamide and cisplatin have nephrotoxic side effects[93, 97, 98], but hypertension is not reported as a consequence of these agents; one study reported a non-significant risk increase[99]. In the general adult population, it is known that treatment of hypertension towards below 140/90mmHg is associated with a reduction in cardiovascular complications[100]. This suggests that identification and treatment of subclinical hypertension in childhood cancer survivors by standard surveillance may decrease morbidity and mortality[93, 94].

Table 1d. Hypertension in childhood cancer survivors

Author	Year	N	Population	Design	Follow-up	% Hypertension	MVA	Prognostic variable
Knijnenburg	2013	4073	CCS	1. Cochrane review	Various	0-18.2%	Yes	Higher BMI, TBI, abdominal radiation, acute kidney injury, stem cell transplantation, growth hormone therapy, older age at screening and male gender
Gibson	2017	3016	CCS	2. Cross-sectional	>10y	22.4	Yes	Older age, nephrectomy (OR 1.68)
Baker	2007	1089	HSCT (adult)	2. Cross-sectional	8.6y M (±5.1y)	18.5	Yes	Allogeneic transplant (OR 2.31)
Chao	2016	652	CCS	2. Cross-sectional	6.2y M (±4.1y)	1.5 vs 1.0; 2.5/1000p-yr; adjusted IRR 1.2 (NS)	Yes	n.a.
Gunn	2016	269	CCS	3. Retrospective	n.a.	19.0	Yes	Male sex, older age, overweight/obesity
Armstrong	2013	10724	CCS	3. Retrospective	25.6y Mdn (7.4-39.3y)	14.9	No	n.a.
Van Laar	2014	3247	CCS	3. Retrospective	>5y	7.75 vs 2.57 / 10.000p.y.	No	n.a.
Kero	2016	2530	CCS	3. Retrospective	10.4y Mdn (0-18y)	HR 4.6	No	ALL, bone tumor
Dekkers	2013	763	CCS	2. Cross-sectional	18.3y Mdn (5.0-58.2)	23.4	No	Renal tumor (31.4%), Abdominal radiation
Essig	2014	556	ALL	3. Retrospective	18.4y Mdn (0.0-33.0)	13	No	n.a.
Felicetti	2015	330	CCS	2. Cross-sectional	16.1y Mdn (5.1-33.0y)	5.3	No	n.a.
Brouwer	2013	277	CCS	2. Cross-sectional	18y Mdn (5-31y)	14	No	n.a.
Wilhelmsson	2014	204	HSCT	3. Retrospective	12y Mdn (4-28y)	7	No	n.a.
Shalitin	2014	139	Non-brain solid	3. Retrospective	9y Mdn (1.2-29.5y)	8.6	No	n.a.
Interiano	2015	75	Wilms	2. Cross-sectional	19.6y Mdn (10.0-32.8)	6.7	No	n.a.
Wei	2016	35	A(L/M)L + HSCT	2. Cross-sectional	12.5y Mdn (3.2-18.2y)	17	No	n.a.

Highest quality evidence above dashed line, based on design (1 = Cochrane review, 2 = cross-sectional, 3 = retrospective) and multivariable analysis. IRR = incidence rate ratio.

THE METABOLIC SYNDROME IN CHILDHOOD CANCER SURVIVORS

Definition

Metabolic syndrome is a cluster of adiposity, IR, dyslipidemia and hypertension[101-103]. It was first described by Reaven in 1988, who found a clustering of symptoms in patients and called this Syndrome X[104]. The symptoms of this cluster are related and interacting in various ways. In general, imbalance in energy intake and consumption results in increased (visceral) adiposity. Secondary effects of adiposity include increased circulating FFA and reduced adiponectin – thus, an increase in IR factors – and increased pro-inflammatory and pro-thrombotic mediators such as IL-6, TNF-alpha and PAI-1. Increased lipid flux into the liver can result in steatosis, which also mediates IR. The liver also produces fibrinogen, enhancing the pro-thrombotic state. IR in liver and muscle leads to hyperinsulinemia, with a result of adipose tissue growth and tissue resistance to insulin. Hyperinsulinemia also contributes to hypertension through enhanced sodium resorption and sympathetic nervous system activation[105, 106]. It is estimated that 20-25% of the world's adult population suffers from MetS[102] and, consequently, are three times more likely to have a heart attack or stroke and twice as likely to die from cardio- and cerebrovascular disease, compared to people without MetS. In addition, patients with MetS are five times more likely to develop DM2 and people with diabetes are three times more likely to develop cardiovascular disease[102, 107, 108] (Figure 1). Metabolic syndrome is also associated with fatty liver disease, gallstones, hepatocellular carcinoma, chronic kidney disease and polycystic ovary syndrome[109-115].

Currently, three definitions of metabolic syndrome are commonly used: those created by the World Health organization (WHO)[103], National Cholesterol Education Program – Third Adult Treatment Panel (NCEP/ATPIII)[101] and the International Diabetes Foundation (IDF)[102] (Supplemental table 2). Although the definition of MetS is based on the principle of clustered components, these components themselves are also independent risk factors for the development of cardiovascular disease[105]. The prevalence of MetS can vary, depending on which definition is used. In young adults, who less frequently meet all MetS criteria, partial clustering of risk factors should be examined. The MetS definitions provide useful guidelines to identify those individuals at risk for development of DM2, atherosclerotic cardiovascular disease, and cardiovascular death. MetS is a "disguised" syndrome; without measurement of blood pressure and lipids, metabolic sequelae can develop for years. This underlines the need for active surveillance.

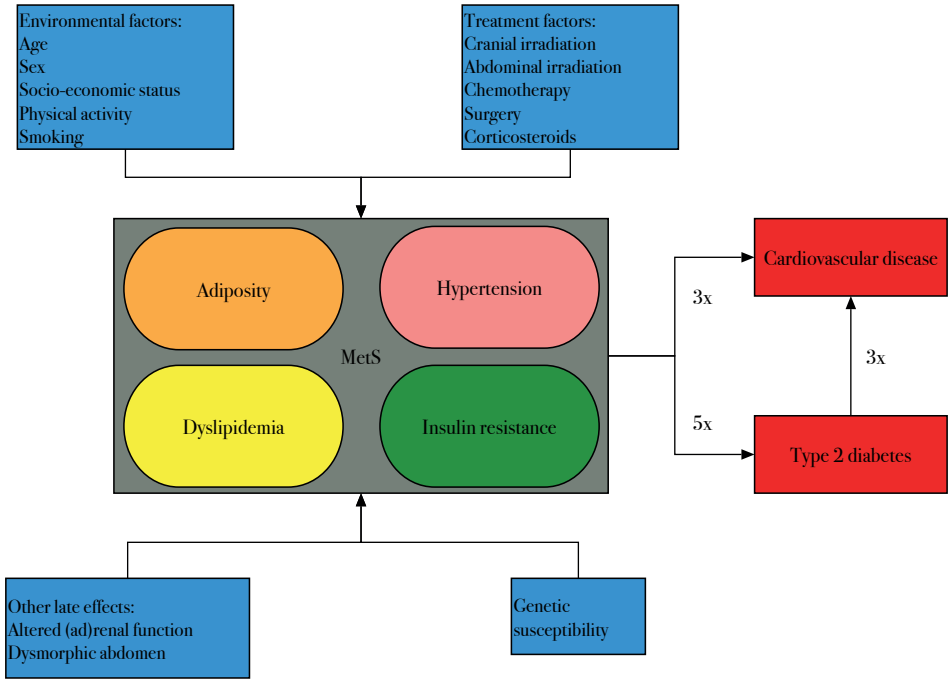

Figure 1. Metabolic syndrome in childhood cancer survivors and the risk of cardiovascular disease
The components of the metabolic syndrome, risk factors for developing the syndrome and the risk for metabolic syndrome patients to develop cardiovascular disease and type 2 diabetes.

Risk and determinants

Several studies have focused on the development of MetS in CCS. Comparison of these studies is hampered by the fact that often small patient groups are analyzed, the heterogeneity of malignancies as well as therapies and the different definitions of MetS that are used. An overview of existing literature on the frequency of MetS and prognostic factors in CCS is presented in Table 2. The first study on this subject was by Talvensaari, reporting a prevalence of 16% in 50 survivors, compared to none of the controls[116]. Since then, reported frequencies of MetS in CCS vary between zero and 39 percent[17, 35, 48, 116-134].

Our literature search retrieved twenty-two studies, six of which performed multivariable analyses in search of risk factors for developing MetS. Only three out of six had a prospective study-design. These were all reports from the French LEA program, a cohort of acute leukemia survivors[117-119]. MetS occurred in 6.9-17.1% of the survivors. In the first study, HSCT with TBI as conditioning regimen was the

only risk factor for metabolic syndrome (OR 3.9). In the second study, TBI was not a risk factor for MetS, nor were gender, total post-transplant steroid dose and follow-up duration. The only risk factor was higher BMI at time of transplantation (OR 1.57). In the third study, male sex (OR 2.64), older age at evaluation and higher BMI at diagnosis were risk factors for MetS, whereas CNS irradiation was not. The three other studies with multivariable analyses had a cross-sectional or retrospective study design. The largest investigated MetS in 784 ALL survivors in the St. Jude Lifetime Cohort, compared to 777 healthy controls[120]. Metabolic syndrome was present in 33.6 percent of survivors (RR 1.43). Risk factors in multivariable analyses were CRT, especially with craniospinal radiation (RR 1.88), and older age at evaluation. Steroid dose was not a risk factor. A smaller study in 74 ALL survivors also revealed HSCT (OR 22.99) as risk factor for MetS[121]. In a large, retrospective study in 648 Indian childhood cancer survivors, not one patient fully met all the criteria for MetS[48]. Only when overweight patients were included (next to obese patients), prevalence was 2.4% for underage survivors and 9.6% for survivors aged 18 years and older. It should be mentioned that follow-up in this study was short (6 and 11.5 years median for survivors below and over 18 years, respectively).

Of the remaining sixteen studies that our search yielded, three had a prospective design. The largest described MetS in a single center cohort of 103 nephro- and neuroblastoma survivors[35]. Survivors had more components of MetS than healthy controls (OR 5.2 in nephroblastoma, 6.5 in neuroblastoma) and frequency was three times higher in patients who received abdominal irradiation (28% vs 9%). A small study in 21 AML survivors reported SCT as risk factor for having more MetS components than healthy controls (OR 24.1), whereas chemotherapy only was not a risk factor[123]. In the third study, none of the 45 survivors of a hematological malignancy treated with HSCT had MetS[122], but this was also a study with a short follow-up time. Risk factors described in the other studies include cranial[17, 125, 132] and abdominal radiation[132], while the other studies found no significant prognostic variables or did not perform this analysis.

Summarizing the studies with the highest quality of data, the following prognostic variables were risk factors for developing the MetS in CCS: the treatment components HSCT, CRT, TBI (although not all studies support this finding) and abdominal radiation, and the patient characteristics male sex (not in all studies), higher BMI at diagnosis or time of transplantation, and older age at evaluation.

Table 2. Metabolic syndrome in childhood cancer survivors

Author	Year	N	Population	Design	Follow-up	MetS definition	% MetS	MVA	Prognostic variable	Control group
Saultier	2016	650	A(L/M)L HSCT-	1. Prospective	16y mean (±6.79y)	NCEP ATP III	6.9	Yes	Male, older age and high BMI at diagnosis	n.a.
Oudin	2011	184	A(L/M)L	1. Prospective	15.4y median (3.4-30.2y)	NCEP ATP III	9.2	Yes	HSCT-TBI	n.a.
Oudin	2015	170	A(L/M)L HSCT+	1. Prospective	14.5y mean (±6.1y)	NCEP ATP III	17.1	Yes	BMI at time of transplantation	n.a.
Nottage	2014	784	ALL	2. Cross-sectional	26.1y median (11-45.3y)	NCEP ATP III	33.6, RR 1.43	Yes	CRT	777 NHANES
Chow	2010	74	ALL	2. Cross-sectional	10y/10.5y median (1-18y)	IDF / NCEP ATP III	10.8	Yes	TBI-HSCT, CRT, positive family history	n.a.
Prasad	2015	648 (471 <18y)	CCS	3. Retrospective	6y median (2-16y) (<18y); 11.5y median (2-41y) (≥18y)	IDF	0	Yes	n.a.	
Van Waas	2012	103	Nephro-/ neuroblast.	1. Prospective	26.2y median (6.4-48.9y)	NCEP ATP III	10 vs 5 (?)	No	Abd RTx	61 HC
Bizzarri	2015	45	Hemat. malig. w. HSCT	1. Prospective	4y mean (±3.2y)/6.9y (±3.1y)	(modified) NCEP ATP III	0	No	n.a.	90 HC
Blijdorp	2013	21	AML	1. Prospective	20y median (9-31y)	NCEP ATP III	10 vs 6.3	No	SCT	48 HC
Smith	2014	1598	CCS	2. Cross-sectional	25.6y mean (±7.6y)	NCEP ATP III	31.8	No	n.a.	n.a.
Van Waas	2010	500	CCS	2. Cross-sectional	19y median (6-49y)	Modified NCEP ATP III	13	No	CRT, ALL, male	MORGEN
Trimis	2007	80	ALL	2. Cross-sectional	6.37 median/5.9y mean (1.1-12.2y)	NCEP ATP III / WHO	11	No	Cranial radiation	n.a.
Mohapatra	2016	76	ALL	2. Cross-sectional	3y median (IQR 2.3-5y)	IDF / NCEP ATP III	5.2	No	n.a.	n.a.
Gurney	2006	75	ALL	2. Cross-sectional	24.6y mean (±4.8y)	NCEP ATP III	16.6 vs 17.5 (NS)	No	n.a.	730 Healthy NHANES
Aldhafiri	2012	56	ALL	2. Cross-sectional	6.2y mean (±3.9y)	IDF / NCEP ATP III	7.1 / 5.4	No	n.a.	n.a.
Talvensaari	1996	50	CCS	2. Cross-sectional	12.6y ave (7.9-21.3y)	Obesity, ↑insulin, ↓HDL	16 vs 0	No	n.a.	50 HC

Table 2. Continued

Author	Year	N	Population	Design	Follow-up	MetS definition	% MetS	MVA	Prognostic variable	Control group
Karakurt	2013	44	ALL	2. Cross-sectional	5.4y mean (3-10y)	IDF	6.8	No	n.a.	Family
Hoffman	2008	32	Sarcoma	2. Cross-sectional	17.3y median (2.9-32.6y)	NCEP ATP III	33	No	n.a.	U.S. population data
Taskinen	2007	31	SCT	2. Cross-sectional	6y median (1-20y)	WHO	39	No	n.a.	n.a.
Van Waas	2013	532	CCS	3. Retrospective	17.9y median (5.0-48.8y)	Modified NCEP ATP III	15	No	CRT, Abd RTx	n.a.
Ness	2005	486	Adult CS	3. Retrospective	n.a.	NCEP ATP III	25.8 vs 18.4	No	Breast cancer history	12,526 HC
Kojima	2013	49	CCS	3. Retrospective	5.1y median (3.0-14.6y)	Japanese criteria	6.1	No	n.a.	n.a.

Highest quality evidence above dashed line, based on design (1 = prospective, 2 = cross-sectional, 3 = retrospective) and multivariable analysis.

PATHOPHYSIOLOGY OF THE METABOLIC SYNDROME IN CHILDHOOD CANCER SURVIVORS

Growth hormone deficiency

Disease as well as treatment, i.e., respectively, brain tumors, CRT[135-139] and brain surgery[140, 141], but also TBI[135, 138, 142] and chemotherapy[143, 144] can damage the hypothalamus and pituitary gland, which leads to several endocrine disorders, the most common being GHD[136, 145, 146]. GHD induces the components of the metabolic syndrome, as shown in several studies: adiposity[147-150], insulin resistance[151, 152], dyslipidemia[149, 153, 154] and hypertension[149, 154, 155]. A recent study in CCS associated GHD with the development of clusters of three or more cardiovascular risk factors[155]. GHD has also been linked to endothelial dysfunction and atherosclerosis[156, 157] and to decreased left ventricular ejection fraction[158], further increasing the risk of cardiovascular complications. Schneider et al. reported an increased ten-year risk of cardiovascular events in ~350 GHD patients compared to healthy controls (4.6% vs. 3.7%)[159].

The hypothalamus, rather than the pituitary gland, is regarded as the primary site of radiation damage[136, 160, 161]. The somatotropic axis is affected first, followed by the gonadal axis, and, least sensitive, the thyroid and adrenal axis[136, 137, 162]. After radiotherapy growth hormone secretion may gradually and irreversibly decrease over the course of years in a dose-dependent manner; at 16 Gy the risk of developing GHD five year off treatment is 50%[163]. The most relevant radiotherapy threshold is not clear: other reported thresholds are 18 Gy[42], 22 Gy[137] and 30 Gy[162]. In a meta-analysis by Mulder the pooled prevalence of GHD after cranial radiation was 35.6%[164].

In non-cancer survivors with GHD, it has been shown that growth hormone replacement has positive effects on cardiac function, cardiovascular risk factors such as body composition, lipid levels and blood pressure, and on the occurrence of cardiovascular events[159, 165-169]. On the other hand, a large study in ~2500 growth hormone deficient adults found no decrease in the prevalence of MetS after three years of replacement[170], and Claessen even reported a substantial increase in MetS after ten years of treatment in 98 patients, from 32.7% to 57.1%[171]. Unfortunately, there is only scarce literature on growth hormone replacement in CCS. Furthermore, clinical interpretation of these studies is commonly complicated by methodologic shortcomings such as lack of a control group and the use of surrogate markers instead of cardiovascular morbidity and mortality. A small study in eighteen ALL survivors with GHD on two years' replacement therapy reported improved cardiac systolic function and reduced incidence of metabolic syndrome[172]. Another small

study with eleven ALL survivors on twelve months' growth hormone replacement reported positive effects on fat mass and fat free mass, but hyperleptinemia and insulin resistance remained unaffected[173]. Van den Heijkant found higher lean mass and lower percentage fat after two years of therapy in 14 ALL survivors[174], and Murray reported beneficial effects on waist-hip ratio, cholesterol and triglycerides in 27 ALL and brain tumor survivors after twelve months' therapy[175].

Gonadal impairment, thyroid morbidity and adrenal insufficiency

The production of other pituitary hormones, and damage to other endocrine end organs seems to be less frequently affected after childhood cancer and therapies. CRT >30Gy causes long-term central hypogonadism in 20-30% of survivors[162], and regimens harming the gonads can be causative factors as well[176-179]. Mainly tested in men, hypogonadism is reported to contribute to MetS and vice versa[180-184]. A few studies associated gonadal impairment with MetS traits in CCS[27, 130, 185-187]. A recent meta-analysis reported that testosterone supplementation in men with testosterone deficiency syndrome had positive effects on body weight and composition and glucose and lipid metabolism[188]. There are no studies available that investigated the effect of sex hormone therapy on the metabolic syndrome in CCS, so far.

Cranial radiation doses of 30Gy and higher cause central hypothyroidism in 3-9% of survivors[162]. Thyroid malignancies (although rare in children) or neck and mantle radiation for other cancer types damage the thyroid and lead to primary hypothyroidism[189, 190]. Metabolic manifestations of hypothyroidism include adiposity, hypertension (due to an increase in peripheral vascular resistance) and dyslipidemia[191-194]. As in the normal population, hypothyroidism after childhood cancer is treated with levothyroxine. Although it is anticipated that levothyroxine treatment has positive effects on the metabolic profile of CCS, no studies have investigated this yet.

Adrenal insufficiency occurs in 3-6% of patients receiving >30Gy CRT[162] and can also temporally be caused by high-dose steroid treatment. Hypocortisolism in itself is not associated with MetS features, but treatment with corticosteroids – especially dexamethasone – is notorious for causing short term adiposity, IR and diabetes[195, 196]. It is conceivable that the use of glucocorticoids in childhood cancer treatment can have these consequences on long term as well. Van Beek showed that treatment with prednisone or dexamethasone is associated with long term increases in BMI and body fat in ALL and Hodgkin lymphoma survivors[26, 197].

General fitness

Another potential mechanism for the development of MetS in CCS is physical inactivity, as this promotes obesity and IR. In the St. Jude Lifetime cohort, 28% of survivors were found not to adhere to lifestyle guidelines. Males and females who did not follow these guidelines were approximately twice more likely to have MetS[124]. Similarly, Warner reported total energy expenditure and physical activity to be lower in 34 ALL survivors compared to 21 survivors of other childhood malignancies, and to healthy controls. This was negatively associated with percentage body fat, but it remains the question whether this is either a cause or a consequence[198]. Additionally, we showed that especially male neuroblastoma survivors might be at risk for reduced physical activity[199]. Visual impairment after certain brain tumors may enhance MetS risk, due to the reduced ability to perform physical activity. For example, in a study in 178 childhood- and adult-onset craniopharyngioma survivors, visual impairment was a borderline significant independent risk factor for MetS[200]. To date, it is not entirely clear yet whether reduced physical activity and sedentary lifestyle play a causative role in development of MetS. However, as it is one of the few modifiable factors that might decrease MetS, it is of great value to initiate intervention studies with regard to physical activity in CCS.

Genetic susceptibility

The role of genetic susceptibility in the development of MetS and cardiovascular disease in childhood cancer survivors has not been extensively studied yet. In our cohort of 532 survivors, we used a candidate gene approach, containing genes previously associated with components of the metabolic syndrome, i.e. JAZF1, THADA, IRS1, TFAP2B, MSRA and ATP2B1[132]. None of the allelic variants was associated with metabolic syndrome, indicating that treatment factors were more dominant than genetic variation. England et al. performed whole-exome sequencing in 209 ALL survivors and reported that variants in BAD and FCRL3 genes were associated with a phenotype of three or more cardiometabolic risk factors[201]. In the St. Jude Lifetime cohort, a genome-wide association study (GWAS) identified single nucleotide polymorphisms associated with obesity in the following genes: FAM155A, which is expressed in the hypothalamus and pituitary, and GLRA3, SOX11 and CDH18, which are involved in neural growth, repair and connectivity[43]. To date, these findings have not been validated, nor has GWAS been performed to identify genetic variants associated with diabetes, dyslipidemia, hypertension and MetS in CCS[202].

SUMMARY AND FUTURE PERSPECTIVE

After almost 25 years of research on childhood cancer survivors, we have gained knowledge on potential late effects, of which the metabolic syndrome so far has been rather disguised. Many CCS are already at risk for cardiovascular disease, for example, due to anthracycline- or radiation-induced cardiotoxicity[203-205]. Additionally, they face an additive risk after CRT, causing GHD and MetS. The role between MetS and other risk factors, such as abdominal radiation, specific chemotherapeutic agents, steroids, gonadal impairment, thyroid morbidity and genetics, warrants further investigation. It is however clear that specific groups of CCS are at higher risk of developing components of the MetS (Figure 1), which underlines the need for close monitoring. These survivors might benefit from early interventions targeting overweight, hypertension and dyslipidemia, for instance lifestyle and diet advice and medication[105]. Since MetS is a cluster of symptoms with heterogeneous presentation among individuals, medical treatment requires a personalized approach[105].

In our opinion, future research may focus on the following three topics: 1) Unravelling the pathophysiologic mechanism of the development of the MetS in specific CCS subgroups, 2) Determining which subgroups of CCS are at risk to develop (components of) MetS by using prediction models, and 3) Determining which preventive and therapeutic interventions are successful in targeting the MetS in CCS – favourably multiple components with the same intervention. As childhood cancer is relatively rare, research will benefit from collaborations between (inter)national cohorts, to enhance effect size and for replication purposes.

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Supplemental table 1. Epidemiological studies on cardiovascular disease in childhood cancer survivors

Author	Year	N	Population	Outcome	Result
Reulen	2010	17.981	CCS	Death due to circulatory disease	SMR 4.0 (95% CI 3.4-4.6)
Tukenova	2010	4.122	CCS	Death due to cardiovascular disease	SMR 5.0 (95% CI 3.3-6.7)
Castellino	2011	2.742	Hodgkin	Death due to cardiovascular disease	EAR 13.1/10,000py
Prasad	2012	9.245	CCS	Death due to cardiovascular disease	SMR 1.9 (95% CI 1.5-2.3)
Armstrong	2013	10.724	CCS	Coronary artery disease	Cum. inc. 5.3% (95% CI 4.4-6.1%)
Perkins	2013	3.627	CNS tumor	Death due to cardiovascular disease	SMR 2.5 (95% CI 1.2-4.8)
Kero	2014	13.860	CCS	Cardiovascular hospitalizations	HR 3.4 (95% CI 2.3-5.1) / 3.3 (95% CI 1.7-6.5)
Van Laar	2014	3.247	CCS	Cardiovascular hospitalizations	RR 2.6 (95% CI 1.9-3.6) / 1.2 (95% CI 0.9-1.5)
Olsen	2014	2.243	CCS	Cardiovascular hospitalizations	HR 2.5 (95% CI 2.1 - 2.9)
Kero	2015	16.769	CCS	Death due to cardiovascular disease	SMR 1.9 (95% CI 1.7-2.1)
Gudmundsdottir	2015	32.308	CCS	Cardiovascular hospitalizations	RR 2.1 (95% CI 2.0-2.2), EAR 324/100,000py
Bhakta	2016	348	Hodgkin	Cardiovascular disease	Cum. burden 100.8 vs 17.0/100 survivors
Schindler	2016	3.965	CCS	Death due to circulatory disease	SMR 12.7 (95% CI 7.8-20.7)
Kero	2016	8.197	CCS	Cardiovascular medication	HR 7.2 (95% CI 5.1-10.1)

SMR = standardized mortality ratio; EAR = excess adverse risk.

Supplemental table 2. WHO, NCEP-ATP III and IDF criteria for metabolic syndrome

		WHO	NCEP-ATP III	IDF
Required for diagnosis		IR + ≥2 others	≥3	Adiposity + ≥2 others
Adiposity	BMI (kg/m^2)	>30	-	-
	Waist (cm)	-	>102*/88**	≥94*/80**
	Waist/hip	>0.90*/0.85**	-	-
Insulin resistance	Fasting glucose (mmol/L)	≥5.5 or DM2	≥5.5 or Rx	≥5.5 or DM2
	IGT (mmol/L)	6.1	-	-
Dyslipidemia	Triglycerides (mmol/L)	≥1.7	≥1.7 or Rx	≥1.7 or Rx
	HDL cholesterol (mmol/L)	<0.9*/1.0**	<1.03*/1.3** or Rx	<1.03*/1.3** or Rx
Blood pressure (mmHg)		≥140/90	≥130/85 or Rx	≥130/85 or Rx
Other		Microalbuminuria	-	-

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Chapter 2

Metabolic syndrome detection with biomarkers in childhood cancer survivors

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ABSTRACT

Purpose:
Augmented childhood nephroblastoma and neuroblastoma survival has increased long-term side effects as metabolic syndrome (MetS). Risk stratification is difficult after abdominal radiation because waist circumference underestimates adiposity. We aimed to develop a strategy for determining MetS in irradiated survivors using an integrated biomarker profile and vascular ultrasonography.

Methods:
The NCEP-ATPIII MetS-components, 14 additional serum biomarkers and 9 vascular measurements were assessed in a single-centre cohort of childhood nephroblastoma (n=67) and neuroblastoma (n=36) survivors and controls (n=61). Multivariable regression models were used to study treatment effects. Principal component analysis (PCA) was used to study all biomarkers in a combined analysis, to identify patterns and correlations.

Results:
After 27.5 years of follow-up, MetS occurred more often in survivors (14%) than controls (3%). Abdominal radiotherapy, and nephrectomy to a lesser extent, were associated with MetS and separate components, and with several biomarker abnormalities. PCA of biomarkers revealed a pattern on PC1 from favourable lipid markers (HDL-cholesterol, adiponectin) towards unfavourable markers (triglycerides, LDL-cholesterol, apo-B, uric acid). Abdominal radiotherapy was associated with the unfavourable biomarker profile (ß=1.45, p=0.001). Vascular measurements were not of added diagnostic value.

Conclusions:
Long-term childhood nephro- and neuroblastoma survivors frequently develop MetS. Additional assessment of biomarkers identified in PCA – adiponectin, LDL, apo-B, uric acid – may be used especially in abdominally irradiated survivors, to classify MetS as alternative for waist circumference. Vascular ultrasonography was not of added value.

INTRODUCTION

Over the past decades, survival rates of childhood nephroblastoma and neuroblastoma have increased to respectively ~90% and ~40-95% (strongly dependent on stadium) [1, 2]. These tumours are of embryonic origin, with a peak incidence under the age of five years, and a presentation predominantly in the abdomen. Treatment often consists of a combination of surgery (nephrectomy and/or adrenalectomy), radiotherapy and/or intensive chemotherapy. Because of increased survival rates, long-term side effects, such as adiposity, insulin resistance, dyslipidaemia and hypertension, have become more prominent, particularly after treatment with abdominal radiotherapy [3-5]. These risk factors for diabetes mellitus and cardiovascular disease interact, and cluster together as metabolic syndrome [6-8].

In order to prevent the development of diabetes mellitus and cardiovascular disease, it is important to identify survivors at risk of developing (components of) MetS and to diagnose and treat them in a timely fashion [9]. Risk stratification in childhood cancer survivors (CCS) with the classic criteria for MetS components can be difficult. This is due to the underestimation of adiposity by waist circumference, waist-hip ratio and body mass index (BMI), in particular after abdominal radiotherapy has been applied [3, 10]. Also, because CCS are relatively young, absolute occurrence rates of cardio- and cerebrovascular events are low, even though they are at higher relative risk [11-14].

There is evidence that measurement of (visceral) fat by DXA-scan is a better indicator of adiposity, and hence a better predictor for cardiovascular disease [3, 10], but this is a costly and time consuming test. Therefore, in addition to the serum biomarkers triglycerides and HDL cholesterol that are already included in the definition of MetS, several other biomarkers have been suggested as surrogate markers for development of MetS and cardiovascular disease. These additional biomarkers include low-density lipoprotein (LDL) cholesterol, adiponectin, uric acid, C-reactive protein and cystatin C [15-17]. Also, it has been proposed that metabolic biomarkers are more clinically useful for risk prediction of diabetes and cardiovascular disease when analysed as a combination reflecting different pathophysiologic pathways, to reveal underlying patterns or clusters of dysmetabolic development [18]. In addition, vascular ultrasound measurements, such as carotid intima media thickness (CIMT), pulse pressure amplification (PPA) and pulse wave velocity (PWV), have also been proposed as surrogate markers for cardiovascular disease [19, 20]. So far, no studies have reported on the value of these additional biomarkers and vascular ultrasound measurements in CCS.

The aim of this cross-sectional study was to develop a strategy for determining MetS even in abdominally irradiated long-term survivors of childhood nephro- and neuroblastoma, using an integrated biomarker profile, based on principal component analysis, and vascular ultrasound measurements.

PATIENTS AND METHODS

Patients

Patients were actively recruited as described before [3]. Briefly, all long-term (five or more years after treatment) adult survivors of childhood nephro- and neuroblastoma (except for survivors of neuroblastoma stage 4s who did not receive surgery, radiotherapy or chemotherapy), treated between 1961 and 2004 in the Erasmus MC/ Sophia Children's Hospital, Rotterdam, The Netherlands, that visited the late effects outpatient clinic regularly were invited to participate in this cross-sectional study. The study was approved by the MREC Erasmus MC Rotterdam (trial NL2685, study period 2009-2012). Survivors were asked to invite potential control subjects such as siblings, friends or neighbours, preferably of the same sex and within an age range of five years. Written informed consent was obtained from all participants.

Data collection

Disease and treatment data were obtained from the medical records. Detailed data regarding surgery were confirmed from the original surgical and pathological reports. Information on medication use (statins, antidiabetic, antihypertensive), smoking and socio-economic status was collected using a self-designed questionnaire. Weight was measured with underwear only to the nearest 0.1kg with a standard clinical balance. Height was measured to the nearest millimetre using a Harpenden Stadiometer. BMI was calculated (weight(kg)/height(cm)^2). Waist circumference was measured between lower rib and iliac crest to the nearest centimetre. Blood pressure was measured with the subject in sitting position after an hour of rest on the right arm with the Dinamap® Procare and was defined as the mean of three measurements. Components of MetS were defined using the NCEP-ATPIII classification: waist ≥102(men)/88(women)cm, triglycerides ≥1.7mmol/L or use of statins, HDL cholesterol ≤1.03(men)/1.29(women) mmol/L or use of statins, blood pressure ≥130/≥85mmHg or use of antihypertensives, fasting glucose ≥5.6mmol/L or antidiabetic treatment, with three or more criteria required for the diagnosis MetS [6]. The occurrence of MetS and components in the current study population have been previously published [3].

Laboratory measurements

Fasting venous blood samples were taken before 10am. In addition to the biomarkers in the NCEP-ATPIII classification, thirteen biomarkers were assessed: free fatty acids (FFA), apolipoprotein(apo)-A1, apo-B, LDL cholesterol (measured, not calculated), leptin, adiponectin, lipoprotein(a) (Lp(a)), insulin, cystatin C, uric acid, urea, creatinine and hsCRP. Homeostasis model assessment (HOMA) was used as an estimate of insulin resistance and beta-cell function calculated from glucose and insulin concentrations [21]. Also, antithrombin, protein C, protein S, diluted Russell's viper venom time and von Willebrandfactor antigen were measured to exclude subjects with possible non-cancer-therapy-related coagulation problems (results not reported as outcome variables).

Vascular ultrasound measurements

Central systolic and diastolic blood pressure were assessed with the SphygmoCor (AtCor Medical, Sydney, Australia), which calculates aortic blood pressure from brachial pulse wave. Brachial and central pulse pressure (PP, the difference between systolic and diastolic blood pressure) were calculated, as well as pulse pressure amplification (PPA, brachial divided by central PP). Measurements of the carotid artery were performed with the subject in supine position, the head tilted slightly towards the contralateral side. After five minutes of rest, diameter of the common carotid artery (CCA), carotid intima media thickness (CIMT) and distensibility were measured with a duplex scanner (operating frequency 7.5MHz, Pie Medical Imaging, Maastricht, The Netherlands) during six non-consecutive heartbeats and reported as mean values. Distensibility coefficient (DC) was calculated using the following formula: ((2000*distensibility)/diameter)/PP*133.22) [22]. Pulse wave velocity (PWV) was also measured with the subject in supine position, with the Complior (Alam Medical, Saint-Quentin-Fallavier, France), which simultaneously records pulse waves at the carotid and femoral arteries (PWV = carotid-femoral distance/time delay).

Statistical analysis

Statistical analysis was performed using R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria). Baseline characteristics were compared between survivors and controls as well as nephroblastoma survivors compared to neuroblastoma survivors, using Fisher's exact test and Chi-squared test for categorical variables and by bootstrapping the difference in median values for continuous variables.

Occurrence of MetS and MetS components was compared between survivors and controls using Fisher's exact test and the Chi-squared test, respectively. Serum biomarkers and vascular ultrasound measurements were compared between survivors

and controls by bootstrapping the difference in median values, for which the 95% confidence interval was calculated with percentiles.

The effect of abdominal radiotherapy and nephrectomy on (components of) MetS, the serum biomarkers and the vascular parameters was tested with univariable logistic and bootstrap linear regression models, and, when significant, also in multivariable regression models, adjusting for age, sex, smoking and socio-economic status. There was no need to adjust for use of steroids, as these are not administered in treatment protocols of these malignancies, nor for adrenalectomy, because this would lead to overcompensation, as we previously published [23].

The serum biomarkers were also analysed by principal component analysis (PCA), to identify correlations and discriminative patterns, and to reduce the effects of multiple testing. PCA is an unsupervised, combined analysis of all biomarkers, that explains most of the variance in two principal components (PC1 and PC2) and the relative contribution of each biomarker to these principal components. With this method, the individual, unbiased, contribution of each biomarker is calculated. PCA was performed on the correlation matrix, which means that all variables are standardized to Z-scores. Missing values were imputed with the median (except for cystatin C, which was missing in 15% of participants and therefore was predicted with R package *mice* based on age, sex and the other kidney function variables). The effect of abdominal radiotherapy and nephrectomy on PC1 and PC2 was tested with linear regression models.

For the analyses of serum biomarkers, vascular ultrasound measurements and PCA, participants using relevant medication were excluded. A p-value <0.05 was considered as statistically significant.

RESULTS

Study population

Eighty-eight nephroblastoma survivors were invited to participate in the study, of whom 67 (39 males) agreed (76%). Fifty-five neuroblastoma survivors were invited, of whom 36 (15 males) agreed to participate (65%). Survivors who did not participate were similar to participating survivors with respect to baseline characteristics. In total, sixty-one controls were included (33 males), 37 of whom were siblings and 24 were partner or friend. Baseline and treatment characteristics are depicted in Table 1. Median age was 30 and 31.8 years for survivors and controls, respectively,

median follow-up time of survivors was 27.5 years (range 6.4 – 48.9 years). Systolic and diastolic blood pressure were higher among survivors, whereas physical activity, smoking behaviour and socio-economic status were not significantly different between survivors and controls. Within survivors, nephroblastoma survivors were older at diagnosis and had been treated more often with nephrectomy and abdominal radiotherapy (Supplemental Table 1). None of the study participants had experienced a cardiac event or stroke at time of inclusion in the study.

Classic MetS components, biomarkers and vascular ultrasound measurements, as compared to controls

MetS, as defined by the presence of at least three of the NCEP-ATPIII criteria, was present in 14 survivors (14%) and 2 controls (3%, p=0.032), as previously described [3]. Thirty-four survivors (33%) revealed at least two MetS criteria, compared to 12 controls (20%, p=0.074). Hypertension and treatment for hypertension occurred significantly more often in survivors than controls, whereas the other MetS components did not differ significantly between groups (Table 2).

Triglycerides (Δ=0.17mmol/L, p=0.036), cystatin C (Δ=0.06mg/L, p=0.002) and creatinine levels (Δ=5mg/mmol, p=0.014) were significantly higher in survivors compared to controls (Table 3). The other additional biomarkers were not different between survivors and controls. All coagulation markers were within the reference range in all participants (data not shown).

All vascular measurements were similar between survivors and controls.

Influence of abdominal radiotherapy and nephrectomy on classic MetS components, biomarkers and vascular ultrasound measurements

Using univariable logistic regression, abdominal radiotherapy was associated with occurrence of MetS (odds ratio (OR)=6.04, 95% CI=2.04-17.89, p=0.001), presence of two or more MetS components (OR=3.36, 95% CI=1.59-7.07, p=0.001), as well as with all separate components of MetS (Table 4). Using multivariable regression analysis, adjusting for age, sex, smoking and socio-economic status, abdominal radiotherapy remained an independent risk factor for MetS occurrence (OR=15.3, 95% CI=3.21-73.36, p<0.001), occurrence of two or more MetS components (OR=3.23, 95% CI=1.35-7.73, p=0.009) as well as the MetS components high triglycerides, low HDL cholesterol and hypertension. Nephrectomy was not a risk factor for MetS occurrence (OR=2.97, 95% CI=0.98-8.97, p=0.054). Using multivariable regression, nephrectomy was a risk factor for having two or more MetS components (OR=2.78, 95% CI=1.26-6.17, p=0.012), high triglycerides or treatment (OR=4.68,

95% CI=1.66-13.19, p=0.004) as well as hypertension or treatment (OR=4.82, 95% CI=2.05-11.29, p<0.001).

With regard to the biomarkers, abdominally irradiated subjects had higher triglycerides, FFA, apo-B, LDL, cystatin C and urea levels (Supplemental Table 2a). Using multivariable linear regression analysis, abdominal radiotherapy remained an independent risk factor for higher triglycerides (ß=0.57, p=0.002), higher FFA (ß=0.15, p=0.008) and higher cystatin C (ß=0.08, p=0.039) (Supplemental Table 2b). Nephrectomy was associated with higher cystatin C, uric acid, urea and creatinine levels (Supplemental Table 2c). Cystatin C (ß=0.12, p<0.001), uric acid (ß=0.05, p=0.006) and creatinine (ß=6.95, p=0.042) remained significantly associated with nephrectomy in multivariable analysis (Supplemental Table 2d).

Ultrasonography revealed that abdominally irradiated survivors had significantly higher central systolic and diastolic blood pressure and PWV and lower DC (Supplemental Table 2a). In multivariable analysis, the association between abdominal radiation and higher central diastolic blood pressure remained significant (ß=5.39, p=0.023) (Supplemental Table 2b). After nephrectomy, survivors had higher central systolic blood pressure, but this association was not significant in linear regression analysis (Supplemental Tables 2c,d). As peripheral blood pressure was higher as well, there was no clear added value of the vascular ultrasound measurements.

Principal component analysis of biomarkers

Principal component analysis of the panel of 17 serum biomarkers in survivors yielded principal component 1 with explained variance of 24.2% and a pattern of "favourable lipids" (high negative loading for HDL and adiponectin) towards "unfavourable lipids" (high positive loading of triglycerides, apo-B and LDL, as well as uric acid). Principal component 2 (PC2, orthogonal on PC1) explained 14.3% variance, with high negative loading reflected by "impaired glucose metabolism" (HOMA and glucose (as well as leptin)), and high positive loading reflected by "kidney disease" (creatinine and cystatin C (as well as HDL)). As all vectors in the biplot originate from the centre, high positive and negative loading on PC2 are two separate entities, i.e. kidney disease is not associated with favourable glucose metabolism and neither is impaired glucose metabolism with good kidney function. This is only applicable to PC1 since one pattern – favourable to unfavourable lipids – can be distinguished along the whole axis.

The effect of abdominal radiotherapy and nephrectomy on the two principal components is depicted in Figure 1. Survivors who received abdominal radiotherapy had a higher positive loading on PC1, constituting the unfavourable profile (ß=1.45,

p=0.001). There was no significant influence of abdominal radiation on PC2 (ß=0.14, p=0.68). Nephrectomy was associated with both higher positive loading on PC1 (unfavourable lipids, ß=1.13, p=0.015) and PC2 (kidney disease, ß=0.75, p=0.037).

DISCUSSION

This is the first report that describes the value of an integrated biomarker profile, defined by principal component analysis, and vascular ultrasound measurements, to estimate metabolic syndrome in long-term survivors of childhood nephroblastoma and neuroblastoma, in addition to classic parameters. We show that survivors more frequently develop MetS, and they have an unfavourable constitution of biomarkers in principal component 1 (PC1), particularly after abdominal radiotherapy. By using a principal component analysis, we could explore new variables better, since this analysis identifies individual contribution with no bias of multiple testing. This would not have been possible with another analysis, such as multiple correlation or regression. This unfavourable constitution of biomarkers consisted of a cluster of low HDL cholesterol and adiponectin and high triglycerides, LDL cholesterol, apo-B and uric acid.

Low HDL and high triglycerides are already classic components of MetS in the NCEP-ATPIII classification; the other biomarkers have been reported as risk predictors for MetS and cardiovascular disease in the general population as well as in CCS [16, 17, 24-26]. Therefore, we propose the addition of adiponectin, LDL, apo-B and uric acid in a surveillance setting, particularly in abdominally irradiated survivors, to classify MetS as alternative for waist circumference.

Our suggestion to add biomarkers to the classical components of MetS is in line with the recently updated dyslipidaemia management guideline from the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS), in which apo-B analysis is recommended for cardiovascular risk assessment, particularly in people with high triglycerides, obesity, MetS and diabetes [27]. Apo-B has been reported as a more sensitive marker of atherogenicity of cholesterol particles, in particular in insulin resistant patients. In those subjects, a relative abundance of dense, more atherogenic LDL particles can be present, which would be reflected by higher apo-B levels [28]. Uric acid is linked to metabolic syndrome in several ways: hyperuricemia contributes to the development of hypertension, insulin resistance and obesity [24]. The observed inverse relation between adiponectin and abdominal radiotherapy is of interest, as it may suggest that local radiation damage leads to decreased endocrine function of the adipose tissue or a lower number of fat cells.

Our finding that abdominal radiotherapy is strongly associated with the development of MetS components in CCS is consistent with other studies, and, more specifically, caused by radiation damage to the pancreas [29-32]. Additionally, abdominal radiotherapy can lead to underdevelopment of belly fat and musculature, and to scoliosis. Hence, measurement of waist circumference underestimates adiposity. Previously, we reported that body composition is more accurately measured in these CCS by DXA-scan [3]. The proposed use of additional biomarkers has the added advantage that this may be a cheaper and less burdensome diagnostic tool.

We found a moderate correlation between PC1 score and waist circumference in non-abdominally irradiated survivors (Pearson's r = 0.64, substantially higher than the correlations of the separate biomarkers) (Supplemental Figure 1), which supports the feasibility of this screening strategy. As a next step, to prove accuracy, sensitivity and cost-effectiveness of this strategy, replication in larger and independent cohorts is needed. Ultimately, for determining how the PC1 biomarkers could be incorporated in MetS classification, longitudinally collected information on solid endpoints (diabetes mellitus, cardio- and cerebrovascular morbidity and mortality) is needed.

Another finding was that principal component 2 was not of added value in determining MetS in abdominally irradiated survivors. By definition, PC2 explains less of the variance. Furthermore, there was no single discriminative pattern reflecting PC2 score and no difference in PC2 constitution was observed between abdominally irradiated and non-irradiated survivors.

Unexpectedly, the vascular ultrasound measurements were neither of evident added value in estimating MetS. We did observe some alterations suggestive of central arterial stiffness after abdominal radiotherapy: elevated central blood pressure and pulse wave velocity (PWV), and lower distensibility coefficient (DC), but after adjustment for potential covariates, only central blood pressure remained significantly associated with abdominal radiotherapy. Although central blood pressure is thought to better reflect cardiovascular risk as this represents the blood pressure in the coronary and cerebral arteries [33], we do not estimate this measurement of substantially added value, with peripherally measured blood pressure already being a classic MetS component. Although vascular abnormalities as observed by ultrasound can be early signs of MetS and its consequences, there can be variation in the development of these consequences, and the type of ultrasound patterns can vary as well. Therefore, in the aforementioned ESC/EAS dyslipidaemia guideline, it is postulated that assessment of arterial plaque burden can be considered as a risk modifier in individuals at low or moderate cardiovascular risk, in addition to standard cardiovascular risk assessment [27]. We think that it is conceivable that this variation, as well as the relatively young age of

our study cohort, may contribute to this unexpected finding. It could be, that these vascular ultrasound measurements will be useful at an older age for early detection of atherosclerosis, so it would be useful to have longitudinal data. The question remains whether asymptomatic atherosclerosis detection would have implications compared to interventions for the other MetS components.

In the separate analysis of the biomarkers, we observed elevated cystatine C in abdominally radiated survivors, even without elevation of creatinine. This discrepant finding may be due to underdeveloped abdominal musculature, and, if so, suggests that cystatine C is a more sensitive marker for assessing renal function in abdominally irradiated survivors [34].

The occurrence of MetS in our control group (3%) was relatively low, as MetS prevalence in The Netherlands at age 30-39 years has been reported as 10-20% [35]. We confirmed the representativeness of our controls by comparing their metabolic profile with other published, similar aged, Dutch reference cohorts [36, 37].

Some limitations of the current study merit consideration. This was a cross-sectional study, providing information at one time point only. As the study population was still relatively young, it is anticipated that the prevalence of MetS will increase when the survivors age. The advantage of diagnosis at younger age is the opportunity to intervene timely, to prevent diabetes and cardiovascular disease. This is particularly beneficial in childhood cancer survivors who received other, direct cardiotoxic treatment, such as anthracyclines and radiotherapy. Furthermore, we did not have information about daily calorie intake and family history of metabolic syndrome, diabetes and cardiovascular disease in our study population. However, we did take siblings (60% of the control group) and partners as control, and took the assumption that the calorie intake would be rather similar since they have a similar background. Family history is also most often similar between survivors and sibling controls.

Future research may focus on the validation of the use of adiponectin, LDL, apo-B and uric acid in larger, independent cohorts of survivors, with longitudinal follow-up. It may also be of interest to study ratios of biomarkers that provide additional diagnostic accuracy of MetS in the general population, such as triglycerides/HDL-ratio, and apoB-/apo-A1-ratio.

In conclusion, (young-)adult long-term survivors of childhood nephroblastoma and neuroblastoma, in particular after abdominal radiotherapy, frequently have MetS, defined by classic components, but also a novel, unfavourable integrated metabolic biomarker profile. This is important as the standard measurement of waist

circumference after abdominal radiation is often infeasible in adult CCS. Our findings suggest that integrating the additional biomarkers identified in PCA – adiponectin, LDL, apo-B and uric acid – may be useful to assess MetS, particularly in abdominally irradiated survivors. In contrast, vascular ultrasound measurements do not seem to be of additional value in estimating MetS at this relatively young age. Validation of our proposed screening strategy will be of importance to elucidate the higher risk of MetS, diabetes mellitus and cardiovascular disease in CCS, after previous intensive cancer treatment, which is still relatively disguised at young age, and to identify subgroups at greater risk at an early stage.

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Conflicts of interest

The authors have no conflicts of interest to declare with regard to this manuscript.

Table 1. Baseline characteristics of included survivors and controls

	Survivors	Controls	Bootstrap 95% CI	P-value
Number	103 (67 nephro-, 36 neuroblastoma)	61		
Male sex	54 (52.4%)	33 (54.1%)	n.a.	0.96[1]
Age at follow-up (years) #	30.0 [25.2 – 37.9]	31.8 [23.3 – 40.0]	[-7.2;2.4]	0.33[2]
Age at diagnosis (years) #	2.3 [0.8 – 5.0]	n.a.		
Follow-up time^ (years) #	27.5 [20.1 – 31.6]	n.a.		
BMI (kg/m^2) #	24.3 [21.3 – 26.3]	24.2 [22.1 – 27.2]	[-1.8;1.6]	0.84[2]
Systolic BP (mmHg) #	124 [117 – 133]	118 [111 – 126]	[0.3;10.0]	0.026[2]*
Diastolic BP (mmHg) #	76 [72 – 83]	72 [66 – 78]	[0.7;7.8]	0.012[2]*
Medication use				
Lipid-lowering	4 (3.9%)	0	n.a.	0.30[3]
Diabetes	6 (5.8%)	0	n.a.	0.085[3]
Antihypertensive	6 (5.8%)	2 (3.3%)	n.a.	0.71[3]
Physical activity score #	7695 [6390 – 10890]	8080 [6465 – 12278]	[-2947;1264]	0.71[2]
Smoking			n.a.	0.62[1]
Non-smoker	62 (60%)	32 (53%)		
Former smoker	15 (14.6%)	10 (16.4%)		
Smoker	26 (25%)	19 (31%)		
Socio-economic status			n.a.	0.31[3]
Low	22 (21.4%)	10 (16.4%)		
Medium	36 (35.0%)	29 (47.5%)		
High	45 (43.7%)	22 (36.1%)		
Nephrectomy	74 (71.8%)	n.a.		
Adrenalectomy	47 (45.6%)	n.a.		
Abdominal radiotherapy	42 (40.8%)	n.a.		
Pancreas	32 (31.1%)			
Flank	17 (17.0%)			
Cumulative dose radiotherapy (Gy) #	21 [20 – 30]	n.a.		
Chemotherapy	90 (87.4%)	n.a.		
Vincristine	65 (63.1%)			
Actinomycine	48 (46.6%)			
Anthracyclines	30 (29.1%)			
Cyclofosfamide	31 (30.1%)			
Cisplatin	7 (6.8%)			
Teniposide	6 (5.8%)			
Dacarbazine	2 (1.9%)			
Ifosfamide	2 (1.9%)			
Corticosteroids	2 (1.9%)	n.a.		

BP = blood pressure; n.a. = not applicable.

Presented as median [IQR]; ^ Time after cessation of treatment.

Significance codes: 0 *** 0.001 ** 0.01 * 0.05

[1] Chi-squared test, [2] Bootstrapped difference in medians, [3] Fisher's exact test

Table 2. Occurrence of MetS and components in survivors and controls

	Survivors (n=103)	Controls (n=61)	p-value
Metabolic syndrome (≥3 components)	14%	3%	0.032*[1]
≥2 MetS components	33%	20%	0.074[1]
Abdominal obesity (waist circumference ≥102(men)/88(women)cm)	8%	11%	0.61[2]
High triglycerides (≥1.7mmol/L) or treatment	23%	10%	0.052[2]
Low HDL cholesterol (≤1.03(men)/1.29(women)mmol/L or treatment	29%	18%	0.16[2]
High blood pressure (≥130/≥85mmHg) or treatment	35%	15%	0.007**[2]
High glucose (≥5.6mmol/L) or treatment	22%	11%	0.20[2]

Significance codes: 0 *** 0.001 ** 0.01 * 0.05

[1] Fisher's exact test, [2] Chi-squared test

Table 3. Comparison of serum biomarkers and vascular parameters between survivors and controls.

Variable	Survivors (n=103) #	Controls (n=61) #	95% CI ^	P-value ^
Biomarkers				
Lipid metabolism[1]				
Triglycerides (mmol/L)	0.96 [0.72 – 1.41]	0.79 [0.63 – 1.20]	[0.01;0.30]	0.036*
HDL (mmol/L)	1.35 [1.11 – 1.52]	1.33 [1.14 – 1.54]	[-0.10;0.12]	0.88
FFA (mmol/L)	0.53 [0.42 – 0.69]	0.49 [0.35 – 0.63]	[-0.05;0.11]	0.36
ApoA1 (g/L)	1.35 [1.23 – 1.53]	1.36 [1.25 – 1.49]	[-0.06;0.07]	0.65
ApoB (g/L)	0.85 [0.71 – 1.06]	0.80 [0.68 – 0.94]	[-0.03;0.16]	0.17
LDL (mmol/L)	2.84 [2.28 – 3.57]	2.83 [2.25 – 3.20]	[-0.26;0.40]	0.84
Leptin (ng/mL)	8.10 [4.23 – 16.15]	7.69 [2.76 – 13.79]	[-2.61;4.33]	0.66
Adiponectin (µg/mL)	2.75 [1.01 – 4.29]	2.74 [1.88 – 4.16]	[-1.02;7.49]	0.91
Lpa (g/L)	0.13 [0.05 – 0.37]	0.11 [0.06 – 0.30]	[-0.03;0.06]	0.45
Glucose metabolism[2]				
Glucose (mmol/L)	5.0 [4.6 – 8.7]	4.9 [4.7 – 5.2]	[-0.1;0.3]	0.42
Insulin (pmol/L)	21.5 [13.0 – 55.0]	25.0 [13.0 – 34.0]	[-12.0;13.0]	0.86
HOMA	0.4 [0.4 – 0.8]	0.5 [0.4 – 0.6]	[-0.1; 0.2]	0.41
Other MetS-associated biomarkers				
Cystatin C (mg/L)	0.86 [0.81 – 0.94]	0.80 [0.74 – 0.86]	[0.03;0.11]	0.002**
Uric acid (mmol/L)	0.31 [0.25 – 0.40]	0.31 [0.26 – 0.34]	[-0.02; 0.05]	0.35
Urea (mmol/L)	5.3 [4.6 – 6.5]	5.1 [4.3 – 5.8]	[-0.2;0.8]	0.21
Creatinine (mg/mmol)	74 [67 – 85]	69 [63 – 78]	[1;10]	0.014*
hsCRP (mg/L)	1.42 [0.55 – 3.47]	1.27 [0.52 – 3.57]	[-0.62;0.97]	0.46
Vascular parameters[3]				
Central SBP (mmHg)	115 [105 – 126]	110 [101 – 122]	[-4;14]	0.28
Central DBP (mmHg)	76 [71 – 84]	77 [70 – 85]	[-5;4]	0.68
Central PP (mmHg)	38 [31 – 45]	33 [28 – 44]	[-1;10]	0.068
PP (mmHg)	46 [40 – 51]	46 [42 – 50]	[-4;2]	0.64
PPA	1.23 [1.04 – 1.44]	1.33 [1.07 – 1.69]	[-0.26;0.09]	0.40
Diameter CCA (mm)	6.38 [5.92 – 6.83]	6.40 [5.82 – 6.68]	[-0.25;0.33]	0.83
CIMT (µm)	523 [477 – 581]	531 [481 – 586]	[-40;21]	0.44
DC	25.6 [18.4 – 32.3]	28.3 [19.6 – 37.6]	[-7.2;3.7]	0.27
PWV (m/s)	6.9 [6.0 – 8.0]	7.0 [6.3 – 7.8]	[-0.7;0.4]	0.39

S/DBP = systolic/diastolic blood pressure; PP = pulse pressure; PPA = pulse pressure amplification; CCA = common carotid artery; CIMT = carotid intima media thickness; DC = distensibility coefficient; PWV = pulse wave velocity.
Presented as median [IQR]; ^ Bootstrapped difference in medians
Significance codes: 0 *** 0.001 ** 0.01 * 0.05
[1] *Subjects using lipid-lowering medication excluded (n=4 survivors)*
[2] *Subjects with diabetes excluded (n=6 survivors)*
[3] *Subjects using antihypertensive medication excluded (n=6 survivors, n=2 controls)*

Table 4. Uni- and multivariable regression of the effect of abdominal radiotherapy and nephrectomy on MetS and components.

The influence of abdominal radiotherapy						
	Univariable analysis			**Multivariable analysis[1]**		
	OR (s.e.)	**95% CI**	**p-value**	**OR (s.e.)**	**95% CI**	**p-value**
MetS	6.04 (0.554)	2.04;17.89	0.001**	15.3 (0.799)	3.21;73.36	<0.001***
≥2 MetS components	3.36 (0.380)	1.59;7.07	0.001**	3.23 (0.445)	1.35;7.73	0.009**
Abdominal obesity	<0.0001 (1659)	-	0.99	-	-	-
High triglycerides or treatment	5.70 (0.430)	2.45;13.24	<0.001***	7.01 (0.548)	2.39;20.52	<0.001 ***
Low HDL cholesterol or treatment	2.39 (0.389)	1.11;5.12	0.025*	2.94 (0.447)	1.23;7.07	0.016*
High blood pressure or treatment	4.24 (0.387)	1.99;9.06	<0.001***	5.11 (0.478)	2.00;13.02	<0.001***
High glucose or treatment	2.38 (0.431)	1.02;5.53	0.044*	2.53 (0.514)	0.92;6.93	0.071

Significance codes: 0 *** 0.001 ** 0.01 * 0.05
[1] Corrected for age, sex, smoking and socio-economic status

The influence of nephrectomy						
	Univariable analysis			**Multivariable analysis[2]**		
	OR (s.e.)	**95%CI**	**p-value**	**OR (s.e.)**	**95%CI**	**p-value**
MetS	2.97 (0.564)	0.98;8.97	0.054	-	-	-
≥2 MetS components	2.15 (0.354)	1.07;4.29	0.031*	2.78 (0.406)	1.26;6.17	0.012*
Abdominal obesity	0.41 (0.607)	0.13;1.35	0.14	-		-
High triglycerides or treatment	2.96 (0.426)	1.29;6.82	0.011*	4.68 (0.528)	1.66;13.19	0.004**
Low HDL cholesterol or treatment	1.22 (0.361)	0.60;2.47	0.59	-	-	-
High blood pressure or treatment	3.95 (0.375)	1.89;8.25	<0.001***	4.82 (0.435)	2.05;11.29	<0.001***
High glucose or treatment	1.55 (0.413)	0.69;3.48	0.29	-		-

Significance codes: 0 *** 0.001 ** 0.01 * 0.05
[2] Corrected for age, sex, smoking and socio-economic status

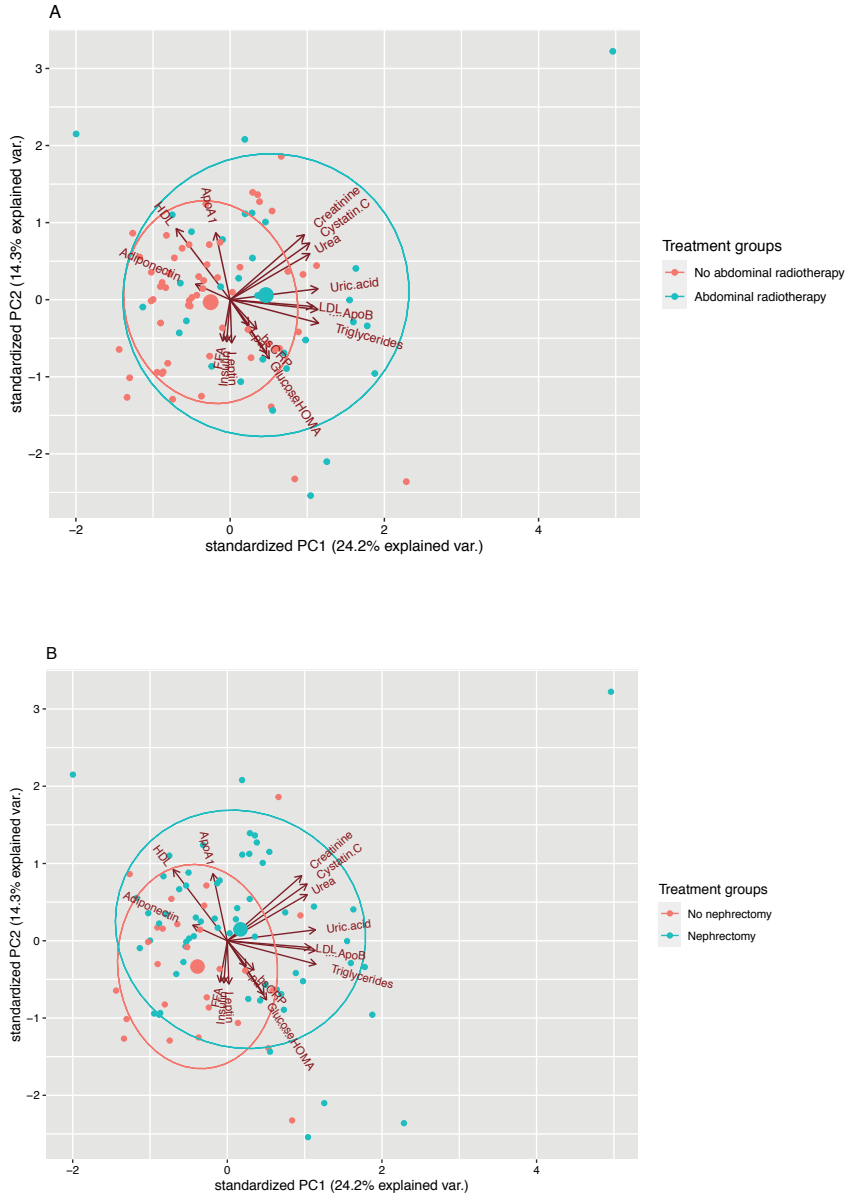


Figure 1. Biplots of PCA (principal components 1 and 2) of serum biomarkers, with the effect of abdominal radiotherapy (A) and nephrectomy (B). Score on PC1 and PC2 is a Z-score, based on loadings and Z-scores of biomarkers.
ApoA1 = apolipoprotein-A1; ApoB = apolipoprotein-B; FFA = free fatty acids; HDL = high density lipoprotein cholesterol; HOMA = homeostasis model assessment; LDL = low density lipoprotein cholesterol; Lpa = lipoprotein(a).

SUPPLEMENTARY MATERIAL

Supplemental Table 1. Baseline characteristics of nephro- and neuroblastoma survivors separated.

	Nephroblastoma	Neuroblastoma	Bootstrap 95% CI	P-value
Number	67	36		
Male sex	39 (58.2%)	15 (41.7%)	n.a.	0.16[1]
Age at follow-up (years) #	30.2 [25.2 – 39.4]	29.6 [25.1 – 33.4]	[-4.0;5.9]	0.85[2]
Age at diagnosis (years) #	3.3 [1.8 – 5.2]	0.8 [0.3 – 1.9]	[1.4;3.8]	<0.001[2]***
Follow-up time^ (years) #	26.3 [19.3 – 32.7]	27.8 [21.7 – 30.9]	[-6.3;3.1]	0.54[2]
BMI (kg/m^2) #	24.6 [21.7 – 27.1]	24.1 [21.1 – 26.0]	[-1.7;2.6]	0.72[2]
Systolic BP (mmHg) #	123 [118 – 133]	124 [115 – 129]	[-6;6]	0.62[2]
Diastolic BP (mmHg) #	77 [73 – 83]	75 [72 – 84]	[-4;5]	0.20[2]
Medication use				
Lipid-lowering	3 (4.5%)	1 (2.8%)	n.a.	1[3]
Diabetes	5 (7.5%)	1 (2.8%)	n.a.	0.66[3]
Antihypertensive	5 (7.5%)	1 (2.8%)	n.a.	0.66[3]
Physical activity score #	8140 [6634 – 12070]	6685 [5805 – 8423]	[-248;2560]	0.10[2]
Smoking			n.a.	0.29[1]
Non-smoker	44 (65.7%)	18 (50.0%)		
Former smoker	8 (11.9%)	7 (19.4%)		
Smoker	15 (22.4%)	11 (30.6%)		
Socio-economic status			n.a.	1[3]
Low	14 (20.9%)	8 (22.2%)		
Medium	24 (35.8%)	12 (33.3%)		
High	29 (43.3%)	16 (44.4%)		
Nephrectomy	67 (100%)	7 (19.4%)	n.a.	<0.001[1]***
Adrenalectomy	33 (49.3%)	14 (38.9%)	n.a.	0.42[1]
Abdominal radiotherapy	35 (52.2%)	7 (19.4%)	n.a.	0.003[1]**
Pancreas	24 (35.8%)	7 (19.4%)		0.23[1]
Flank	17 (26.6%)	0		0.002[1]**
Cumulative dose radiotherapy (Gy) #	21 [20 – 30]	20 [19.6 – 20.5]	[-0.7;8.6]	0.064[2]
Chemotherapy	59 (88.1%)	31 (86.1%)	n.a.	0.76[3]
Vincristine	50 (74.6%)	15 (41.7%)		0.002[1]**
Actinomycine	48 (71.6%)	0		<0.001[1]***
Anthracyclines	18 (26.9%)	12 (33.3%)		0.64[1]
Cyclofosfamide	2 (3.0%)	29 (80.6%)		<0.001[1]***
Cisplatin	0	7 (19.4%)		<0.001[3]
Teniposide	0	6 (16.7%)		0.001[3]
Dacarbazine	2 (3.0%)	0		0.54[3]
Ifosfamide	2 (3.0%)	0		0.54[3]
Corticosteroids	0	2 (5.6%)	n.a.	0.12[3]

BP = blood pressure; n.a. = not applicable.

Presented as median [IQR]; ^ Time after cessation of treatment.

Significance codes: 0 *** 0.001 ** 0.01 * 0.05

[1] Chi-squared test, [2] Bootstrapped difference in medians, [3] Fisher's exact test

Supplemental Table 2a. The effect of abdominal radiotherapy on biomarkers and vascular parameters.

Variable	Abdominal radiotherapy (n=42) #	No abdominal radiotherapy (n=61) #	95% CI ^	P-value ^
Biomarkers				
Lipid metabolism[1]				
Triglycerides (mmol/L)	1.35 [0.81 – 2.09]	0.91 [0.70 – 1.08]	[0.06;0.88]	0.024*
HDL (mmol/L)	1.32 [1.08 – 1.52]	1.35 [1.12 – 1.52]	[-0.19;0.13]	0.74
FFA (mmol/L)	0.57 [0.44 – 0.74]	0.50 [0.38 – 0.64]	[0.01;0.17]	0.026*
ApoA1 (g/L)	1.35 [1.21 – 1.58]	1.35 [1.25 – 1.49]	[-0.10;0.16]	0.75
ApoB (g/L)	0.99 [0.81 – 1.18]	0.81 [0.65 – 0.97]	[0.07;0.30]	0.004**
LDL (mmol/L)	3.19 [2.71 – 3.79]	2.55 [2.15 – 3.18]	[0.15;1.09]	0.004**
Leptin (ng/mL)	9.00 [4.98 – 18.38]	7.60 [3.67 – 12.90]	[-2.95;6.81]	0.51
Adiponectin (µg/mL)	2.30 [0.56 – 4.31]	2.83 [1.53 – 4.15]	[-2.09;1.22]	0.64
Lpa (g/L)	0.09 [0.03 – 0.45]	0.13 [0.05 – 0.35]	[-0.10;0.17]	0.65
Glucose metabolism[2]				
Glucose (mmol/L)	5.0 [4.8 – 5.5]	4.9 [4.6 – 5.3]	[-0.2;0.4]	0.36
Insulin (pmol/L)	18.0 [13.0 – 44.0]	24.0 [13.0 – 58.0]	[-22.0;13.0]	0.48
HOMA	0.4 [0.4 – 0.8]	0.4 [0.4 – 0.8]	[-0.3; 0.3]	0.46
Other MetS-associated biomarkers				
Cystatin C (mg/L)	0.88 [0.83 – 0.97]	0.85 [0.77 – 0.90]	[0.00;0.09]	0.048*
Uric acid (mmol/L)	0.35 [0.29 – 0.44]	0.30 [0.24 – 0.38]	[-0.01;0.10]	0.094
Urea (mmol/L)	5.7 [5.1 – 6.8]	5.0 [4.5 – 5.9]	[0.1;1.5]	0.006**
Creatinine (mg/mmol)	75 [68 – 84]	74 [66 – 85]	[-7;9]	0.83
hsCRP (mg/L)	1.62 [0.77 – 3.35]	1.42 [0.39 – 3.79]	[-0.90;1.61]	0.65
Vascular parameters[3]				
Central SBP (mmHg)	124 [114 – 132]	109 [101 – 122]	[8;20]	<0.001***
Central DBP (mmHg)	84 [76 – 80]	74 [69 – 77]	[4;15]	<0.001***
Central PP (mmHg)	40 [34 – 47]	37 [30 – 44]	[-2;9]	0.19
PP (mmHg)	45 [41 – 53]	46 [40 – 49]	[-4;6]	0.95
PPA	1.23 [1.06 – 1.40]	1.30 [1.03 – 1.48]	[-0.21;0.15]	0.52
Diameter CCA (mm)	6.46 [5.93 – 6.90]	6.32 [5.92 – 6.83]	[-0.30;0.53]	0.47
CIMT (µm)	552 [482 – 595]	509 [458 – 569]	[-2;71]	0.052
DC	20.6 [15.7 – 26.1]	30.3 [21.3 – 39.8]	[-13.9;3.1]	0.004**
PWV (m/s)	8.0 [6.1 – 8.8]	6.6 [6.0 – 7.3]	[0.2;1.9]	0.016*

S/DBP = systolic/diastolic blood pressure; PP = pulse pressure; PPA = pulse pressure amplification; CCA = common carotid artery; CIMT = carotid intima media thickness; DC = distensibility coefficient; PWV = pulse wave velocity.
Presented as median [IQR]; ^ Bootstrapped difference in medians
Significance codes: 0 *** 0.001 ** 0.01 * 0.05

[1] *Subjects using lipid-lowering medication excluded (n=3 abdominal radiotherapy, n=1 no abdominal radiotherapy)*

[2] *Subjects with diabetes excluded (n=5 abdominal radiotherapy, n=1 no abdominal radiotherapy)*

[3] *Subjects using antihypertensive medication excluded (n=4 abdominal radiotherapy, n=2 no abdominal radiotherapy)*

Supplemental Table 2b. Uni- and multivariable bootstrap linear regression analysis of the influence of abdominal radiotherapy on biomarkers and vascular parameters.

		Univariable analysis		Multivariable analysis[1]	
Variable	**P-value bootstrap difference medians**	**Beta (s.e.)**	**P-value**	**Beta (s.e.)**	**P-value**
Triglycerides	0.024	0.614 (0.165)	<0.001***	0.572 (0.154)	0.002**
FFA	0.026	0.123 (0.049)	0.008**	0.151 (0.059)	0.008**
ApoB	0.004	0.168 (0.054)	0.002**	0.083 (0.068)	0.19
LDL	0.004	0.469 (0.170)	0.008**	0.043 (0.235)	0.83
Cystatin C	0.048	0.065 (0.032)	0.041*	0.076 (0.039)	0.039*
Urea	0.006	0.895 (0.373)	0.011*	0.690 (0.465)	0.10
Central SBP	<0.001	10.226 (3.687)	0.008**	6.029 (5.033)	0.19
Central DBP	<0.001	9.122 (2.024)	<0.001***	5.385 (2.929)	0.023*
DC	0.004	-8.578 (2.810)	0.004**	-2.672 (3.279)	0.44
PWV	0.016	0.935 (0.451)	0.025*	0.294 (0.456)	0.53

Significance codes: 0 *** 0.001 ** 0.01 * 0.05

[1] Corrected for age, sex, smoking and socio-economic status.

Supplemental Table 2c. The effect of nephrectomy on biomarkers and vascular parameters.

Variable	**Nephrectomy (n=74) #**	**No nephrectomy (n=29) #**	**95% CI ^**	**P-value ^**
Biomarkers				
Lipid metabolism[1]				
Triglycerides (mmol/L)	0.98 [0.77 – 1.58]	0.90 [0.70 – 1.19]	[-0.16;0.37]	0.43
HDL (mmol/L)	1.35 [1.09 – 1.55]	1.32 [1.16 – 1.48]	[-0.13;0.16]	0.72
FFA (mmol/L)	0.50 [0.40 – 0.61]	0.65 [0.49 – 0.77]	[-0.26;0.01]	0.054
ApoA1 (g/L)	1.38 [1.24 – 1.54]	1.31 [1.20 – 1.51]	[-0.04;0.16]	0.18
ApoB (g/L)	0.85 [0.70 – 1.05]	0.86 [0.74 – 1.08]	[-0.17;0.13]	0.79
LDL (mmol/L)	2.83 [2.20 – 3.54]	2.92 [2.45 – 3.63]	[-0.69;0.28]	0.43
Leptin (ng/mL)	8.37 [3.64 – 18.38]	8.10 [5.44 – 12.90]	[-5.47;3.66]	0.86
Adiponectin (µg/mL)	2.47 [0.98 – 4.31]	3.02 [1.81 – 4.13]	[-1.58;0.55]	0.26
Lpa (g/L)	0.13 [0.05 – 0.35]	0.11 [0.05 – 0.39]	[-0.11;0.08]	0.80
Glucose metabolism[2]				
Glucose (mmol/L)	5.0 [4.7 – 5.3]	4.8 [4.5 – 5.4]	[-0.1;0.5]	0.086
Insulin (pmol/L)	29.0 [13.0 – 55.0]	13.0 [13.0 – 49.0]	[-6.0;23.0]	0.088
HOMA	0.6 [0.4 – 0.9]	0.4 [0.4 – 0.8]	[-0.1; 0.3]	0.060
Other MetS-associated biomarkers				
Cystatin C (mg/L)	0.88 [0.84 – 0.99]	0.81 [0.72 – 0.86]	[0.03;0.16]	0.002**
Uric acid (mmol/L)	0.35 [0.27 – 0.43]	0.26 [0.21 – 0.31]	[0.02;0.13]	0.002**
Urea (mmol/L)	5.4 [4.8 – 6.6]	5.0 [4.2 – 5.7]	[-0.2;1.0]	0.084
Creatinine (mg/mmol)	79 [69 – 89]	69 [63 – 75]	[2;16]	0.004**
hsCRP (mg/L)	1.62 [0.72 – 3.48]	1.42 [0.39 – 3.09]	[-0.93;1.45]	0.46

Supplemental Table 2c. Continued

Variable	Nephrectomy (n=74) #	No nephrectomy (n=29) #	95% CI ^	P-value ^
Vascular parameters[3]				
Central SBP (mmHg)	118 [108 – 127]	108 [100 – 123]	[0;19]	0.050*
Central DBP (mmHg)	77 [72 – 85]	75 [71 – 79]	[-2;7]	0.17
Central PP (mmHg)	39 [34 – 47]	34 [30 – 43]	[-3;10]	0.14
PP (mmHg)	46 [40 – 53]	45 [41 – 48]	[-3;5]	0.62
PPA	1.23 [0.99 – 1.41]	1.31 [1.13 – 1.54]	[-0.30;0.09]	0.43
Diameter CCA (mm)	6.30 [5.87 – 6.82]	6.57 [6.13 – 6.93]	[-0.72;0.21]	0.32
CIMT (μm)	521 [479 – 582]	523 [461 – 570]	[-43;53]	0.81
DC	24.0 [15.8 – 39.8]	29.9 [24.0 – 31.9]	[-10.7;1.5]	0.13
PWV (m/s)	6.8 [6.0 – 8.0]	6.9 [6.1 – 7.5]	[-0.7;0.7]	0.79

S/DBP = systolic/diastolic blood pressure; PP = pulse pressure; PPA = pulse pressure amplification; CCA = common carotid artery; CIMT = carotid intima media thickness; DC = distensibility coefficient; PWV = pulse wave velocity.
Presented as median [IQR]; ^ Bootstrapped difference in medians
Significance codes: 0 *** 0.001 ** 0.01 * 0.05

[1] *Subjects using lipid-lowering medication excluded (n=3 nephrectomy, n=1 no nephrectomy)*

[2] *Subjects with diabetes excluded (n=5 nephrectomy, n=1 no nephrectomy)*

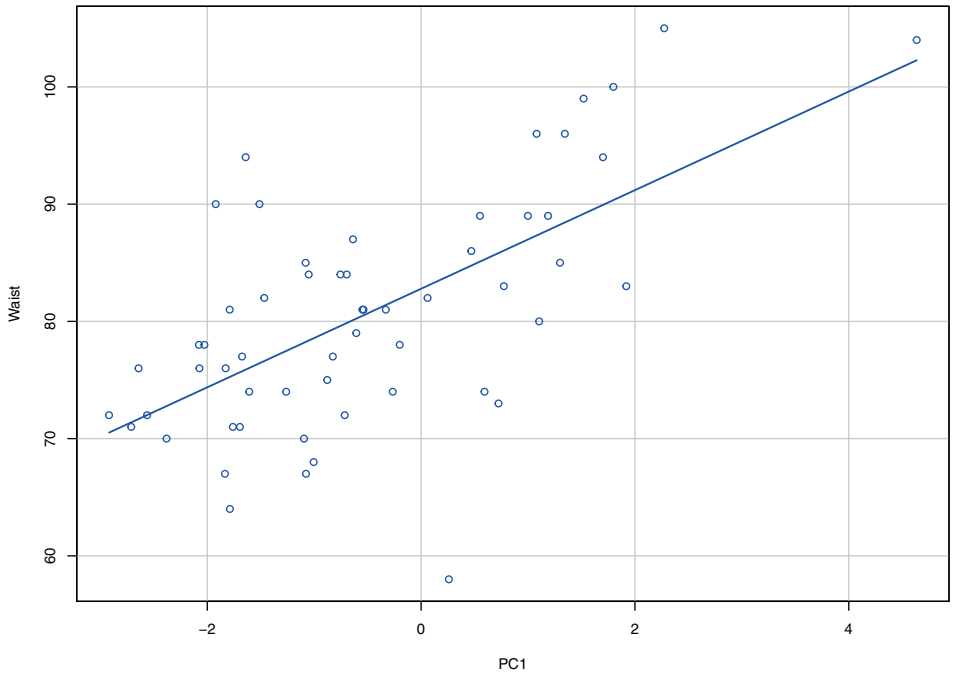
[3] *Subjects using antihypertensive medication excluded (n=6 nephrectomy)*

Supplemental Table 2d. Uni- and multivariable bootstrap linear regression analysis of the influence of nephrectomy on biomarkers and vascular parameters.

		Univariable analysis		Multivariable analysis[1]	
Variable	**P-value bootstrap difference medians**	**Beta (s.e.)**	**P-value**	**Beta (s.e.)**	**P-value**
Cystatin C	0.002	0.128 (0.027)	<0.001***	0.115 (0.027)	<0.001***
Uric acid	0.002	0.075 (0.017)	<0.001***	0.045 (0.013)	0.006**
Creatinine	0.004	12.062 (2.899)	0.002**	6.954 (2.640)	0.042*
Central SBP	0.050	8.233 (4.089)	0.054	-	-

Significance codes: 0 *** 0.001 ** 0.01 * 0.05

[1] Corrected for age, sex, smoking and socio-economic status



Supplemental Figure 1. Correlation between waist and PC1 score in non-abdominally irradiated survivors. Pearson's correlation coefficient: 0.64

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Chapter 3

Can biomarkers be used to improve diagnosis and prediction of metabolic syndrome in childhood cancer survivors? A systematic review

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ABSTRACT

Background: Childhood cancer survivors (CCS) are at increased risk to develop metabolic syndrome (MetS), diabetes and cardiovascular disease. Common criteria underestimate adiposity and possibly underdiagnose MetS, particularly after abdominal radiotherapy.

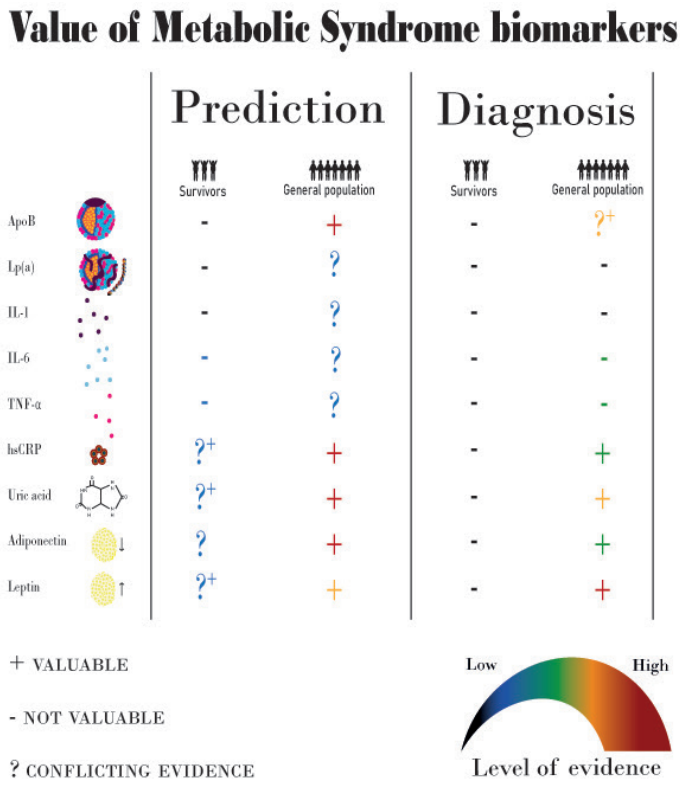
Design: A systematic literature review and meta-analysis on the diagnostic and predictive value of nine newer MetS related biomarkers (adiponectin, leptin, uric acid, hsCRP, TNF-alpha, IL-1, IL-6, apolipoprotein B (apoB), and lipoprotein(a) [lp(a)]) in survivors and adult non-cancer survivors was performed by searching PubMed and Embase. Evidence was summarized with GRADE after risk of bias evaluation (QUADAS-2/QUIPS). Eligible studies on promising biomarkers were pooled.

Results: We identified 175 general population, and 5 CCS studies. In the general population, valuable predictive biomarkers are uric acid, adiponectin, hsCRP and apoB (high level of evidence) and leptin (moderate level of evidence). Valuable diagnostic biomarkers are hsCRP, adiponectin, uric acid and leptin (low, low, moderate and high level of evidence, respectively). Meta-analysis showed OR for hyperuricemia of 2.94 (age-/sex-adjusted), OR per unit uric acid increase of 1.086 (unadjusted), and AUC for hsCRP of 0.71 (unadjusted).

Conclusions: Uric acid, adiponectin, hsCRP, leptin, and apoB can be alternative biomarkers in the screening setting for MetS in survivors, to enhance early identification of those at high risk of subsequent complications.

Abbreviations: CVD = cardiovascular disease; T2DM = type 2 diabetes mellitus; MetS = metabolic syndrome; CCS = childhood cancer survivors; DXA = Dual-energy X-ray Absorptiometry; hsCRP = high sensitivity C-reactive protein; TNF-alpha = Tumor Necrosis Factor alpha; IL-1 = interleukin 1; IL-6 = interleukin 6; apoB = apolipoprotein B; Lp(a) = lipoprotein(a); AUC = area under the curve; ROC = receiver operating characteristic; OR = odds ratio; HR = hazard ratio; GRADE = Grading of Recommendations Assessment Development and Evaluation; BMI = body mass index; ALL = acute lymphoblastic leukemia; apoA1 = apolipoprotein A1; LDL = low density lipoproteins; HOMA-IR = Homeostatic Model Assessment of Insulin Resistance; HDL = high density lipoproteins.

GRAPHICAL ABSTRACT



Graphical abstract of systematic review for novel biomarkers for diagnosis and prediction of the metabolic syndrome in childhood cancer survivors and a young general population. Conclusions per biomarker are categorized as valuable, not valuable or conflicting evidence, and the level of evidence is expressed per color from low to high (black-blue-green-yellow-red; see figure on the lower right).

Key words: childhood cancer survivors, the metabolic syndrome, biomarker, systematic review

INTRODUCTION

Childhood cancer 5-year survival rates have increased from 5-30% in early seventies to more than 80% in the present time.[1, 2] Deployed therapies, such as chemotherapy, radiotherapy, and stem cell transplantation, better stratification and enhanced supportive care regimens, are responsible for increase in survival rates. However, intensification of treatment is also associated with long-term excess mortality and morbidities in survivors.[3] Survivors have a high level of frailty, suggesting their biological age progresses faster than their actual age. Consequently, survivors with an actual mean age of 33 have a biological age of 65 if they are compared to the general population.[4-9] At the age of 45-50 years, the prevalence of any chronic health condition is very high, from 95% up to 99%.[3, 10] One of these severe conditions is represented by cardiovascular disease (CVD), which is an important cause of premature death beyond 5 years cancer survival; the standardized mortality risk for CVD ranges from 1.9 to 12.7.[11-25]

This high risk of cardiovascular death is not only due to treatment effects, such as anthracycline exposure and cardiac irradiation;[26] survivors are also at high risk of type II diabetes mellitus (T2DM) and the metabolic syndrome (MetS).[11] These diseases are independent predictors of CVD and associated with factors such as adiposity, dyslipidemia, insulin resistance, and hypertension. These factors cluster together and form the 'deadly quartet', a MetS concept developed by Reaven in 1988.[27] The MetS had many definitions ever since.[11, 27-37] Patients with MetS carry a doubled risk of dying from cardio- and cerebrovascular disease.[11, 38] In addition, patients with the MetS are five times more likely to develop T2DM, which subsequently triples the risk of CVD.[11, 39-41]

As survivors develop cardiovascular complications at a relatively young age, there is a need for early diagnosis of MetS, to possibly prevent T2DM and CVD, and to improve long-term survival.[11] The occurrence of MetS may be underestimated especially in abdominally irradiated childhood cancer survivors (CCS), who have an unreliable waist circumference, while their MetS risk is even higher.[11, 42-44] Body mass index and bioimpedance are alternative methods for body composition measurement, but do not specifically measure abdominal fat, rely on hydration status and often underestimate body fat.[42, 45-47] Obviously, another alternative option to evaluate adiposity is measuring fat percentage by Dual-energy X-ray Absorptiometry (DXA) scan, which is the gold standard in case of suspected discordance of anthropomorphic measurements and adiposity.[42, 48, 49] However, performing DXA scans in all survivors on a routine basis is time-consuming and costly.[11] Additionally, there is currently no consensus for the threshold of fat percentage for diagnosing obesity.[50] Newer serum

biomarkers may serve as another alternative for accurate early diagnosis or prediction of (disguised) MetS in CCS. Adult cardiologists currently apply multiple biomarkers that have been shown to improve risk estimation for CVD.[51]

Therefore, our primary objectives were to evaluate the value of the use of these newer serum biomarkers as (1) diagnostic marker, and as (2) additional independent predictor for the occurrence of MetS later in life, in survivors of childhood cancer specifically, as well as in a relatively young general, non-cancer population (studies with >75% of participants below 65 years). By including this selection of general population studies as well, we aimed to cover all available literature applicable and generalizable to young-adult survivors. To accomplish this, we performed a systematic literature search on adipokines adiponectin and leptin, uric acid, the inflammatory markers high sensitivity C-reactive protein (hsCRP), Tumor Necrosis Factor alpha (TNF-alpha), interleukin 1 (IL-1), and interleukin 6 (IL-6), and the lipid markers apolipoprotein B (apoB) and lipoprotein(a) [Lp(a)], and performed a meta-analysis of these outcomes for relevant recurrently published biomarkers. As secondary purpose, we screened for other new biomarkers that are not enlisted above, in order to reveal additional, potentially useful biomarkers.

METHODS

The Systematic Search

A systematic literature review was performed in PubMed and Embase, to gather all published literature published between the first of October 2009 and September 3, 2020. Details of the search terms are available in Supplementary Table 1; in general, the search terms were related to adults/general population, as well as to (childhood) cancer survivors, and combined with all enlisted 9 separate biomarkers (adiponectin, leptin, uric acid, hsCRP, TNF-alpha, IL-1, IL-6, apoB and lp(a)) and the MetS. The AMSTAR checklist for systematic reviews was followed.[52] All titles and abstracts were screened by two independent reviewers (VP and SSvS), who were blinded to each other's judgement. Studies were included if they had the MetS as outcome, and one or more newer biomarker(s) as independent variable included in the model in predictive studies, or as discriminative variable in diagnostic studies. For studies performed in CCS, no limits were set for sample size or age. General population studies were eligible if the sample size was roughly 250 or larger and if 75% or more of this population was below 65 years of age, as they have comparable levels of frailty to a young adult survivor population.[5, 7, 8] We excluded studies with older adults since they are expected to have higher levels of frailty, comorbidities and aging factors,

which may be confounders in the correlation between the newer biomarker and the metabolic syndrome. Multivariable analysis was mandatory for article inclusion of studies that investigated the prediction of MetS.

Studies were excluded if all included patients had an elevated biomarker; if all or none of the subjects had the MetS; if it was a selected cohort with pre-existing comorbidities (i.e. familial hypercholesterolemia, psoriasis, schizophrenia, polycystic ovary syndrome, obesity, hypertension); if all patients suffered from MetS or endpoint(s) such as T2DM, cardio- or cerebrovascular disease, or non-alcoholic fatty liver disease; if the article was a review, case study, expert opinion or conference abstract; if the article was written in a language other than English or Dutch, or if the full text was unavailable (see Appendix A for an overview of selection criteria). Studies were only included if the outcome was presence or absence of MetS; those with separate MetS components or MetS risk score as outcome were out of the scope of this review. After all articles were screened based on title and abstract, the judgements were unblinded. Discrepancies were discussed and resolved by the two reviewers (VP and SSvS) and where necessary, two senior experts were consulted (MMvdHE and SJCMMN). A cross-reference check was performed with Scopus, to screen all forward and backward citations of included studies. The articles found by the cross-reference check were screened likewise. A flow diagram with the number of in- and excluded articles and reasons for exclusion illustrates this process (Figure 1).

Risk of bias assessment

The QUIPS tool was applied for critical appraisal of predictor studies[53, 54] (Supplementary Table 2) and QUADAS-2 tool for diagnostic studies (Supplementary Table 3). Definitions for low risk of bias judgement are shown in Appendix A. In case of doubt, the study was discussed with both reviewers and senior experts (VP, SSvS, MMvdHE, SJCMMN).

Data extraction enlisted novel biomarkers

Data of all included articles were extracted and summarized; the summaries of the enlisted newer biomarkers (adiponectin, leptin, uric acid, hsCRP, TNF-alpha, IL-1, IL-6, apoB and lp(a)) are depicted in Supplementary Table 4A-V. Data of interest are details regarding the size of the population and its type (survivors and their previous diagnosis, or general population), the study design (cross-sectional or longitudinal and retrospective or prospective), the biomarker (which and how it was measured), the exact outcome (MetS definition) and statistical analysis of choice. For studies investigating the diagnostic value of the biomarker for MetS, outcomes of interest were area under the curve (AUC) of receiver operating characteristic (ROC) curves, sensitivity and specificity. For the studies evaluating the predictive value of the

biomarker of later development of the MetS, odds ratios (OR's) or beta-coefficients of multivariable logistic regression models, or hazard ratios (HR's) from multivariable Cox Proportional Hazards analysis were extracted from the publications.

Summary of evidence

The Grading of Recommendations Assessment Development and Evaluation (GRADE) tool was applied to summarize the quality of the evidence for each biomarker, per clinical research question (diagnosing or predicting MetS) and per population (general population and CCS).[55] The level of evidence was classified as insufficient, very low, low, moderate and high (Supplementary Table 4).[55] The applied thresholds for biomarkers are shown in Supplementary Table 5. An overview was made for studies assessing the same independent variables and outcome (Supplementary Table 6).

Data extraction non-enlisted biomarkers

As secondary objective we screened all articles for other biomarkers than the above enlisted nine biomarkers of our main interest (non-enlisted biomarkers). Details are discussed in Part 2 of the Appendix . These non-enlisted biomarkers were evaluated for presence of an effect if there were 4 or more publications with this biomarker in our search. As we did not search for these biomarkers systematically, evidence quality was not assessed with GRADE.

Meta-analysis

A meta-analysis was performed of relevant enlisted biomarkers with at least three publications on the same outcome measures and, if applicable, adjusted for the same covariates. Dichotomous outcomes were considered as comparable if the applied threshold differed less than the intra- and inter-assay variability for the biomarker as reported in literature. A random effects model with inverse variance weighting was used to estimate a pooled overall outcome measure. Overall heterogeneity (I-squared) and between-study variance (tau-squared) were calculated.[56] Meta-analysis was performed with the package *meta* in R.[57]

RESULTS

Study selection

As shown in the flow chart (Figure 1), the literature search in PubMed and Embase yielded a total of 4,510 unique records. After title and abstract screening, 650 full-text articles were reviewed, after which 162 relevant studies remained. Backward and

forward citation searching identified 18 additional studies. Hence, a total of 180 studies were identified that reported on the diagnostic and/or predictive value of one or more of the enlisted nine biomarkers of interest. Only five studies among the 180 were performed among a population of CCS.[58-62] All other studies were performed in the general population.

Among 180 studies which included data regarding the 9 enlisted biomarkers, 60 also reported the value of other, non-enlisted newer biomarkers. Furthermore, we identified 119 other studies that only investigated non-enlisted newer biomarkers (other than the nine of our main interest), yielding a total of 179 studies for our secondary objective.

A detailed description of the critical appraisal of each of the 180 included studies for the nine predefined biomarkers is provided in the supplementary material (Supplementary Table 2 and 3).

Used metabolic syndrome definitions

In the included studies, a variety of MetS definitions was used of which the most common are described in Table 1 and the applied definition per study is depicted in Supplementary Table 4. The applied biomarker thresholds are summarized in Supplementary Table 5.

Evidence for newer, enlisted biomarkers as (additional) diagnostic criterion for metabolic syndrome

Twenty-nine studies reported on the diagnostic value of one or more of the nine enlisted newer biomarkers. These were all performed in the general population without a history of cancer. Six studies had a Caucasian study population.[63-68] The number of studies per biomarker ranged between zero [IL-1 and lp(a)] and twelve (adiponectin). The biomarker studied in the largest total number of participants was uric acid (73,190 participants). The relevant data extracted from each study, as well as the summary of evidence scored with the GRADE tool for each biomarker, are provided in the supplementary material (Supplementary Table 4). For each biomarker, a description of the number of studies and participants, and a summary of the several diagnostic outcomes, are provided in Table 2.

Whereas, ideally, the additional diagnostic value of a biomarker would be tested by comparing the AUC, sensitivity and specificity for a model containing only relevant covariates, versus a model containing covariates and the newer biomarker, this method was used in only two of the 29 studies.[65, 69] One study compared the AUC of the biomarker with the AUC of waist circumference.[69] Most studies, however, only

reported the AUC of the biomarker, either unadjusted or adjusted for age, sex, and sometimes body mass index (BMI) or waist circumference. Therefore, interpretation of the additional value is limited by detection and confounding bias for most of the biomarkers.

The overall summary of our findings, with a conclusion about the diagnostic value of each biomarker in the general population and in survivors based on the GRADE assessment, is shown in Figure 2. Of the nine investigated biomarkers, four were identified as valuable diagnostic biomarkers for MetS: leptin (high quality of evidence), uric acid (moderate quality), adiponectin, and hsCRP (both low quality). In addition, apoB may be valuable, although based on only one study with moderate quality of evidence. TNF-alpha and IL-6 appeared to be unusable, based on one low quality study testing both biomarkers. For IL-1 and lp(a), no studies were found.

Evidence for newer, enlisted biomarkers as independent predictor of metabolic syndrome

In total, 162 general population studies, and 5 survivor studies [two in acute lymphoblastic leukemia (ALL) survivors, two in survivors of hematological malignancies, one in survivors of heterogeneous tumors],[58-62] investigated the role of one or more of the nine enlisted, newer biomarkers as independent predictors of MetS. Twenty-six of the general population studies had a Western/Caucasian study population.[65, 67, 68, 70-92] The number of general population studies per biomarker ranged between 3 (TNF-alpha, 1,458 participants in total) and 78 (uric acid, 447,559 participants in total). Two of the survivors studies had a Western/Caucasian study population,[58, 59] the others were performed in Japan,[60] Malaysia,[61] and Mexico.[62] The number of survivors studies per biomarker ranged between zero [IL-1, apoB, and lp(a)] and 3 (adiponectin and leptin). The biomarker studied in the largest total number of survivors was uric acid (390 survivors). The relevant data extracted from each study, as well as the summary of evidence scored with the GRADE tool for each biomarker, are provided in the supplementary material (Supplementary Table 4). For each biomarker, a description of the number of studies and participants, and a summary of the several prognostic outcomes, are provided in Table 2.

A common analysis strategy in these studies was to divide the biomarker value in quantiles, with thresholds that may differ per study. Not all participants in the highest or lowest quantile always had a biomarker value that would be classified as abnormal according to reference values. This may attenuate its value in predicting MetS. On the other hand, this bias towards the null hypothesis increases the effect of true positive findings. Also, several studies tested a dose-response effect by comparing the effect on

MetS across the quantiles. Studies can be compared on whether a dose-response effect was observed or not.

Figure 2 shows the overall summary of our findings, consisting of a conclusion about the role of each biomarker as independent predictor of MetS in the general population and in survivors, after GRADE assessment. Five biomarkers were identified as independent predictors of MetS in the general population: uric acid, adiponectin, hsCRP, apoB (all high quality of evidence), and leptin (moderate quality). There is conflicting evidence for the value of TNF-alpha, IL-1, IL-6, and lp(a) (very low quality of evidence). Among survivors, uric acid and hsCRP may be valuable as prognostic biomarkers, based on two and one studies, respectively, with very low quality of evidence. There is conflicting evidence for the prognostic value of adiponectin and leptin (very low quality). TNF-alpha and IL-6 appear not to be independent predictors, based on one very low quality study testing both biomarkers. For IL-1, apoB, and lp(a), no studies were found.

Meta-analysis of most relevant findings of enlisted biomarkers

We aimed to perform a meta-analysis of the most promising biomarkers: uric acid, adiponectin, leptin, hsCRP, and apoB. For diagnostic studies, only the AUC is suitable for meta-analysis, due to different thresholds used for sensitivity and specificity (Supplementary Table 6). For predictor studies, only dichotomous and continuous (per unit, or per unit log-transformed) studies are useful. Many studies use quantiles but these are unsuited for meta-analysis: cut-offs between the quantiles depend on the range and distribution in each study population, and are therefore insufficiently comparable between studies to perform a meta-analysis.

A wide variety of outcome measures was used in the studies, and many studies performed an analysis that was unsuited for meta-analysis. Also, there was variance in thresholds used for dichotomous outcomes, as well as in covariates in multivariable models. Therefore, we were unable to retain at least three sufficiently comparable studies for most biomarkers, and for most outcomes, in order to perform a meta-analysis. For a few biomarkers, enough studies were eligible for meta-analysis, because the authors also published crude outcomes, and outcomes that were only age- and sex-adjusted (Supplementary Table 6).

We were able to perform a meta-analysis for the prognostic value of uric acid (hyperuricemia and continuous uric acid levels), and for the diagnostic value of hsCRP. We estimated the pooled OR for the association between hyperuricemia and MetS, adjusted for age and sex (four studies,[93-96] with threshold variability accepted of 10%,[97] OR 2.94, 95%CI 2.08-4.15), the pooled OR per unit increase in uric acid,

unadjusted (three studies,[90, 98, 99] OR 1.086, 95%CI 1.066-1.106), and the pooled AUC for hsCRP, also unadjusted (three studies,[91, 100, 101] AUC 0.71, 95%CI 0.67-0.74).[90, 99] Forest plots are shown in Figure 3. Unfortunately, many studies could not be included, and the reported estimators are not adjusted for relevant covariates, in particular age and sex for some, and overweight, insulin resistance, and smoking for all.

Other, non-enlisted biomarkers

In Supplementary Table 7, 179 articles for all other biomarkers for diagnosis or prognosis of MetS are enlisted and the main data is summarized. These included ratios of our studied biomarkers. All studies investigating leptin/adiponectin ratio as prognostic[62, 68, 73, 102-105] or diagnostic study[68, 102, 104-107] showed a possible relevance. Apolipoprotein A1 (apoA1) and apoB/apoA1 ratio seem valuable in predicting the MetS (6 studies with a protective effect of apoA1[82, 86, 87, 108, 109], and 8 studies with an effect of increasing risk of increasing apoB/apoA1 ratio[82, 108-114]). There are two studies reporting a diagnostic value of apoB/apoA1 ratio.[108, 111] Other recurrently reported, potentially useful biomarkers were Gamma GT, (non-high sensitivity) CRP, ferritin, leukocyte count, hemoglobin and urine pH and sodium excretion.

DISCUSSION

This is the first systematic literature review investigating newer biomarkers for metabolic syndrome (MetS) in CCS, with the aim to obtain the highest level of evidence by including validated tools for risk of bias assessment and summary of evidence, and by performing a meta-analysis.

For five biomarkers, numerous studies with moderate to high quality of evidence were found for diagnosing and predicting MetS: uric acid, adiponectin, leptin, hsCRP, and apoB. The evidence was not sufficient to confirm the value of candidate biomarkers lp(a), IL-1, IL-6, and TNF-alpha.

Meta-analysis of eligible studies showed a predictive value of uric acid for MetS, with a positive association, and a diagnostic value for hsCRP.

These findings suggest that uric acid, adiponectin, leptin, hsCRP, and apoB may be used in a screening setting for CCS, in addition to standard MetS criteria, in order to provide better diagnosis and prediction of MetS (risk). Systematic reviews in other populations have identified not only elevated leptin,[115] uric acid,[115-118] and low

(HWM) adiponectin,[115, 119, 120] but also Il-6[115] and TNF-alpha[115] as potential MetS biomarkers.

As anticipated, the number of publications for survivors on this topic was rather limited: we identified only five studies in CCS specifically, which found a possible predictive value for hsCRP and uric acid, and conflicting or no evidence for the value of adiponectin, leptin and TNF-alpha. Disadvantages of these survivor studies were low patient numbers and moderate to high (detection and confounding) bias risk. No studies investigated the diagnostic value of newer biomarkers. Survivor studies with information on altered biomarker values but no direct comparison between biomarker and MetS occurrence, were excluded.[60, 121-130] We expected to miss many relevant studies when designing the study, if we based our conclusions only on survivor studies. Therefore, evidence in the younger general adult population without childhood cancer history was included in our search as well, leading to 175 general population studies with relevant data which were generalizable to young adult survivors.

CCS can have an increased risk to develop MetS, in particular after treatment with cranial and/or abdominal radiotherapy, intensive chemotherapy, nephrectomy, adrenalectomy, or stem cell transplantation.[43, 131-139] These therapies can lead to several underlying conditions that can increase the risk for (components of) MetS, such as hypothalamic damage, growth hormone deficiency, pancreatic beta cell dysfunction, hypogonadism, hypothyroidism, and altered body composition with increased abdominal fat.[43, 131-139]

Furthermore, it is well acknowledged, that in CCS the biological age progresses faster than their true age, as can be derived from their high level of frailty.[4-9] Previous studies have shown, that the physiologic reserve of CCS with a median age of 33 is similar to that of adults in the general population who are aged 65 years.[6] For this reason, we included studies investigating biomarkers for MetS in the general population, with >75% of participants aged below 65 years, as may be very well applicable to CCS. We excluded studies investigating MetS biomarkers among elderly people on purpose, since they have an even higher level of frailty than CCS, comorbidities and aging factors, which may be confounders in the association between the newer biomarker and metabolic syndrome. We considered that extrapolating conclusions from a general elderly population to CCS could draw invalid conclusions . Based on this approach, all available literature applicable to survivors is now discussed in this review, as it includes both survivor studies as well as all generalizable data from a reasoned selection of the general population studies.

On the other hand, several studies excluded people with certain chronic illnesses.[73, 101, 140-150] This may limit applicability of results to the population of CCS, in which the prevalence of comorbidities is high.[3, 25, 126, 151] This was taken into account when scoring the risk of bias. Additionally, childhood cancer (treatment) related long-term side effects, such as altered fat distribution, sarcopenic obesity, and hormonal disbalances, may play a survivor specific role in the pathogenesis of MetS;[11] development of future studies that apply the use of biomarkers in large cohorts of CCS is therefore important.

Due to differences in study designs and statistical analyses, a wide variety of outcome measures was used. There was also substantial diversity in follow-up time in longitudinal studies. By employing the GRADE tool for summarizing evidence, we were able to draw conclusions for each biomarker from this heterogeneity of results. The meta-analysis was based on few studies, as many studies could not be included. Also, heterogeneity was high in the meta-analysis on uric acid per unit increase, as the study of Liu et al. had a remarkably higher OR than the other two studies.[98]

Furthermore, although the ability of different MetS definitions to predict diabetes and CVD appears to be similar,[152, 153] the use of different definitions (Table 1) can lead to differences in occurrence of MetS. There are subtle differences between the definitions that were mostly used in the included studies (Table 1). The potential consequence of choice of definition is illustrated by studies that tested the biomarker use in diagnosing or predicting MetS according to multiple definitions, and sometimes found different results depending on the definition used.[67, 143, 154, 155] Therefore, comparing different studies and interpreting results of the meta-analysis requires some caution, as a full comparison of the studies is often not possible.

Adiposity, and hence the MetS, can be underdiagnosed in survivors, due to altered body composition after radiotherapy, stem cell transplantation, or amputations. For clinical applicability to survivors, it is important that newer biomarkers play an independent role in MetS, and measurement of newer biomarkers is only useful when their effect is not yet captured by established MetS components. Therefore, we did not investigate routine dyslipidemia and insulin resistance markers in our search (e.g., LDL, HOMA-IR). Although apoB and lp(a) are also lipid markers, they are of interest because they are better predictors of atherogenicity than triglycerides, HDL and LDL – particularly apoB, because it gives an estimate of the total number of circulating atherogenic particles.[156-158]

In this light, it is also favorable that studies adjust for MetS components, such as adiposity and insulin resistance, in order to adjust for potentially major correlations

and interactions,[159-162] and to yield the independent/additional diagnostic and predictive value of the biomarker. Furthermore, it remains important to evaluate other traditional risk factors, including smoking, physical activity, socio-economic status, and family history[78].[163] In addition, genetic profile may still be relevant for MetS risk, although so far this is not included in standard screening.[164-166] Risk of detection and confounding bias remains high, especially in the diagnostic studies, as many studies did not adjust for MetS components and traditional risk factors. In particular for the diagnostic studies, a risk of (detection and) confounding bias remained.

The MetS is defined as a cluster of symptoms such as obesity, hypertension, impaired glucose tolerance and dyslipidemia.[11] These clustered symptoms are related to each other: an imbalance in energy intake and consumption causes a cascade of increased (visceral) adiposity, increased circulating free fatty acids and decreased adiponectin (which causes also an increase in insulin resistance), and high levels of pro-inflammatory and pro-thrombotic mediators, such as TNF-alpha, IL-1 and IL-6.[11, 34] Insulin resistance is associated with a lowered excretion of uric acid by the kidneys, and higher uric acid production.[167, 168] The adipokines leptin and adiponectin are produced by adipocytes.[169] Low leptin values trigger metabolic, behavioral and endocrine responses that aim at a preservation of the fuel reserves of the body.[170] Adiponectin enhances insulin sensitization and suppresses inflammation and cell death.[170, 171].[172, 173] Another important molecule is apoB: all atherogenic lipoproteins carry one single apoB molecule as their structural protein, and therefore apoB represents the atherogenic burden.[174] Serum apoB is a strong predictor of cardiovascular risks[156, 175, 176] and comes in as an important player for the MetS in this review as well. One of the low density lipoproteins carrying an apoB molecule, is Lp(a).[177] The interpretation of Lp(a) values in an individual can be difficult due to a high heterogeneity and wide distribution of Lp(a) concentrations.[178] Although evidence for relevance of Lp(a) for MetS evaluation in survivors was unavailable, it remains a marker of interest, since elevated Lp(a) levels were an independent predictor for cardio- and cerebrovascular outcomes[179-187] and were inversely associated with T2DM.[188]

An important inflammatory marker is (hs)CRP, which is synthesized by hepatocytes[189, 190] in response to infection, inflammation, tissue damage and malignant neoplasia.[189, 190] CRP binds to LDL[189, 191] and may have a causal role in atherogenesis,[189] as it is present in atherosclerotic plaques.[189, 192] Inflammatory markers may reflect a transient state instead of chronic state of inflammation.[193] Still, in the study of Oda et al., the diagnostic value of hsCRP was reproducible when the measurement was repeated after one year.[194] Many studies had a high CRP,[70, 101, 195, 196] or infection[100, 197-200] as exclusion criterion. Regarding inflammation; smooth muscle cells, endothelial cells and macrophages produce cytokines such as IL-1 and IL-6[201-203] in reaction to

metabolic stress,[203, 204] by other inflammatory mediators such as interferon-gamma and TNF, and cholesterol itself.[203] Still, the evidence for the usefulness as marker for the MetS is rather limited.

Due to the systemic nature of MetS, our secondary objective to reveal other interesting biomarkers yielded many markers. Interesting markers for further research include Gamma GT, ferritin, leukocytes and hemoglobin. In several studies biomarkers were related to each other, as MetS components are related as well.[205] In one study, leptin was inversely associated with uric acid excretion;[205] in another, a synergistic effect between hsCRP and high molecular weight adiponectin was found.[206] Also, ratios of biomarkers (e.g. leptin/adiponectin, apoB/apoA1) include extra information and may be better diagnostic or prognostic agents than single biomarkers. Future studies may investigate the value of combining biomarkers.

Some limitations are present in this systematic literature.

Many of the included studies had a cross-sectional design, which is suboptimal to investigate causality; this was taken into account for the GRADE and level of evidence. Some authors conducted prospective longitudinal studies[81, 193, 207, 208] and associated MetS risk at end of follow-up with baseline and/or change in biomarker level. Study designs even more suitable for determining prediction and causality include prediction models and Mendelian randomization.[209-212] These study designs require more time and financial resources, and large cohorts. These types of studies where either not performed or unsuitable for our research question.

Many studies were performed among Asian cohorts. Asian people are more susceptible to insulin resistance,[213, 214] which is accounted for in lower waist circumference thresholds. Additionally, there may be an ethnicity specific component in the relationship between biomarker and MetS.[215-222] This may limit the applicability to a Caucasian population.

For this literature study, we focused on diagnosis and prediction of the full MetS; other outcomes such as resolution of the MetS,[223] components of the MetS, CVD or T2DM were out of scope.[186, 224-235] Therefore, our findings do not provide a complete overview of the use of the newer biomarkers in diagnosing and predicting cardiovascular risk factors in CCS.

We have two suggestions for future research that are relevant for the implementations of our findings in the follow-up of CCS. The newer biomarkers could be added as a sixth criterion for MetS. This application can be especially of value in cases of doubt

of MetS diagnosis for individuals who had abdominal irradiation: it may be valuable to replace waist circumference with the adipokines leptin or adiponectin. This may identify MetS in more survivors, and can potentially improve the predictive ability for T2DM and CVD.[231]

An important requirement for the applicability of these newer biomarkers in such a screening setting for MetS (risk) in CCS, is the determination of a threshold. For uric acid, this is relatively well-established (Supplementary Table 5); for other biomarkers, this is less clear, as is illustrated by the range of applied thresholds (Supplementary Table 5). This is partly because of the use of different assays and testing of subfractions of a biomarker, such as high molecular weight adiponectin. Also, a tradeoff between sensitivity and specificity may influence the determination of an optimal threshold.

In conclusion, based on this systematic literature search, we suggest to consider the additional use of uric acid, adiponectin, hsCRP, leptin, and apoB in the screening setting for metabolic syndrome in CCS. As our conclusions are largely based on general population studies, studies in CCS are needed. Furthermore, future studies may specifically test the use of newer biomarkers as additional MetS components, and define optimal thresholds. The addition of one or more of these newer biomarkers as a criterion for MetS may lead to a newer and better classification and enhanced identification of risk of developing T2DM and CVD, especially in CCS in whom components are difficult to evaluate in the currently applied definitions. Early intervention can delay or prevent complications, and hence improve very long-term survival outcomes and quality of life.

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TABLES AND FIGURES

Table 1. Commonly used metabolic syndrome definitions in selected studies

	NCEP ATP III	IDF 2006	Joint interim statement/ Harmonized definition	Japanese Obesity Society	Chinese Diabetes Society	Children and adolescents	Modified with BMI instead of waist circumference
Required for MetS diagnosis	3 or more criteria	Obesity plus 2 or more criteria	3 or more criteria	Obesity plus 2 or more criteria	3 or more criteria	3 or more criteria	
Obesity	Waist circumference >102cm (men) or >88cm (women)	Waist circumference >90cm (men) or >80cm (women)	Waist circumference with ethnic-specific thresholds	Waist circumference >85cm (men) or >90cm (women)	Body mass index ≥25kg/m^2	Waist circumference ≥90th percentile	Body mass index ≥30 (Caucasians) or ≥25 (Asians) kg/m^2
Insulin resistance	Fasting plasma glucose ≥5.6 mmol/L or treatment	Fasting plasma glucose ≥5.6 mmol/L or treatment	Fasting plasma glucose ≥5.6 mmol/L or treatment	Fasting plasma glucose ≥6.1 mmol/L or treatment	Fasting plasma glucose ≥6.1 mmol/L or treatment	Fasting plasma glucose ≥5.6 mmol/L or treatment	
Dyslipidemia	Triglycerides ≥1.7mmol/L or treatment	Triglycerides ≥1.7mmol/L or treatment	Triglycerides ≥1.7mmol/L or treatment	Triglycerides ≥1.7mmol/L, or HDL cholesterol <1mmol/L (men) or <1.3mmol/L (women), or treatment	Triglycerides ≥1.7mmol/L, or HDL cholesterol <0.9mmol/L (men) or <1.0mmol/L (women), or treatment	Triglycerides ≥1.7mmol/L or treatment	
	HDL cholesterol <1mmol/L (men) or <1.3mmol/L (women) or treatment	HDL cholesterol <1mmol/L (men) or <1.3mmol/L (women) or treatment	HDL cholesterol <1mmol/L (men) or <1.3mmol/L (women) or treatment			HDL cholesterol <1mmol/L or treatment	
Hypertension	≥130/85mmHg or treatment	≥130/85mmHg or treatment	≥130/85mmHg or treatment	≥130/85mmHg or treatment	≥140/90mmHg or treatment	≥130/85mmHg or treatment	

Table 2. Summary of outcomes

Summary of outcomes in diagnostic studies				
Biomarker	**Total number of studies and participants**	**Outcome**	**Number of studies**	**Range**
Leptin, in general population	6 studies, 8,209 participants[68, 102, 106, 154, 236, 237]	AUC	5[68, 102, 106, 236, 237]	0.68-0.93
		Sensitivity	3[102, 154, 237]	48.0-92.6%
		Specificity	3[102, 154, 237]	56.3-72.0%
Uric acid, in general population	9 studies, 73,190 participants[66, 101, 150, 237-242]	AUC	7[66, 101, 150, 237, 239, 240, 242]	0.56-0.85
		Sensitivity	3[237-239]	38.0-76.0%
		Specificity	3[237-239]	56.0-85.0%
Adiponectin, in general population	12 studies, 21,888 participants[63, 65, 67-69, 102, 106, 140, 143, 243-245]	AUC	12[63, 65, 67-69, 102, 106, 140, 143, 243-245]	0.55-0.92
		Sensitivity	2[102, 243]	64.7-69.3%
		Specificity	2[102, 243]	56.0-66.0%
hsCRP, in general population	7 studies, 18,211 participants[64, 91, 100, 101, 208, 246, 247]	AUC	6[64, 91, 100, 101, 208, 247]	0.55-0.74
		Sensitivity	3[208, 246, 247]	51.0-69.0%
		Specificity	3[208, 246, 247]	56.6-72.0%
ApoB, in general population	1 study, 8,120 participants[111]	AUC	1[111]	0.68
TNF-alpha, in general population	1 study, 976 participants[64]	AUC	1[64]	0.54
IL-6, in general population	1 study, 976 participants[64]	AUC	1[64]	0.56
IL-1 and lp(a), in general population	No studies	n.a.	n.a.	n.a.
All biomarkers, in survivors	No studies	n.a.	n.a.	n.a.

Summary of outcomes in prognostic studies				
Biomarker	**Total number of studies and participants**	**Outcome**	**Number of studies**	**Range**
Uric acid, in general population	78 studies, 447,559 participants[71, 72, 75, 77, 82, 88, 90, 92-96, 98, 99, 101, 150, 207, 210, 238, 239, 241, 242, 248-303]	OR dichotomous	21[77, 82, 93-96, 98, 150, 248, 254, 257, 258, 264, 266, 267, 274, 280, 287, 296, 297, 304]	1.00-5.17
		OR per unit	19[90, 92, 98, 99, 101, 238, 249, 250, 255, 260, 262, 263, 265, 283, 285, 288, 289, 298]	1.001-2.14
		OR per unit log-transformed	2[239, 302]	1.16, 2.08
		OR highest quantile	24[72, 95, 251, 253, 256-259, 261, 268, 271, 272, 275, 277-279, 281, 282, 290-292, 296, 301, 303]	1.00-8.04
		HR dichotomous	5[207, 239, 242, 270, 300]	1.06-2.99
		HR per unit	4[241, 285, 286, 294]	1.10-2.35
		HR per SD	3[207, 210, 295]	0.86-1.36
		HR highest quantile	8[207, 241, 252, 269, 286, 293-295]	0.74-3.47
		HR per unit longitudinal increase	2[207, 285]	1.05, 1.31
		RR per unit log-transformed	1[276]	7.25 for men, 13.26 for women
		RR per SD	1[71]	1.10
		RR per 1.4mg/dl	1[88]	1.54 for men, 1.82 for women
		RR highest quantile	2[71, 299]	1.69, 1.76
		PR	2[75, 284]	1.47, 2.10
		IRR	1[75]	1.73

Table 2. Continued

Summary of outcomes in prognostic studies				
Biomarker	**Total number of studies and participants**	**Outcome**	**Number of studies**	**Range**
Uric acid, in survivors	2 studies, 390 survivors[58, 59]	MetS prevalence in uric acid Q4 vs Q1-3	1[59]	28.5% vs 12.5% (p=0.0044)
		MetS component(s) prevalence high vs low uric acid	1[58]	60% vs 24% (p=0.04)
Adiponectin, in general population	38 studies, 56,656 participants [65, 67, 69, 70, 73, 74, 76, 78, 81, 102, 103, 105, 140-148, 159, 245, 305-319]	OR dichotomous (low adiponectin)	2[78, 103]	0.90, 2.68
		OR per unit	9[67, 73, 76, 141, 144, 148, 159, 309, 314]	0.66-1.08
		OR per 5 units	1[69]	0.82 for men, 0.90 for women
		OR per unit log-transformed	4[74, 146, 245, 306]	0.10-0.67
		OR per SD	2[315, 317]	0.50-0.91
		OR per unit log-transformed Z-score	1[102]	0.76 for boys, 0.69 for girls
		OR highest quantile	13[69, 105, 140, 142, 143, 145, 147, 305, 308, 315, 317-319]	0.10-0.67
		OR lowest quantile	6[65, 81, 307, 312, 313, 316]	1.82-18.6
		HR high baseline and increase during follow-up vs low baseline and decrease	1[311]	0.33
		HR decreased at follow-up	1[81]	4.37
		Time ratio of developing MetS Q1 vs Q4	1[310]	0.15 (=85% shorter time to develop MetS)
		Baseline ratio (value in MetS subjects divided by value in non-MetS, adjusted for covariates)	1[70]	1.27
Adiponectin, in survivors	3 studies, 139 survivors[58, 60, 62]	OR highest quantile at baseline and follow-up	1[62]	0.5 (n.s.) for baseline, 0.9 (n.s.) for follow-up
		HR dichotomous (low adiponectin)	1[58]	6.7
		P-value of Kruskal-Wallis test median adiponectin in 0, 1, 2-4 MetS components	1[60]	n.s.

Table 2. Continued

Summary of outcomes in prognostic studies				
Biomarker	**Total number of studies and participants**	**Outcome**	**Number of studies**	**Range**
hsCRP, in general population	32 studies, 119,138 participants[70, 74, 83-85, 88, 90, 91, 147, 155, 193, 195-199, 208, 246, 249, 265, 269, 320-330]	OR dichotomous	2[155, 196]	1.20, 2.74
		OR per unit	7[90, 91, 198, 199, 249, 328, 330]	1.007-2.97
		OR per unit log-transformed	4[74, 246, 265, 324]	1.15-3.2
		OR per SD	1[85]	1.21
		OR per SD log-transformed	2[208, 325]	0.96, 1.07
		OR highest quantile	11[84, 147, 193, 197, 321-323, 325-327, 329]	1.07-7.11
		OR highest of three groups (<1.0, 1.0-3.0 and >3 µg/ml)	3[195, 320, 324]	1.65-18.86
		HR per unit log-transformed	1[269]	1.15
		RR per threefold increase	1[88]	1.13
		Baseline ratio	1[70]	0.80 (n.s.)
		P-value of likelihood test in multivariable model	1[83]	n.s.
hsCRP, in survivors	1 study, 87 survivors and 87 controls[61]	OR dichotomous	1[61]	7.26
ApoB, in general population	10 studies, 66,924 participants[74, 79, 82, 86, 87, 108, 109, 331-333]	OR dichotomous	1[82]	2.55
		OR per unit	1[74]	2.99
		OR per 30mg/dl	1[87]	1.76 for men, 2.10 for women
		OR per SD	1[331]	1.56
		OR highest quantile	6[79, 108, 109, 331-333]	0.96-6.03
		OR highest of three groups (<90, 90-119 and ≥120 mg/dl)	1[86]	2.69 for men, 1.69 for women
		RR per SD	1[331]	1.17 (n.s.)
		RR highest quantile	1[331]	1.79
ApoB, in survivors	No studies	n.a.	n.a.	n.a.
Leptin, in general population	17 studies, 28,797 participants [68, 73, 74, 102, 103, 147-149, 236, 306, 314, 315, 319, 334-337]	OR dichotomous	1[103]	2.39
		OR per unit	4[73, 148, 314, 336]	0.96-1.91
		OR per 10ng/ml	1[149]	1.06 (adjusted for WC), 1.22 (adjusted for BMI)
		OR per unit log-transformed	2[74, 306]	1.47, 2.76
		OR per SD	3[68, 315, 335]	1.01-1.31
		OR per unit log-transformed Z-score	1[102]	1.81 for boys, 1.32 for girls
		OR highest quantile	6[147, 236, 315, 319, 334, 337]	1.16-3.02

Table 2. Continued

Summary of outcomes in prognostic studies				
Biomarker	**Total number of studies and participants**	**Outcome**	**Number of studies**	**Range**
Leptin, in survivors	3 studies, 139 survivors [58, 60, 62]	OR highest quantile at baseline and follow-up	1 [62]	4.8 for baseline, 5.7 for follow-up
		MetS component(s) prevalence high vs low leptin	1 [58]	54% vs 17% (p=0.03)
		P-value of Kruskal-Wallis test median adiponectin in 0, 1, 2-4 MetS components	1 [60]	n.s.
IL-6, in general population	5 studies, 3,370 participants [67, 80, 143, 199, 200]	OR per unit	2 [67, 199]	0.98-1.47
		OR highest quantile	2 [143, 200]	0.98 (n.s.), 4.10
		P-value in multivariable model	1 [80]	n.s.
IL-6, in survivors	1 study, 87 survivors and 87 controls [61]	OR dichotomous	1 [61]	1.53 (n.s.)
Lp(a), in general population	5 studies, 15,162 participants [89, 320, 338-340]	OR dichotomous	1 [320]	8.27
		OR highest of three groups (<18.40, 18.40-33.84 and ≥33.85 μg/ml)	1 [338]	0.82 (n.s.)
		OR per unit	1 [339]	1.0 (n.s.)
		OR highest quantile	1 [340]	0.45
		HR highest quantile	1 [89]	1.01 (n.s.)
Lp(a), in survivors	No studies	n.a.	n.a.	n.a.
IL-1, in general population	4 studies, 1,594 participants [70, 199, 200, 341]	OR per unit	2 [199, 341]	2.28 (IL-1alpha), 1.009, 2.01 (IL-1beta)
		OR highest quartile	1 [200]	0.98 (n.s.)
		Baseline ratio	1 [70]	1.17 (suggests effect in other direction)
TNF-alpha, in general population	3 studies, 1,458 participants [80, 199, 200]	OR per unit	1 [199]	1.45
		OR highest quartile	1 [200]	0.78 (n.s.)
		P-value in multivariable model	1 [80]	n.s.
TNF-alpha, in survivors	1 study, 87 survivors and 87 controls [61]	OR dichotomous	1 [61]	0.52 (n.s.)

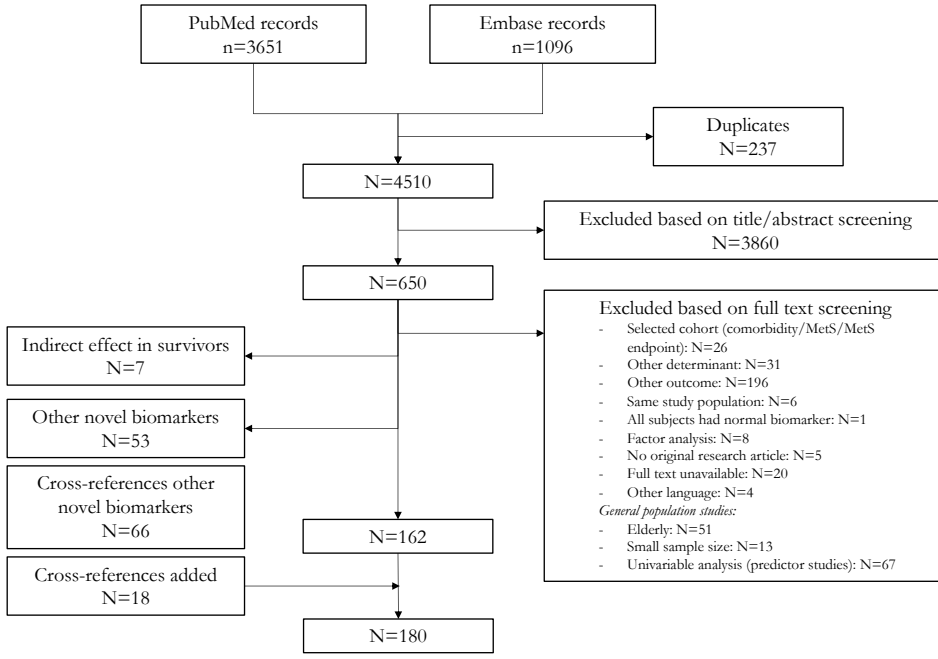

Figure 1 Flow chart of in- and excluded articles from the systematic literature search

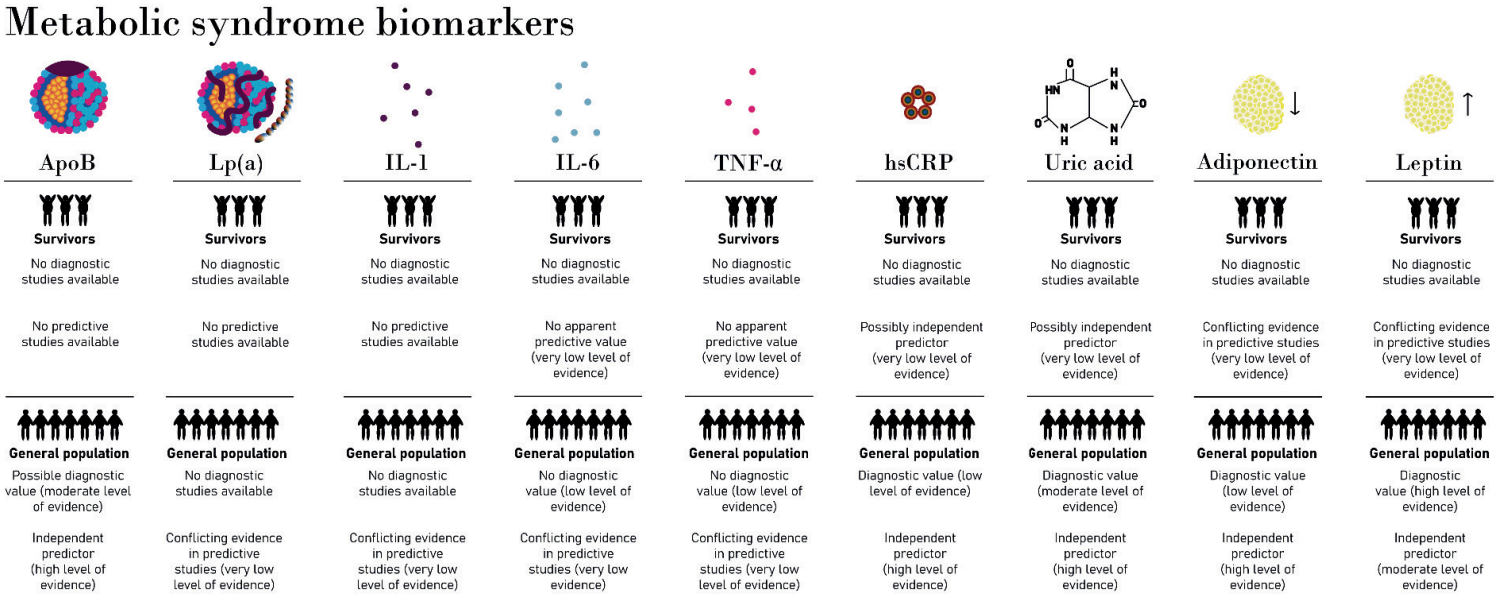

Figure 2 Summary of conclusions: predictive and diagnostic value of novel biomarkers for the MetS

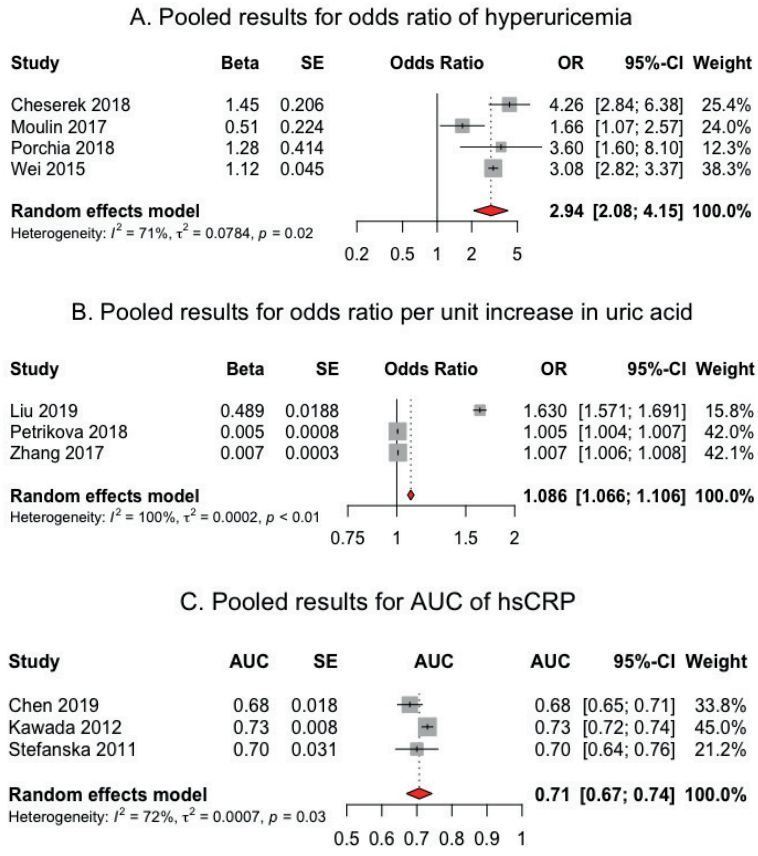

Figure 3 Forest plots for different study-specific outcomes.

A. Odds ratio (OR) for hyperuricemia. B. OR for per unit increase in uric acid. C. Area under the curve (AUC) of hsCRP. The sizes of the square boxes on the forest plots are proportional to the total number of patients in the selected trials

SUPPLEMENTARY MATERIAL

Part 1 Enlisted biomarkers

Summary of evidence diagnostic and predictive value of biomarkers for MetS

Part 2 Other biomarkers (not enlisted in advance)

The supplementary material is available online: https://onlinelibrary.wiley.com/doi/full/10.1111/obr.13312

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Chapter 4

Metabolic syndrome parameters, determinants, and biomarkers in adult survivors of childhood cancer: protocol of the Dutch Childhood Cancer Survivor Study Metabolic Syndrome (Dutch LATER METS study)

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ABSTRACT

Background

Potential late effects of treatment for childhood cancer include adiposity, insulin resistance, dyslipidemia and hypertension. These risk factors cluster together as metabolic syndrome (MetS) and increase the risk for development of diabetes mellitus and cardio- and cerebrovascular disease. Knowledge on risk factors, timely diagnosis and preventive strategies is of importance to prevent cardio- and cerebrovascular complications and improve quality of life. Currently, no studies in national cohorts on prevalence and determinants of MetS in childhood cancer survivors including biomarkers and genetic predisposition are available.

Objectives

The objectives of the Dutch LATER METS study are to assess 1) the prevalence and risk factors of MetS and its separate components, and 2) the potential value of additional biomarkers, in the national cohort of adult long-term survivors of childhood cancer.

Methods

This is a cross-sectional study, based on recruitment of all survivors treated in the Netherlands between 1963 and 2002. MetS will be classified according to the definitions of the National Cholesterol Education Program (NCEP-ATP III) as well as the Joint Interim Statement (JIS), and compared to reference data. Dual-energy X-ray absorptiometry (DXA) scans were performed to assess body composition in more detail. The effect of patient characteristics, previous treatment, and genetic variation on the risk of MetS will be assessed. The diagnostic and predictive value of novel biomarkers will be tested.

Results

Patient accrual started in 2016 and lasted until April 2020. A total of 2380 survivors has participated, in seven pediatric oncology hospitals. From July 2020, biomarker testing, SNP analysis and data analysis will be performed.

Conclusions

The Dutch LATER METS study will provide knowledge on clinical and genetic determinants of MetS, and the diagnostic value of biomarkers, in childhood cancer survivors. The results of this study will be used to optimize surveillance guidelines for MetS in survivors, based on enhanced risk stratification and screening strategies. This will improve diagnosis of MetS, and prevent complications.

INTRODUCTION

Because of increasing survival of patients with childhood cancer, late side effects have become more prominent. Potential late effects include adiposity, insulin resistance, dyslipidemia and hypertension, which cluster together as metabolic syndrome (MetS). MetS is associated with a higher risk of diabetes mellitus, as well as cardio- and cerebrovascular morbidity and mortality later in life [1-3]. The separate components are in itself risk factors for diabetes and cardiovascular disease, but when coexisting, the components can aggravate each other, leading to an even higher risk [4, 5].

Studies in childhood cancer survivors have reported a prevalence of MetS of over 30% after 25 years follow-up, substantially higher compared to age- and sex-matched controls (odds ratio 1.76) [6, 7]. This apparent risk difference for MetS further increases the elevated risks for cardiovascular outcomes and endothelial damage from anthracyclines, alkylating agents and irradiation [8, 9]. Consequently, the mortality due to coronary and cerebrovascular disease in long-term survivors is up to 12.7 times higher than the general population [10-13]. The fact that MetS can be subclinical for many years, emphasizes the need for timely identification of MetS in survivors and early intervention strategies. Lifestyle and diet advices, exercise and medication may prevent the development of diabetes and cardio- and cerebrovascular disease, improving survival rates and quality of life.

Several underlying conditions have been reported to increase the risk for (components of) MetS in survivors: growth hormone deficiency, pancreatic beta cell dysfunction, hypogonadism, hypothyroidism, and altered body composition with increased intra-abdominal fat [14-19]. Hence, an increased risk of MetS might be associated with treatment for a brain tumor, treatment with radiotherapy, intensive chemotherapy, nephrectomy, adrenalectomy, or stem cell transplantation (SCT) [7, 16, 20-32]. The effects of other potentially harmful treatments, for example corticosteroids, and patient-related factors such as sex, age, BMI at diagnosis, and lifestyle, are still not clear [3]. Also, heterogeneity in incidence of MetS among homogeneously treated survivors, suggests a role of genetic susceptibility [33, 34]. A few studies, using candidate gene approach as well as one genome-wide association study, have identified genetic variants that might be associated with development of MetS and its components in survivors [24, 35, 36]. Results based on these studies have not yet been replicated nor functionally validated.

Multiple definitions of MetS have been developed over the past years. The two most commonly used are those of the National Cholesterol Education Program (Adult Treatment Panel, third report: NCEP-ATPIII) and the Joint Interim Statement (JIS)

of the International Diabetes Federation (IDF), National Heart, Lung, and Blood Institute (NHLBI), and the American Heart Association (AHA). Both definitions overlap largely but they differ in waist circumference cut-off point (Table 1) [37, 38]. Apart from the four components, pro-inflammatory and pro-thrombotic markers have been reported to be relevant biomarkers of MetS, as well as hyperuricemia [39, 40].

Adequate assessment of MetS in survivors using the NCEP-ATP III and JIS definitions has specific challenges, particularly after abdominal radiotherapy. It has been shown that BMI and waist circumference underestimate adiposity, due to deformation of spine, muscles and fat, particularly in past treatment eras when higher radiotherapy doses and larger fields were used [21, 41, 42]. Similarly, adiposity can be disguised due to sarcopenic obesity after SCT [43, 44]. Body composition can be more reliably measured by Dual-energy X-ray absorptiometry (DXA scan), but this is time consuming and expensive to be implemented for standard follow-up of all survivors. Serum biomarkers may be more cost-effective surrogate markers for MetS. In smaller survivor cohorts, and in the general population, biomarkers – other than triglycerides and high-density lipoprotein (HDL) cholesterol – that have been proposed as predictors of MetS include low-density lipoprotein (LDL), apolipoprotein-B (apo-B), leptin, adiponectin, uric acid, and C-reactive protein (CRP) [39, 45-50].

So far, large studies on clinically diagnosed MetS in survivors are scarce. Two large multi-center cohort studies with clinically diagnosed MetS are the American St. Jude Lifetime (SJLIFE, all types of childhood cancer) [6, 7] and the French Leucémies de l'Enfant et l'Adolescent (leukemia) [31, 51, 52] studies. Other studies have yielded heterogeneous and sometimes conflicting results, and can be difficult to compare. This may be due to MetS components being analyzed only separately, or due to small patient cohorts, a questionnaire based or retrospective design, insufficient treatment data (e.g., only childhood cancer diagnosis is known, not treatment), and short follow-up (MetS risk increases continuously with age, so a follow-up of 10 to 20 years likely underestimates this) [22, 32, 53-55]. In addition, comparison of study outcomes can be difficult due to the use of different classifications. Currently, no studies in national cohorts on prevalence and determinants of MetS in childhood cancer survivors, including biomarkers and genetic predisposition to MetS, are available.

Here we describe the methodology of the Dutch LATER METS study in the adult cohort of survivors treated between 1963 and 2002. This nationwide study assesses MetS prevalence, clinical and genetic risk factors, and the diagnostic and predictive value of additional biomarkers. The results of this study will be used to identify survivors at risk and to optimize surveillance guidelines.

Table 1. NCEP-ATP III and JIS classifications of metabolic syndrome, and alternative classification with adiposity measured by DXA scan.

		NCEP-ATP III [37]	JIS [38]	Alternative with DXA scan
Required for diagnosis			**≥3**	
Adiposity	Waist circumference (cm)	>102[a]/88[b]	≥94[a]/80[b c]	Body fat Z-score >2
Insulin resistance	Fasting glucose (mmol/L)		≥5.5 or treatment	
Dyslipidemia	Triglycerides (mmol/L)		≥1.7 or treatment	
	HDL cholesterol (mmol/L)		<1.0*/1.3** or treatment	
Hypertension	Blood pressure (mmHg)		≥130/85 or treatment	

[a] men; [b] women; [c] cut-off for Caucasian population

NCEP-ATP III = National Cholesterol Education Program Adult Treatment Panel III; JIS = Joint Interim Statement of IDF, NHLBI and AHA.

METHODS

Objectives

The objectives of this study are to assess 1) the prevalence and risk factors (patient characteristics, previous treatment, and genetic variation) of MetS and its separate components, compared to reference data, and 2) the potential diagnostic and predictive value of novel biomarkers for surveillance for MetS, in the national cohort of adult long-term survivors of childhood cancer.

Study population and design

The current study is part of the nationwide Dutch LATER study (Figure 1). This study started accrual in all seven pediatric oncology centers in The Netherlands in 2016, thereby inviting the national cohort of all survivors treated in these hospitals between 1963 and 2002 to participate. Survivors were identified from registries of children with newly diagnosed cancer, that are maintained in each of the seven pediatric oncology centers in The Netherlands. For the current study, this information was merged to a specific childhood cancer survivors registry, containing all registered survivors. Dependent on completeness of these sources in the centers, the starting year varied from 1963 to 1977. The LATER METS study was approved by the Medical Research Ethics Committee of the Amsterdam UMC, The Netherlands (registered at toetsingonline.nl, NL32117.018.10)

In the Dutch LATER study, data for fifteen sub-studies of late effects were collected, including cardiotoxicity, bone density, frailty, growth hormone deficiency, renal toxicity, fatigue, and psychological late effects. Individuals who survived at least five years after diagnosis of histologically confirmed malignancies defined in the

International Classification of Childhood Cancer, edition 3 [56], or Langerhans cell histiocytosis treated with chemotherapy and/or radiotherapy, who were between 0 and 17 years of age at diagnosis, were invited. Exclusion criteria were treatment for a malignancy in the past year, and living abroad.

For all eligible survivors, prior to the visit of the late-effects clinic, sex, date of birth, date of cancer diagnosis, and detailed data on cancer type and treatment, including chemotherapy regimens and doses, radiotherapy fields and (fractionated) dose, SCT and corticosteroids, have been collected in a pseudonymized, web-based, central database. This includes primary diagnosis as well as, if present, recurrences and subsequent malignancies.

Subsequently, data collection for all studies was combined with the survivors' regular care visit to the late-effects clinic for the majority of survivors. Before the visit, survivors received information about the study, sent by mail by the study personnel. If they agreed to participate, study data was collected by the treating physician and/or the study personnel.

The entire cohort, at formation in 2008, contained 6165 eligible survivors. By mail, survivors were provided the option to opt-out of future study participation. For the Dutch LATER study, the cleaned cohort was frozen in 2016, leaving 5160 subjects eligible. For the LATER METS study, only adults (n=4741) were invited. Inclusion took place until April 2020. Written informed consent was obtained from all study participants.

Reference population

Normative data from the Dutch Lifelines study cohort will serve as reference population [57]. This is a three-generational cohort of 167,000 inhabitants (10%) of the north of the Netherlands, of whom, among other data, the following parameters relevant to our study were collected between 2006 and 2013: age, sex, height, weight, waist and hip circumference, blood pressure, co-morbidities, medication use, smoking, physical activity, HDL, triglycerides, glucose, apo-B, LDL, total cholesterol, uric acid, and high sensitivity CRP (hsCRP). We aim to use a subset of this reference cohort of controls that have the same age and sex distribution as our study cohort.

Data collection

Data collected before visit of late-effects clinic

An overview of collected variables is presented in Table 2. In addition to the previously mentioned data, the following variables relevant for the METS study were extracted

from the medical records: height and weight at cancer diagnosis, and relevant comorbidities.

Data collected at visit of late-effects clinic

Weight was measured without shoes and with light clothing, on an electronic scale to the nearest 0.1kg. Height was measured without shoes to the nearest centimeter. Body mass index (BMI) was calculated from weight and height. Waist circumference was measured in the middle between the lower rib and iliac crest to the nearest centimeter. Hip circumference was measured at the greater trochanter to the nearest centimeter. Waist/hip ratio was calculated. Blood pressure was measured after at least five minutes rest with an electronic oscillometric meter (the mean of two measurements).

Survivors completed a general health questionnaire, containing questions about co-morbidities, current medication use, smoking and alcohol habits, education level, and family history of diabetes mellitus and cardiovascular disease. They also completed the SQUASH questionnaire on physical activity [58].

Total body DXA scans (Hologic and Lunar types) were used to assess body composition [41]. These measurements include fat percentage and lean body mass.

The 6-minutes walking test was performed in a subset of the survivors (those treated in the Sophia children's hospital/Erasmus MC, Rotterdam), as a measure of functional exercise capacity [59, 60].

Data determined from stored samples

Venous blood samples were drawn after overnight fasting, and stored at -80°C in the biobank. To assess dyslipidemia, a lipid spectrum will be measured, consisting of triglycerides, HDL, LDL, total cholesterol, and apo-B. Insulin resistance will be assessed by measuring glucose and insulin. Additionally, adiponectin, leptin and uric acid will be measured. Inflammatory markers include hsCRP, interleukin-6 (IL-6), high sensitivity tumor necrosis factor alpha (hsTNFα) and IL-1. The following possible confounders will be measured: IGF-1, kidney function (creatinine, urea), sex hormones (luteinizing hormone (LH), follicle stimulating hormone (FSH), anti-Müllerian hormone (AMH) (women), estradiol (women), testosterone (men)), thyroid function (thyroid-stimulating hormone (TSH), free T4), cortisol.

DNA for analysis of single nucleotide polymorphisms (SNPs) will be isolated from blood or, in survivors who received allogeneic SCT, saliva. Saliva was obtained by spitting into a collection tube (Oragene kit), after not drinking or eating for 30 minutes.

Metabolic syndrome definition

MetS will be classified according to the NCEP-ATPIII and JIS definitions [37, 38] (Table 1). Should these criteria be updated during our analysis, we will strive to take these adjustments into account.

Risk of bias

Sex, date of birth, date of cancer diagnosis, and disease and treatment data are also available for non-participating survivors. Hence, comparing participating and non-participating survivors is feasible, in order to determine the risk of selection bias. We will also compare these data between survivors with complete and incomplete data, to judge the risk of attrition bias. Neither physician nor study personnel were blinded to the exposures of the survivors. Objectively measurable outcomes will reduce the risk of bias in this setting.

Statistical analysis

Prevalence of MetS

The percentage of subjects with MetS and the separate components will be assessed in survivors and in the Lifelines reference cohort according to both aforementioned MetS definitions. Both cohorts will be compared by chi-square (or Fisher's exact) test. The relative risk for survivors to develop MetS, compared to Lifelines reference data, will be calculated by employing a log-binomial regression model. The agreement between both MetS definitions will be investigated with *kappa* statistic, in the whole cohort and stratified by sex.

A total body fat percentage of more than two standard deviations above the mean, as assessed by DXA, will be used as most reliable marker for adiposity. We will estimate the correlation between waist circumference and fat percentage measured by DXA scan, and we will compare overweight classification with both definitions.

Risk factors

Treatment-related risk factors for occurrence of MetS and the separate components will be assessed using multiple uni- and multivariable logistic regression models. Based on literature, an initial model will be built with cranial radiotherapy, abdominal radiotherapy, and alkylating agents (total alkylating dose calculated using cyclophosphamide equivalent dose [61]) as treatment-related independent variables, and age, sex, follow-up time, and smoking as patient-related independent variables. The effect of potential additional risk factors will be assessed by adding them to the initial model, and variables with a p-value <0.20 will be kept in the final model. These potential risk factors include all other chemotherapy agents (type and total cumulative

dose), other radiotherapy fields (body location and dose), corticosteroids, education level, family history, physical activity, functional exercise capacity, and comorbidities.

We will also investigate different abdominal radiotherapy fields involved (pancreas, liver), and the influence of SCT conditioning regimens. We will also study patient- and treatment-related risk factors for the outcome underdiagnosis of overweight measured by waist circumference.

Biomarkers

Biomarker values will be reported, with reference values from the local laboratory where the samples are measured. This will be compared to Lifelines reference data by chi-square (or Fisher's exact) test. A risk factor analysis of altered biomarker values will be performed similar to the abovementioned strategy for risk factor analysis of MetS occurrence.

The diagnostic and predictive value of the biomarkers to detect MetS will be investigated in multiple steps. We will stratify the survivors by MetS presence or absence, and compare mean or median values with the *t*-test or Mann-Whitney U test. We will evaluate sensitivity and specificity, and positive and negative predictive value, based on the reference values of the local laboratory where the samples are measured. We will compare the area under the curve for a model with MetS components and for a model with each biomarker added, in order to investigate the additional diagnostic value of the novel biomarkers. We will build multivariable logistic regression models with MetS as dependent variable, and the biomarker as independent variable. In these models, we will also include MetS components as covariates, in order to investigate the independent predictive value of the novel biomarkers. We will estimate the MetS risk by including the biomarker as categorical as well as continuous variable.

Correlation (Pearson or Spearman) between biomarkers and fat percentage by DXA scan will be used to measure the potential use as surrogate markers for adiposity.

Genetic susceptibility analysis

Genotyping will be performed with the Infinium Global Screening Array (Illumina, San Diego, California, USA [62]), on DNA isolated from blood or, in post-SCT survivors, saliva. Quality control of the genotype data will be performed following a standardized protocol [63] including filtering based on call rate (excluded when <0.975 for either SNP or individual call rate), Hardy-Weinberg equilibrium, excess heterozygosity, gender mismatches, and familial relationships. Genetic ancestry will be assessed based on principal component analysis. Imputation will be performed

with the Michigan Imputation Server using standard settings [64] with reference panel Haplotype Reference Consortium version r1.1 [65].

The SNP analysis will be performed with the RVtests software package [66], using multiple logistic regression models with MetS and its separate components as outcomes. The initial analysis will be adjusted for age at follow-up, sex, and genetic ancestry. Then, potentially relevant covariates will be added to the model using forward selection, to study whether they influence the SNP analysis; if so, they will be kept in the model. These covariates include: BMI at follow-up, comorbidities (growth hormone deficiency, hypogonadism, diabetes mellitus, and hypothyroidism), cranial and abdominal radiotherapy, and alkylating agents (cyclophosphamide equivalent dose). We will also perform a time-to-event analysis (with left-censoring) on identified hits in order to get clinically relevant effect estimates.

Quality control of the SNP analysis will be performed with the EasyQC package using standard settings [67]. This includes filtering based on minor allele frequency (MAF, excluded when <0.05) and imputation quality (excluded when <0.3).

Visualization of the genetic associations, and annotation of biological function for the top SNPs, will be performed with the FUMA platform [68]. Findings will be replicated in available independent international cohorts.

RESULTS

Patient accrual

Patient accrual started in 2016 and lasted until April 2020. A total of 2380 survivors has participated (participation rate 50.2%). From July 2020, biomarker testing, SNP analysis and data analysis will be performed.

Power calculation

We performed a power calculation with an expected prevalence of MetS in our study cohort of 30%. This percentage is based on results from the SJLIFE cohort, in which the prevalence of clinically diagnosed MetS in 1598 survivors, after a mean of 25.6 years since diagnosis, was 31.8% [6]. This is the only large cohort study so far with clinically diagnosed MetS in survivors of heterogeneous malignancies, with a follow-up time comparable to our cohort.

Based on the sample size of 2380 survivors, expected MetS prevalence of 30%, power of 80%, and type I error of 0.05, we will have sufficient power to detect a ~3% difference in MetS prevalence with the reference cohort. For risk factor analysis among survivors, depending on in how many survivors the risk factor (for example, a treatment regimen) is present, e.g. in 10, 25 and 50% of survivors, a minimum difference of ~9%, ~7%, and ~6%, respectively, can be detected.

A genetic power calculator was used to estimate the relative risk that can be found in the genetic susceptibility analysis for an assumed MAF of 0.25 [69]. Based on the sample size of 2380, MetS population prevalence of 15% [70], a power of 80%, a type I error of $5*10^{-8}$, and a case-control ratio of 1:2, the relative risk per high risk allele that can be found is 1.5.

DISCUSSION

In the current study, we will assess the prevalence and patient and treatment-related risk factors for MetS and its separate components, in adult survivors of childhood cancer, as well as the additional diagnostic value of novel biomarkers for surveillance, and the genetic susceptibility to (treatment-related) MetS by SNP analysis.

A total of 2380 survivors has participated in the study. This corresponds to 38.6% of all survivors in the Dutch LATER cohort, and a participation rate of 50.2% of invited adult survivors. The definitive numbers of refusals, non-responders, deaths or otherwise excluded subjects are not available yet. We will report these in the paper with the results of our study.

Strengths of this study include the availability of a national cohort of survivors, the availability of comprehensive disease and treatment data, and the clinical assessment of late effects, in addition to questionnaire based endpoints. So far, the role of biomarkers and genetic susceptibility to MetS has not been well defined in survivors. We specifically intend to use DXA scans and relevant biomarkers (those with a high independent diagnostic or prognostic value, and a high correlation with fat percentage on DXA scan), to enable identification of survivors at risk for MetS, in whom waist circumference measurement is not feasible due to abdominal radiotherapy.

In conclusion, our study will provide knowledge on clinical and genetic determinants of MetS, and the diagnostic value of biomarkers, in adult childhood cancer survivors. The results of this study will be used to optimize surveillance guidelines for MetS among survivors, based on enhanced risk stratification and screening strategies. This

will improve the diagnosis of MetS, and prevent complications, thereby improving quality of life.

Table 2. Overview of collected variables

Category	Variable	Unit(s) or categories
Collected before visit of late-effects clinic		
Childhood cancer type and treatment	Primary childhood cancer diagnosis	ICCC-3 classification
	Treatment protocol	Name and arm
	Chemotherapy, per regimen	TCD
	Radiotherapy fields…	TCD, fractions (if applicable)
	Cranial/craniospinal	
	Total body	
	Abdominal	
	Pancreas involvement	
	Surgery procedure	
	Autologous SCT	Yes / No, conditioning regimen
	Allogeneic SCT	Yes / No, conditioning regimen
	Relapse	Yes / No
Patient characteristics	Sex	Male / Female
	Date of birth	Date
	Date of childhood cancer diagnosis	Date
	Date of study measurements (follow-up date)	Date
Medical history	Height at cancer diagnosis	Centimeter
	Weight at cancer diagnosis	Kilogram
	Growth hormone deficiency	Yes / No
	Growth hormone replacement	Yes / No
	Hypothyroidism	Yes / No
	Hypogonadism	Yes / No
	Hypocortisolism with steroid replacement	Yes / No
Collected at visit of late-effects clinic		
Physical examination	Height	Centimeter
	Weight	Kilogram
	Waist circumference	Centimeter
	Hip circumference	Centimeter
	Blood pressure	mmHg
General health questionnaire	Does the survivor have or has the survivor experienced…	Yes / No, age at diagnosis
	High cholesterol	
	Hypertension	
	Diabetes mellitus	
	Myocardial infarction	
	Stroke	
	Medication use	Type, dose, age at start
	Smoking status	Yes / Former / No
	Cardiovascular disease in family	Relative, type of disease, age at diagnosis
SQUASH questionnaire on physical activity		

Table 2. Continued

Category	Variable	Unit(s) or categories
DXA scan	Total body fat	Percentage
	Z-score total body fat	Z-score
	Lean body mass	Kilogram per m^2
	Appendicular lean body mass	Kilogram per m^2
6-minutes walking test		Meter
Data determined from stored samples		
Serum biomarkers	HDL	mmol/L
	LDL	mmol/L
	Total cholesterol	mmol/L
	Apo-B	g/L
	Glucose	mmol/L
	Insulin	pmol/L
	Adiponectin	ug/mL
	Leptin	ng/mL
	Uric acid	mmol/L
	hsCRP	mg/L
	IL-6	pg/mL
	hsTNFα	pg/mL
	IL-1	pg/mL
	IGF-1	ug/L
	Creatinine	mg/mmol
	Urea	mmol/L
	LH	U/L
	FSH	U/L
	AMH	ug/L
	Estradiol	pmol/L
	Testosterone	nmol/L
DNA from blood/saliva		

ICCC-3= International Classification of Childhood Cancer, edition 3; TCD=total cumulative dose; SCT=stem cell transplantation; DXA=Dual-energy X-ray absorptiometry; HDL=high-density lipoprotein; LDL=low-density lipoprotein; Apo-B=apolipoprotein B; hsCRP=high-sensitivity C-reactive protein; IL-6=interleukin-6; hsTNFα=high-sensitivity tumor necrosis factor alpha; IL-1=interleukin-1; IGF-1=insulin-like growth factor 1; LH=luteinizing hormone; FSH=follicle stimulating hormone; AMH=anti-Müllerian hormone.

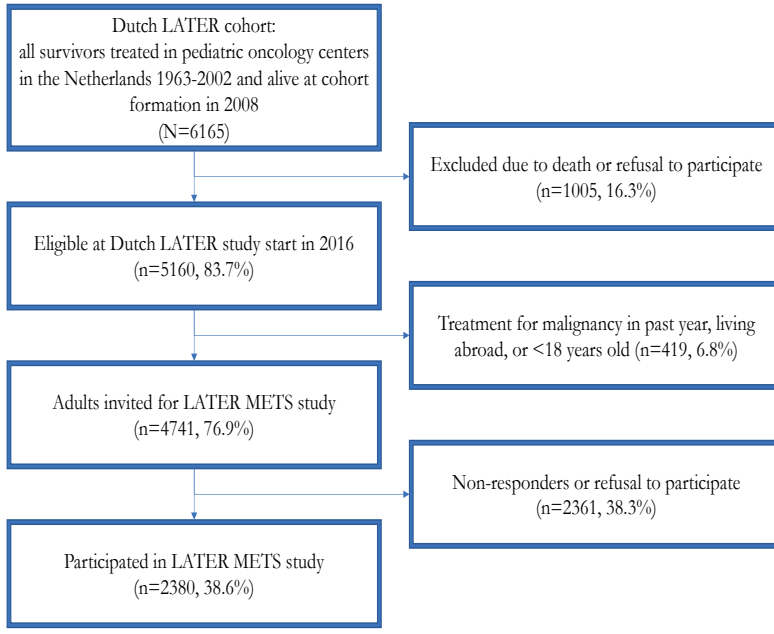

Figure 1. Overview of the Dutch LATER cohort and embeddedness of the METS study cohort within the underlying cohort. Percentages indicate proportion of Dutch LATER cohort (n=6165).

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Chapter 5

Prevalence, risk factors and optimal way to determine overweight, obesity and morbid obesity, in the first Dutch cohort of 2,338 very long-term survivors of childhood cancer: a DCCSS-LATER study

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ABSTRACT

Objective

Overweight and obesity are common challenges among childhood cancer survivors. Overweight may be disguised, as survivors can have normal weight but high fat percentage (fat%) on dual-energy X-ray absorptiometry (DXA). We aimed to assess prevalence, identify determinants and assess which method captures overweight best, in a nationwide cohort.

Research design and methods

Prevalence of overweight and obesity, primarily defined by body mass index, was assessed in the DCCSS-LATER cohort of adult survivors treated 1963-2002, with the LifeLines cohort as reference. Associations between risk factors and outcomes were investigated using logistic regression. Additional overweight metrics included DXA-fat%, waist circumference (WC), waist/hip-ratio (WHR), waist/height-ratio (WHtR), and high-molecular-weight-(HMW)-adiponectin.

Results

2,338 (mean age 35.5y, follow-up 28.3y) survivors participated. Overweight prevalence was 46.3% in men and 44.3% in women (obesity 11.2% and 15.9%, morbid obesity 2.4% and 5.4%), with highest rates among brain tumor survivors. Compared to controls, overweight rate was higher in women >50y, morbid obesity in men >50y. Overweight at cancer diagnosis (adjusted odds ratio (aOR)=3.83, 95%CI=2.19-6.69), cranial radiotherapy (aOR=3.21, 95%CI=1.99-5.18) and growth hormone deficiency (separate model, aOR=1.61, 95%CI=1.00-2.59) were associated with overweight. Using BMI, WC, WHR and WHtR, overweight prevalence was similar. Low HMW-adiponectin, present in only 4.5% of survivors, was an insensitive overweight marker. DXA-based classification identified overweight in an additional 30%, particularly after abdominal radiotherapy, total body irradiation, anthracyclines and platinum.

Conclusions

Overweight occurs in almost half of long-term survivors. We identified factors associated with overweight, as well as subgroups in whom DXA can more reliably assess overweight.

INTRODUCTION

Although childhood cancer survival rates have increased impressively, excess treatment-related morbidity and mortality among childhood cancer survivors are observed(1). Overweight and obesity are examples of long-term morbidity after treatment. These are components of metabolic syndrome and risk factors for diabetes mellitus, atherosclerotic disease and consequent mortality.

While in the general population overweight is a common problem, among survivors it is even more frequent. The reported prevalence of overweight (8.5-40.7%) and obesity (1.4-42%) in survivors varies due to different follow-up times, size and selection of cohorts(2-6). Except for Switzerland(4), overweight prevalence has so far not been assessed in a nationwide, unselected cohort of survivors.

Risk factors for overweight in the general population are related to lifestyle (unhealthy diet, lack of exercise and smoking) and genetic susceptibility(7, 8). Additionally, in survivors, increased overweight risk can be of endocrine origin: cranial and abdominal radiotherapy and alkylating chemotherapy can cause growth hormone deficiency (GHD), hypogonadism and hypothyroidism(2-6). Also, abdominal radiotherapy and stem cell transplantation (SCT) have been shown to be associated with an altered body composition, i.e. increased abdominal fat(9, 10). For other potential risk factors, e.g. corticosteroid use, study results are conflicting(5, 6). Lastly, survivors may experience visual, neurologic or orthopedic problems causing decreased ability to exercise.

Overweight and obesity are mostly reported as high body mass index (BMI)(7). BMI can however underestimate the true adiposity status, or overestimate overweight in muscular people. Other overweight measurements include waist circumference (WC) (11-13), the ratio of waist to hip circumference (WHR)(13) and the ratio of waist to height (WHtR)(14). These methods involve easily performed assessments that are specifically directed at measuring abdominal fat, which in the general population correlates better with fat% than BMI(11-13). Still, these methods are not ideal, and can be more challenging in survivors treated with abdominal radiotherapy, in whom due to tissue damage waist circumference does not reflect the total fat%(9, 15, 16). Fat percentage (fat%) on dual-energy X-ray absorptiometry (DXA) is regarded as a more accurate method for overweight assessment(17), but performing it as standard of care can be a logistic and financial challenge. Therefore, BMI is still the primary method to measure overweight in the current Dutch surveillance guideline for survivors. Serum adiponectin might serve as another overweight diagnostic. Low adiponectin is associated with overweight and higher intra-abdominal fat in the general population(18), but studies on its diagnostic value for assessing overweight

in survivors are lacking(19). The underestimation of overweight in survivors who in fact may have an increased risk of developing subsequent health problems is a major challenge in surveillance of survivors and prohibits adequate counseling.

We studied overweight in the first treated Dutch national cohort of long-term survivors of childhood cancer, aiming to assess prevalence based on a nationwide survivor cohort and compare this to the general population, to further clarify risk factors for developing overweight, and to assess optimal overweight measurement methods for future survivor surveillance.

METHODS

Study cohort

This study is part of the nationwide Dutch Childhood Cancer Survivor Study – Long Term Effects (DCCSS-LATER) study(20). The first national cohort of all adult survivors treated in a pediatric oncology center in The Netherlands between 1963 and 2002 was invited (N=4,671, Figure 1). This study was approved by the Amsterdam UMC Medical Research Ethics Committee, The Netherlands (toetsingonline.nl, NL32117.018.10). Written informed consent was obtained from all participants.

Reference cohort

Data on BMI from the Dutch LifeLines study cohort served as reference population(21). This is a large three-generational cohort of which we included all members aged between 18 and 65 years, without a history of cancer.

Data collection and definitions of overweight

An overview of definitions of outcomes and covariates is provided in Supplemental Table 1. During a late effects clinic visit (2016-2020), height, weight (adjusted for amputation when applicable) and waist and hip circumference were measured. From this data BMI, WHR and WHtR were calculated. Total body DXA scans were performed in survivors <40 years to measure fat% (converted to Hologic values when applicable). Overweight, obesity and morbid obesity, as primarily defined by BMI, were defined as ≥25, ≥30 and ≥35kg/m^2, respectively(7). Thresholds for overweight for the other modalities were (in men/women) WC ≥94/≥80cm(11) and ≥102/88cm(12), WHR ≥0.90/0.85(13), WHtR ≥0.50/0.50(14) and DXA fat% ≥25/30%(22). During outpatient clinic visit, venous samples were drawn after overnight fasting, for assessment of high-molecular-weight-(HMW)-adiponectin and insulin-like growth factor 1 (IGF-1) levels. Smoking habits were collected in

a questionnaire and physical activity information was acquired with the SQUASH questionnaire. From the medical records we extracted height and weight at cancer diagnosis to calculate BMI at diagnosis. If present, we also extracted data on GHD tests and treatment. Childhood cancer treatment data were collected on a national level in our central database.

Statistical analysis

Analyses were conducted in R version 3.6.3 (R Foundation, Vienna, Austria)(23). Demographic and treatment characteristics were compared between participants and non-participants. Sex-specific prevalence of overweight, obesity and morbid obesity in men and women was compared to that in the Lifelines reference population using chi-squared or Fisher's exact test. Risk factors for these outcomes were assessed using logistic regression. Significant variables ($p<0.05$) in univariable analysis and patient factors known to be relevant from literature (age, sex, smoking, physical activity) were included in the multivariable model. Multicollinearity was inspected with the variance inflation factor. As GHD is a potential mechanism for overweight caused by therapies, it was analyzed in a separate model. Sensitivity analyses were performed to inspect the role of missing data. The influence of overweight measurement methods was evaluated by assessing overweight prevalence according to each, and discrepancies were calculated with BMI <25kg/m^2 and high fat% as references. We studied what factors were associated with disguised overweight, in logistic regression models with all treatment groups as predictors.

RESULTS

Study cohort description

In total, 2,338 very long-term survivors (52.1% male) participated (50.1% participation rate) (Figure 1, Table 1). Mean age was 35.5 (standard deviation ±9.3) years, and mean follow-up time was 28.3 (±8.4) years. The most common childhood cancer diagnosis groups had been leukemias (35.5%), lymphomas (19.2%), renal tumors (11.5%) and central nervous system (CNS) tumors (9.1%). Participants had more often received cranial and abdominal radiotherapy, alkylating agents, anthracyclines, platinum derivatives and vinca alkaloids (compared to non-responders (n=1,599) only, this data was unavailable for survivors who declined participation), but did not differ regarding age at diagnosis, treatment period, age at invitation and follow-up time (Supplemental Table 2).

The Lifelines reference cohort consisted of 132,150 subjects (58.6% female), with mean age of 42.0, SD ±11.0 years (Supplemental Table 3).

Prevalence of overweight, obesity and morbid obesity

Based on BMI values, overweight prevalence was 46.3% (males) and 44.3% (females). For obesity this was 11.2% and 15.9%, and for morbid obesity 2.4% and 5.4%, respectively. Compared to LifeLines, there was a higher overweight rate among women aged 50+ (68.7 vs 57.0%, p=0.032) and a higher morbid obesity rate among men aged 50+ (6.7 vs 2.5%, p=0.040) (Figure 2). Lower rates were observed for overweight in male survivors aged 30-40 (44.0 vs 56.3%, p<0.001) and 40-50 (59.7 vs 66.9%, p=0.012) and for obesity in male survivors aged 30-40 (8.5 vs 12.2%, p=0.019).

Overweight was most common in survivors of a CNS tumor (52.3%), retinoblastoma (50.0%) and lymphomas (49.4%). Obesity and morbid obesity rates were particularly high after CNS tumor (22.1% and 10.8%, respectively) and retinoblastoma (30.0% and 10.0%) (Supplemental figure 1).

Risk factors for overweight, obesity and morbid obesity

The strongest risk factor for overweight in univariable analysis was overweight at cancer diagnosis (odds ratio (OR) 3.16, 95%CI 2.08-4.79) (Supplemental table 4). CrRT was another risk factor, with stronger association for lower dose CrRT (OR for 1-25 Gy 3.58 (95%CI 2.60-4.94), for >25 Gy 1.75 (95%CI 1.31-2.34)) as was GHD (OR 2.28, 95%CI 1.59-3.27). Corticosteroids use was not associated with overweight. In a sex-stratified analysis we observed that treatment-related risk estimates were similar in men and women (data not shown).

In multivariable analysis, risk factors for overweight were overweight at diagnosis (OR 3.83, 95%CI 2.19-6.69), CrRT 1-25 Gy (OR 3.21, 95%CI 1.99-5.18) and older age at clinic visit (OR 1.03, 95%CI 1.01-1.05 per year) (Table 2). In the separate model GHD was also associated with overweight (OR 1.61, 95%CI 1.00-2.59).

Female sex, CrRT and GHD were identified as independent risk factors for obesity and morbid obesity (Supplemental table 5). Inspection of variance inflation in all multivariable models suggested that multicollinearity was not present. Sensitivity analyses showed similar results (data not shown).

Assessment of overweight using different methods

In the overall cohort, BMI, WC (≥94/80cm), WHR and WHtR revealed an overweight prevalence in the range 45.3%-49.0% (Supplemental table 6). When stratified by sex, high WC was observed less in men (33.6%) and more in women (60.8%). High fat%

on DXA identified overweight in 83.7% of women and 58.4% of men as well as in 77.7% of abdominally irradiated survivors. When using Gallagher's threshold for high fat%, higher prevalence was observed particularly in men (82.3%, and 66.1% in women). When using Heo's threshold, prevalence remained similar to BMI, WHR and WHtR.

There were differences in the classification of overweight according to different methods. High WC was observed in 11.2% of survivors with normal BMI, high WHR in 18.8%, and high WHtR in 9.3%. DXA identified an additional 31.6% survivors with normal BMI as overweight, and this was 39.3% in the abdominally irradiated group.

When compared to fat% on DXA scan, underestimation of overweight was comparable with BMI, WC, WHR and WHtR for the whole cohort (range 29.9-32.9%) (Figure 2). BMI, WHR and WHtR underestimated overweight more often in women (up to 47.4%). After abdominal irradiation, the percentage of underestimation of overweight was highest for the methods that use waist circumference (range 45.9-63.1%).

Consequently, in the regression model with outcome underestimation of overweight with BMI, female sex was a strong risk factor (OR 3.02, 95%CI 2.27-4.03) (Table 3, Supplemental table 7). Therapies significantly associated with underestimation were total body irradiation (TBI, OR 9.06, 95%CI 2.41-34.04) and anthracyclines (second tertile OR 1.57 (95%CI 1.02-2.24), a trend for the highest tertile 1.48 (95%CI 0.99-2.23)). Abdominal radiotherapy was a major risk factor for misclassification with the methods that use WC (for WC OR up to 3.06 (95%CI 1.64-5.72, data for WHR and WHtR were similar (data not shown)). In this model anthracyclines (OR highest tertile 1.58, 95%CI 1.12-2.23) and platinum (OR 1.66, 95%CI 1.21-2.29) also emerged as independent risk factors.

Adiponectin as marker for overweight

Low HMW-adiponectin was present in only 4.5% of survivors. Consequently, only 1.9% of survivors with normal BMI were additionally diagnosed as overweight. Also, when compared to fat%, low adiponectin underestimated overweight in 66.1% of survivors, with higher rates in women (78.9%) and abdominally irradiated survivors (72.3%). Sensitivity and specificity for low adiponectin compared to high BMI were 6.2% and 96.5%, respectively. When compared to high fat% on DXA, sensitivity was 6.1% and specificity 97.1%.

DISCUSSION

This study shows that overweight occurs in almost half of all adult long-term childhood cancer survivors, and that associated factors include overweight at diagnosis, CrRT and GHD. We also show that DXA scans identified overweight in an additional 30% of survivors not identified with conventional methods such as BMI and WC.

There was a significantly higher prevalence in our cohort for overweight among women aged 50+ and for morbid obesity among men aged 50+. Our findings may suggest that the increase in prevalence per age category is more pronounced in survivors than in the general population. This was particularly the case in women, which may be partly attributed to the effect of menopause. So, while aging, prevalence of overweight, obesity and morbid obesity may be higher in survivors, increasing their risk of overweight-associated comorbidity and mortality. It could also be that this increase slows down later on, as was observed for cardiac disease in survivors(24). Another potential reason for increased prevalence in the oldest age groups, is that younger participants were treated more recently, and may therefore suffer from less treatment-related side effects. ALL is the most prevalent cancer type in this cohort. Use of prophylactic CrRT was reduced in the 1980-1990s with the introduction of the ALL-6 and ALL-9 protocols. Accordingly, ALL survivors who underwent CrRT are overrepresented among the oldest survivors in this study. Longitudinal follow-up is required to elucidate this.

The only other study so far on overweight in a heterogeneous nationwide survivor cohort, the Swiss Childhood Cancer Survivor Study (N=2,365), found a prevalence of 26% after fifteen years, which was not different from sibling and general population controls(4). The lower prevalence compared to our study might be due to the shorter follow-up (15 v 28 years), since overweight prevalence increases with aging. The Childhood Cancer Survivor Study (CCSS)(3) and the St Jude Lifetime Cohort Study (SJLIFE)(5), had a comparable follow-up time (~24 years). Higher overweight rates in these studies seem in part to be due to an already higher general population overweight prevalence. The CCSS found no difference with siblings. In SJLIFE, the general population obesity prevalence was already twice as high as in our control group. Still, the authors observed more obesity among survivors (standardized morbidity ratio 1.14). It is clear that follow-up time and general population risk impede a full comparison between studies.

We observed that overweight prevalence was highest among survivors of CNS tumors, but differences between diagnosis groups were small. Obesity and morbid obesity prevalence was clearly higher after CNS tumors. This is in line with previous

findings(4, 25, 26) and likely related to damage to the hypothalamus and pituitary gland due to tumor and treatment. Whereas other studies also observed a higher overweight prevalence after ALL(6), we did not, as there was no excess overweight in ALL survivors unexposed to CrRT.

We further explored the role of CrRT and confirmed the association with overweight, obesity and morbid obesity, as has been reported multiple times(3-6). In multivariable analysis, the effect of dosages <25Gy on overweight was stronger. This group most likely consists of survivors who received craniospinal radiotherapy. The higher dose group more likely received local radiotherapy to a brain tumor, with the exception of medulloblastoma and a few ALL survivors who received higher dose craniospinal radiation. In the low dose group the hypothalamus and pituitary may therefore have been in the radiation field more often. Radiotherapy affects the somatotropic axis first, with GHD occurring from 15-20 Gy(27). In our regression models GHD was independently associated with overweight, obesity and morbid obesity. Another potential mechanism is hypogonadism, which can occur after hypothalamic and gonadal radiation and alkylating chemotherapy(28). Alkylating agents were no independent risk factor in our analysis. Unfortunately no data were available yet on presence of hypogonadism. This and other potential mechanisms will be further explored in additional studies in this cohort.

In our cohort, corticosteroids use during cancer treatment did not impact overweight or obesity. Apparently, the short-term metabolic side effects of these compounds, have not led to overweight on the long-term. Two previous studies among ALL survivors, 10 and 12.7 years after treatment, still observed an association with corticosteroids and overweight(29, 30). This may suggest that steroids exposure does influence the middle-long-term, but that this resolves later on. In the SJLIFE cohort, the association between glucocorticoid treatment and obesity was significant even after 24.6 years(5). This difference with our cohort might be explained by environmental and lifestyle factors in the United States that make it harder to lose weight after weight gain during adolescence. Our study is the first to investigate the effect of total cumulative dose steroids on development of overweight, obesity and morbid obesity in a large, unselected, national cohort of very long-term survivors of all types of childhood cancer. The absence of an association therefore appears to be convincing.

Overweight at cancer diagnosis emerged as a strong patient factor associated with overweight and obesity. Other studies have observed similar results(5, 6). This may reflect a genetic susceptibility to weight gain, socio-economic status and associated lifestyle, and for some brain tumors a hypothalamus/pituitary damaging effect prior to diagnosis. Smoking was also an independent risk factor for overweight, as is in the

general population. Our findings show that these patient factors have an additional effect on top of historical treatment and emphasize that they need to be acknowledged in surveillance.

Our third aim was to explore how adequate currently used methods assess overweight in survivors. After abdominal irradiation, WC and WHR do not provide optimal overweight assessment, and that fat% assessment with DXA may be more valuable(9, 15, 16). Furthermore, short stature due to GHD, reduced bone mineral density, sarcopenia, and amputations can hinder the estimation of overweight with BMI, WC, WHR and WHtR. We show that waist-circumference-based methods classified a substantial number of survivors with normal BMI as overweight. Moreover, we show that DXA scan measured overweight in an additional 30% of survivors, and even 40% in the subgroup of abdominally irradiated survivors. Underestimation occurred more often in females, which is also observed in the general population(31). The underestimation rate was similar for all types of childhood cancer except hepatic tumors (Supplemental figure 2). Previous studies observed similar underestimation rates when comparing anthropometric measurements to DXA measured fat%(9, 15, 16). Karlage used the obesity threshold for BMI, hence, the observed discrepancy was higher(16). Subsequently, we identified subgroups of survivors that may benefit from assessing overweight with DXA. These include survivors treated with abdominal irradiation and TBI. Altered fat distribution has been described after SCT preconditioned with TBI(10). Furthermore, for the first time, we identified anthracyclines and platinum chemotherapy to be associated with disguised overweight. How these therapies might lead to an altered body composition is yet unknown. Anthracyclines were associated with low BMI in a previous study in Dutch survivors, but the mechanism, e.g., sarcopenia, was not clear(32). In another study in the DCCSS-LATER cohort, an association between platinum and meningioma appeared to be confounded by medulloblastoma survivors also receiving high dose CrRT(33), but in a sensitivity analysis excluding these survivors the effect remained. Hence, survivors with BMI or WC near the upper limit of normal and who received abdominal radiation or TBI, and possibly anthracyclines and platinum chemotherapy, may benefit from a DXA scan as most reliable diagnostic method.

It was remarkable how our analysis of the three previously reported thresholds for high fat% influenced overweight prevalence. The common use of 25% for men and 30% for women may in part be caused by a misinterpretation of a World Health Organization statement on body fat(34). In the two other studies we compared, the authors attempted to calibrate BMI values of 25 and 30kg/m^2 to corresponding DXA fat% values(35, 36). This yields a grey area until 28% in men and 40% in women with somewhat unclear overweight diagnosis.

Low HMW-adiponectin could serve as alternative marker for overweight. HMW-adiponectin is the most biologically active isoform of adiponectin, an adipokine that enhances insulin sensitization and suppresses inflammation and cell death(37). In the general population low adiponectin is associated with overweight, increased intra-abdominal fat, as well as metabolic syndrome, diabetes and atherosclerotic disease(18). In our recent systematic literature review we proposed that it may be used to replace the overweight component of metabolic syndrome in survivors with unreliable WC after abdominal radiotherapy(19). However, less than 5% of the cohort had low adiponectin, so it was not a sensitive marker for overweight, and particularly compared to DXA many overweight survivors are missed. An explanation of this finding could be that the study cohort was still relatively young and that as the cohort ages, low adiponectin levels may develop. Alternatively, some underlying mechanisms for overweight development may be different in survivors and less correlated with adiponectin than in the general population.

A few limitations of this study require consideration. First, prescribed radiotherapy dose is not the same as dose received by organs involved in metabolic side effects. For full CrRT the prescribed dose can be assumed to reflect dose received by the hypothalamus and pituitary, but for other malignancies such dosimetric data were not available yet. Second, DXA scans were intentionally only performed in survivors <40 years to avoid bias caused by menopause, but it may limit full generalizability of our DXA related findings. Third, we did not have data on hypogonadism. Fourth, due to historic changes in treatment protocols and the cross-sectional design of this study, treatment exposure is correlated with attained age at study participation, and interpretation of findings regarding the impact of attained age require caution.

To further deepen our understanding of late effects of childhood cancer, future perspectives may include longitudinal designs shedding more light on potential causative mechanisms, dosimetry for specific organs, and further elucidated pathophysiological mechanisms. Also, obesity is often not a sole side effect, but related to other cardiovascular risk factors – insulin resistance, dyslipidemia and hypertension – as metabolic syndrome, further increasing the risk of diabetes and cardiovascular disease(11, 12). This will be further explored in our study. Lastly, it is important not only to identify risk factors for overweight and other metabolic sequelae, but also to invest in lifestyle interventions.

In conclusion, in this study in our nationwide Dutch cohort of the first treated (1963-2002) childhood cancer survivors, we show that overweight occurs in almost half of all long-term survivors, and that overweight at diagnosis, CrRT and GHD, but not corticosteroids, are associated with long-term overweight. Of several assessment

methods, DXA was most sensitive, as it identified overweight in an additional 30% of survivors, particularly those treated with abdominal irradiation, TBI, anthracyclines and platinum chemotherapy. HMW-adiponectin did not have added diagnostic value.

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Conflict of Interest

All authors have no conflicts of interest to declare.

Author Contributions and Guarantor Statement

VGP, DTCdW, MMvdH-E and SJCMMN contributed to the study funding, concept, and design. VGP, JEcA, DTCdW, RAJN, DB, MvdH-vdL, ACHdV, ML, HJvdP, LCMK, JJL, EcD-dB, WJET, MMvdH-E and SJCMMN contributed to data acquisition. VGP, SJCMMN and MMvdH-E contributed to data analysis and interpretation. VGP, MMvdH-E and SJCMMN drafted the manuscript. VGP, JEvA, DTCdW, MF, MB, RAJN, GORJ, DB, MvdH-vdL, ACHdV, ML, HJvdP, SMFP, CMR, ABV, LCMK, JJL, EvDdB, WJET, HMvS, MMvdH-E and SJCMMN contributed to manuscript revision and approval. VGP, JEvA, DTCdW, MB, MF, MvdH-vdL, SJCMMN, and MMvdH-E had full access to all data in the study and take responsibility for data integrity, verification, and analysis. All authors had access to all the data reported in the study and accept responsibility to submit for publication.

Data Sharing Statement

The data underlying this Article were provided by the DCCSS-LATER consortium under license. Data will be shared on reasonable request to the corresponding author with permission of the DCCSS-LATER consortium. The data are not publicly available due to privacy or ethical restrictions.

TABLES AND FIGURES

Table 1. Baseline characteristics of the study cohort

	Entire cohort	Male	Female	Comparison male vs female
Number of participants	2338	1198 (51.2%)	1140 (48.8%)	
Patient, cancer and treatment characteristics				
Age and follow-up time				
Age at clinic visit (y) (mean (SD))	35.5 (9.3)	35.5 (9.0)	35.5 (9.5)	0.87
Age at clinic visit categorized (y) (%)				0.77
18/30	728 (31.3)	371 (31.0)	357 (31.6)	
30/39	883 (38.0)	462 (38.7)	421 (37.2)	
40+	715 (30.7)	362 (30.3)	353 (31.2)	
Follow-up time (y) (mean (SD))	28.3 (8.4)	28.0 (8.2)	28.5 (8.7)	0.17
Follow-up time categorized (y) (%)				0.63
10/19	472 (20.2)	241 (20.1)	231 (20.3)	
20/29	928 (39.7)	489 (40.8)	439 (38.5)	
30/39	693 (29.6)	352 (29.4)	341 (29.9)	
40/49	220 (9.4)	103 (8.6)	117 (10.3)	
50/59	25 (1.1)	13 (1.1)	12 (1.1)	
Childhood cancer characteristics				
Childhood cancer diagnosis per ICCC-3 site group (%)				**<0.001**
1 Leukemias, myeloproliferative diseases and myelodysplastic diseases	831 (35.5)	437 (36.5)	394 (34.6)	
2 Lymphomas and reticuloendothelial neoplasms	448 (19.2)	288 (24.0)	160 (14.0)	
3 CNS and miscellaneous intracranial and intraspinal neoplasms	213 (9.1)	107 (8.9)	106 (9.3)	
4 Neuroblastoma and other peripheral nervous cell tumors	135 (5.8)	44 (3.7)	91 (8.0)	
5 Retinoblastoma	11 (0.5)	5 (0.4)	6 (0.5)	
6 Renal tumors	269 (11.5)	111 (9.3)	158 (13.9)	
7 Hepatic tumors	18 (0.8)	9 (0.8)	9 (0.8)	
8 Bone tumors	136 (5.8)	63 (5.3)	73 (6.4)	
9 Soft tissue and other extraosseous sarcomas	167 (7.1)	93 (7.8)	74 (6.5)	
10 Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	76 (3.3)	28 (2.3)	48 (4.2)	
11 Other malignant epithelial neoplasms and malignant melanomas	31 (1.3)	12 (1.0)	19 (1.7)	
12 Other and unspecified malignant neoplasms	3 (0.1)	1 (0.1)	2 (0.2)	
Age at diagnosis (y) (mean (SD))	6.71 (4.69)	6.90 (4.63)	6.51 (4.74)	**0.045**
Age at diagnosis categorized (y) (%)				0.14

Table 1. Continued

	Entire cohort	Male	Female	Comparison male vs female
0 /5	1071 (45.8)	525 (43.8)	546 (47.9)	
5/10	651 (27.8)	347 (29.0)	304 (26.7)	
10/15	481 (20.6)	261 (21.8)	220 (19.3)	
15/18	135 (5.8)	65 (5.4)	70 (6.1)	
Treatment period (%)				0.15
1960/69	33 (1.4)	15 (1.3)	18 (1.6)	
1970/79	317 (13.6)	142 (11.9)	175 (15.4)	
1980/89	732 (31.3)	386 (32.2)	346 (30.4)	
1990/99	1013 (43.3)	528 (44.1)	485 (42.5)	
2000/09	243 (10.4)	127 (10.6)	116 (10.2)	
Height at cancer diagnosis (cm) (mean (SD))	120.4 (30.6)	122.0 (30.5)	118.6 (30.7)	**0.016**
Weight at cancer diagnosis (kg) (mean (SD))	25.6 (15.4)	26.1 (15.2)	25.1 (15.7)	0.16
BMI at cancer diagnosis (kg/m2) (mean (SD))	16.22 (2.88)	16.14 (2.90)	16.30 (2.85)	0.26
Overweight at cancer diagnosis	116 (6.1)	51 (5.1)	65 (6.8)	0.11
Obesity at cancer diagnosis	22 (1.1)	12 (1.2)	10 (1.1)	0.75
Cancer treatment characteristics				
Cranial radiotherapy (%)	432 (18.5)	239 (20.1)	193 (16.9)	0.053
Cranial radiotherapy categorized (%)				**0.008**
No	1898 (81.6)	952 (80.1)	946 (83.2)	
1-25 Gy	204 (8.8)	100 (8.4)	104 (9.1)	
25+ Gy	223 (9.6)	136 (11.4)	87 (7.7)	
Total body irradiation	88 (3.8)	63 (5.3)	25 (2.2)	**<0.001**
Abdominal/pelvic radiotherapy (%)	201 (8.6)	84 (7.1)	117 (10.3)	**0.006**
Abdominal/pelvic radiotherapy categorized (%)				**0.006**
No	2126 (91.4)	1104 (93.0)	1022 (89.8)	
1-29 Gy	112 (4.8)	41 (3.5)	71 (6.2)	
30+ Gy	87 (3.7)	42 (3.5)	45 (4.0)	
Alkylating agents (CED) (%)	1175 (55.3)	648 (59.0)	527 (51.3)	**<0.001**
Cyclophosphamide equivalent dose categorized (%)				**0.001**
No	951 (44.7)	451 (41.0)	500 (48.7)	
1-4000mg/m2	445 (20.9)	229 (20.8)	216 (21.0)	
4000-8000mg/m2	340 (16.0)	201 (18.3)	139 (13.5)	
8000+ mg/m2	390 (18.3)	218 (19.8)	172 (16.7)	
Anthracyclines (DED) (%)	1172 (53.2)	635 (55.7)	537 (50.5)	**0.014**
Doxorubicin equivalent dose categorized (%)				**0.041**
No	1032 (46.8)	505 (44.3)	527 (49.5)	
tertile 1 (range 9-138mg/m2)	391 (17.7)	200 (17.5)	191 (18.0)	
tertile 2 (range 139-273mg/m2)	391 (17.7)	216 (18.9)	175 (16.4)	

Table 1. Continued

	Entire cohort	Male	Female	Comparison male vs female
tertile 3 (range 275-1764mg/m2)	390 (17.7)	219 (19.2)	171 (16.1)	
Corticosteroids (SED) (%)	1190 (50.9)	675 (56.3)	515 (45.2)	**<0.001**
Steroid equivalent dose categorized (%)				**<0.001**
No	1148 (49.1)	523 (43.7)	625 (54.8)	
1-10g/m2	1083 (46.3)	598 (49.9)	485 (42.5)	
10+ g/m2	107 (4.6)	77 (6.4)	30 (2.6)	
Asparaginase (%)	584 (25.0)	316 (26.4)	268 (23.6)	0.11
Platinum derivatives (%)	311 (13.3)	142 (11.9)	169 (14.9)	**0.03**
Vinca alkaloids (%)	1829 (78.3)	966 (80.7)	863 (75.8)	**0.004**
Amputation (%)	67 (2.9)	24 (2.0)	43 (3.8)	**0.010**
Amputation type				0.084
Elbow/upper arm	3 (0.1)	1 (0.1)	2 (0.2)	
Shoulder/scapula	4 (0.2)	3 (0.3)	1 (0.1)	
Ankle/lower leg	9 (0.4)	3 (0.3)	6 (0.5)	
Knee/upper leg	42 (1.8)	13 (1.1)	29 (2.5)	
Hip/pelvis	9 (0.4)	4 (0.3)	5 (0.4)	
Allogeneic SCT (%)	99 (4.3)	65 (5.4)	34 (3.0)	**0.004**
Measurements assessed at clinic visit Physcial examination				
Height (cm) (mean (SD))	173.6 (10.1)	179.5 (8.8)	167.2 (7.2)	**<0.001**
Weight (kg) (mean (SD))	76.1 (16.0)	81.1 (15.3)	70.8 (15.0)	**<0.001**
BMI (kg/m2) (mean (SD))	25.20 (4.65)	25.10 (4.15)	25.30 (5.12)	0.33
BMI >25kg/m2	1020 (45.3)	535 (46.3)	485 (44.3)	0.33
BMI >30kg/m2	303 (13.5)	129 (11.2)	174 (15.9)	**0.001**
BMI >35kg/m2	87 (3.9)	28 (2.4)	59 (5.4)	**<0.001**
Waist circumference (cm) (mean (SD))	87.2 (12.7)	89.8 (11.6)	84.5 (13.3)	**<0.001**
Hip circumference (cm) (mean (SD))	99.2 (10.3)	98.5 (8.8)	99.9 (11.7)	**0.001**
High waist circumference JIS	1040 (46.8)	382 (33.6)	658 (60.8)	**<0.001**
High waist circumference NCEP	543 (24.5)	159 (14.0)	384 (35.5)	**<0.001**
Waist hip ratio (mean (SD))	0.88 (0.09)	0.91 (0.07)	0.85 (0.09)	**<0.001**
High waist hip ratio	1082 (49.0)	608 (53.8)	474 (43.9)	**<0.001**
Waist height ratio (mean (SD))	0.50 (0.07)	0.50 (0.06)	0.51 (0.08)	0.087
High waist height ratio	1042 (47.0)	524 (46.1)	518 (47.9)	0.41
Mean systolic blood pressure (mmHg) (mean (SD))	124 (16)	126 (15)	121 (16)	**<0.001**
Mean diastolic blood pressure (mmHg) (mean (SD))	76 (10)	77 (11)	75 (10)	**<0.001**
Hypertension (%)	806 (36.1)	470 (41.2)	336 (30.8)	**<0.001**
DXA scan, laboratory and questionnaire data				

Table 1. Continued

	Entire cohort	Male	Female	Comparison male vs female
Fat% on DXA scan (mean (SD))	31.1 (7.7)	26.3 (5.3)	36.4 (6.2)	**<0.001**
High fat% on DXA 25/30% (%)	1150 (70.4)	503 (58.4)	647 (83.7)	**<0.001**
High fat% on DXA Gallagher (%)	649 (39.7)	395 (45.9)	254 (32.9)	**<0.001**
High fat% on DXA Heo	346 (21.2)	206 (23.9)	140 (18.1)	**0.004**
Adiponectin (µg/ml) (mean (SD))	3.96 (2.31)	3.17 (1.78)	4.79 (2.50)	**<0.001**
Low adiponectin (%)	106 (4.5)	57 (4.8)	49 (4.3)	0.59
IGF1 (nmol/l) (mean (SD))	24.40 (8.04)	24.86 (7.66)	23.91 (8.41)	**0.006**
Low IGF1 (%)	27 (1.2)	15 (1.3)	12 (1.1)	0.67
Growth hormone deficiency (%)	116 (5.0)	55 (4.6)	61 (5.4)	0.40
Smoking (current or former) (%)	665 (32.5)	372 (35.4)	293 (29.4)	**0.003**
Minutes per week of moderate activity (median [IQR])	390 [135, 960]	450 [180, 1132.5]	352.50 [120, 845]	**<0.001**
Low physical activity (%)	436 (25.5)	191 (21.6)	245 (29.8)	**<0.001**

BMI was available for 2,252 (96.3%) survivors, WC for 2,220 (95.0%), WHR for 2,210 (94.5%), WHtR for 2,218 (94.9%), a DXA scan was performed in 1,652 (70.7%), and adiponectin was measured in 2,219 (94.9%) survivors.
Abbreviations: BMI = body mass index; IGF-1 = insulin-like growth factor 1; ICCC-3 = International Classification of Childhood Cancer, Third edition; CNS = central nervous system; SCT = stem cell transplantation

Table 2. Multivariable logistic regression analysis of variables associated with overweight (BMI ≥25kg/m^2)

	Frequency of high BMI (n (%))	Multivariable model 1			Multivariable model 2		
		OR	95% CI	P-value	OR	95% CI	P-value
--Patient characteristics							
Age at clinic visit		1.03	1.01 - 1.05	**0.006**	1.04	1.02 - 1.06	**<0.001**
First tertile	258 (34.1%)						
Second tertile	326 (43.8%)						
Third tertile	435 (58.1%)						
Sex		0.88	0.69 - 1.13	0.32	0.96	0.76 - 1.20	0.70
Female	485 (44.3%)						
Male	535 (46.3%)						
Age at diagnosis		1.02	0.99 - 1.05	0.27	1.00	0.98 - 1.03	0.77
First tertile	299 (40.0%)						
Second tertile	340 (45.0%)						
Third tertile	381 (50.9%)						
Overweight at cancer diagnosis		3.83	2.19 - 6.69	**<0.001**	3.44	2.04 - 5.78	**<0.001**
Yes	80 (70.8%)						
No	782 (43.4%)						
Smoking (current or former)		1.26	0.97 - 1.63	0.079	1.24	0.97 - 1.58	0.086
Yes	338 (52.7%)						
No	590 (44.2%)						
Low physical activity		1.12	0.85 - 1.47	0.42	1.14	0.88 - 1.48	0.31
Yes	188 (44.8%)						
No	500 (40.3%)						
--Treatment characteristics							
Cranial radiotherapy							
Yes	260 (63.1%)						
No	755 (41.2%)						
Cranial radiotherapy categorized				<0.001			
No	755 (41.2%)	Ref					
1-25 Gy	143 (71.5%)	3.21	1.99 - 5.18	<0.001			
25+ Gy	114 (55.1%)	1.67	0.98 - 2.87	0.061			
Total body irradiation		0.39	0.12 - 1.31	0.13			
Yes	12 (13.8%)						
No	1001 (46.5%)						
Abdominal/pelvic radiotherapy							
Yes	89 (44.5%)						
No	924 (45.2%)						
Abdominal/pelvic radiotherapy categorized							
No	924 (45.2%)						
1-29 Gy	52 (46.4%)						
30+ Gy	35 (41.4%)						
Alkylating agents (CED)							
Yes	477 (41.7%)						
No	431 (47.3%)						

Table 2. Continued

	Frequency of high BMI (n (%))	Multivariable model 1			Multivariable model 2		
		OR	95% CI	P-value	OR	95% CI	P-value
Cyclophosphamide equivalent dose categorized				0.050			
No	431 (47.3%)	Ref					
1-4000mg/m^2	183 (42.3%)	0.99	0.73 - 1.35	0.95			
4000-8000mg/m^2	140 (42.9%)	0.92	0.65 - 1.32	0.67			
8000+ mg/m^2	154 (40.1%)	0.62	0.44 - 0.88	0.008			
Corticosteroids							
Yes	536 (46.7%)						
No	484 (43.8%)						
Corticosteroids categorized							
No	484 (43.8%)						
0-10g/m^2	490 (46.9%)						
10+ g/m^2	46 (44.2%)						
Allogeneic SCT		0.56	0.16 - 1.91	0.35			
Yes	17 (17.7%)						
No	996 (46.5%)						
--Comorbidity							
Growth hormone deficiency					1.61	1.00 - 2.59	**0.048**
Yes	88 (64.2%)						
No	932 (44.1%)						

Model 1: patient and cancer treatment characteristics. Model 2: patient characteristics and growth hormone deficiency. For variables with more than two categories the overall p-value was calculated with the Wald test. Significant variables in univariable analysis and patient factors known to be relevant from literature (age, sex, smoking, physical activity) were included in the multivariable model. If for a treatment factor both the dichotomous and categorized variable were significant, only the latter was included.

Abbreviations: CI = confidence interval; OR = odds ratio; CI = confidence interval; Ref = reference

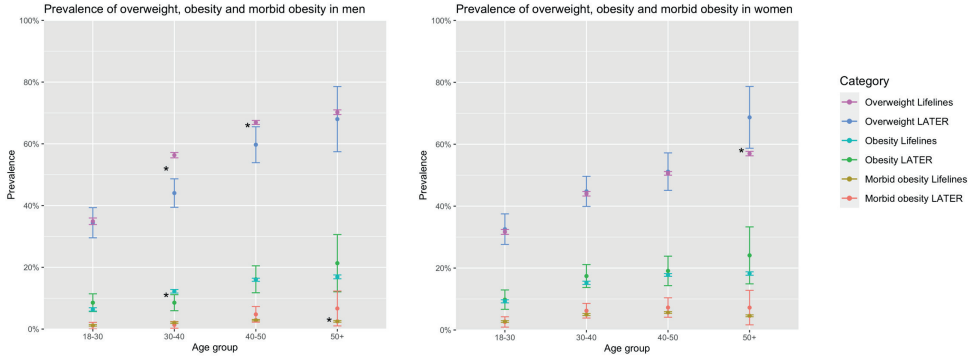

Figure 1. Comparison of overweight, obesity and morbid obesity between our survivor cohort and the LifeLines control cohort.

Prevalence of overweight (BMI >25kg/m2), obesity (>30kg/m2) and morbid obesity (>35kg/m2) in the LATER study cohort and LifeLines control cohort in men and women. Significantly different proportions are indicated by an asterisk.

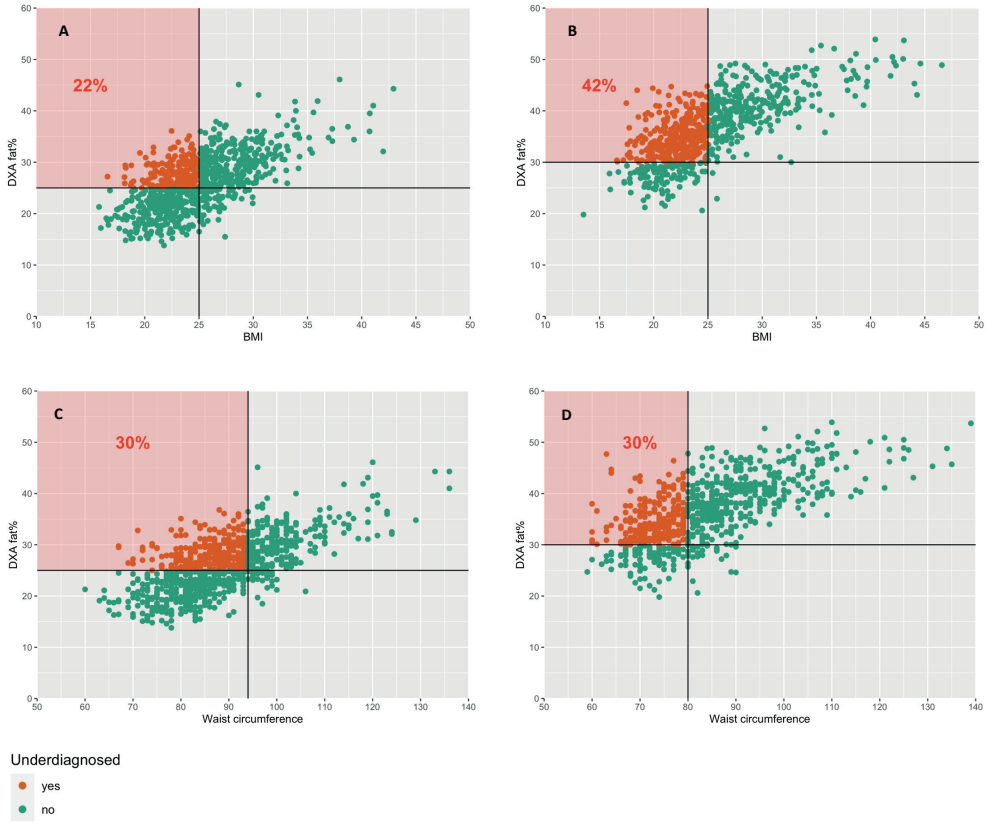

Figure 2. Comparison of overweight classification using BMI and waist circumference versus DXA scan

Underdiagnosis of overweight, when compared to fat percentage on DXA scan, with BMI in men (figure A) and in women (figure B), and with waist circumference in men (figure C) and in women (figure D). The red square and percentage in each figure indicate the survivors with high fat percentage and normal waist circumference or BMI.

SUPPLEMENTAL MATERIAL:SUPPLEMENTAL TABLE 1. DEFINITIONS OF OUTCOMES AND COVARIATES

Supplemental table 1. Definitions of outcomes and covariates

Outcome or covariate	Measurement, calculation or derivation	Definition of aberrant value	Source
Body mass index (kg/m^2)	Weight(kg)/Height(m)^2	Overweight ≥25, obesity ≥30 and morbid obesity ≥35kg/m^2	World Health Organization[1]
Weight adjusted for amputation (kg)	Percentage added to body weight based on amputation level	n.a.	The Amputee Coalition[2]
Waist circumference (cm)	n.a.	≥94cm in European men, ≥80cm in European women ≥102cm in men, ≥88cm in women	Joint Interim Statement[3] National Cholesterol Education Program ATP III[4]
Waist hip ratio	Waist circumference(cm)/Hip circumference(cm)	≥0.90 in men, ≥0.85 in women	World Health Organization[5]
Waist height ratio	Waist circumference(cm)/Height(cm)	≥0.50 in both sexes	Ashwell et al.[6]
DXA fat% Lunar conversion to Hologic (%)	Conversion factor to account for difference in beam techniques	n.a.	Shepherd et al.[7]
DXA fat% (%)	n.a.	Primary threshold used in main analyses: ≥25% in men, ≥30% in women Age-dependent, ≥21.0-23.0% in white men, ≥33.0-35.0% in white women Age-dependent, ≥24.9-28.0% in white men, ≥37.0-39.9% in white women	Sommer et al.[8], Shah et al.[9] Gallagher et al.[10] Heo et al.[11]
HMW adiponectin (µg/ml)	Lumipulse G immunoassay (Fujirebio, Tokyo, Japan)	≤1.46µg/ml in men, 0.90µg/ml in women	Reference values provided by the manufacturer
IGF-1	IDS-iSYS immunoassay (Immunodiagnostic Systems, Tyne & Wear, United Kingdom)	Age and sex-dependent reference values	Bidlingmaier et al.[12]
GHD tests or treatment	Insulin tolerance, arginine, clonidine or levodopa-propranolol test, or treatment with growth hormone	n.a.	Medical records
Growth hormone deficiency		1. Low IGF-1 and having had a brain tumor, CrRT or TBI, in order to include low IGF-1 due to hypothalamic and pituitary damage only, or 2. Diagnosed with GHD or receiving treatment according to medical records	Consensus between authors

Supplemental table 1. Continued

Outcome or covariate	Measurement, calculation or derivation	Definition of aberrant value	Source
Smoking status (yes or no)	General health questionnaire	Any (current or former) smoking	Consensus between authors
Physical activity	SQUASH questionnaire data, converted to average weekly exercise capacity following the corresponding protocol[13-15]	Not fulfilling norm of 150 minutes of medium exercise per week	World Health Organization[16]
BMI at diagnosis (kg/m^2)	Weight at diagnosis(kg)/Height at diagnosis(m)^2	Age and sex-dependent reference values for overweight and obesity	Obesity Taskforce Classification for childhood obesity[17]
Cyclophosphamide equivalent dose (mg/m^2)	Equivalence ratios for alkylating agents	Categorization based on literature	Green et al.[18]
Doxorubicin equivalent dose (mg/m^2)	Equivalence ratios for anthracyclines	Categorization based on tertiles	Feijen et al.[19]
Steroid equivalent dose (mg/m^2)	Equivalence ratios for steroids	Categorization based on literature	Parente et al.[20], Christoffersen et al.[21]
Cranial radiotherapy dose (Gy)	n.a.	Categorized as lower or higher than 25Gy, in order to distinguish between irradiation to the whole cranium and local, higher dose radiotherapy	Sherlock et al.[22], Crowne et al.[23], Blijdorp et al.[24]

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Supplemental table 2. Childhood cancer and treatment characteristics of study participants, non-participants and underlying cohort

Characteristics	Participants (n=2,338)	Non-participants (n=2,333)	Underlying cohort (N=6,165)	Comparison participants vs non-participants	Comparison participants vs cohort
Sex					
Male	1198 (51.2)	1418 (60.8)	3433 (55.7)	**P<0.001**	**P<0.001**
Female	1140 (48.8)	915 (39.2)	2731 (44.3)		
Transgender	0	0	1 (0.0)		
Primary childhood cancer (ICCC)					
Leukemias, myeloproliferative diseases and myelodysplastic diseases	831 (35.5)	779 (33.4)	2094 (34.0)	**P<0.001**	**P<0.001**
Lymphomas and reticulo endothelial neoplasms	448 (19.2)	416 (17.8)	1062 (17.2)		
CNS and miscellaneous intracranial and intraspinal neoplasms	213 (9.1)	341 (14.6)	844 (13.7)		
Neuroblastoma and other peripheral nervous cell tumors	135 (5.8)	103 (4.4)	324 (5.3)		
Retinoblastoma	11 (0.5)	15 (0.6)	3 (0.5)		
Renal tumors	269 (11.5)	222 (9.5)	596 (9.7)		
Hepatic tumors	18 (0.8)	28 (1.2)	52 (0.8)		
Bone tumors	136 (5.8)	126 (5.4)	370 (6.0)		
Soft tissue and other extraosseous sarcomas	167 (7.1)	170 (7.3)	450 (7.3)		
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	76 (3.3)	97 (4.2)	232 (3.8)		
Other malignant epithelial neoplasms and malignant melanomas	31 (1.3)	35 (1.5)	102 (1.7)		
Other and unspecified malignant neoplasms	3 (0.1)	1 (0.0)	6 (0.1)		
Age at diagnosis (yr)					
0-4	1071 (45.8)	1073 (46.1)	2727 (45.3)	P=0.94	P=0.66
5-9	651 (27.8)	630 (27.1)	1628 (27.1)		
10-14	481 (20.6)	489 (21.0)	1285 (21.4)		
15-17	135 (5.8)	136 (5.8)	376 (6.2)		
Treatment period					
1963-1969	33 (1.4)	34 (1.5)	119 (1.9)	P=0.72	**P=0.02**
1970-1979	317 (13.6)	311 (13.3)	978 (15.9)		
1980-1989	732 (31.3)	722 (30.9)	1931 (31.3)		

Supplemental table 2. Continued

Characteristics	Participants (n=2,338)	Non-participants (n=2,333)	Underlying cohort (N=6,165)	Comparison participants vs non-participants	Comparison participants vs cohort
1990-1999	1013 (43.3)	1048 (44.9)	2541 (41.2)		
2000-2001	243 (10.4)	218 (9.3)	596 (9.7)		
Age at invitation					
<18	NA	NA	49 (0.8)	P=0.43	**P<0.001**
18-30	784 (33.5)	526 (22.5)	1313 (21.3)		
30-40	878 (37.6)	632 (27.1)	1511 (24.5)		
>40	676 (28.9)	441 (18.9)	1118 (18.1)		
Follow-up time since childhood cancer diagnosis					
10-20	472 (20.2)	433 (18.6)	981 (15.9)	P=0.48	P=0.96
20-30	928 (39.7)	968 (41.5)	1931 (31.3)		
30-40	693 (29.6)	680 (29.1)	1393 (22.6)		
40-50	220 (9.4)	232 (9.9)	460 (7.5)		
50-60	25 (1.1)	20 (0.9)	46 (0.7)		
Surgery[a]					
Yes	1145 (49.1)	1212 (52.0)	3185 (51.7)	**P=0.03**	**P=0.01**
Amputation	67 (2.9)	41 (2.6)	-	P=0.57	
Radiotherapy[a]					
Yes	905 (38.7)	86 (33.7)	2527 (41.0)	**P<0.001**	**P=0.04**
Cranial	432 (18.5)	248 (15.6)	-	**P=0.016**	
Total body	88 (3.8)	30 (1.9)	-	**P=0.001**	
Abdominal/pelvic	201 (8.6)	93 (5.8)	-	**P=0.001**	
Chemotherapy[a]					
Yes	2057 (88.0)	1824 (78.2)	5005 (81.2)	**P<0.001**	**P<0.001**
Alkylating agents	1176 (55.3)	657 (45.6)	-	**P<0.001**	
Anthracyclines	1172 (53.2)	676 (45.7)	-	**P<0.001**	
Corticosteroids	1190 (50.9)	757 (47.3)	-	P=0.088	
Asparaginase	584 (25.0)	357 (22.4)	-	P=0.057	
Platinum	311 (13.3)	175 (11.0)	-	**P=0.028**	

Supplemental table 2. Continued

Characteristics	Participants (n=2,338)	Non-participants (n=2,333)	Underlying cohort (N=6,165)	Comparison participants vs non-participants	Comparison participants vs cohort
Vinca alkaloids	1829 (78.3)	1128 (70.7)	-	**P<0.001**	
Bone marrow transplant[a]					
Allogeneic	99 (4.3)	59 (2.5)	231 (3.7)	**P<0.001**	P=0.70
Autologous	57 (2.5)	34 (1.5)	155 (2.5)		
Therapy					
No treatment	10 (0.4)	28 (1.2)	61 (1.0)	**P<0.001**	**P<0.001**
Surgery only	150 (6.4)	295 (12.6)	575 (9.3)		
Chemotherapy only (± surgery)	1272 (54.4)	1216 (52.1)	2967 (48.1)		
Radiotherapy only (± surgery)	121 (5.2)	178 (7.6)	484 (7.9)		
Chemotherapy and radiotherapy (± surgery)	784 (33.5)	607 (26.0)	2030 (32.9)		

[a] For primary cancer and recurrences.

Not all numbers and percentages add up to group total because data for refusers is not available.

NA Not applicable, no inclusion <18y

We compared participants with non-participants and with the underlying cohort, which contains all invited survivors as well as survivors who refused participation in an earlier phase of the study and survivors who died after the formation of the cohort.

Supplemental table 3. Baseline characteristics of Lifelines reference cohort

	Total	Men	Women
Number of participants	132,150	54,733 (41.4%)	77,417 (58.6%)
Age (mean/SD)	42.0 (11.0)	42.5 (10.9)	41.7 (11.1)
Age (median/IQR)	43.0 (34.0-49.0)	43.0 (35.0-50.0)	43.0 (34.0-49.0)
Age range	18.0-65.0	18.0-65.0	18.0-65.0
Age 18-30y	20,812 (15.7%)	7,793 (14.2%)	13,019 (16.8%)
Age 30-40y	30,028 (22.7%)	12,488 (22.8%)	17,540 (22.7%)
Age 40-50y	49,006 (37.1%)	20,469 (37.4%)	28,537 (36.9%)
Age 50+y	32,304 (24.4%)	13,983 (25.5%)	18,321 (23.7%)
BMI (mean/SD)	25.93 (4.35)	26.29 (3.72)	25.68 (4.73)
BMI (median/IQR)	25.28 (22.95-28.09)	25.88 (23.81-28.28)	24.74 (22.39-27.97)
BMI range	13.38-73.57	14.43-73.57	13.38-66.26
Overweight	69,989 (53.0%)	33,274 (60.8%)	36,715 (47.4%)
Obesity	19,941 (15.1%)	7,656 (14.0%)	12,285 (15.9%)
Morbid obesity	4,966 (3.8%)	1,289 (2.4%)	3,677 (4.7%)

Supplemental table 4. Univariable logistic regression analysis of variables associated with overweight (BMI ≥25kg/m^2)

Dependent variable	Frequency of high BMI (n (%))	Univariable models		
		OR	95% CI	P-value
Patient characteristics				
Age at clinic visit		1.05	1.04 - 1.06	**<0.001**
First tertile	258 (34.1%)			
Second tertile	326 (43.8%)			
Third tertile	435 (58.1%)			
Sex		0.92	0.78 - 1.09	0.33
Female	485 (44.3%)			
Male	535 (46.3%)			
Age at diagnosis		1.04	1.02 - 1.06	**<0.001**
First tertile	299 (40.0%)			
Second tertile	340 (45.0%)			
Third tertile	381 (50.9%)			
Overweight at cancer diagnosis		3.16	2.08 - 4.79	**<0.001**
Yes	80 (70.8%)			
No	782 (43.4%)			
Smoking (current or former)		1.41	1.17 - 1.70	**<0.001**
Yes	338 (52.7%)			
No	590 (44.2%)			
Low physical activity		1.20	0.96 - 1.50	0.11
Yes	188 (44.8%)			
No	500 (40.3%)			
Treatment characteristics				
Cranial radiotherapy		2.44	1.96 - 3.05	**<0.001**
Yes	260 (63.1%)			
No	755 (41.2%)			
Cranial radiotherapy categorized				**<0.001**
No	755 (41.2%)	Ref		
1-25 Gy	143 (71.5%)	3.58	2.60 - 4.94	

Supplemental table 4. Continued

Dependent variable	Frequency of high BMI (n (%))	Univariable models		
		OR	**95% CI**	**P-value**
25+ Gy	114 (55.1%)	1.75	1.31 - 2.34	
Total body irradiation		0.18	0.10 - 0.34	**<0.001**
Yes	12 (13.8%)			
No	1001 (46.5%)			
Abdominal/pelvic radiotherapy		0.97	0.72 - 1.30	0.84
Yes	89 (44.5%)			
No	924 (45.2%)			
Abdominal/pelvic radiotherapy categorized				0.70
No	924 (45.2%)	Ref		
1-29 Gy	52 (46.4%)	1.05	0.72 - 1.54	
30+ Gy	35 (41.4%)	0.83	0.54 - 1.29	
Alkylating agents (CED)		0.80	0.67 - 0.95	**0.012**
Yes	477 (41.7%)			
No	431 (47.3%)			
Cyclophosphamide equivalent dose categorized				0.081
No	431 (47.3%)	Ref		
1-4000mg/m^2	183 (42.3%)	0.82	0.65 - 1.03	
4000-8000mg/m^2	140 (42.9%)	0.84	0.65 - 1.08	
8000+ mg/m^2	154 (40.1%)	0.75	0.59 - 0.95	
Corticosteroids		1.12	0.95 - 1.32	0.18
Yes	536 (46.7%)			
No	484 (43.8%)			
Corticosteroids categorized				0.35
No	484 (43.8%)	Ref		
0-10g/m^2	490 (46.9%)	1.13	0.96 - 1.34	
10+ g/m^2	46 (44.2%)	1.02	0.68 - 1.52	
Allogeneic SCT		0.25	0.15 - 0.42	**<0.001**
Yes	17 (17.7%)			
No	996 (46.5%)			
Comorbidity				
Growth hormone deficiency		2.28	1.59 - 3.27	**<0.001**
Yes	73 (64.0%)			
No	947 (44.3%)			

Supplemental table 5. Multivariable logistic regression models for outcomes obesity (BMI ≥30kg/m2) and morbid obesity (BMI ≥35kg/m2)

Dependent variable	Outcome BMI >30kg/m^2			Outcome BMI >35kg/m^2		
	OR	**95% CI**	**P-value**	**OR**	**95% CI**	**P-value**
Age at clinic visit	1.02	0.99 - 1.05	0.12	1.04	0.98 - 1.09	0.18
Female sex	2.05	1.44 - 2.94	**<0.001**	3.33	1.58 - 7.02	**0.002**
Overweight at cancer diagnosis	2.85	1.60 - 5.08	**<0.001**	5.83	2.53 - 13.45	**<0.001**
Smoking (current or former)	1.29	0.90 - 1.86	0.17	1.32	0.66 - 2.63	0.43
Cranial radiotherapy categorized			**<0.001**			**0.036**
No	Ref			Ref		
1-25 Gy	2.48	1.45 - 4.23		3.06	1.24 - 7.50	
25+ Gy	1.94	1.11 - 3.39		1.97	0.70 - 5.55	
Total body irradiation	0.11	0.01 - 1.50	0.097			
Allogeneic SCT	1.52	0.22 - 10.61	0.67			
Growth hormone deficiency*	2.40	1.37 – 4.21	**0.002**	1.11	1.23 – 7.50	**0.016**

** tested in separate model*

Supplemental table 6. Prevalence of overweight according to different definitions, and percentage of false-negative when compared to BMI or fat% on DXA

Column "Prevalence of overweight" is the percentage of overweight survivors according to each method, with percentage for the total cohort, men and women separately, and for the subgroup of abdominally irradiated survivors. Column "Additionally diagnosed as overweight" is the percentage of survivors with normal BMI but overweight according to each other method. Column "Underdiagnosis of overweight" has fat% on DXA as reference and shows the percentage of survivors with overweight on DXA not captured by each other method.

Measurement	Prevalence of overweight				Additionally diagnosed as overweight				Underdiagnosis of overweight			
	All	Men	Women	Abd. irr.	All	Men	Women	Abd. irr.	All	Men	Women	Abd. irr.
High BMI (≥25kg/m^2)	45.3%	46.3%	44.3%	44.5%	Ref	Ref	Ref	Ref	31.6%	21.9%	42.4%	39.3%
High waist JIS (≥80/94cm)	46.8%	33.6%	60.8%	42.6%	11.2%	3.5%	19.3%	10.2%	30.1%	30.0%	30.2%	46.8%
High waist NCEP ATP III (≥88/102cm)	24.5%	14.0%	35.5%	18.8%	2.3%	0.1%	4.6%	1.5%	48.7%	46.5%	51.1%	63.1%
High waist hip ratio (≥0.85/0.90)	49.0%	53.8%	43.9%	35.5%	18.8%	20.4%	17.0%	13.7%	32.9%	19.9%	47.4%	53.2%
High waist height ratio (≥0.50)	47.0%	46.1%	47.9%	40.1%	9.3%	8.5%	10.2%	6.6%	29.9%	20.2%	40.7%	45.9%
High fat% on DXA (≥30/25%)	70.4%	58.4%	83.7%	77.7%	31.6%	21.9%	42.4%	39.3%	Ref	Ref	Ref	Ref
High fat% on DXA (≥33-35/21-23%)	74.7%	82.3%	66.1%	74.1%	33.6%	39.5%	27.1%	35.7%	8.3%	0.0%	17.6%	10.7%
High fat% on DXA (≥37.0-39.9%/24.9-28.0%)	51.4%	56.7%	45.5%	58.0%	16.2%	20.8%	11.0%	22.3%	19.0%	1.7%	38.2%	19.6%
Low adiponectin (≤1.46/0.90µg/ml)	4.5%	4.8%	4.3%	4.5%	1.9%	2.2%	1.6%	2.0%	66.1%	54.6%	78.9%	72.3%

Abbreviations: BMI = body mass index; JIS = Joint Interim Statement; NCEP ATP III = National Cholesterol Education Program Adult Treatment Panel III.

Supplemental table 7. Univariable logistic regression of factors associated with outcome false-negative classification of overweight based on BMI and waist circumference, when compared to overweight based on fat% on DXA scan.

Dependent variable	Outcome false-negative classification with BMI			
	Frequency of false-negative classification with BMI (n (%))	Univariable models		
		OR	95% CI	P-value
Patient characteristics				
Age and sex				
Age at clinic visit		1.00	0.98 - 1.01	0.52
First tertile	203 (32.2%)			
Second tertile	199 (31.9%)			
Third tertile	111 (29.9%)			
Sex		2.62	2.11 - 3.26	**<0.001**
Female	327 (42.4%)			
Male	187 (21.9%)			
Age and weight at diagnosis				
Age at diagnosis		1.00	0.98 - 1.03	0.90
First tertile	183 (30.9%)			
Second tertile	177 (31.4%)			
Third tertile	154 (32.7%)			
Overweight at cancer diagnosis		0.56	0.33 - 0.96	**0.035**
Yes	18 (21.4%)			
No	437 (32.6%)			
Lifestyle				
Smoking (current or former)		0.73	0.57 - 0.93	**0.011**
Yes	128 (26.8%)			
No	321 (33.4%)			
Low physical activity		1.54	1.20 - 1.97	**0.001**
Yes	143 (39.7%)			
No	323 (30.0%)			
Treatment characteristics				
Cranial radiotherapy		0.76	0.56 - 1.02	0.068
Yes	68 (26.8%)			
No	445 (32.6%)			
Cranial radiotherapy categorized				0.12
No	445 (32.6%)	Ref		
1-25 Gy	28 (23.5%)	0.64	0.41 - 0.99	
25+ Gy	39 (30.0%)	0.89	0.60 - 1.31	
Total body irradiation		3.85	2.32 - 6.39	**<0.001**
Yes	42 (62.7%)			
No	471 (30.4%)			
Abdominal/pelvic radiotherapy		1.43	0.96 - 2.12	0.076
Yes	44 (39.3%)			
No	469 (31.2%)			
Abdominal/pelvic radiotherapy categorized				0.19
No	469 (31.2%)	Ref		
1-29 Gy	25 (37.9%)	1.35	0.81 - 2.24	
30+ Gy	19 (41.3%)	1.55	0.86 - 2.82	

Supplemental table 7. Continued

Dependent variable	Outcome false-negative classification with BMI			
	Frequency of false-negative classification with BMI (n (%))	Univariable models		
		OR	95% CI	P-value
Alkylating agents (CED)		1.36	1.09 - 1.70	**0.006**
Yes	293 (34.4%)			
No	188 (27.8%)			
Cyclophosphamide equivalent dose categorized				**0.021**
No	188 (27.8%)	Ref		
1-4000mg/m^2	114 (32.7%)	1.26	0.95 - 1.67	
4000-8000mg/m^2	76 (33.0%)	1.28	0.93 - 1.77	
8000+ mg/m^2	103 (37.7%)	1.58	1.17 - 2.12	
Anthracyclines (DED)		1.35	1.09 - 1.68	**0.007**
Yes	296 (34.3%)			
No	195 (27.9%)			
Doxorubicin equivalent dose categorized				**0.0022**
No	195 (27.9%)	Ref		
First tertile	86 (28.7%)	1.04	0.77 - 1.40	
Second tertile	110 (35.8%)	1.45	1.09 - 1.92	
Third tertile	100 (38.9%)	1.65	1.22 - 2.23	
Corticosteroids		0.86	0.70 - 1.06	0.16
Yes	260 (30.1%)			
No	254 (33.3%)			
Corticosteroids categorized				0.17
No	254 (33.3%)	Ref		
0-10g/m^2	234 (29.5%)	0.84	0.67 - 1.04	
10+ g/m^2	26 (36.7%)	1.16	0.70 - 1.92	
Asparaginase		0.85	0.67 - 1.08	0.19
Yes	129 (29.1%)			
No	384 (32.5%)			
Platinum derivatives		1.37	1.03 - 1.82	**0.029**
Yes	91 (37.6%)			
No	422 (30.5%)			
Vinca alkaloids		1.02	0.79 - 1.33	0.88
Yes	410 (31.7%)			
No	103 (31.2%)			
Allogeneic SCT		2.92	1.83 - 4.66	**<0.001**
Yes	42 (56.0%)			
No	469 (30.4%)			
Comorbidity				
Growth hormone deficiency		0.94	0.59 - 1.50	0.79
Yes	27 (30.3%)			
No	487 (31.7%)			

Dependent variable	Outcome false-negative classification with WC			
	Frequency of false-negative classification with WC (n (%))	Univariable models		
		OR	95% CI	P-value
Patient characteristics				
Age and sex				
Age at clinic visit		0.98	0.97 - 1.00	**0.014**
First tertile	203 (32.2%)			
Second tertile	190 (30.5%)			
Third tertile	94 (25.6%)			
Sex		1.01	0.82 - 1.25	0.92
Female	231 (30.2%)			
Male	256 (30.0%)			
Age and weight at diagnosis				
Age at diagnosis		0.97	0.95 - 1.00	**0.035**
First tertile	184 (31.1%)			
Second tertile	179 (31.9%)			
Third tertile	124 (26.6%)			
Overweight at cancer diagnosis		0.70	0.42 - 1.17	0.17
Yes	20 (23.8%)			
No	413 (31.0%)			
Lifestyle		0.83	0.65 - 1.06	0.13
Smoking (current or former)				
Yes	128 (26.9%)			
No	294 (30.8%)			
Low physical activity		1.10	0.85 - 1.43	0.45
Yes	117 (32.9%)			
No	330 (30.7%)			
Treatment characteristics				
Cranial radiotherapy		0.79	0.59 - 1.08	0.14
Yes	66 (26.2%)			
No	420 (30.9%)			
Cranial radiotherapy categorized				0.15
No	420 (30.9%)	Ref		
1-25 Gy	26 (22.2%)	0.64	0.41 - 1.00	
25+ Gy	40 (30.8%)	0.99	0.67 - 1.47	
Total body irradiation		3.20	1.95 - 5.25	**<0.001**
Yes	38 (56.7%)			
No	448 (29.1%)			
Abdominal/pelvic radiotherapy		2.16	1.46 - 3.19	**<0.001**
Yes	52 (46.8%)			
No	434 (29.0%)			
Abdominal/pelvic radiotherapy categorized				**<0.001**
No	434 (29.0%)	Ref		
1-29 Gy	28 (43.1%)	1.86	1.12 - 3.07	
30+ Gy	24 (52.2%)	2.67	1.48 - 4.82	
Alkylating agents (CED)		1.47	1.18 - 1.85	**0.001**
Yes	285 (33.5%)			

Supplemental table 7. Continued

Dependent variable	Outcome false-negative classification with WC			
	Frequency of false-negative classification with WC (n (%))	Univariable models		
		OR	95% CI	P-value
No	171 (25.5%)			
Cyclophosphamide equivalent dose categorized				**0.0027**
No	171 (25.5%)	Ref		
1-4000mg/m^2	106 (30.6%)	1.29	0.97 - 1.72	
4000-8000mg/m^2	85 (36.8%)	1.70	1.24 - 2.34	
8000+ mg/m^2	94 (34.4%)	1.54	1.13 - 2.08	
Anthracyclines (DED)		1.49	1.19 - 1.86	**<0.001**
Yes	290 (33.7%)			
No	177 (25.5%)			
Doxorubicin equivalent dose categorized				**<0.001**
No	177 (25.5%)	Ref		
First tertile	89 (29.7%)	1.23	0.91 - 1.67	
Second tertile	102 (33.7%)	1.49	1.11 - 1.99	
Third tertile	99 (38.5%)	1.83	1.35 - 2.48	
Corticosteroids		0.89	0.72 - 1.10	0.29
Yes	248 (28.9%)			
No	239 (31.4%)			
Corticosteroids categorized				0.064
No	239 (31.4%)	Ref		
0-10g/m^2	220 (28.0%)	0.85	0.68 - 1.06	0.14
10+ g/m^2	28 (40.0%)	1.46	0.88 - 2.41	0.14
Asparaginase		0.89	0.70 - 1.13	0.33
Yes	124 (28.3%)			
No	363 (30.8%)			
Platinum derivatives		1.63	1.23 - 2.17	**0.001**
Yes	95 (39.4%)			
No	392 (28.5%)			
Vinca alkaloids		1.06	0.82 - 1.39	0.65
Yes	391 (30.4%)			
No	96 (29.1%)			
Allogeneic SCT		2.37	1.49 - 3.78	**<0.001**
Yes	37 (49.3%)			
No	447 (29.1%)			
Comorbidity				
Growth hormone deficiency		1.07	0.68 - 1.70	0.77
Yes	28 (31.5%)			
No	459 (30.0%)			

For variables with more than two categories the overall p-value was calculated with the Wald test. Significant variables in univariable analysis were included in the multivariable model. If for a treatment factor both the dichotomous and categorized variable were significant, only the latter was included.

Abbreviations: CI = confidence interval; OR = odds ratio; CI = confidence interval; Ref = reference

Supplemental table 8. Multivariable logistic regression of factors associated with outcome false-negative classification of overweight based on BMI and waist circumference, when compared to overweight based on fat% on DXA scan.

Dependent variable	Outcome false-negative classification with BMI			
	Frequency of false-negative classification with BMI (n (%))	Multivariable model		
		OR	95% CI	P-value
Patient characteristics				
Age and sex				
Age at clinic visit		1	0.98 - 1.03	0.74
First tertile	203 (32.2%)			
Second tertile	199 (31.9%)			
Third tertile	111 (29.9%)			
Sex		3.02	2.27 - 4.03	**<0.001**
Female	327 (42.4%)			
Male	187 (21.9%)			
Weight at diagnosis				
Overweight at cancer diagnosis		0.57	0.29 - 1.11	0.098
Yes	18 (21.4%)			
No	437 (32.6%)			
Lifestyle				
Smoking (current or former)		0.95	0.70 - 1.29	0.76
Yes	128 (26.8%)			
No	321 (33.4%)			
Low physical activity		1.36	1.00 - 1.85	0.052
Yes	143 (39.7%)			
No	323 (30.0%)			
Treatment characteristics				
Cranial radiotherapy				
Yes	68 (26.8%)			
No	445 (32.6%)			
Cranial radiotherapy categorized				**0.036**
No	445 (32.6%)	Ref		
1-25 Gy	28 (23.5%)	0.47	0.26 - 0.83	
25+ Gy	39 (30.0%)	0.89	0.45 - 1.76	
Total body irradiation		9.06	2.41 - 34.04	**0.001**
Yes	42 (62.7%)			
No	471 (30.4%)			
Cyclophosphamide equivalent dose categorized				0.55
No	188 (27.8%)	Ref		
1-4000mg/m^2	114 (32.7%)	1.13	0.72 - 1.77	
4000-8000mg/m^2	76 (33.0%)	1.15	0.72 - 1.84	

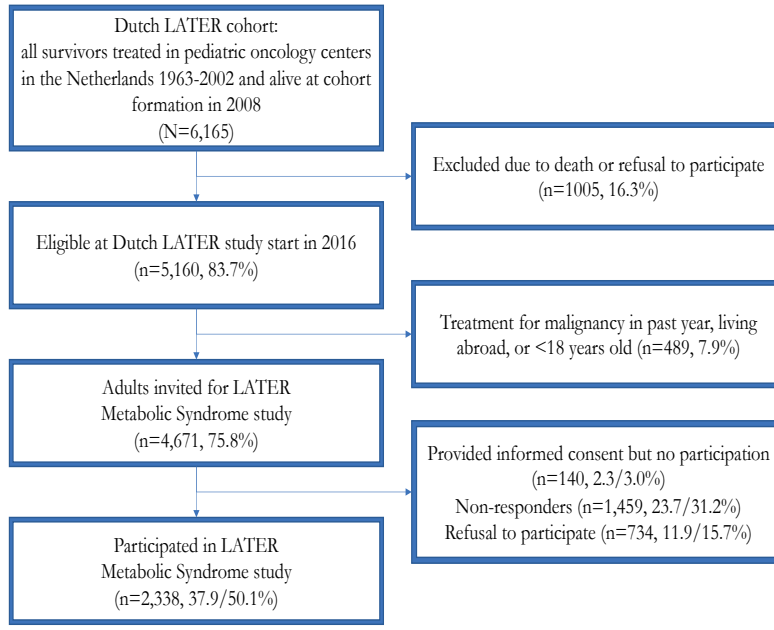
Supplemental table 8. Continued

Dependent variable	Outcome false-negative classification with BMI			
	Frequency of false-negative classification with BMI (n (%))	Multivariable model		
		OR	95% CI	P-value
8000+ mg/m^2	103 (37.7%)	1.36	0.90 - 2.05	
Doxorubicin equivalent dose categorized				0.13
No	195 (27.9%)	Ref		
First tertile	86 (28.7%)	1.29	0.80 - 2.09	
Second tertile	110 (35.8%)	1.57	1.02 - 2.42	
Third tertile	100 (38.9%)	1.48	0.99 - 2.23	
Platinum derivatives		1.35	0.89 - 2.04	0.16
Yes	91 (37.6%)			
No	422 (30.5%)			
Allogeneic SCT		0.63	0.16 - 2.44	0.5
Yes	42 (56.0%)			
No	469 (30.4%)			
Dependent variable	**Outcome false-negative classification with WC**			
	Frequency of false-negative classification with WC (n (%))	**Multivariable model**		
		OR	**95% CI**	**P-value**
Patient characteristics				
Age at clinic visit		0.99	0.97 - 1.01	0.28
First tertile	203 (32.2%)			
Second tertile	190 (30.5%)			
Third tertile	94 (25.6%)			
Age at diagnosis		0.97	0.94 - 1.00	**0.023**
First tertile	184 (31.1%)			
Second tertile	179 (31.9%)			
Third tertile	124 (26.6%)			
Treatment characteristics				
Total body irradiation		4.35	1.78 - 10.67	**<0.001**
Yes	38 (56.7%)			
No	448 (29.1%)			
Abdominal/pelvic radiotherapy categorized				**<0.001**
No	434 (29.0%)	Ref		
1-29 Gy	28 (43.1%)	2.05	1.17 - 3.56	
30+ Gy	24 (52.2%)	3.06	1.64 - 5.72	
Cyclophosphamide equivalent dose categorized				0.71

Supplemental table 8. Continued

Dependent variable	Outcome false-negative classification with WC			
	Frequency of false-negative classification with WC (n (%))	Multivariable model		
		OR	95% CI	P-value
No	171 (25.5%)	Ref		
1-4000mg/m^2	106 (30.6%)	1.07	0.74 - 1.56	
4000-8000mg/m^2	85 (36.8%)	1.25	0.85 - 1.83	
8000+ mg/m^2	94 (34.4%)	1.11	0.79 - 1.57	
Doxorubicin equivalent dose categorized				**0.049**
No	177 (25.5%)	Ref		
First tertile	89 (29.7%)	1.33	0.90 - 1.97	
Second tertile	102 (33.7%)	1.43	0.99 - 2.05	
Third tertile	99 (38.5%)	1.58	1.12 - 2.23	
Platinum derivatives		1.66	1.21 - 2.29	**0.002**
Yes	95 (39.4%)			
No	392 (28.5%)			
Allogeneic SCT		1.08	0.46 - 2.51	0.86
Yes	37 (49.3%)			
No	447 (29.1%)			

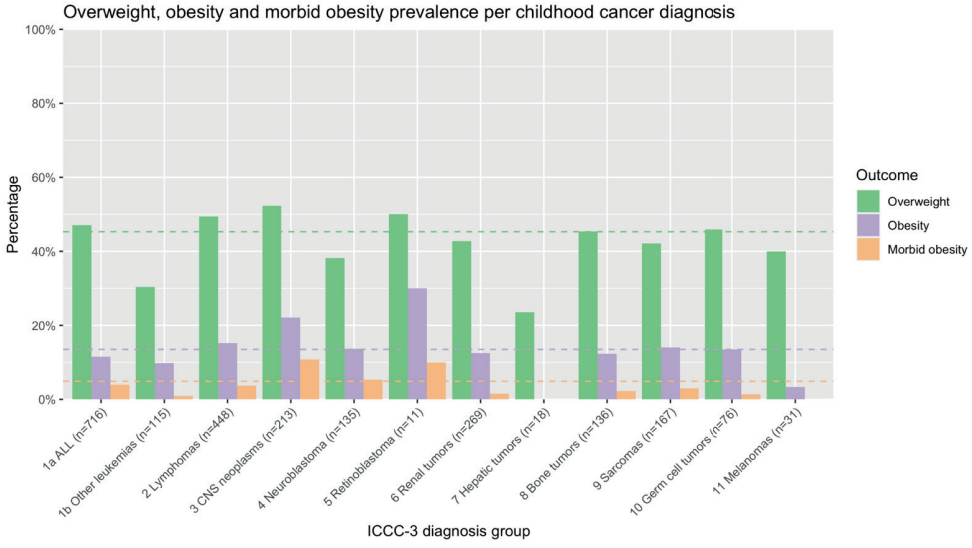
For variables with more than two categories the overall p-value was calculated with the Wald test. Significant variables in univariable analysis were included in the multivariable model. If for a treatment factor both the dichotomous and categorized variable were significant, only the latter was included.
Abbreviations: CI = confidence interval; OR = odds ratio; CI = confidence interval; Ref = reference



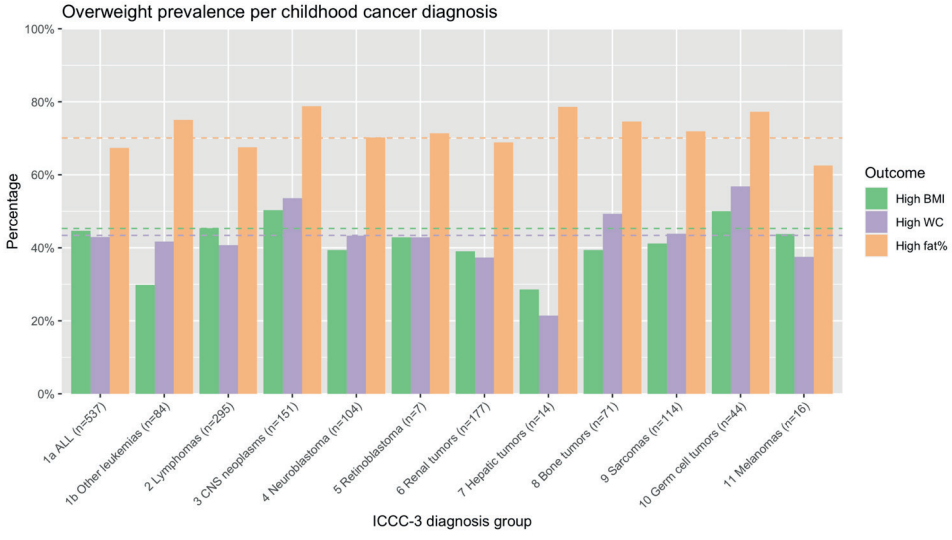
Supplemental figure 1. Flow chart of study participants

The current study used the cohort of the Metabolic Syndrome sub-study of the DCCSS-LATER study. This flow chart shows the number of participants in the current study and how they are embedded in the overall cohort. Percentages indicate the proportion of the overall cohort (N=6,165). In the lower two blocks, there are two percentages: the first indicates the proportion of the overall cohort, the second is the proportion of the invited survivors, indicating the (non-) participation rate.

At formation in 2008, the entire survivors cohort contained 6,165 eligible survivors. By mail, survivors were provided the option to opt-out of future study participation. At the start of the DCCSS-LATER study, the cleaned cohort was frozen in 2016, leaving 5,160 subjects eligible. For the Metabolic Syndrome sub-study, only adults (n=4,671) were invited.



Supplemental figure 2. Overweight, obesity and morbid obesity prevalence per childhood cancer diagnosis group
ICCC-3 group "Other and unspecified" is not included. Dashed lines indicate the overall mean for each outcome.
Abbreviations: ALL = acute lymphoid leukemia; CNS = central nervous system



Supplemental figure 3. Overweight outcomes per childhood cancer diagnosis group
Only survivors with all three measurements available are shown in this figure. ICCC-3 group "Other and unspecified" is not included. Dashed lines indicate the overall mean for each outcome.
Abbreviations: ALL = acute lymphoid leukemia; CNS = central nervous system

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Chapter 6

Genetic susceptibility to treatment related dyslipidemia in adult long-term survivors of childhood cancer: a report from the Childhood Cancer Survivor Study and the St Jude Lifetime cohorts

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In preparation

ABSTRACT

Background

Dyslipidemia can occur as a long-term side effect of childhood cancer treatment. Difference in occurrence in children receiving comparable treatment may suggest a role for genetic variation. We performed the first genome-wide association study on dyslipidemia in three large childhood cancer survivor cohorts.

Methods

Discovery analysis was performed in the Original (diagnosis 1970-1986) Cohort of the Childhood Cancer Survivor Study (CCSS, N=4,332). Replication analyses were performed in the St Jude Lifetime Cohort (SJLIFE, N=2,274) and the CCSS Expansion (diagnosis 1986-1999) Cohort (N=2,212). In the CCSS cohorts, dyslipidemia was defined as CTCAE grade 2 self-reported high cholesterol or high triglycerides, in the SJLIFE this was based on serum lipid assessment. Association was studied in the entire cohort as well as by treatment stratification. Additionally, we performed a candidate SNP approach of three variants identified in the only available genetic study on dyslipidemia in acute lymphoblastic leukemia (ALL) survivors.

Results

The initial discovery analysis yielded one significant ($p<5x10^{-8}$) and 16 suggestive ($p<5x10^{-6}$) loci. Nine with biological plausibility were included for replication analysis but none replicated. Using a treatment-stratified analysis of nine additional significant loci, variant rs114017774 was statistically significant among cranial radiotherapy (CRT) exposed survivors when pooling CCSS and SJLIFE (OR=11.30, 95%CI=5.03-25.40, $p=4.5x10^{-9}$), but did not individually replicate in the CCSS Expansion or SJLIFE cohorts. This variant had an interaction with CRT (genotypexCRT OR=14.61, 95%CI=4.59-56.55) independent of BMI. This variant could potentially alter *LRRTM4* or *CTNNA2* gene expression or function, making survivors more susceptible to hypothalamic damage caused by CRT. From the candidate SNP study we replicated rs676210 in the *APOB* gene in the CCSS cohort (p=0.0061 in ALL survivors, p=0.0049 in the total cohort).

Discussion

We identified rs114017774 on chromosome 2 as a potential genetic variant for dyslipidemia, specifically in cranially irradiated survivors of childhood cancer. We replicated for the first time variant rs676210 in a heterogeneous survivor cohort.

INTRODUCTION

The survival rates of childhood cancer have increased tremendously over the past decades. Currently, more than 80% of children with cancer will attain 5-year survival[1]. This number, however, fails to reflect cancer treatment related morbidity and consequent mortality occur[2]. Dyslipidemia, an established risk factor for atherosclerotic disease and mortality, is among the chronic health conditions experienced by long-term childhood cancer survivors [3]. Dyslipidemia is also a component of the metabolic syndrome, together with adiposity, insulin resistance and hypertension, a synergistic cluster strongly associated with subsequent diabetes mellitus and cardiovascular disease[4].

Previous studies have revealed that childhood cancer survivors have an early increased risk for developing dyslipidemia and metabolic syndrome[5-11]. Several factors have been associated with dyslipidemia, either directly or consequent to obesity. Endocrinopathies such as growth hormone deficiency (GHD), hypothyroidism and hypogonadism can lead to dyslipidemia and obesity. Disease and treatment related risk factors for dyslipidemia include diagnosis of brain tumor, brain surgery, cranial radiotherapy (CRT), and radiation to other relevant organs involved in metabolism, e.g., pancreas, gonads, thyroid and abdominal fat tissue[6, 11-16].

Variation in the occurrence and severity of dyslipidemia that is observed in comparably treated survivors suggests a potential role for genetic susceptibility[17, 18]. This genetic susceptibility could be explained by carrier status of genetic variants known to be associated with dyslipidemia in the general population, unique variants interacting with cancer treatment (e.g., drug metabolism), or a combination of the two. Although genome-wide association studies (GWAS) in the general population have revealed numerous loci associated with different lipid traits[19-23], evidence for relevance in survivors and for unique variants in survivors is scarce. So far, only one candidate SNP study in a cohort of 209 acute lymphoblastic leukemia (ALL) survivors has been performed, identifying dyslipidemia associated single nucleotide polymorphisms (SNP) in three genes[24]. These findings have, however, not been replicated in other survivors.

In order to identify unique genetic variants, we performed the first GWAS on dyslipidemia in childhood cancer survivors, aiming to identify SNPs uniquely associated with dyslipidemia in this group, with discovery and replication analyses in three large survivor cohorts: the Childhood Cancer Survivor Study (CCSS) Original and Expansion cohort and the St. Jude Lifetime Cohort (SJLIFE). We also performed a candidate SNP analysis of the three variants found in ALL survivors.

METHODS

Discovery cohort

The discovery analysis was performed on the original cohort of the CCSS. The CCSS original cohort consists of 14,361 5-year survivors, diagnosed between 1970 and 1986[25]. Participants in the study completed a baseline and follow-up questionnaire to capture self-reported outcomes. Of these, 5,739 survivors were genotyped (Supplemental Figure 1). This was done with DNA from blood, saliva or buccal cells, using the HumanOmni5Exome array (Illumina, San Diego, CA, USA) and imputed with the 1000Genomes phase 3 version 5 reference panel[26]. We restricted our analysis to participants with European genetic ancestry. Survivors participating in SJLIFE were excluded, to avoid double inclusion. Survivors who received stem cell transplantation were also excluded. Hence, the cohort for the discovery analysis consisted of 4,332 survivors, of whom 759 (17.5%) had developed dyslipidemia.

Replication cohorts

The SJLIFE and CCSS Expansion Cohorts both served as replication cohorts. SJLIFE is a cohort of 4,713 survivors, diagnosed between 1962 and 2012, who completed health questionnaires and underwent a comprehensive clinical assessment at a late effects clinic[27]. The CCSS Expansion Cohort is questionnaire-based and consists of 11,304 5-year survivors diagnosed between 1987 and 1999. For both replication cohorts genotyping was performed with whole genome sequencing using HiSeq X10 and NovaSeq sequencers (Illumina, San Diego, USA). Replication analyses were also restricted to European genetic ancestry, and survivors who received stem cell transplantation were excluded. Participants in both SJLIFE and CCSS Expansion Cohort were excluded from the latter. This resulted in 2,274 SJLIFE survivors and 2,212 CCSS Expansion Cohort survivors included as our replication cohorts (Supplemental Figure 1). Participants in all study cohorts provided informed consent for the use of genetic data for future analyses.

Phenotype definition

In all three cohorts, dyslipidemia was defined as high total cholesterol or hypertriglyceridemia grade 2 (=moderate) or higher, according to the modified CTCAE criteria[28] (Supplemental Table 1). In the CCSS and CCSS Expansion Cohorts, this is self-reported dyslipidemia, requiring medication, based on health questionnaires[29]. In the SJLIFE Cohort, grade 2 or higher dyslipidemia was defined as total cholesterol >300 mg/dL (7.8 mmol/L) or triglycerides >300 mg/dL (3.4 mmol/L) or treatment with one or more lipid lowering agents.

GWAS analysis

Discovery and replication analysis

A genetic power calculator was used to estimate the minimally detectable odds ratio (OR) that could be achieved in the discovery analysis for variants to reach the genome-wide significance threshold of $p<5x10^{-8}$ for several minor allele frequencies (MAF) [30]. For a survivor population dyslipidemia prevalence of 15%[16], under an allelic model with additive risk, with the required power of 80%, the minimal detectable ORs per high risk allele with MAF = 0.01, 0.05, 0.1 and 0.25 were 2.7, 1.8, 1.5 and 1.2, respectively. In addition to the genome-wide significance threshold, we set a suggestive threshold of $p<5x10^{-6}$. To reach the suggestive threshold, the minimum ORs per high risk allele with the four MAFs were 2.4, 1.6, 1.4 and 1.1, respectively.

GWAS was performed in *Rvtests*[31], using the score test option. This first analysis used a logistic regression model adjusting for age at follow-up, sex and the first four genetic principal components. Additionally, treatment-stratified GWAS were performed, in order to identify SNP effects in survivors with a specific cancer treatment, as well as to find SNPs whose effects are otherwise outweighed/masked by cancer treatment and relevant only in non-exposed survivors[13, 32-36]. To identify treatment factors to stratify by, hypothesized relevant factors (i.e., CRT, abdominal radiotherapy, asparaginase, prednisone and dexamethasone)[6, 11-15] were examined by a logistic regression model and those with a p-value less than 0.10 were used as stratification variables.

Quality control of the GWAS analysis was performed in R (R Core Foundation, Vienna, Austria)[37], with the package *EasyQC*[38], using an imputation quality filter of 0.4 and otherwise default settings. Genetic associations were visualized in a Manhattan plot using the R package *EasySTRATA*[39].

For replication analysis we included initial discovery analysis SNPs that either reached the genome-wide significance threshold, or that reached the suggestive threshold and had biological plausibility to be associated with dyslipidemia (see section Functional evidence below). For treatment-stratified models we were more stringent and only included genome-wide significant SNPs for replication analysis. For the replication analysis the same logistic regression model was used. For a significant replication, the SNP effect on the outcome needed to be in the same direction, with meeting the statistical significance threshold at p-value of 0.05 divided by the number of SNPs tested (Bonferroni correction).

The results of the discovery and replication analyses were pooled using the R package *rmeta*[40], with a fixed effects model or, in case of significant heterogeneity ($p<0.05$), a

random effects model. Due to its relatively young age, lower dyslipidemia prevalence and differences in treatment characteristics reflecting newer protocols, the CCSS Expansion cohort was judged differently from the CCSS and SJLIFE cohorts. It was therefore decided to first pool the CCSS original and SJLIFE cohorts in meta-analysis, and as the next step add the CCSS Expansion results. The results of the meta-analysis were regarded as significant if the p-value became lower.

Functional evidence

We used the UCSC Genome Browser[41], Locus Zoom[42], Functional Mapping and Annotation of GWAS (FUMA-GWAS)[43], ROADMAP[44], GWAS catalog[45], GTEx[46], Mouse Genome Informatics[47], International Mouse Phenotyping Consortium[48] and published literature to explore supportive information that links the discovered loci to potential genes, and these genes to dyslipidemia. This was performed for the variant as well as variants in high LD (R^2>0.80).

Further analyses of replicated SNP

For the SNP replicated in treatment-stratified analysis, additional analyses were performed by adding terms to the regression model, to further explore a potential gene-treatment exposure interaction. First, the interaction term (i.e., SNPxTherapy) was included. Furthermore, a dose-effect relation was evaluated by adding the dose variable to the model instead of the dichotomous variable. For cranial radiotherapy, an additional variable was available specifying the dose received by several brain regions (Supplemental figure 2). This variable was added to the model to find out whether the gene-radiotherapy interaction was associated with radiation to the hypothalamic-pituitary region specifically[49, 50]. In order to evaluate whether the effect was restricted to this treatment exposure only, the association for this locus in the other treatment-stratified analyses and the full cohort analysis was looked up in the GWAS results. Finally, we evaluated whether the effect of the SNP on dyslipidemia was a direct effect or mediated by overweight, by adding body mass index (BMI) to the model, as well as by building a model with BMI, overweight or obesity as outcomes. The additional models were built in R and tested with the Wald test.

Comparison with general population

To verify that the genetic signal is specific to survivors, we performed a look-up of the identified variant and variants in high linkage disequilibrium with it (LD, according to FUMA-GWAS results and LD proxy[51]) in two of the largest GWASs in the general population published to date, that had the complete summary data publicly available[22, 23]. The genome-wide significance threshold ($p<5x10^{-8}$) was set for association in the general population.

Candidate SNP analysis

We looked up associations for the three variants (rs676210, rs2286615, rs62079523) previously identified in the candidate SNP study by England et al. among ALL survivors[24] in the largest of our three cohorts, i.e., the CCSS Original cohort (overall cohort and ALL subgroup). The significance threshold after Bonferroni correction of 0.05/3=0.017 was used for this analysis.

RESULTS

Cohort description

In the CCSS discovery cohort (mean age 43.3 years old, mean follow-up time 35.3 years), the prevalence of dyslipidemia was 17.5% (Table 1). The most prevalent childhood cancer diagnoses included ALL (26.9%), Hodgkin lymphoma (13.3%), and kidney tumors (9.8%). Survivors with dyslipidemia were on average 6.5 years older and were more often overweight or obese. Growth hormone deficiency, hypogonadism, diabetes and hypothyroidism were more prevalent among dyslipidemia cases. Administration of abdominal radiotherapy and alkylating agents was more frequent among cases, whereas controls had more often received asparaginase.

In SJLIFE (mean age 36.2 years old, mean follow-up 28.1 years) the dyslipidemia prevalence was 19.7% and in CCSS Expansion (mean age 30.0 years old, mean follow-up 21.3 years) this was 5.4%. Participants in CCSS Expansion had significantly less often received cranial and abdominal radiotherapy.

GWAS analysis

Discovery and replication analysis

In the discovery analysis of the full CCSS Original cohort, one locus with a genome-wide significant association and 16 suggestive loci were identified (Table 2, Figure 1, Supplemental Figure 3). For nine of these 17 loci, we were able to find supportive biological information for an association with dyslipidemia. However, in both replication cohorts none of these variants replicated with statistical significance. In the meta-analysis with CCSS Original and SJLIFE, and with all three cohorts pooled, none of the variants were significant either.

Next, in the model without genetic factors (Supplemental Table 2), in order to determine the stratification variables, an independent positive association with dyslipidemia was found for administration of CRT (OR=1.57, 95%CI=1.26-1.94) and

abdominal radiotherapy (1.86, 1.53-2.25) and a negative association for asparaginase (0.68, 0.50-0.92). Prednisone (1.23, 0.98-1.55) was also included in the treatment-stratified analyses. By this treatment-stratified approach, nine additional genome-wide significant loci were identified (Table 3). In the abdominal radiotherapy exposed group, one locus on chromosome 6 showed a statistically significant association in SJLIFE but the effect was in the opposite direction so the meta-analysis did not reveal a significant association. In the CRT exposed group (Table 3, Figure 2, Supplemental table 3, Supplemental figure 3 and 4), one locus on chromosome 2, rs114017774, revealed a trend for a positive association with dyslipidemia in the SJLIFE cohort (OR=4.27, 95%CI=0.97-22.11, p=0.055) and was significant in the meta-analysis of the two cohorts (OR=11.30, 95%CI=5.03-25.40, $p=4.46x10^{-9}$). This locus was, however, not associated in the CCSS Expansion cohort and not significant in the meta-analysis of all three cohorts. The other seven loci were not replicated in either cohort and not significant in the meta-analysis.

Functional evidence

Additional analyses were performed for our top locus, rs114017774 on chromosome 2. We could not identify any expression quantitative trait locus between our top locus and genes in the region. Chromatin interactions have been observed with the genes Leucine Rich Repeat Transmembrane Neuronal 4 (*LRRTM4*), Catenin Alpha 2 (*CTNNA2*) and Regenerating Islet-derived 1 Alpha, 3 Alpha, 1 Beta and 3 Gamma (*REG1A, 3A, 1B* and *3G*) (Supplemental table 4). Based on protein expression in the brain, protein function including nervous system development and functioning, and associated phenotypes for genetic variants, including high-density-lipoprotein (HDL) and very-low-density-lipoprotein (VLDL) cholesterol levels, *LRRTM4* and *CTNNA2* are the most promising to be the causal genes. However, genetic variants studied were not in LD with our identified variant, and knockout models for these genes leading to a dyslipidemia phenotype are not available yet.

Further analyses of replicated SNP

When the interaction term of the top locus genotypexCRT was added to the model, it was significantly associated with dyslipidemia (OR=14.61, 95%CI=4.59-46.55, $p=5.75x10^{-6}$, Table 4). When CRT dose was added to the model, a dose-response effect was observed: as continuous variable the OR for the interaction term, per Gy increase, was 1.06 (95%CI=1.03-1.09, $p=1.88x10^{-5}$, Supplemental table 5). When categorized, only the interaction term with high dose radiotherapy was significant (OR=12.51, 95%CI=2.24-69.86, p=0.004). In dosimetry analysis of different brain regions, only radiation dose to the frontal region was continuously associated with dyslipidemia (OR per Gy 1.01, 95%CI=1.00-1.03, p=0.043). High dose to the hypothalamic/pituitary region was associated with dyslipidemia (OR=1.50, 95%CI=1.10-2.04,

p=0.0096), as was high dose to the other three regions. The association was identified in the CRT exposed group only (Supplemental table 6). Overweight and obesity, when added as covariates, were significantly associated with dyslipidemia, but the genotype-dyslipidemia association remained (Supplemental table 5). Also, when the model was built with overweight or obesity as outcomes, the genetic variant was not associated with these.

Comparison with general population

rs114017774 on chromosome 2 has not been reported as a susceptibility variant for dyslipidemia in prior general population GWASs. We were also unable to find this locus in the entire GWAS datasets that were made available by Teslovich et al.[22] and Liu et al.[23].

Candidate SNP analysis

The protective SNP rs676210 in the *APOB* gene identified by England et al.[24] was associated with a lower prevalence of dyslipidemia in our study as well, in both the overall cohort (p=0.0049) and the ALL subgroup (p=0.0061) (Table 5). The exact effect sizes are not comparable due to differences in included covariates that we did not have available including additional treatment and lifestyle variables. The two other variants did not replicate in the overall cohort nor the ALL subgroup.

DISCUSSION

We performed the first GWAS on dyslipidemia in three large survivor cohorts: the CCSS Original, SJLIFE and CCSS Expansion cohorts. In the analysis of the entire cohort, none of nine SNPs replicated. Next, in survivors exposed to cranial radiotherapy, we found one locus, rs114017774 on chromosome 2, which was genome-wide significant after pooling results of the CCSS and SJLIFE cohorts. Our analyses show that survivors carrying the genetic polymorphism and treated with CRT have a significantly higher risk of developing dyslipidemia, with a pooled OR of 11.30 (95%CI=5.03-25.40, $p=4.46x10^{-9}$). Sequential inclusion of the CCSS Expansion Cohort in meta-analysis was not significant, which may be due to younger age, lower dyslipidemia prevalence and cancer treatment differences. This genetic variant was not reported in large general population GWASs on dyslipidemia, potentially suggesting a survivor-specific effect. Survivors carrying this variant may be more susceptible to damage caused by CRT and therefore have an increased dyslipidemia risk.

Potential candidate genes for rs114017774 to cause dyslipidemia are *LRRTM4* and *CTNNA2. LRRTM4* is expressed in the hypothalamus and other brain parts

and involved in development and maintenance of the nervous system and synaptic functioning[52]. In the Framingham Heart Study, a variant in this gene was associated with high VLDL cholesterol levels, but this was not genome-wide significant and this variant is not in LD with our variant[53]. Knock-out mice were found to have decreased system synapse formation and abnormal excitatory postsynaptic potential, but development of dyslipidemia was not studied[54]. *CTNNA2* is also expressed in the hypothalamus and other brain parts, and involved in neuronal growth and migration[55]. A non-genome-wide significant association was found between a variant in *CTNNA2* and HDL cholesterol in HIV patients[56] and genome-wide significant associations were observed for coronary artery disease[57], body fat distribution[58] and BMI[59], but all these variants were not in LD with our variant. An *in vitro* model showed that CTNNA2 deficiency led to a cortical neuronal migration defect[55]. Mice with CTNNA2 knock-out have been observed to have impaired cerebellar and body growth, but dyslipidemia was not described[60]. Therefore, for both *LRRTM4* and *CTNNA2,* more evidence is required that the variant affects gene expression in relevant (i.e., brain) tissue, and that this leads to dyslipidemia. Mouse models with mutations in the *REG* genes, for which chromatin interactions with rs114017774 were also observed, showed interesting phenotypes: mice with a mutation in *REG3* showed increased levels of circulating total, LDL and HDL cholesterol[61] and were at increased risk of developing fatty liver[62], and mice with overexpression of *REG1A* showed delayed development of autoimmune diabetes[63]. However, these genes are mostly expressed in the pancreas, so a relationship with CRT is hard to argue.

We performed treatment-stratified GWASs because we hypothesized that the genetic effect may be different (present/absent) depending on exposure to certain treatments. Therefore, adding them as a covariate to a regression model would not capture this effect. Adding several interaction terms to a regression model would be another strategy, but this could lead to a complex and difficult to understand model. Also, *Rvtests* was unable to add interaction terms to the model. Therefore, we decided to first test whether potentially important variables (from literature) were associated with dyslipidemia in our study cohort to a certain significance ($p<0.10$) and as next step included these variables for stratified analyses.

The regression models that we additionally built suggest a significant interaction effect between genotype and CRT, with a stronger effect for higher dose. Previous studies observed that CRT was associated with dyslipidemia[6, 64, 65] and this is likely caused by disruption of the hypothalamic-pituitary axis leading to hormonal deficiencies, particularly growth and sex hormone[49, 66, 67]. CRT was also a risk factor for obesity in previous studies[12, 13, 68-70], which can in turn lead to dyslipidemia[4], but in our analysis the genetic effect on dyslipidemia was direct, not through overweight.

Conceivably, high dose radiotherapy (>25Gy), with the exception of medulloblastoma survivors, is most often local radiotherapy for a brain tumor, whereas low dose is likely radiotherapy to the whole cranium for leukemia treatment. We could take this difference into account in our dosimetry analysis of four brain regions. We observed that the frontal region, rather than hypothalamic pituitary region, appeared to be mostly associated with dyslipidemia. We did observe an association with higher dose radiation to the hypothalamic-pituitary region and dyslipidemia, as in fact for all four brain regions. An association between frontal lobe radiation damage and dyslipidemia has not been previously reported in literature.

Our results suggest that survivors carrying the variant rs114017774 are more vulnerable to CRT, supported by a high pooled odds ratio of 11.30 for this effect. It may be noted that the true risk can be lower, as genetic discovery analyses tend to overestimate the true effect size as a consequence of the "winner's curse"[71]. The fact that this variant is not reported in general population GWASs may suggest a survivor-specific effect, although its absence in the two available datasets may also suggest that it failed quality control. Replication in another cohort of cranially irradiated survivors is required to further argue its relevance. Preferably, as our cohorts only had survivors with dyslipidemia that were heterozygous for the variant, this additional cohort would contain homozygous survivors as well. If this locus turns out to be relevant, assessment of a survivor's genotype for this variant, in addition to clinical risk factors, may facilitate personalized risk stratification.

The protective variant rs676210 in the *APOB* gene, which encodes apolipoprotein B, that we replicated from the ALL survivors study, is also known in the general population[72]. We could not replicate the variants in *BAD* and *OGFOD3*. This is the first time that a genetic variant related to dyslipidemia, in addition to ALL survivors, was replicated in an independent cohort of survivors of heterogeneous malignancies. In addition to treatment-related dyslipidemia, accelerated aging phenotypes are observed in survivors[73], suggesting that general population SNPs may also be relevant, and potentially earlier in life.

Three aspects of our study merit consideration. First, for suggestive loci to be included for replication analysis, supportive information for relatedness to dyslipidemia was required. This requirement lowers the risk of false-positive findings, but it excludes the possibility of identifying new pathways involved in the phenotype. In a next replication step, it may therefore still be interesting to also replicate the other seven SNPs identified in the initial discovery analysis. Second, the CCSS was a questionnaire-based study. In larger genetic studies it has been shown that self-reported phenotypes, including high cholesterol, perform better than one may expect[74, 75], but in smaller samples as

ours self-report might lead to bias as survivors may be unaware of having dyslipidemia or be using lipid lowering medication as secondary prevention. At this age the first is more likely, leading to a bias towards the null hypothesis, hence, there may be other variants that we did not find due to the self-reported nature of the data. Third, we do not know whether non-replication in the CCSS Expansion cohort resulted from a power issue due to low dyslipidemia prevalence in CRT exposed survivors, or valid non-replication.

In conclusion, in our GWAS we identified variant rs114017774 on chromosome 2 as a potential genetic predisposing factor for dyslipidemia specifically in cranially irradiated survivors. We also, for the first time, replicated variant rs676210 in *APOB* in a heterogeneous survivor cohort, indicating that this general population variant is relevant in survivors as well.

Acknowledgments

We thank all survivors that participated in the CCSS, SJLIFE and CCSS Expansion cohort studies.

Conflicts of interest

The authors have no conflicts of interest to declare.

TABLES AND FIGURES

Table 1. Demographic and treatment characteristics of the discovery and replication cohorts

Characteristics	CCSS Original				SJLIFE				
	Entire cohort	Cases	Controls	Cases v controls	Entire cohort	Cases	Controls	Cases v controls	CCSS Original vs SJLIFE
N	4,332	759 (17.5%)	3,573 (82.5%)	**p<0.001**	2,274	448 (19.7%)	1826 (80.3%)	**p<0.001**	**p=0.03°**
Male sex	2,081 (48.0%)	444 (58.5%)	1,637 (45.8%)	**p<0.001**	1,194 (52.5%)	278 (62.1%)	916 (50.2%)	**p<0.001**	**p<0.001**
Age at dyslipidemia diagnosis*	37.3 (8.8)	37.3 (8.8)	n.a.	n.a.	37.2 (10.5)	37.2 (10.5)	n.a.	n.a.	p=0.70
Age at follow-up*	43.3 (9.1)	48.7 (7.4)	42.2 (9.0)	**p<0.001**	36.2 (9.8)	42.2 (9.4)	34.8 (9.3)	**p<0.001**	**p<0.001**
Follow-up time*	35.3 (6.8)	38.1 (5.6)	34.7 (6.8)	**p<0.001**	28.1 (9.3)	32.8 (8.8)	27.0 (9.1)	**p<0.001**	**p<0.001**
BMI at follow-up*	27.2 (6.8)	28.5 (6.0)	26.9 (6.9)	**p<0.001**	29.4 (7.7)	32.0 (7.1)	28.8 (7.7)	**p<0.001**	**p<0.001**
Overweight or obesity	2,521 (58.3%)	559 (73.6%)	1,962 (55.1%)	**p<0.001**	1,573 (69.2%)	383 (85.5%)	1,190 (65.2%)	**p<0.001**	**p<0.001**
Obesity	1,109 (25.7%)	251 (33.1%)	858 (24.1%)	**p<0.001**	914 (40.2%)	261 (58.3%)	653 (35.8%)	**p<0.001**	**p<0.001**
Comorbidities (CTCAE grade)									
GHD gr3	0	0	0	n.a.	2	0	2	n.a.	n.a.
GHD gr2	196 (4.5%)	50 (6.6%)	146 (4.1%)	**p=0.003**	190 (8.4%)	47 (10.5%)	143 (7.8%)	p=0.06	**p<0.001**
GHD gr1	119 (2.7%)	27 (3.6%)	92 (2.6%)	p=0.13	288 (12.7%)	88 (19.6%)	200 (11.0%)	**p<0.001**	**p<0.001**
Hypogon gr3	232 (5.4%)	58 (7.6%)	174 (4.9%)	**p=0.002**	134 (5.9%)	45 (10.0%)	89 (4.9%)	**p<0.001**	p=0.36
Hypogon gr2	1	0	1	n.a	224 (9.9%)	103 (23.0%)	121 (6.6%)	**p<0.001**	**p<0.001**
Hypogon gr1	12 (0.3%)	2 (0.3%)	10 (0.3%)	p=0.94	250 (11.0%)	57 (12.7%)	193 (10.6%)	p=0.19	**p<0.001**
Diabetes gr3	119 (2.7%)	56 (7.4%)	63 (1.8%)	**p<0.001**	89 (3.9%)	34 (7.6%)	55 (3.0%)	**p<0.001**	**p=0.01**
Diabetes gr2	125 (2.9%)	83 (10.9%)	42 (1.2%)	**p<0.001**	104 (4.6%)	49 (10.9%)	55 (3.0%)	**p<0.001**	**p<0.001**
Diabetes gr1	55 (1.3%)	19 (2.5%)	36 (1.0%)	**p=0.0008**	464 (20.4%)	130 (29.0%)	334 (18.3%)	**p<0.001**	**p<0.001**
Hypothyr gr3	0	0	0	n.a.	3	1	2	p=0.48	n.a.
Hypothyr gr2	678 (15.7%)	209 (27.5%)	469 (13.1%)	**p<0.001**	513 (22.6%)	151 (33.7%)	362 (19.8%)	**p<0.001**	**p<0.001**
Hypothyr gr1	100 (2.3%)	20 (2.6%)	80 (2.2%)	p=0.51	16 (0.7%)	2	14 (0.8%)	p=0.75	**p<0.001**
Cardiomyo gr5	3	0	3 (0.1%)	n.a.	0	0	0	n.a.	n.a.
Cardiomyo gr4	2	1	1	n.a.	2	2	0	n.a.	p=0.61
Cardiomyo gr3	6 (0.1%)	1	5 (0.1%)	p=0.94	87 (3.8%)	39 (8.7%)	48 (2.6%)	**p<0.001**	**p<0.001**
Cardiomyo gr2	0	0	0	n.a.	225 (9.9%)	61 (13.6%)	164 (9.0%)	**p=0.003**	n.a.
Cardiomyo gr1	17 (0.4%)	7 (0.9%)	10 (0.3%)	**p=0.01**	0	0	0	n.a.	n.a.

Table 1. Continued

Characteristics	CCSS Original				SJLIFE				
	Entire cohort	Cases	Controls	Cases v controls	Entire cohort	Cases	Controls	Cases v controls	CCSS Original vs SJLIFE
Smoking	881 (21.0%)	164 (21.8%)	717 (20.9%)	p=0.34	826 (36.3%)	174 (38.8%)	652 (35.7%)	p=0.22	**p<0.001**
Cancer diagnosis									
ALL	1165 (26.9%)	148 (19.5%)	1017 (28.5%)	**p<0.001**	792 (34.8%)	162 (36.2%)	630 (34.5%)	p=0.51	**p<0.001**
AML	83 (1.9%)	11 (1.4%)	72 (2.0%)	p=0.30	45 (2.0%)	7 (1.6%)	38 (2.1%)	p=0.48	p=0.86
Astrocytoma	386 (8.9%)	49 (6.5%)	337 (9.4%)	**p=0.009**	0	0	0	n.a.	n.a.
Ewing sarcoma	124 (2.9%)	17 (2.2%)	107 (3.0%)	p=0.26	81 (3.6%)	13 (2.9%)	68 (3.7%)	p=0.40	p=0.12
Hodgkin	575 (13.3%)	189 (24.9%)	386 (10.8%)	**p<0.001**	285 (12.5%)	79 (17.6%)	206 (11.3%)	**p<0.001**	p=0.40
Kidney tumor	426 (9.8%)	51 (6.7%)	375 (10.5%)	**p=0.002**	150 (6.6%)	24 (5.4%)	126 (6.9%)	p=0.24	**p<0.001**
Medullo/PNET	134 (3.1%)	41 (5.4%)	93 (2.6%)	**p<0.001**	0	0	0	n.a.	n.a.
Neuroblast	323 (7.5%)	29 (3.8%)	294 (8.2%)	**p<0.001**	108 (4.7%)	10 (2.2%)	98 (5.4%)	**p=0.005**	**p<0.001**
Non-Hodgkin	346 (8.0%)	78 (10.3%)	268 (7.5%)	**p=0.01**	171 (7.5%)	34 (7.6%)	137 (7.5%)	p=0.95	p=0.50
Osteosarc	237 (5.5%)	54 (7.1%)	183 (5.1%)	**p=0.03**	80 (3.5%)	27 (6.0%)	53 (2.9%)	**p=0.001**	**p<0.001**
Other bone	15 (0.3%)	5 (0.7%)	10 (0.3%)	p=0.11	0	0	0	n.a.	n.a.
Other CNS	91 (2.1%)	19 (2.5%)	72 (2.0%)	p=0.39	227 (10.0%)	46 (10.3%)	181 (9.9%)	p=0.82	**p<0.001**
Other leukem	15 (3.5%)	5 (0.7%)	10 (0.3%)	p=0.11	4 (0.2%)	1 (0.2%)	3 (0.2%)	p=0.58	p=0.33
Soft tissue sarc	412 (9.5%)	63 (8.3%)	349 (9.8%)	p=0.21	61 (2.7%)	6 (1.3%)	55 (3.0%)	**p=0.050**	**p<0.001**
Rhabdomyosarc	0	0	0	n.a.	74 (3.3%)	14 (3.1%)	60 (3.3%)	p=0.86	n.a.
Germ cell	0	0	0	n.a.	45 (2.0%)	3 (0.7%)	42 (2.3%)	**p=0.02**	n.a.
Histiocytosis	0	0	0	n.a.	17 (0.7%)	1 (0.2%)	16 (0.9%)	p=0.22	n.a.
Liver	0	0	0	n.a.	16 (0.7%)	2 (0.4%)	14 (0.8%)	p=0.75	n.a.
Melanoma	0	0	0	n.a.	14 (0.6%)	3 (0.7%)	11 (0.6%)	p=0.75	n.a.
Retinoblastoma	0	0	0	n.a.	62 (2.7%)	9 (2.0%)	53 (2.9%)	p=0.30	n.a.
Other	0	0	0	n.a.	42 (1.8%)	7 (1.6%)	35 (1.9%)	p=0.62	n.a.

Table 1. Continued

Characteristics	CCSS Original				SJLIFE				
	Entire cohort	Cases	Controls	Cases v controls	Entire cohort	Cases	Controls	Cases v controls	CCSS Original vs SJLIFE
Radiotherapy									
Cranial	1242 (30.5%)	236 (33.0%)	1006 (30.0%)	p=0.10	669 (29.4%)	193 (43.1%)	476 (26.1%)	**p<0.001**	p=0.52
Dose (cGy)**	200 (20-2400)	20 (20-2400)	200 (20-2400)	p=0.066	1800 (20-2400)	1800 (20-2400)	1250 (20-2400)	***	***
Region 1	1050 (25.8%)	270 (37.7%)	780 (23.3%)	**p<0.001**		135 (30.1%)	283 (15.5%)	**p<0.01**	**p<0.001**
Region 2	200 (20-2400)	200 (20-3100)	200 (20-2100)	**p<0.001**	n.a.	200 (20-2100)	20 (20-1500)	***	***
Region 3	15 (0.4%)	6 (0.8%)	9 (0.3)	**p=0.02**	n.a.	0	0	n.a.	n.a.
Region 4					n.a.				
Abdominal					n.a.				
Dose (cGy)**					418 (18.4%)				
Total body					20 (20-1800)				
					0				
Chemotherapy									
Anthrac (DED)	1444 (34.9%)	239 (32.7%)	1205 (35.4%)	p=0.24	1320 (58.0%)	240 (53.6%)	1080 (59.1%)	**p=0.03**	**p<0.001**
Dose (mg/m^2)**	258 (153-370)	277 (170-397)	253 (152-363)	**p=0.037**	154 (52-241)	174 (75-306)	152 (52-213)	***	***
Alkyl (CED)	1959 (47.4%)	378 (51.9%)	1581 (46.5%)	**p=0.005**	1302 (57.3%)	288 (64.3%)	1014 (44.5%)	**p<0.001**	**p<0.001**
Dose (g/m^2)**	8.7 (4.4-13.9)	10.5 (5.9-15.0)	8.2 (4.0-13.6)	**p<0.001**	8.6 (5.4-12.5)	8.6 (5.6-14.1)	8.5 (5.3-12.2)	***	***
Asparagin	1016 (25.6%)	129 (18.5%)	887 (27.1%)	**p<0.001**	774 (34.0%)	135 (30.4%)	639 (35.0%)	p=0.052	**p<0.001**
Steroids									
Predniso(lo)ne, m-predn	1547 (39.3%)	270 (39.1%)	1277 (39.4%)	P=0.93	1129 (49.6%)	231 (51.6%)	898 (49.2%)	p=0.37	**p<0.001**
Dexameth	278 (6.8%)	45 (6.2%)	233 (6.9%)	P=0.55	182 (8.0%)	6 (1.3%)	176 (9.6%)	**p<0.001**	**p=0.02**

Table 1. Continued

Characteristics	CCSS Expansion				
	Entire cohort	Cases	Controls	Cases v controls	CCSS Original v CCSS Expansion
N	2,212	119 (5.4%)	2093 (94.6%)	**p<0.001**	**p<0.001°**
Male sex	1051 (47.5%)	79 (66.4%)	972 (46.4%)	**p<0.001**	p=0.69
Age at dyslipidemia diagnosis*	27.5 (7.1)	27.5 (7.1)	n.a.	n.a.	**p<0.001**
Age at follow-up*	30.0 (6.3)	34.0 (6.4)	29.8 (6.3)	**p<0.001**	**p<0.001**
Follow-up time	21.3 (3.9)	22.5 (3.8)	21.3 (3.9)	**p=0.001**	**p<0.001**
BMI at follow-up*	26.4 (6.0)	28.8 (6.4)	26.3 (6.0)	**p<0.001**	**p<0.001**
Overweight or obesity	1144 (51.7%)	84 (70.6%)	1060 (50.6%)	**p<0.001**	**p<0.001**
Obesity	496 (22.4%)	41 (34.5%)	455 (21.7%)	**p=0.0012**	**p=0.005**
Comorbidities (CTCAE grade)					
GHD gr3	0	0	0	n.a.	n.a.
GHD gr2	159 (7.2%)	22 (18.5%)	137 (6.5%)	**p<0.001**	**p<0.001**
GHD gr1	47 (2.1%)	7 (5.9%)	40 (1.9%)	**p=0.003**	p=0.13
Hypogon gr3	61 (2.8%)	9 (7.6%)	52 (2.5%)	**p<0.001**	**p<0.001**
Hypogon gr2	0	0	0	n.a.	n.a.
Hypogon gr1	5 (0.2%)	1 (0.8%)	4 (0.2%)	p=0.24	p=0.70
Diabetes gr3	14 (0.6%)	2 (1.7%)	12 (0.6%)	p=0.17	**p<0.001**
Diabetes gr2	16 (0.7%)	5 (4.2%)	11 (0.5%)	**p<0.001**	**p<0.001**
Diabetes gr1	38 (1.7%)	9 (7.6%)	29 (1.4%)	**p<0.001**	p=0.15
Hypothyr gr3	0	0	0	n.a.	n.a.
Hypothyr gr2	318 (14.4%)	50 (42.0%)	268 (12.8%)	**p<0.001**	p=0.17
Hypothyr gr1	52 (2.4%)	5 (4.2%)	47 (2.2%)	p=0.17	p=0.92
Cardiomyo gr5	0	0	0	n.a.	n.a.
Cardiomyo gr4	0	0	0	n.a.	n.a.
Cardiomyo gr3	0	0	0	n.a.	n.a.
Cardiomyo gr2	66 (3.0%)	12 (10.1%)	54 (2.6%)	**p<0.001**	n.a.
Cardiomyo gr1	0	0	0	n.a.	n.a.

Table 1. Continued

Characteristics	CCSS Expansion				
	Entire cohort	Cases	Controls	Cases v controls	CCSS Original v CCSS Expansion
Smoking	674 (30.5%)	42 (35.3%)	632 (30.2%)	p=0.24	**p<0.001**
Cancer diagnosis					
ALL	400 (18.1%)	14 (11.8%)	386 (18.4%)	p=0.066	**p<0.001**
AML	49 (2.2%)	1 (0.8%)	48 (2.3%)	p=0.52	p=0.42
Astrocytoma	352 (15.9%)	13 (10.9%)	339 (16.2%)	p=0.13	**p<0.001**
Ewing sarcoma	74 (3.3%)	3 (2.5%)	71 (3.4%)	p=0.80	p=0.28
Hodgkin	274 (12.4%)	31 (26.1%)	243 (11.6%)	**p<0.001**	p=0.31
Kidney tumor	217 (9.8%)	6 (5.0%)	211 (10.1%)	p=0.072	p=0.98
Medullo/PNET	140 (6.3%)	19 (16.0%)	121 (5.8%)	**p<0.001**	**p<0.001**
Neuroblast	160 (7.2%)	2 (1.7%)	158 (7.5%)	**p=0.01**	p=0.74
Non-Hodgkin	208 (9.4%)	7 (5.9%)	201 (9.6%)	p=0.18	p=0.052
Osteosarc	101 (4.6%)	10 (8.4%)	91 (4.3%)	**p=0.04**	p=0.12
Other bone	17 (0.8%)	0	17 (0.8%)	n.a.	**p=0.02**
Other CNS	92 (4.2%)	4 (3.4%)	88 (4.2%)	p=0.82	**p<0.001**
Other leukem	17 (0.8%)	0	17 (0.8%)	n.a.	**p=0.02**
Soft tissue sarc	111 (5.0%)	9 (7.6%)	102 (4.9%)	p=0.19	**p<0.001**
Rhabdomyosarc	0	0	0	n.a.	n.a.
Germ cell	0	0	0	n.a.	n.a.
Histiocytosis	0	0	0	n.a.	n.a.
Liver	0	0	0	n.a.	n.a.
Melanoma	0	0	0	n.a.	n.a.
Retinoblastoma	0	0	0	n.a.	n.a.
Other	0	0	0	n.a.	n.a.

Table 1. Continued

Characteristics	CCSS Expansion				
	Entire cohort	Cases	Controls	Cases v controls	CCSS Original v CCSS Expansion
Radiotherapy					
Cranial	408 (18.4%)	41 (34.5%)	367 (17.5%)	**p<0.001**	**p<0.001**
dose**	1150 (20-5300)	1800 (20-5400)	830 (20-5200)	***	***
Region 1	n.a.	32 (26.9%)	306 (14.6%)	**p<0.001**	**p<0.001**
Region 2	n.a.	200 (20-2900)	200 (20-2100)	***	***
Region 3	n.a.	0	0	n.a.	**P=0.006**
Region 4	n.a.				
Abdominal	338 (15.3%)				
dose**	200 (20-2100)				
Total body	0				
Chemotherapy					
Anthrac (DED)	1158 (52.4%)	62 (52.1%)	1096 (52.4%)	p=0.96	**p<0.001**
dose**	165 (120-280)	200 (135-303)	163 (119-273)	***	***
Alkyl (CED)	1140 (51.5%)	72 (60.5%)	1068 (51.0%)	**p=0.04**	**p<0.001**
dose**	6.9 (3.8-11.5)	8.4 (4.9-11.9)	6.9 (3.6-11.5)	***	***
Asparagin	433 (19.6%)	17 (14.3%)	71 (3.4%)	**p<0.001**	**p<0.001**
Steroids					
Prednisone and/or dexam	875 (39.6%)	47 (39.5%)	828 (39.6%)	p=0.99	n.a.°°

* mean (SD) ** median (IQR). Chi2 test for categorical variables, Fisher exact test for categorical variables with less than five observations in one or more cells, two sample t-test for normally distributed continuous variables, Mann-Whitney U test for non-normally distributed continuous variables. *** Not possible to compare Mann-Whitney U test from summary statistics without raw data. ° Comparison of dyslipidemia prevalence between cohorts. °° in CCSS Expansion prednisone and dexamethasone are registered as one variable.
Due to differences in childhood cancer classification some tumors have no occurrence. Smoking is defined as ever smoking, including current and former. Radiotherapy dose includes registration of 20cGy for low stray exposure and 200cGy for high stray exposure.

Table 2. Genome-wide significant and suggestive hits in discovery analysis, results from replication analyses and meta-analysis, and functional annotation of loci

					Discovery CCSS Original						Replication SJLIFE					Replication CCSS Expansion					Meta-analysis three cohorts		
SNP	**Interesting gene(s)**	**Biological function(s)**	**RA**	**EA**	**N**	**EAF**	**Imp.**	**OR**	**95%CI**	**P**	**N**	**EAF**	**OR**	**95%CI**	**P**	**N**	**EAF**	**OR**	**95%CI**	**P**	**OR**	**95%CI**	**P**
1:97869183	LPPR5 (c.i.)	Free fatty acid synthesis	T	C	4,323	0.016	1	3.00	1.88-4.76	3.60E-6	2,274	0.013	1.26	0.64-2.36	0.49	2,210	0.014	2.60	0.90-7.54	0.079	2.26†	1.58-3.24	7.63E-6
2:49370715	-	-	G	A	4,323	0.014	0.922	3.55	2.13-5.90	1.10E-6	-	-	-	-	-	-	-	-	-	-	-	-	-
2:222263469*	SGPP2 (c.i.)	Lipid metabolism	A	G	4,323	0.020	1	3.62	2.40-5.48	1.02E-9	2,274	0.015	1.34	0.72-2.37	0.34	2,212	0.020	1.79	0.83-3.88	0.14	2.13‡	1.08-4.18	0.028
6:32472999	-	-	I	D	4,323	0.011	0.669	5.69	2.89-11.20	4.86E-7	-	-	-	-	-	-	-	-	-	-	-	-	-
6:52486401	-	-	A	G	4,323	0.157	0.999	0.69	0.59-0.81	4.94E-6	-	-	-	-	-	-	-	-	-	-	-	-	-
7:5187718	-	-	A	G	4,323	0.955	0.937	0.50	0.38-0.68	4.67E-6	-	-	-	-	-	-	-	-	-	-	-	-	-
8:29262306	LEPROTL1 (c.i.) MBOAT4 (c.i.)	Growth hormone signaling in liver Ghrelin and leptin function	G	C	4,323	0.204	0.948	0.71	0.61-0.82	3.33E-6	2,203	0.199	1.00	0.82-1.21	1.00	2,209	0.211	0.87	0.59-1.28	0.48	0.84‡	0.65-1.09	0.19
10:3302154	IDI1 (c.i.)	Cholesterol synthesis	C	T	4,323	0.633	0.987	0.75	0.67-0.85	4.43E-6	2,271	0.638	1.09	0.93-1.28	0.29	2,212	0.644	0.84	0.62-1.15	0.28	0.89‡	0.68-1.16	0.39
10:60175440	TFAM (n.g.) CISD1 (eQTL) SLC16A9 (c.i.)	Mitochondrial transcription regulation Adipogenesis and energy metabolism Lipid transport	C	T	4,323	0.110	1	1.55	1.29-1.87	3.13E-6	2,272	0.098	1.17	0.91-1.50	0.22	2,212	0.102	1.01	0.61-1.66	0.97	1.38†	1.20-1.59	5.69E-6
10:74473228	-	-	G	A	4,323	0.016	0.981	3.13	1.92-5.09	4.46E-6	-	-	-	-	-	-	-	-	-	-	-	-	-
15:70769440	UACA (n.g.) THSD4 (c.i.)	Apoptosis in pancreas and adipose tissue Free fatty acid synthesis	G	C	4,323	0.011	0.618	5.86	2.79-12.32	3.16E-6	2,273	0.013	0.87	0.43-1.63	0.67	2,211	0.012	0.63	0.17-2.38	0.50	1.55‡	0.37-6.52	0.55
16:82711821	CDH13 (n.g.) S1P/MBTPS1 (c.i.) HSD17B2 (c.i.) SDR42E1 (c.i.)	Adiponectin receptor Lipid metabolism Androgen and estrogen metabolism Steroid hormone and lipid metabolism	G	A	4,323	0.093	1	0.60	0.49-0.73	6.25E-7	2,273	0.095	0.91	0.69-1.17	0.46	2,212	0.101	1.31	0.89-1.93	0.17	0.87‡	0.56-1.33	0.52
17:34109023	CCLs (c.i.)	Pro-inflammatory pathway	A	T	4,323	0.020	0.883	2.93	1.90-4.54	1.32E-6	2,199	0.018	0.90	0.47-1.60	0.73	2,205	0.018	0.53	0.16-1.74	0.29	1.22‡	0.44-3.45	0.70
19:53876108	NLRP12 (c.i.)	Pro-inflammatory pathway	A	G	4,323	0.711	1	1.38	1.21-1.57	8.60E-7	2,271	0.708	0.94	0.80-1.12	0.51	2,212	0.718	1.09	0.74-1.61	0.66	1.13‡	0.85-1.51	0.39
19:54163026	-	-	C	T	4,323	0.031	0.613	2.92	1.88-4.53	1.78E-6	-	-	-	-	-	-	-	-	-	-	-	-	-
20:52550194	-	-	A	G	4,323	0.036	0.994	2.09	1.53-2.85	3.40E-6	-	-	-	-	-	-	-	-	-	-	-	-	-
22:16924826	-	-	C	T	4,323	0.017	0.858	3.25	1.99-5.29	2.20E-6	-	-	-	-	-	-	-	-	-	-	-	-	-

SNP position is according to 1000Genomes build 37.

*Hit that reached significant threshold; RA = reference allele; EA = effective allele; EAF = effective allele frequency; imp. = imputation quality; n.g. = nearest gene; eQTL = expression quantitative trait locus; c.i. = chromatin interaction; † fixed effects model; ‡ random effects model

Logistic regression analysis adjusted for first four genetic ancestry principal components, age at last follow-up (continuous) and sex.

Table 3. Treatment-stratified GWASs

				Discovery CCSS Original						Replication SJLIFE					Replication CCSS Expansion					Meta-analysis CCSS Original + SJLIFE		
Subgroup	**SNP**	**RA**	**EA**	**N**	**EAF**	**Imp.**	**OR**	**95%CI**	**P**	**N**	**EAF**	**OR**	**95%CI**	**P**	**N**	**EAF**	**OR**	**95%CI**	**P**	**OR**	**95%CI**	**P**
Abd RT +	6:15911191	G	A	1,044	0.068	0.903	3.82	2.47-5.91	1.65E-9	418	0.084	0.53	0.27-0.96	**0.036**	338	0.089	1.02	0.42-2.45	0.97	1.44	0.21-10.00	0.71
CRT +	**2:76547279**	**C**	**T**	**1,239**	**0.012**	**1**	**16.80**	**6.36-44.22**	**1.18E-8**	**669**	**0.006**	4.27	0.97-22.11	0.055	407	0.014	0.98	0.13-7.55	0.99	**11.30**	**5.03-25.40**	**4.46E-9**
CRT -	8:49202507	D	I	3,084	0.707	0.946	0.62	0.53-0.73	6.39E-9	n.a.*	-	-	-	-	n.a.*	-	-	-	-	-	-	-
CRT -	16:54120330	T	G	3,084	0.325	1	1.53	1.131-1.77	3.30E-8	1586	0.329	0.97	0.78-1.20	0.77	1,687	0.33	1.08	0.73-1.58	0.71	1.22	0.79-1.90	0.37
Asp +	5:73438634	G	A	1,016	0.011	1	50.30	12.30-205.26	4.88E-8	774	0.009	1.51	0.32-5.40	0.57	433	0.007	2.81	0.13-61.94	0.56	8.68	0.28-271.00	0.22
Asp +	8:138526638	A	G	1,016	0.872	1	0.30	0.20-0.46	3.71E-8	773	0.878	1.24	0.82-1.93	0.32	433	0.857	1.29	0.37-4.45	0.68	0.61	0.16-2.42	0.48
Asp +	22:49419475	G	A	1,016	0.017	0.94	24.9	8.30-74.55	9.42E-9	774	0.01	0.45	0.02-2.40	0.40	433	0.009	n.a.**	-	-	3.69	0.07-187.00	0.51
Pred +	2:166087768	C	T	1,545	0.019	0.877	8.55	4.03-18.15	2.30E-8	1,122	0.014	0.81	0.28-2.01	0.66	875	0.016	0.55	0.05-5.93	0.59	2.70	0.27-27.20	0.40
Pred -	17:77873943	C***	T	2,778	0.04	0.903	3.17	2.11-4.76	2.66E-8	1,151	0.053	0.76	0.43-1.26	0.30	1,257	0.048	0.69	0.27-1.79	0.43	3.16	2.09-4.77	4.35E-8

SNP position is according to 1000Genomes build 37.

RA = reference allele; EA = effective allele; EAF = effective allele frequency; imp. = imputation quality

* Failed quality control; ** No dyslipidemia cases with genetic variant; *** The reference allele C vs effect allele T in CCSS Original corresponded to an INDEL in SJLIFE and CCSS Expansion at this position with reference allele TTAAC vs effect allele T. The C vs T SNP also passed quality control but had a much lower EAF compared to the CCSS Original data.

Logistic regression analysis adjusted for first four genetic ancestry principal components, age at last follow-up (continuous) and sex.

Table 4. Interaction analysis between genotype and cranial radiotherapy

Results of logistic regression analysis with outcome dyslipidemia and as predictors genotype, CRT, interaction between genotype and CRT, age, sex and genetic ancestry

Variable	OR	95% CI	P-value
Genotype dosage	0.59	0.26 - 1.36	0.22
CRT yes	1.31	1.08 - 1.58	**0.005**
Genotype*CRT	14.61	4.59 - 46.55	**$5.75*10^{-6}$**
Age (per year)	1.1	1.09 - 1.11	**<0.001**
Female sex	0.54	0.45 - 0.64	**<0.001**
PC1	27.45	0.15 - 5051.4	0.21
PC2	5.6	0.02 - 1561.6	0.55
PC3	0.37	0 - 105.5	0.73
PC4	0.01	0 - 3.28	0.12

Table 5. Replication analysis of SNPs identified in candidate SNP study by England et al.

					ALL study England et al					CCSS Entire cohort					CCSS ALL subgroup				
rsID	**chr: pos37**	**REF**	**ALT**	**Gene**	**N**	**EAF**	**OR**	**95%CI**	**P**	**N**	**EAF**	**OR**	**95%CI**	**P**	**N**	**EAF**	**OR**	**95%CI**	**P**
rs676210	2:21231524	G	A	APOB	209	0.23	0.43	0.22-0.88	**0.020**	4,323	0.223	0.82	0.71-0.94	**0.0049**	1,165	0.212	0.64	0.46 – 0.88	**0.0061**
rs2286615	11:64039175	G	A	BAD	209	0.10	4.02	1.44-11.23	**0.008**	4,323	0.163	1.08	0.92-1.26	0.37	1,165	0.164	1.29	0.91 – 1.83	0.16
rs62079523	17:80352303	G	A	OGFOD3	209	0.33	2.71	1.35-5.44	**0.005**	4,323	0.355	0.97	0.86-1.10	0.65	1,165	0.369	1.01	0.77 – 1.31	0.96

England et al used a different regression model including more treatment and lifestyle covariables. So only direction of effect and significance are comparable.

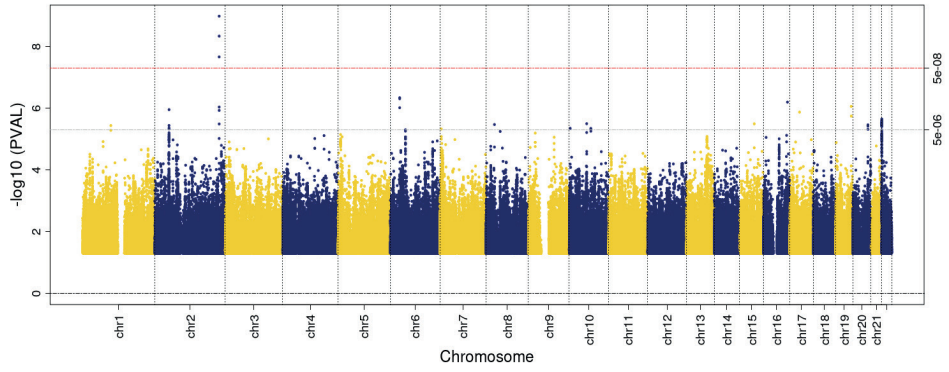

Figure 1. Manhattan plot of the initial discovery analysis

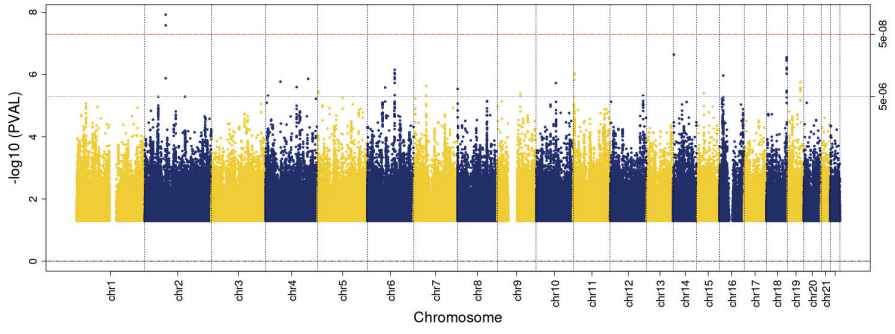

Figure 2. Manhattan plot of the CRT+ discovery analysis

SUPPLEMENTAL DATA

Supplemental table 1. Definition of dyslipidemia in study cohorts

CCSS & CCSS Expansion	SJLIFE
CTCAE high total cholesterol or hypertriglyceridemia grade 2: self-reported high cholesterol or triglycerides and on medication	CTCAE high total cholesterol or hypertriglyceridemia grade 2: total cholesterol >300 mg/dL or triglycerides >300 mg/dL or treatment with >=1 lipid lowering agent

Supplemental table 2. Clinical model to determine treatment factors for stratified GWASs

Predictor	OR	95% CI	P-value
Male sex	1.90	1.59-2.28	**<0.001**
Age (per year)	1.09	1.09-1.11	**<0.001**
Cranial radiotherapy	1.57	1.26-1.94	**<0.001**
Abdominal radiotherapy	1.86	1.53-2.25	**<0.001**
Asparaginase	0.68	0.50-0.92	**0.012**
Prednisone	1.23	0.98-1.55	0.069
Dexamethasone	1.12	0.78-1.62	0.54

Supplemental table 3. Baseline characteristics of survivors carrying the alternative allele of the significant locus in CRT exposed analysis.

n	29
Heterozygous	29 (100%)
Dyslipidemia	17 (58.6%)
Female	15 (51.7%)
Age at dyslipidemia dx (mean (SD))	34.0 (9.8)
Age (mean (SD))	41.8 (7.0)
BMI at follow-up (mean (SD))	31.0 (7.2)
Overweight at follow-up = yes (%)	24 (82.8%)
Obese at follow-up = yes (%)	12 (41.4%)
Diagnosis (%)	
ALL	11 (37.9%)
AML	12 (41.4%)
Astrocytoma	3 (10.3%)
Hodgkin	2 (6.9%)
Non-Hodgkin	1 (3.4%)
CRT dose (median [IQR]) (Gy)	34 [18-52]
Abd RT	8 (27.6%)
TBI	1 (3.4%)
Anthracyclines	9 (31.0%)
Alkylating agents	16 (55.2%)
Asparaginase	9 (31.0%)
Prednisone	15 (51.7%)
Dexamethasone	2 (6.9%)
Growth hormone deficiency (%)	
No	17 (58.6%)
Grade 1	6 (20.7%)
Grade 2	6 (20.7%)
Hypogonadism (%)	

Supplemental table 3. Continued

No	26 (89.7%)
Grade 3	3 (10.3%)
Diabetes (%)	
No	24 (82.8%)
Grade 1	2 (6.9%)
Grade 2	2 (6.9%)
Grade 3	1 (3.4%)
Hypothyroidism (%)	
No	18 (62.1%)
Grade 1	1 (3.4%)
Grade 2	10 (34.5%)
Cardiomyopathy (%)	
No	29 (100%)
Smoking (%)	4 (13.8%)

Supplemental table 4. Biological information of significant locus

SNP (rs / CHR:POS_ REF/ALT)	R^2	SNP in study	SNP in cell line	Chromatin state (ROADMAP)	eQTL	Chromatin interaction (dist)	Nearest protein coding gene (dist)	Protein function	Protein expression in tissue (GTeX)	Gene polymorphisms in study on lipids	Gene polymorphisms in study on other outcome	Gene knockout in mouse	Gene knockout in vitro
rs114017774, 2:76547279_C/T (lead SNP); rs150423417/ 2:76566559_C/T (R^2=1); rs144942742/ 2:76612183_T/C (R^2=0.86)	n.a.	No		9/15 in primary NK cell, foreskin fibroblast, epidermal keratinocyte, 14/15 in liver, right atrium, foreskin melanocyte 15/15 in all other tissues	No								
						CTNNA2 (3Mbp)		Linker between cadherin adhesion receptors and cytoskeleton, regulation of cell-cell adhesion and differentiation in nervous system; neuronal growth and migration[1]	Brain, incl hypothalamus	HDL in HIV patients ($p=5*10^{-6}$)[2] (R^2=0.012)	Coronary artery disease ($p=2*10^{-14}$)[3] (R^2=0.005), body fat distribution ($p=7*10^{-12}$)[4] (R^2=0.001), body mass index ($p=5*10^{-10}$)[5] (R^2=0.001)	Impaired cerebellar and body growth (50% less weight than littermate controls)[6]	Cortical neuronal migration defect[1]
						LRRTM4 (430kbp)	LRRTM4 (430kbp)	Development and maintenance of nervous sytem, protein-protein interactions at and transmission across synpases	Brain, incl hypothalamus[7]	VLDL ($p=2*10^{-5}$)[8] (R^2=0.001)	Insulin resistance ($p=5*10^{-9}$)[9] (R^2=0.0) Chronotype (circadian rhythm)[10] (R^2=0.001)	Decreased central nervous system synpase formation, abnormal excitatory postsynaptic potential[11]	
						REG1A (2.57Mbp)		Neuronal sprouting and differentiation in brain, brain and pancreas regeneration, associated with inflammatory bowel disease, higher expression in brain in Alzheimer patients[12]	Pancreas, small intestine/terminal ileum, brain		Increased risk of nasopharyngeal carcinoma[13] (R^2=0.001) Radiation sensitivity and survival of nasopharyngeal carcinoma[14] (R^2=0.001)	Reduced pancreatic islet proliferation, reduced insulin-producing cell mass. Transgenic mice had increased islet volume and delayed development of autoimmune diabetes[15] Overexpression in mice with induces colitis led to increased intestinal mucosa barrier integrity[16]	Reduced levels of pro-inflammatory cytokines, accelerated cell proliferation, reduced apoptosis[16] Enhanced chemo- and radiosensitivity of squamous esophageal cancer cells[17]

Supplemental table 4. Continued

SNP (rs / CHR:POS_ REF/ALT)	R^2	SNP in study	SNP in cell line	Chromatin state (ROADMAP)	eQTL	Chromatin interaction (dist)	Nearest protein coding gene (dist)	Protein function	Protein expression in tissue (GTeX)	Gene polymorphisms in study on lipids	Gene polymorphisms in study on other outcome	Gene knockout in mouse	Gene knockout in vitro
						REG1B (2.76Mbp)		Associated with pancreatitis and inflammation-associated pancreatic carcinogenesis	Pancreas				
						REG3A (2.84Mbp)		Associated with pancreatitis and inflammation-associated pancreatic carcinogenesis	Pancreas				
						REG3G (2.71Mbp)		Associated with pancreatitis and inflammation-associated pancreatic carcinogenesis	Pancreas, liver			Increased levels of circulating LDL and HDL cholesterol[18] Alcoholic steatohepatitis[19]	

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Supplemental table 5. Additional regression analyses

Additional regression models were built to study the interaction between genotype and CRT and the influence of BMI.

Model	Variable	OR	95% CI	P-value
CRT added (whole cohort)	Genotype dosage	1.89	1.14 – 3.14	**0.014**
	CRT yes	1.41	1.17 – 1.70	**<0.001**
Interaction with CRT added (whole cohort)	Genotype dosage	0.59	0.26 – 1.36	0.22
	CRT yes	1.31	1.08 – 1.58	**0.005**
	Genotype*CRT	14.61	4.59 – 46.55	**<0.001**
CRT dose added (continuous) (whole cohort)	Genotype dosage	1.87	1.13 – 3.11	**0.015**
	CRT dose (cont.), per Gy	1.01	1.005 – 1.015	**<0.001**
Interaction with CRT dose added (continuous) (whole cohort)	Genotype dosage	0.76	0.37 – 1.57	0.46
	CRT dose (cont.), per Gy	1.01	1.003 – 1.013	**0.002**
	Genotype*CRT dose (cont.)	1.06	1.03 – 1.09	**<0.001**
CRT dose added (categorized) (whole cohort)	Genotype dosage	1.82	1.09 – 3.03	**0.021**
	CRT dose 0-2500cGy	2.00	1.63 – 2.46	**<0.001**
	CRT dose >2500cGy	2.64	1.98 – 3.53	**<0.001**
Interaction with CRT dose added (categorized) (whole cohort)	Genotype dosage	1.01	0.28 – 3.64	0.98
	CRT dose 0-2500cGy	2.00	1.63 – 2.47	**<0.001**
	CRT dose >2500cGy	2.40	1.78 – 3.23	**<0.001**
	Genotype*CRT dose 0-2500cGy	1.07	0.25 – 4.61	0.93
	Genotype*CRT dose>2500cGy	12.51	2.24 – 69.86	**0.004**
Brain radiotherapy region added (CRT+ only)	Genotype dosage	7.97	3.56 – 17.84	**$4.57*10^{-7}$**
	Region 1	1.01	1.00 – 1.02	0.23
	Region 2	1.00	0.99 – 1.02	0.48
	Region 3	1.01	1.00 – 1.03	**0.043**
	Region 4	0.99	0.98 – 1.01	0.40
Brain radiotherapy region 2 dose added (categorized) (CRT+ only)	Genotype dosage	7.21	3.25 – 15.96	**$1.13*10^{-6}$**
	Region 2 dose >2500cGy	1.50	1.10 – 2.04	**0.0096**
BMI added (continuous) (CRT+ only)	Genotype dosage	7.51	3.39 – 16.67	**$7.05*10^{-7}$**
	BMI (cont.)	1.01	0.99 – 1.04	0.21
Overweight added (CRT+ only)	Genotype dosage	7.34	3.31 – 16.30	**$9.68*10^{-7}$**
	Overweight	1.68	1.17 – 2.42	**0.005**
Obesity added (CRT+ only)	Genotype dosage	7.66	3.45 – 16.98	**$5.41*10^{-7}$**
	Obesity	1.41	1.03 – 1.92	**0.027**
Outcome BMI (cont.) (CRT+ only)*	Genotype dosage	Beta=1.84	-0.74 – 4.43	0.16
Outcome overweight (CRT+ only)	Genotype dosage	1.93	0.73 – 5.14	0.19
Outcome obesity (CRT+ only)	Genotype dosage	1.19	0.56 – 2.53	0.66

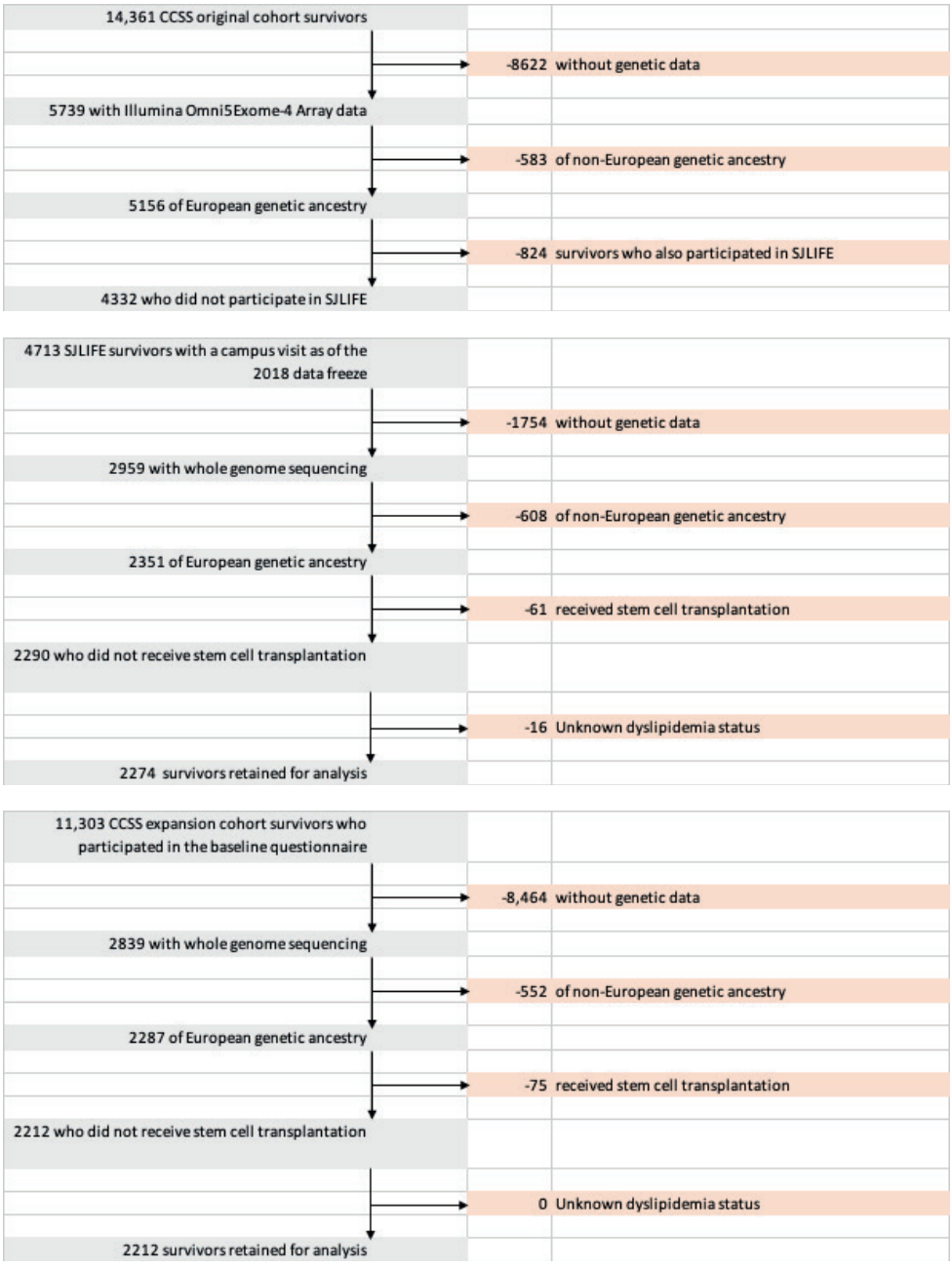
* Linear regression model, so beta reported instead of odds ratio.

All models are also adjusted for age, sex and the first four genetic principal components

Supplemental table 6. Significant locus in other GWASs

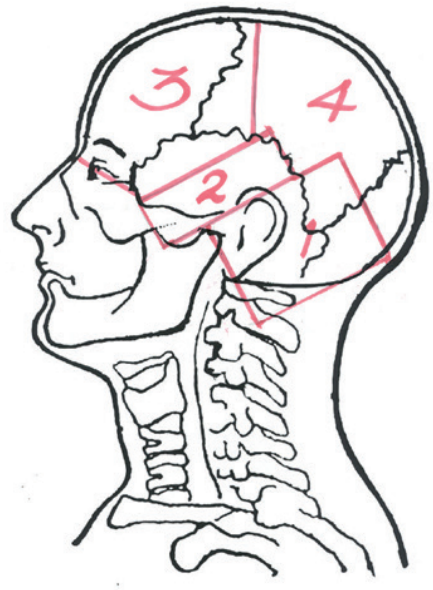
GWAS	MAF	Beta	SE	OR	Lower 95%CI	Upper 95% CI	P-value
CRT+	0.012	2.82	0.49	16.7768507	6.42	43.83	1.18E-08
Overall	0.010	0.69	0.29	1.99371553	1.13	3.52	0.018
CRT -	0.0099*	-0.59	0.42	0.55432728	0.24	1.26	0.17
Abd RT +	0.012	0.54	0.50	1.71600686	0.64	4.57	0.27
Abd RT -	0.0099*	0.63	0.31	1.87761058	1.02	3.45	0.043
Asparaginase +	0.010	0.68	0.71	1.97387773	0.49	7.94	0.34
Asparaginase -	0.010	0.71	0.32	2.03399126	1.09	3.81	0.027
Prednisone +	0.012	0.83	0.45	2.29331874	0.95	5.54	0.066
Prednisone -	0.0097*	0.51	0.34	1.66529119	0.86	3.24	0.13

*Excluded by Rvtest due to MAF<0.01, analysis was rerun in R (Wald test instead of score test)

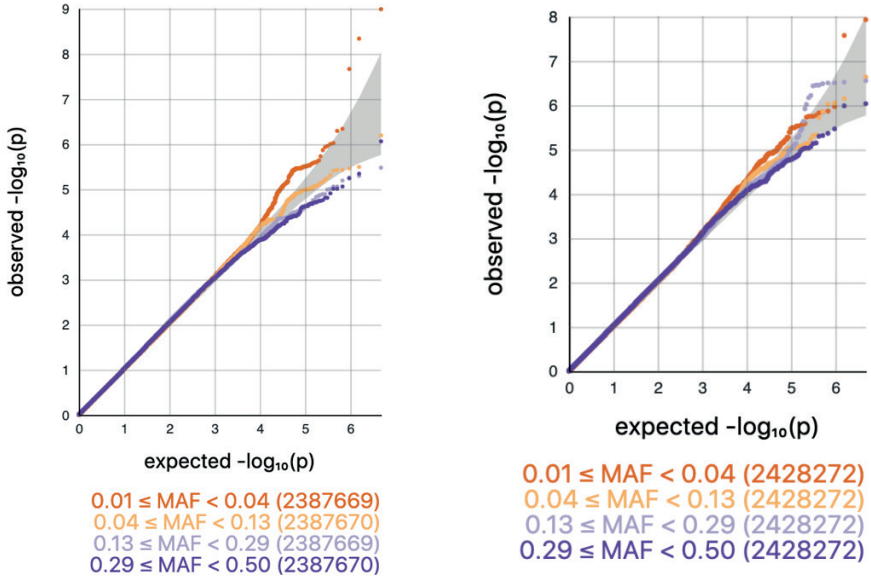


Supplemental figure 1. Flow charts of the CCSS Original, SJLIFE and CCSS Expansion cohorts, indicating included and excluded subjects.

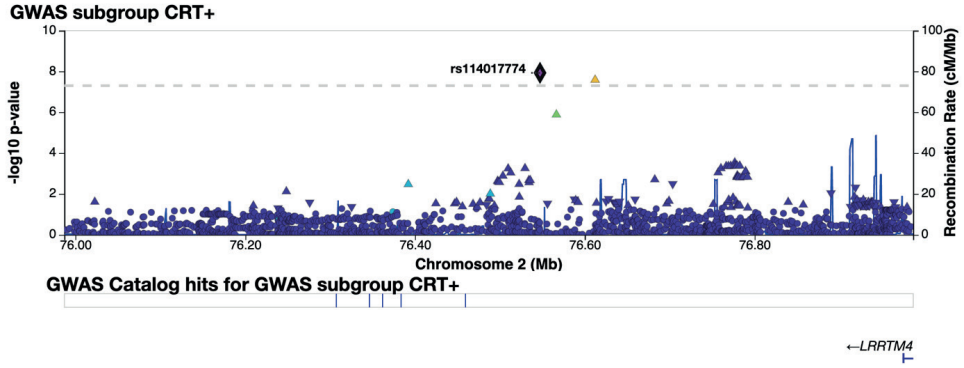
Exclusion because of absence of genetic data had various reasons, including non-consent, unusable samples (old or not enough DNA), consented but did not supply a sample.



Supplemental figure 2. Four brain regions for which dosimetry has been calculated in the CCSS cohort and included in post-GWAS analysis.



Supplemental figure 3. QQ plots of the initial discovery analysis and the CRT+ analysis.
QQ plots of the overall analysis (left) and CRT+ stratified analysis (right). Separate curves are shown for four categories of minor allele frequency (MAF).



Supplemental figure 4. LocusZoom of significant variant rs114017774 on chromosome 2.

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Chapter 7

Perspectives on follow-up care and research for childhood cancer survivors: results from an international SIOP meet-the-expert questionnaire in Kyoto, 2018

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ABSTRACT

Introduction

Survival of childhood cancer has increased over the past decades. This has led to the development of strategies aiming to enhance follow-up care and research, for which priorities may vary globally. We explored perspectives of an international healthcare workers panel.

Methods

Attendants of a meet-the-expert session on childhood cancer survivorship at the 2018 SIOP conference completed a survey about their view on important follow-up care and research aspects for survivors below and over 18 years. We analyzed overarching categories and subtopics, and compared Asian versus European and North American healthcare workers.

Results

Fifty-eight participants from different medical specialties (67.2% pediatric oncologists) and continents (48.3% Asia, 39.7% Europe/North America) responded. Follow-up care priorities for survivors below and over 18 years included physical care (39.3% ≤18 years, 35.9% >18 years) and healthcare structure (29.4%, 26.0%). Physical care was also the most important research aspect for both age groups (52.5%, 50.7%). Psychological support was the most frequently reported subtopic. Asian clinicians (n=22) primarily prioritized physical care aspects of follow-up care, whereas European/North American (n=19) clinicians underscored the importance of healthcare structure.

Conclusion

Physical care is the most important aspect of survivorship care and research according to clinicians from several continents. Asian and European/North American respondents shared most priorities, however, healthcare structure was a more important category for European/North American clinicians. The most common subtopic was psychological support, underlining also the need to involve psychologists in follow-up.

MINI ABSTRACT

Physical care is the most important survivor follow-up aspect, according to clinicians from several continents. Asian and European/North American respondents shared most priorities. Healthcare structure was more important for European/North American clinicians.

INTRODUCTION

Over the past decades, childhood cancer survival rates have increased, from less than 30% in the sixties to current rates exceeding 80% in developed countries [1]. This is due to improvement of chemotherapy, radiotherapy, surgery, stem cell transplantation, and immunotherapy, as well as better treatment stratification and enhanced supportive care.

However, childhood cancer survivors can suffer from serious long-term treatment-related side effects, leading to a significant symptom burden and increased mortality rates [2-4]. It has been shown that 70% of survivors develop at least one side effect, and 40% develop at least one severe side effect [5, 6]. A recent study even showed that, at the age of 50, 96% of survivors have developed one or more severe chronic health conditions [7]. These morbidities – physical sequelae such as secondary malignancies, cardiovascular disease, metabolic syndrome, adverse bone health, fertility problems, chronic lung and kidney disease, hearing loss, frailty, but also psychological side effects – are of detrimental influence on later life.

Increased awareness of late effects and knowledge on risk factors have led to the development of surveillance guidelines for survivors in different parts of the world, including the USA, Canada, European countries, and Japan [8-10]. Goals of these care and research strategies are optimal surveillance, development of interventions, and adjustments in treatment protocols for newly diagnosed childhood cancer patients. Although surveillance guidelines are being harmonized, different cultural values may lead to varying perspectives on which part of survivor care is most important, and what the focus of survivorship research should be. In addition, discrepancies in available resources, access to healthcare and insurance, as well as expertise to organize late effects care, may give rise to differences in follow-up care and research facilities across countries and continents. In some countries, follow-up care is currently embedded in specialized clinics with follow-up protocols, while in others this is uncommon.

Studies comparing perspectives on what is important for improving follow-up in different geographical areas, including Asia, are not yet available. The aim of this observational study was to evaluate survivorship care and research priorities across participants from different parts of the world.

METHODS

Attendants of a meet the expert (MTE) session on childhood cancer survivorship at the 2018 conference of the International Society of Pediatric Oncology (SIOP) in Kyoto, Japan, were invited to complete a survey created by the hosts of the MTE (MM, MMvdHE, YI, CK, KY, VP, and JvA; Supplemental Figure 1). This survey included characteristics of the respondents and continent of residence. Furthermore, respondents were asked how much of their employment time is spent on late effects care and research. Information regarding access to and setting of follow-up care in their respective country was retrieved.

In four open-ended questions, the most important aspects (maximum of 4 answers) of care and research for childhood and adult survivorship were evaluated. We did not request to rank these aspects. In this way, priorities were gathered for the following four areas: 1) follow-up care for survivors under 18 years old; 2) follow-up care for survivors over 18 years old; 3) follow-up research for survivors under 18 years old; and 4) research for survivors over 18 years old. In addition, the respondents were invited to explain what they considered the most important obstacle in establishing or improving survivorship care. All included respondents provided written permission for reporting on the outcome of the survey.

The answers to each of the open-ended questions were analyzed in two ways: in five overarching categories, and as separate, uncategorized subtopics (Supplemental Figure 2). The overarching categories included 'physical care', 'psychological care', and 'quality of life' (all components of care), a category 'healthcare structure', and a remaining category 'other'. Two authors (M-CB and VP) independently categorized the answers. Disagreements were resolved by discussion with each other or with the other authors. In the case of persistent ambiguity, an answer was placed in two categories. In the primary analysis, we focused on the overarching categories that were addressed by the respondents. The frequency of these categories was calculated as a percentage of the total number of answers. In the secondary analysis, in which we focused on the respondents, the frequency of the uncategorized subtopics was calculated as a percentage of the total number of respondents. Finally, the separate answers to the four questions combined were ranked according to frequency, to provide an overview of all reported subtopics.

All items of the survey were analyzed for the total cohort. We also broadly assessed shared and differing opinions by the largest continental subsets of respondents, i.e. European/North American versus Asian clinicians. We grouped European and North

American respondents, because these are Western high income countries with the longest experience in survivor follow-up care.

RESULTS

Participants

Sixty-seven participants of the MTE completed the questionnaire. Nine participants that did not provide written consent were excluded. The remaining 58 respondents included a variety of professions, ages, and nationalities (Table 1). The two largest groups were Asian (n=22) and European/North American clinicians (n=19). The other respondents included clinicians from the Middle East (n=3), Middle and South America (n=2), and Africa (n=1), as well as people with a different relationship to survivors: researchers (n=3), survivors (n=2), a parent, and 'other' (n=5).

The majority of respondents were pediatric oncologists (n=39). Other healthcare professions included nurses (n=3), a pediatrician, a social worker, a pediatric psychologist, a radiologist, and a pharmacist. Most respondents were 30 to 50 years old (n=29), or older than 50 years (n=25). Most respondents were female (n=40).

Thirty-nine respondents spent 1% to 30% of their employment on late effects care, 9 respondents spent 31% to 60%, and 7 respondents spent no time on late effects care. Thirty respondents reported to spend no employment time on late effects research and 22 respondents spent 1% to 30%. Almost all respondents answered that survivors in their country had access to survivorship care, either in a late effects clinic (n=25) or in a non-specialized clinic (n=30).

Priority categories and subtopics of follow-up care and research

Aspects of care for survivors under the age of 18 years

A total of 201 topics were reported (Table 2a). Physical care was the most frequently prioritized category (n=79, 39.3%). This was followed by health care structure (n=59, 29.4%), psychological care (n=39, 19.4%), quality of life (n=17, 8.5%), and other (n=7, 3.5%).

When analyzing all subtopics separately, the most frequently reported priorities were psychological support (answered by 32.8% of respondents), education (15.5%), and growth (12.1%) (Table 3).

Aspects of care for survivors over the age of 18 years

For adult survivors, a similar pattern was observed (Table 2b). Also for these survivors, physical care was the most frequently prioritized category (n=65/181 topics, 35.9%). This was followed by healthcare structure (n=47, 26.0%), psychological care (n=39, 21.5%), quality of life (n=26, 14.4%), and other (n=4, 2.2%).

The most frequently reported subtopics included psychological support (36.2% of respondents), fertility (29.3%), and continuing study/work (15.5%).

Aspects of research for survivors under the age of 18 years

A total of 160 subtopics was reported for this age group (Table 2c). The majority fell into the physical care category (n=84, 52.5%), followed by psychological care (n=37, 23.1%), other (n=15, 9.4%), healthcare structure (n=13, 8.1%), and quality of life (n=11, 8.1%).

The most frequently reported subtopics included psychological support (29.3% of respondents), second malignancy, genetics, and neurocognitive (all 12.1%).

Aspects of research for survivors over the age of 18 years

For survivors over 18 years old, the research priorities were similar (Table 2d). Of a total of 152 reported subtopics, 77 (50.7%) were related to physical care, followed by psychological care (n=30, 19.7%), other (n=19, 12.5%), quality of life (n=14, 9.2%), and healthcare structure (n=12, 7.9%).

The most frequently reported subtopics were fertility (34.5% of respondents), psychological support (24.1%), subsequent malignancy, and endocrinology (both 10.3%).

Overview of the frequency of subtopics

When the answers to the four questions were combined, psychological support was the most frequently prioritized subtopic of follow-up care and research for survivors (n=71, 10.2% of all answers) (Figure 1). This was followed by fertility (n=48, 6.9%), continuing study/work (n=30, 4.3%), and social support (n=29, 4.2%).

Comparison between Asian and European/North American clinicians

The two largest groups of respondents were Asian clinicians (n=22) and European/North American clinicians (n=19), which enabled a descriptive, broad comparison of the answers reported by these groups of respondents. A detailed description of the two groups is supplied in Supplementary table 1. In both groups, most respondents were pediatric oncologists, involved in late effects care for some part of their employment.

All but two respondents from Asia reported that survivors in their countries have access to survivorship care, and half of each group indicated that this care is embedded in specialized follow-up clinics.

The Asian clinicians primarily prioritized physical care aspects of follow-up care for both age groups of survivors, whereas the European/North American clinicians underscored the importance of healthcare structure. In the analysis of the uncategorized subtopics, psychological support was the most frequently answered subtopic for both groups of clinicians for survivors below as well as over 18 years.

The main research priority category concerned physical care for Asian as well as European/North American respondents. Psychological support was the most frequently reported subtopic for survivors below 18 years, although for Asian respondents this first place was shared with cardiology. For adult survivors, both groups of respondents predominantly reported fertility. For Asian respondents this was again a shared first place, with psychological support.

In the combined analysis, it is clear that psychological support was the most frequently reported aspect in survivor follow-up for the Asian as well as European/North American respondents, followed by fertility (Supplemental figure 3). Psychological support was mentioned 31 times (11.3% of a total of 274 answers) by Asian respondents and 32 times (14.4% of 222 answers) by European/North American. Fertility was reported 22 times (8.0%) and 21 (9.5%) times, respectively. Social support and continuing study/work were also answered frequently by both groups. Cardiology, second malignancy and endocrinology were mentioned often by Asian respondents, but not by European/North American respondents.

Obstacles in survivorship care

Thirty-nine participants provided one or more obstacles in establishing or improving survivorship care. Commonly reported obstacles included lack of financial resources (n=10) and lack of manpower (n=9). Other obstacles were logistic difficulties, lack of knowledge of the importance of follow-up, lack of cooperation, lack of time, difficulties in organizing a multidisciplinary team, lack of support by the hospital, lack of communication with primary care, and difficulties in reaching survivors. None of the participants answered that there are no obstacles.

DISCUSSION

We gathered views from clinicians on follow-up care and research priorities for childhood cancer survivors on an international level, based on a survey distributed at a meet-the-expert session on childhood cancer survivorship on the 2018 SIOP conference.

Enhancement of components of care (i.e. physical care, psychological care, and quality of life) was regarded the most important challenge in follow-up care for survivors by the total group of respondents. This was felt to be relevant for survivors under as well as over 18 years old. Improving physical care was also the most important aspect of follow-up research, for both juvenile and adult survivors. This is likely a result of the many persisting knowledge gaps in the field of late effects surveillance [9]. Research initiatives continuously discover new insights on the risks of specific treatments based on large cohort studies, including questionnaire investigations and recruitment studies such as the Childhood Cancer Survivor Study, St Jude Lifetime (both from the USA), the British Childhood Cancer Survivor Study, the DCCSS LATER cohort (The Netherlands), and the Japanese NCCHD Lifetime cohort. Psychological late effects are often not the first priority in care and research pursued by oncologists. Since the majority of our participants were oncologists, this topic may be less frequently addressed.

Nevertheless, when the uncategorized subtopics for care and research were combined, psychological support was the most frequently issued subtopic in the total group of respondents. This illustrates that psychological support is also an important topic for physicians. Still, this is a broad term, so involvement of psychologists in late effects care and research is desired, to address tailored support for survivors (e.g., anxiety, depression, concentration, empowerment, relationships, psychosexuality). By asking respondents' visions for survivors under and over 18 years old, we were able to analyze whether age was an important factor for determining the focus. In general, this was not the case: respondents answered largely similar for both age groups. One exception was fertility, which was particularly mentioned for adult survivors. This seems logical because fertility becomes a more important issue when survivors reach reproductive age.

The distribution of this survey on the international SIOP conference provided us the opportunity to collect and compare visions of an international group of respondents. Still, this is a selected cohort consisting of people who decided to attend the MTE and mainly comprised of pediatric oncologists. These factors may limit generalizability of our results to larger groups of healthcare professionals from the different countries

and continents. Replication in a larger, non-selected cohort is therefore an interesting future step.

We broadly compared the results from Asian and European/North American respondents. Whereas physical care was addressed most frequently by the Asian respondents, enhancement of healthcare structure was more prominently mentioned by European/North American respondents. We realize that these differences may be due to selection bias of attendants of the MTE, and also due to an age difference, as the Asian group was younger. Additionally, the priority for healthcare structure among European/North American respondents may also be due to the fact that in Europe, the USA and Canada, physicians have focused for a longer time on setting up late effects clinics, and are more involved in developing follow-up guidelines, which were unavailable in Japan until 2013 [8, 9, 11, 12]. Since then, there has been an ongoing development to address cancer survivorship issues in clinical care, and the development of follow-up guidelines has continued in Japan [12-15]. As a result, a general Japanese long-term follow-up guideline, as well as specific guidelines for after stem cell transplantation, and for fertility preservation have become available. Given the relative recency of these developments, it is reasonable that improving physical care in follow-up had highest priority among Asian respondents. This emphasizes the need to implement these guidelines and disseminate knowledge. Additionally, the fact that these guidelines are only available in Japanese language limits accessibility to the international public. Therefore, we opt to involve Japan, and possibly other countries, in international consensus meetings on childhood cancer survivorship.

To our knowledge, there is no other survey comparing Asian and European/North American perspectives of survivorship care and research among clinicians. Opinions of the two groups have been studied separately, although only a few of these studies included Asian physicians [11, 12, 16-18]. Similar to our study, survey-based studies among European or American physicians revealed aspects of healthcare structure improvement, such as specialized clinics and a smooth transfer after reaching adulthood [16-18]. In contrast, the psychological aspect was not highlighted in any of these studies.

We also asked participants about obstacles in establishing and improving survivorship care and research in their country. Several obstacles were mentioned, including lack of manpower, insufficient awareness among survivors of the importance of follow-up, and lack of cooperation, time and money. Similar obstacles were identified in surveys among US, UK and Swiss physicians [11, 16, 17, 19, 20]. Interestingly, in the light of financial obstacles, a recent study by Kaal et al. found that coordinated survivor care can lead to a reduction in healthcare costs of 300-900 Canadian dollar per patient [21].

In our study, two survivors and one parent were among the respondents. Unfortunately, this group is too small to draw firm conclusions. In future research, it remains of particular interest to include the opinion of more parents and survivors about important aspects of follow-up care and research.

Because the questions in our survey were open-ended, respondents were able to give their opinions in their own words. Although this is a good aspect, we experienced some difficulties with categorizing answers. For instance, some answers were not readable and could therefore not be included in our analysis. More importantly, we realize that categorization depends on the authors' interpretation of the answers. Two reviewers independently categorized the answers, and discussed disagreements with the other authors. Persistent ambiguous terms were placed in two categories, for example, the term 'puberty' may refer to physical changes during puberty, or psychological aspects of puberty. This way, we aimed to minimize the influence of the reviewers' interpretation of answers.

In conclusion, our survey revealed priorities of clinicians from several continents regarding aspects of childhood cancer survivor follow-up care and research. We identified shared as well as differing opinions between Asian and European/North American clinicians. Important priorities were physical care and healthcare structure, the latter being more prominent among European/North American respondents. The most frequent uncategorized answer was psychological support, underlining the need to involve psychologists in follow-up care. Therefore, specialized follow-up clinics are preferred, in order to deliver tailored care in a structured way. Although this may be challenging given the identified obstacles, the results of our survey emphasize the need for a structured approach, covering physical as well as psychological care. Our findings can aid healthcare workers in improving surveillance guidelines, adjusted to local settings and with good accessibility. Including experts from various continents, thereby including perspectives from various cultural angles, can lead to the international harmonization and optimization of follow-up, to provide survivors worldwide with optimal quality of survival.

Conflicts of interest:
None.

Acknowledgment:
Marie-Christine Bakker is a medical student participating in the Honours programme of the Faculty of Medicine, University Medical Center Utrecht, The Netherlands.

TABLES AND FIGURE

Table 1. Description of study population

Question	Answer	Number of respondents	Percentage of total (N=58)
I am…	Pediatric oncologist	39	67.2
	Nurse	3	5.2
	Pediatrician	1	1.7
	Social worker pediatric oncology	1	1.7
	Pediatric psychologist	1	1.7
	Radiologist	1	1.7
	Pharmacist	1	1.7
	Researcher	3	5.2
	Survivor	2	3.4
	Parent	1	1.7
	Other	5	8.6
Age	<30 years	4	6.9
	30-50 years	29	50.0
	>50 years	25	43.1
Gender	Female	40	69.0
I live/work in…	Asia	28	48.3
	Europe	19	32.8
	USA/Canada	4	6.9
	Middle East	3	5.2
	Middle or South America	3	5.2
	Africa	1	1.7
I spend … of my employment on late effects care	0%	7	12.1
	1-30%	39	67.2
	31-60%	9	15.5
	>60%	2	3.4
	Not answered	1	1.7
I spend … of my employment on late effects research	0%	30	51.7
	1-30%	22	37.9
	31-60%	3	5.2
	>60%	2	3.4
	Not answered	1	1.7
Childhood cancer survivors in my country have access to survivorship care	Yes, in a late effects clinic	25	43.1
	Yes, but not in a late effects clinic	30	51.7
	No	1	1.7
	Not answered	2	3.4

Table 2a. Important aspects of late effects care for childhood cancer survivors ≤18 years

Category	Number of answers	Percentage of total number of answers (N=201)
Components of care	135	67.2
--Physical care	--79	--39.3
--Psychological care	--39	--19.4
--Quality of life	--17	--8.5
Health care structure	59	29.4
Other	7	3.5
--Genetics	--1	--0.5

Table 2b. Important aspects of late effects care for childhood cancer survivors >18 years

Category	Number of answers	Percentage of total number of answers (N=181)
Components of care	130	71.8
--Physical care	--65	--35.9
--Psychological care	--39	--21.5
--Quality of life	--26	--14.4
Health care structure	47	26.0
Other	4	2.2
--Genetics	--1	--0.6

Table 2c. Important aspects of late effects research for childhood cancer survivors ≤18 years

Category	Number of answers	Percentage of total number of answers (N=160)
Components of care	132	82.5
--Physical care	--84	--52.5
--Psychological care	--37	--23.1
--Quality of life	--11	--6.9
Health care structure	13	8.1
Other	15	9.4
--Genetics	--7	--4,4

Table 2d. Important aspects of late effects research for childhood cancer survivors >18 years

Category	Number of answers	Percentage of total number of answers (N=152)
Components of care	121	79.6
--Physical care	--77	--50.7
--Psychological care	--30	--19.7
--Quality of life	--14	--9.2
Health care structure	12	7.9
Other	19	12.5
--Genetics	--7	--4.6

Table 3. Most frequent answers to open-ended questions

Category	Most frequent answers	Number of respondents that gave answer	Percentage of total number of respondents (N=58)
Late effects care ≤18 years	Psychological support	19	32.8
	Education	9	15.5
	Growth	7	12.1
Late effects care >18 years	Psychological support	21	36.2
	Fertility	17	29.3
	Continuing study/work	9	15.5
Late effects research ≤18 years	Psychological support	17	29.3
	Second malignancy	7	12.1
	Genetics, inheritance	7	12.1
	Neurocognitive	7	12.1
Late effects research >18 years	Fertility	20	34.5
	Psychological support	14	24.1
	Second malignancy	6	10.3
	Endocrinology	6	10.3

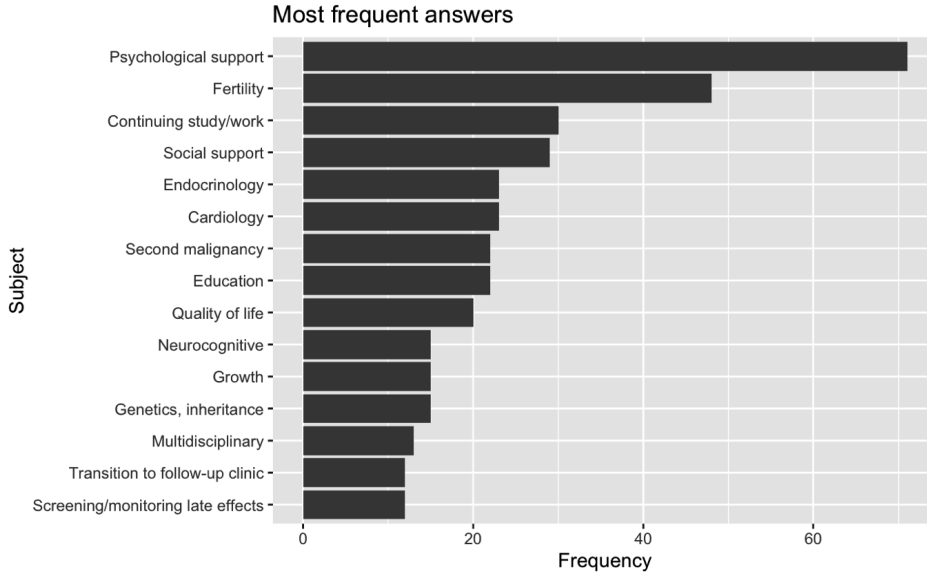
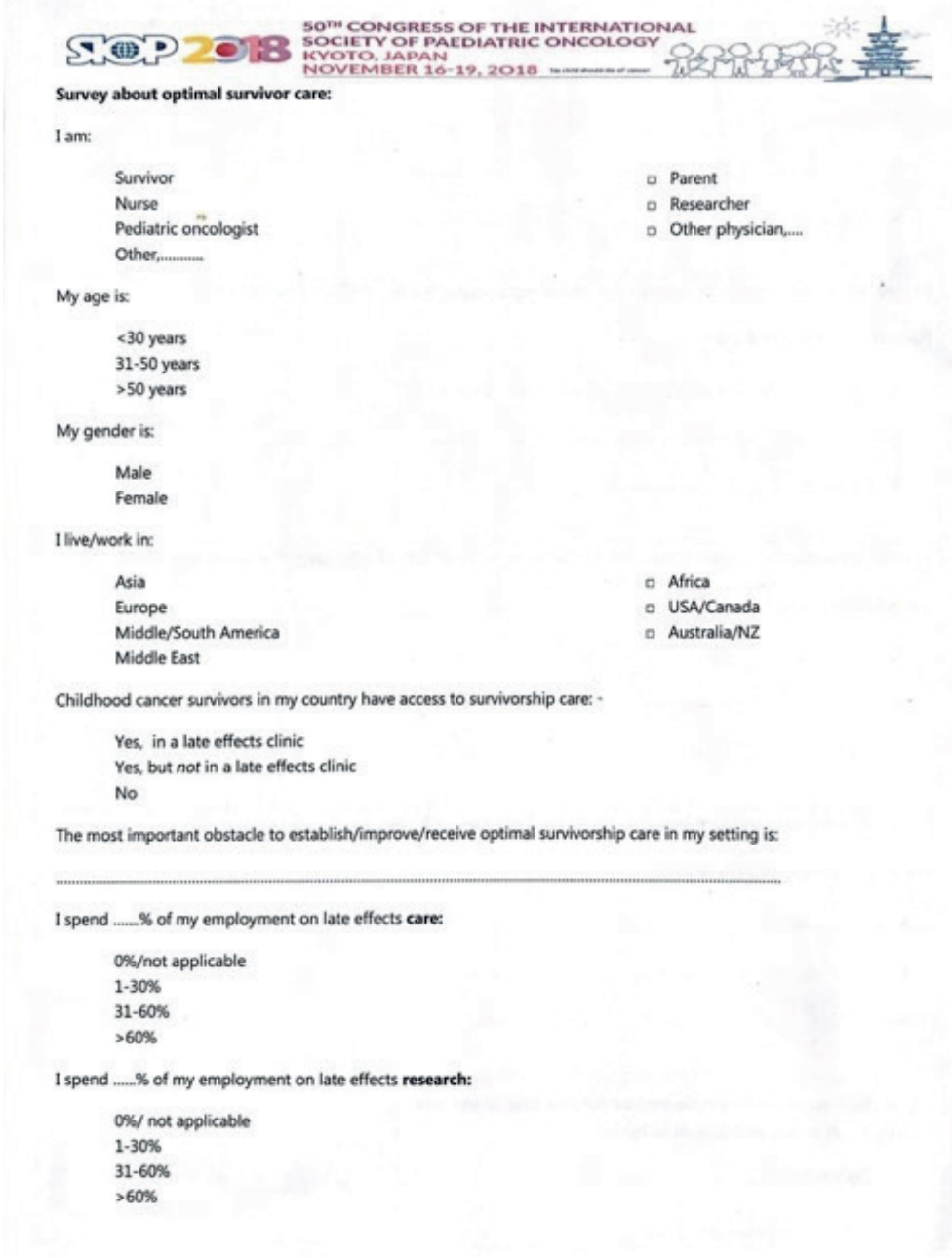


Figure 1. Top 15 most frequent answers on all open-ended questions combined

SUPPLEMENTAL MATERIAL

Supplemental table 1. Description of the Asian and European/American healthcare workers in the study population

Question	Answer	Asian respondents (N=22)	European/American respondents (N=19)
I am…	Pediatric oncologist	19 (86.4%)	14 (73.7%)
	Nurse	0	3 (15.8%)
	Pediatrician	1 (4.5%)	0
	Social worker pediatric oncology	1 (4.5%)	0
	Pediatric psychologist	0	1 (5.3%)
	Radiologist	0	1 (5.3%)
	Pharmacist	1 (4.5%)	0
Age	<30 years	1 (4.5%)	0
	30-50 years	14 (63.6%)	6 (31.6%)
	>50 years	7 (31.8%)	13 (68.4%)
Gender	Female	13 (59.1%)	15 (78.9%)
I live/work in…	Asia	22 (100%)	-
	Europe	-	15 (78.9%)
	USA/Canada	-	4 (21.1%)
I spend … of my employment on late effects care	0%	0	2 (10.5%)
	1-30%	18 (81.8%)	13 (68.4%)
	31-60%	4 (18.2%)	3 (15.8%)
	>60%	0	1 (5.3%)
I spend … of my employment on late effects research	0%	12 (54.5%)	9 (47.4%)
	1-30%	10 (45.5%)	8 (42.1%)
	31-60%	0	1 (5.3%)
	>60%	0	1 (5.3%)
Childhood cancer survivors in my country have access to survivorship care	Yes, in a late effects clinic	9 (40.9%)	10 (52.6%)
	Yes, but not in a late effects clinic	11 (50.0%)	9 (47.4%)
	No	1 (4.5%)	0
	Not answered	1 (4.5%)	0



50TH CONGRESS OF THE INTERNATIONAL SOCIETY OF PAEDIATRIC ONCOLOGY
KYOTO, JAPAN
NOVEMBER 16-19, 2018

Survey about optimal survivor care:

I am:

- Survivor
- Nurse
- Pediatric oncologist
- Other,.....
- Parent
- Researcher
- Other physician,....

My age is:

- <30 years
- 31-50 years
- >50 years

My gender is:

- Male
- Female

I live/work in:

- Asia
- Europe
- Middle/South America
- Middle East
- Africa
- USA/Canada
- Australia/NZ

Childhood cancer survivors in my country have access to survivorship care: -

- Yes, in a late effects clinic
- Yes, but *not* in a late effects clinic
- No

The most important obstacle to establish/improve/receive optimal survivorship care in my setting is:

.....

I spend .....% of my employment on late effects **care:**

- 0%/not applicable
- 1-30%
- 31-60%
- >60%

I spend .....% of my employment on late effects **research:**

- 0%/ not applicable
- 1-30%
- 31-60%
- >60%

Supplemental figure 1: The questionnaire.

For me, the 4 most important topics that characterize excellent **late effects care** after childhood cancer are:

For survivors aged 5-18 years:

X .....

X .....

X .....

X .....

For me, the 4 most important priorities for **late effects research** after childhood cancer are:

For survivors aged 5-18 years:

X .....

X .....

X .....

X .....

For me, the 4 most important topics that characterize excellent **late effects care** after childhood cancer are:

For adult survivors, aged >18 years:

X .....

X .....

X .....

X .....

For me, the 4 most important priorities for **late effects research** after childhood cancer are:

For adult survivors, aged >18 years:

X .....

X .....

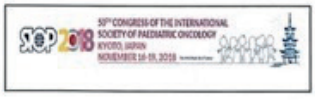
X .....

X .....

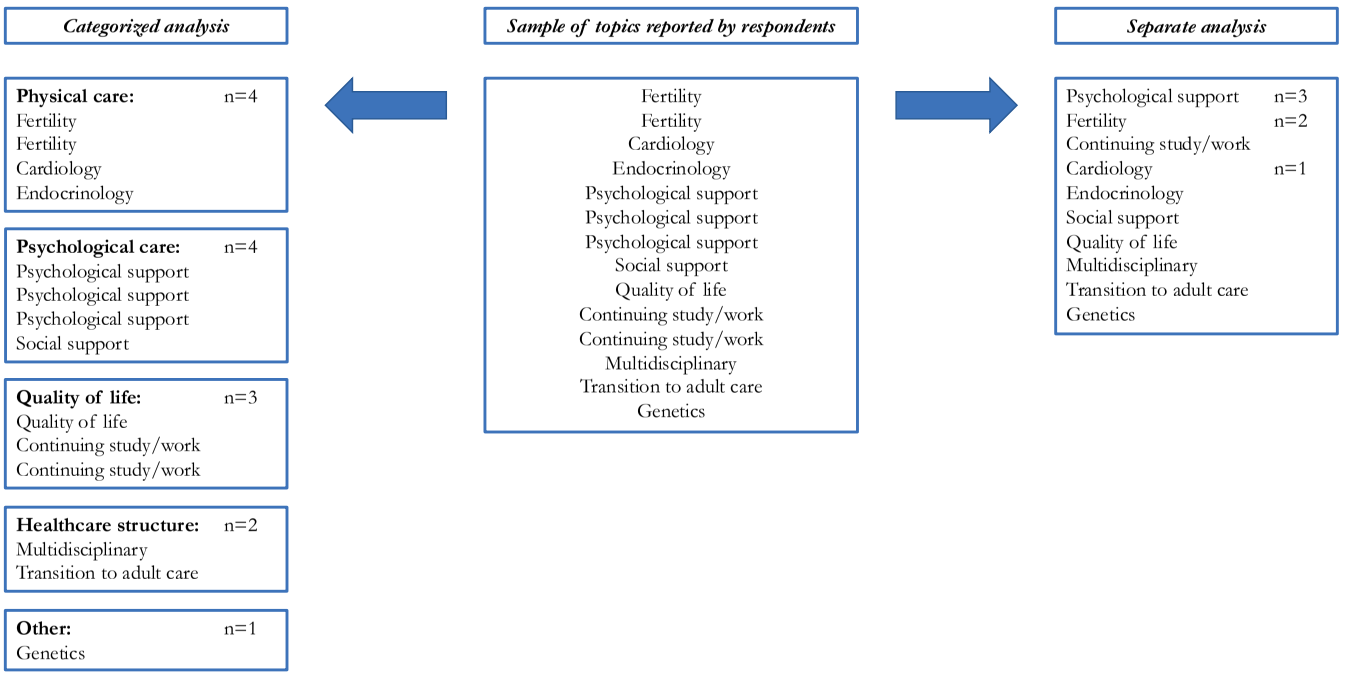
I give permission to the organizers of the meeting to use this survey for analyses and scientific report

Signature,.....

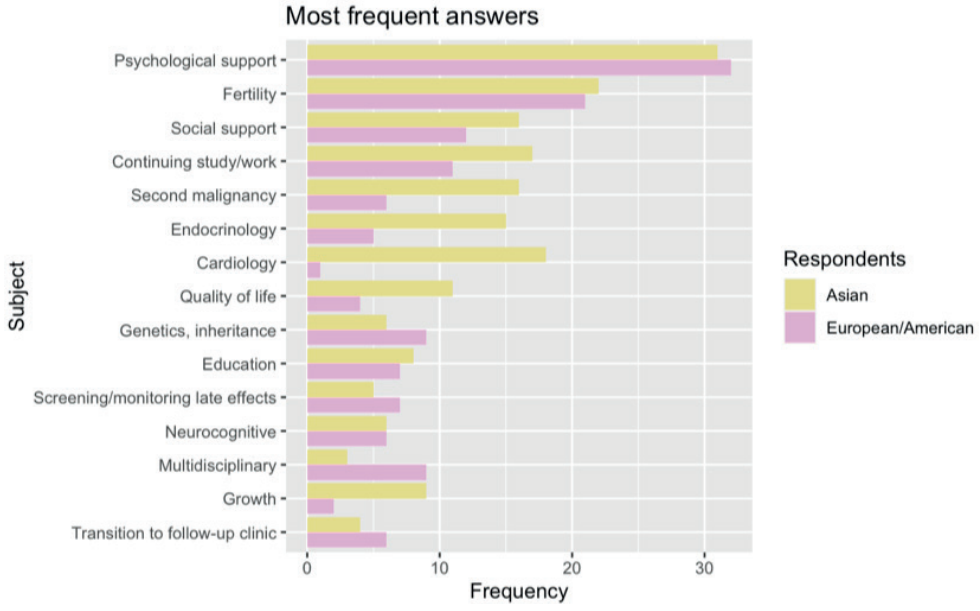
Date, 17/18 November 2018



Supplemental figure 2. Explanation of the two ways that the topics reported by respondents were analyzed: in five overarching categories (on the left), and as separate topics (on the right).



Supplemental figure 3. Top 8 most frequent answers by Asian and European/American respondents, on all questions regarding both late effects care and research.



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Chapter 8

Discussion and future perspectives

DISCUSSION AND FUTURE PERSPECTIVES

Over the past decades, curing children with cancer has become increasingly feasible. Nevertheless, on the long term, in adult survivors of childhood cancer excess morbidity and mortality due to cancer treatment are observed. Metabolic syndrome, which is a risk factor for early death due to cardio- and cerebrovascular disease and diabetes mellitus, is more prevalent among childhood cancer survivors (CCS), even at a very young age. Therefore, survivors need to be closely monitored, but metabolic syndrome and also the separate components can be underdiagnosed with the current criteria in (subgroups of) survivors. In addition, knowledge on prevalence as well as on clinical and genetic risk factors, based on large national cohort studies, is lacking. The studies described in this thesis aimed to address these challenges with regard to metabolic syndrome and the components overweight and dyslipidemia.

Determinants of developing metabolic syndrome, and better metabolic syndrome (bio)markers in childhood cancer survivors

Effective follow-up includes proactive screening of CCS at risk of developing metabolic syndrome, as well as avoidance of unnecessary screening of those who are not. Established risk factors, as identified in our literature review (*Chapter 1*), include brain tumors, cranial and abdominal radiotherapy, and alkylating chemotherapy. Knowledge is still incomplete, as most studies so far are based on small and selected survivor cohorts, have insufficient treatment data, are questionnaire-based, or have short follow-up. Additionally, more reliable metabolic syndrome (bio)markers are required, most importantly because waist circumference underestimates adiposity in survivors who received abdominal radiotherapy. Consequently, this may underdiagnose metabolic syndrome. During this thesis project, data from a national cohort was acquired that will increase insight in these subjects.

In the preparation phase for the national cohort study, a single-center study among 103 survivors of nephroblastoma and neuroblastoma (described in *Chapter 2*) was performed, showing that abdominal radiotherapy was significantly associated with occurrence of metabolic syndrome and the components high triglycerides, low HDL cholesterol and arterial hypertension. The mechanisms by which abdominal radiotherapy leads to metabolic syndrome are multifactorial and not yet fully elucidated. It has been shown that radiation to the pancreas area, most importantly the tail where the Langerhans islet density is highest, leads to insulin resistance and metabolic syndrome in a dose dependent manner[1, 2]. Females experience an additional risk, as radiation damage to the ovaries can lead to premature ovarian insufficiency (POI) and subsequent metabolic syndrome risk[3, 4]. This is because estrogen deficiency in POI , in addition to a directly increased cardiovascular disease risk[5], leads to body

fat redistribution with increased central adiposity, reduced pancreatic insulin synthesis and secretion and beta cell survival, and increased low-density lipoprotein cholesterol (LDL) and apolipoprotein B (apoB) production in the liver[6, 7]. Furthermore, radiation to the liver contributes to the dyslipidemia component of metabolic syndrome[8]. Abdominal adipose tissue may also be affected by irradiation. Previous *in vitro* and mouse experiments have suggested that irradiation could trigger fat accumulation in adipose tissue[9], and that increase of fibrous adipose tissue – which radiation can induce – is associated with insulin resistance, but also with a favorable plasma lipid profile[10]. We could hypothesize that fibrous abdominal adipose tissue has less potential to serve as a fat reservoir, with negative consequences including hypertrophy of remaining adipocytes, and intra-abdominal and liver fat accumulation. Future research may therefore focus on how adipose tissue is affected by irradiation, and on identifying mechanisms by which this is associated with overweight, altered lipid metabolism and metabolic syndrome in CCS.

In order to further unravel the multifactorial manner in which abdominal radiotherapy may lead to metabolic syndrome, data on radiation dose received by different organs and tissues will be more informative than the prescribed field and dose. This may even allow analysis of organ parts and structures, which has revealed insights in radiation-induced diabetes, stroke and cardiac disease in recent survivor studies[2, 11, 12]. In the DCCSS-LATER study, such dosimetry data are currently analyzed. Based on this, dosimetry based surveillance strategies could be developed, which could reduce the amount of diagnostic testing[13]. In addition, such analyses may not be pursued only in survivors, but already start during and from the end of treatment, to identify early damage, to better understand the mechanisms, and to use interventions at an early stage.

Another next step may be to build prediction models that quantify the risk increase for treatment-related factors. To establish these risk scores, larger cohorts are required, enabling the concurrent analysis of several factors. Such prediction models need to be validated in independent cohorts. Prediction models were recently developed to predict ischemic heart disease and stroke in survivors[14]. A model predicting metabolic syndrome can be even more clinically relevant, as this is the preceding condition. Several studies have suggested that CCS with metabolic syndrome have a higher risk of developing cardiovascular disease and diabetes mellitus than the general population, due to an additive or even synergistic effect of factors including cardio- and vasculotoxic therapies, comorbidities including endocrinopathies, and sedentary lifestyle[15-18]. Using prediction models for metabolic syndrome (components) could therefore enable earlier intervention with potential great benefits. Still, it needs to be proven that intervention also avoids cardiovascular disease and diabetes mellitus in

CCS. This includes medical interventions, e.g., the use of GLP1 agonists for weight loss, and lifestyle interventions, including diet, smoking and physical exercise[19]. Such survivor specific intervention programs are currently being developed[20-22]. This may become challenging, as in the general population, it has been shown that hormonal, metabolic and neurochemical adaptations in metabolic syndrome hamper the effectiveness of interventions[23].

In the aforementioned single-center study, we also noticed that almost none of the survivors had the metabolic syndrome component "increased waist circumference", which is remarkable when taking the normal population into account. This may be explained by the historic administration of abdominal or flank radiotherapy, leading to a damaged and underdeveloped waist region. This underlines the troublesome situation that, while abdominal irradiation is a risk factor for metabolic syndrome, at the same time it hampers its assessment, as three of the four remaining diagnostic criteria are required. We therefore suggest that replacing the overweight criterion with an alternative component, may be an option. In a previous study, replacement of waist circumference with body composition, expressed as fat% on dual-energy X-ray absorptiometry (DXA), revealed a more accurate prevalence of metabolic syndrome[8]. We investigated the potential use of biomarkers for this purpose, among the nephro- and neuroblastoma survivors in our single center study. Four serum biomarkers appeared to be potentially useful candidates to assess metabolic syndrome for this purpose: adiponectin, LDL cholesterol, apoB and uric acid.

Additionally, we summarized the available literature for the use of biomarkers in improving diagnosis and prediction of metabolic syndrome (*Chapter 3*). Five out of nine reviewed biomarkers were identified to be useful: uric acid, leptin, high sensitivity C-reactive protein (hsCRP), adiponectin and apoB. However, studies among survivors were scarce and often had high risk of bias. Based on general population studies high and moderate quality evidence for the use of these five biomarkers was found. Evidence from this older general population may be applicable to young-adult childhood cancer survivors, because accelerated aging was observed in several other studies, with frailty levels in 30 year old survivors comparable to 65 year old adults from the normal population[24-26]. Even so, it could be that survivor-specific comorbidity influences the pathophysiology of metabolic syndrome and therefore the relationship between biomarker and metabolic syndrome.

Advantages of biomarkers include that they are mostly easy and cheap to determine on a routine basis in clinical practice. We feel that LDL may be suboptimal as additional criterion, because the components "high triglycerides" and "low high-density lipoprotein" already capture most of this effect. ApoB is also a lipid marker

but evidence is increasing that it is a more accurate marker of atherogenic risk[27-29]. Measurement of hyperuricemia is a true separate component and may therefore be interesting. The same accounts for measurement of chronic inflammation with hsCRP. We suggest that leptin and particularly adiponectin, for which most evidence was found, may be the most interesting surrogate markers in survivors with unreliable waist circumference, given their relation with adipose tissue.

To further study the usefulness of these biomarkers, these need to be assessed in a prospective study, as additional metabolic syndrome components, in larger cohorts of survivors of heterogeneous malignancies. Furthermore, although reference values for these biomarkers are available, a stricter threshold value may be required when analyzed concurrently with the correlated metabolic syndrome components. This is illustrated in current metabolic syndrome definitions, that use a lower threshold for hypertension than commonly used when blood pressure is measured solely (130/85 vs 140/90mmHg)[30-32]. In the DCCSS-LATER study we are currently evaluating the use of biomarkers for this purpose.

We also studied the potential value of vascular ultrasound among the nephro- and neuroblastoma survivors, in enhancing metabolic syndrome diagnosis. Although vascular ultrasound can identify early vascular consequences of metabolic syndrome, we were unable to find a clear benefit. This is different from a few previous studies in adult CCS, that included other cancer types and treatments including testicular cancer and mediastinal and total body irradiation (TBI)[33-36]. It could be that the survivors in our study were still too young to develop vascular effects. Recent evidence from the general population suggests short-term progression of subclinical, ultrasound assessed, atherosclerosis between the age of 40 and 50[37]. The median age of the nephro- and neuroblastoma survivors was 30, so if accelerated vascular aging occurs, and if CCS develop similar vascular consequences, these effects may be identified in their next decade of life. Such a vascular study might also include carotid or femoral artery plaque burden on arterial ultrasound, as well as coronary artery calcium (CAC) score on CT scan of the heart. These modalities are more predictive of cardio- and cerebrovascular events than other radiologic measurements, according to the European Society of Cardiology (ESC) guideline on dyslipidemia[38].

Prevalence and determinants of overweight in childhood cancer survivors and the influence of measurement modalities

The DCCSS-LATER Metabolic syndrome study aims to provide knowledge on clinical and genetic determinants of metabolic syndrome and the separate components, and the diagnostic value of DXA scan and biomarkers, in a nationwide study cohort that comprises the first treated (1963-2002) survivors in The Netherlands (*Chapter 4*). This

thesis describes the results of the overweight component and the results of the total picture of metabolic syndrome are currently under analysis. Overweight occurred in almost half of all long-term survivors. The increase in overweight prevalence per age group was steeper in survivors compared to the general population. This may suggest a more rapid increase in overweight when survivors age, indicating the need for follow-up on the very long-term for all survivors. Or it may be an effect of the earlier treatment eras, when children with cancer received more harmful therapies, e.g., cranial radiotherapy (CrRT). This would imply that only treatment-based subgroups require follow-up. Given these contrary implications, a longitudinal follow-up study until elderly age will be relevant.

Overweight at diagnosis, CrRT and growth hormone deficiency (GHD) were associated with long-term overweight in this study. Overweight at diagnosis may in part reflect a genetic predisposition. In current additional analyses this genetic variation is also studied. As the relationship between GHD and overweight is bidirectional[39], it cannot yet be concluded that GHD is a risk factor for overweight development, because of the cross-sectional design of the study. A longitudinal study from childhood cancer treatment onwards would be required to fully elucidate which of the two occurred first. Additionally, hypogonadism may play a role in overweight development. Hypogonadism has been reported to be associated with overweight in previous studies[4], and it often co-occurs with GHD in survivors after treatment with cranial radiotherapy[40-42]. Data on hypogonadism in our study cohort will become available. Hence, in future analyses it will be interesting to include data on gonadal status, to further elucidate these mechanisms. Future analyses could also benefit from the use of dosimetry data of parts of the brain. Whereas with full CrRT the hypothalamus and pituitary gland will receive the entire dose, with local radiotherapy these important structures may or may not be in the field. These nuances could not be incorporated yet in the current analyses.

Prevalence of overweight using several assessment methods was compared, of which the DXA scan was most sensitive. It identified overweight in an additional 30% of survivors, as compared to BMI, particularly in those treated with abdominal irradiation, TBI, anthracyclines and platinum chemotherapy. For abdominal irradiation and TBI this higher discrepancy rate between BMI and DXA has been reported in previous studies[8, 43] but anthracycline and platinum chemotherapy treated survivors were never identified to be at risk, so far. This more sensitive method may therefore be relevant in these subgroups. In a previous study in Dutch survivors, there was an association between anthracyclines and low BMI, but fat% was not measured[44]. In future studies it will be interesting to unravel why altered body composition seems to occur in these survivors. Sarcopenia frequently occurs among CCS[45] and could

be an explanation why anthropometric measurements that include lean mass, such as BMI, underestimate overweight. It is known that anthracyclines can induce cardiac myocyte cell death, leading to heart failure[46]. This could lead to decreased exercise ability and less muscle mass, although in our analyses we adjusted for physical activity. Toxicity of anthracyclines so far appears to be limited to the heart, but it could be that also the skeletal myocytes are damaged, leading to sarcopenia and subsequent underestimation of overweight. Such a mechanism was observed in a rat study, which showed that doxorubicin reduced fiber size and satellite cell and capillary density of the soleus muscle[47]. The question remains whether this accounts for CCS as well. As for platinum chemotherapy, this can induce skeletal muscle wasting[48]. While this muscle damage and dysfunction during treatment has been frequently reported, a long-term effect has not been described. Therefore, whether anthracyclines and platinum lead to clinically relevant altered body composition remain to be demonstrated.

In this study we also observed that high molecular weight-adiponectin did not seem to add diagnostic value in assessing overweight. This was not what we expected based on our systematic review, although in this review we looked at metabolic syndrome as endpoint, not overweight specifically. Less than five percent of the national cohort had low adiponectin, so overweight development in CCS may be less correlated with adiponectin than in the general population. TBI and CrRT were associated with low adiponectin, and adiponectin levels were inversely associated with high BMI and high waist circumference. This does suggest its potential as a surrogate marker for overweight, but perhaps only in subgroups of CCS. Therefore, this remains interesting for further analysis, as we are currently performing in this cohort.

Genetic polymorphisms associated with dyslipidemia in survivors

Similarly treated CCS can have a different risk to develop metabolic syndrome and separate components. This may be due to environmental factors but also to genetic susceptibility. For the dyslipidemia component, only one candidate gene study in survivors has been performed so far, which identified three loci to be relevant in acute lymphoblastic leukemia (ALL) survivors (in *APOB, BAD* and *OGFOD3*)[49]. By genome-wide association approach, numerous loci associated with dyslipidemia have been identified in the general population[50, 51].

We performed a genome-wide association study (*Chapter 6*) in the Childhood Cancer Survivor Study cohort, which identified variant rs114017774 on chromosome 2 as a potential genetic variant associated with dyslipidemia, specifically in cranially irradiated survivors. After meta-analysis of the results of this cohort and the St Jude Lifetime cohort a higher risk of developing dyslipidemia was observed, with OR 11.30 (95%CI=5.03-25.40, $p=4.46*10^{-9}$). Because of the low minor allele frequency

of the variant rs114017774, our results remain based on a very small sample size, of only heterozygous subjects. Therefore, additional replication and meta-analysis in other cohorts of cranially irradiated survivors is required. We are currently performing a replication analysis using the Dutch CCS cohort, to further validate this finding. The potential candidate genes affected by rs114017774 are *LRRTM4* and *CTNNA2*, although further functional evidence is required. No expression quantitative trait locus between the top locus and genes in the area was identified. These candidate genes were identified based on chromatin interactions. Future experiments will need to show whether this locus, which is in a non-coding region, influences expression of one of these genes, in relevant tissue (i.e., brain)[52]. Also, additional evidence for development of dyslipidemia phenotype is required. *LRRTM4* and *CTNNA2* are expressed in the brain and involved in development and maintenance of the nervous system and synaptic functioning. Genetic variants inside these genes were associated with high BMI and coronary artery disease, which may suggest a link with metabolic phenotypes. Knock-out mice showed decreased brain synapse formation and growth and also decreased body growth[53, 54], but knock-out mice in general show a phenotype involving reduced weight[55]. Growth hormone deficiency would also show a phenotype with increased fat but this was not described in these mice. Mouse models with knock-out of the *REG3* gene, for which chromatin interactions with rs114017774 were also observed, did develop dyslipidemia[56]. However, this gene is mostly expressed in the pancreas, so one would expect to find this association in the abdominal radiotherapy exposed subgroup analysis, which was not the case. Based on current evidence, we may hypothesize that survivors carrying the variant rs114017774 identified in our GWAS, when treated with cranial radiotherapy, are more vulnerable to aberrant functioning of the hormonal pathways arising in the hypothalamus. Hence, at increased risk of developing dyslipidemia (and other metabolic syndrome components).

A remarkable finding in our analyses was that radiotherapy to the frontal brain region appeared to be even more associated with dyslipidemia than radiotherapy to the hypothalamic and pituitary region. This is a good example of how dosimetry data of organ structures can yield more detailed observations. An association between frontal lobe radiation damage and dyslipidemia was not reported in literature before. Among other functions, the frontal lobe is involved in impulse regulation[57]. Although highly speculative, it could be that dysfunction of impulse control due to genetic predisposition and cranial radiotherapy leads to an unhealthier lifestyle and consequent dyslipidemia.

Additional genetic variants may be discovered by increasing power using a genome-wide meta-analysis of multiple survivor cohorts. Unlike one might expect, it has been

shown that with a large enough sample size a self-reported phenotype, including high cholesterol, is very well able to serve as outcome in GWAS[58, 59]. So for this strategy cohorts with either self-reported or lab data could both be included.

This study for the first time replicated the protective variant rs676210 in the apoB encoding *APOB* gene, which was identified in the aforementioned ALL study[49], in a more heterogeneous cohort of adult CCS. This general population variant alters the structure of apoB in a way that decreases oxidation of LDL cholesterol[60]. In a large GWAS this variant was associated with lower triglycerides, LDL cholesterol and total cholesterol levels and with higher HDL cholesterol[50]. The two other general population variants replicated in the ALL study, rs2286615 in the *BAD* gene and rs62079523 in the *OGFOD3*, were not replicated. *BAD* encodes a protein that has both a pro-apoptotic function and is involved in the insulin secretion pathway[61]. Dysfunction of *BAD* caused by rs2286615 is thought to have an impact on insulin resistance, and the authors of the study in ALL survivors suggest a subsequent effect on dyslipidemia development. For *OGFOD3*, which encodes a part of an enzyme involved in iron binding, no specific role in lipid metabolism is known. The authors included it as a candidate gene based on their gene ontology search terms and it increased dyslipidemia risk but the mechanism remained unclear. For the variant in *BAD* it may be interesting to follow-up insulin-resistant survivors and study whether in this apparent susceptible group rs2286615 carriers develop dyslipidemia faster.

Studying the effect of known polymorphisms in the general population associated with metabolic syndrome components in CCS is interesting in the context of accelerated aging[25]. In CCS, these genetic predispositions can become relevant at an earlier age. A next step is to determine the effect of multiple general population genes predisposing to dyslipidemia in survivors, based on a polygenic risk score. This may also be relevant because of the polygenic nature of dyslipidemia, where multiple genes have small effects[62]. This approach was recently performed for another metabolic syndrome component, hypertension[63], and for severe obesity (BMI≥40kg/m^2)[64]. The study on hypertension revealed that survivors with the lowest polygenic risk score still had a higher hypertension risk compared to the general population, suggesting treatment-related risk. Survivors with the highest polygenic risk score had a 2.5 fold higher hypertension risk compared to the lowest, and the authors determined that this polygenic risk score contributed about one quarter of the hypertension risk. The study on severe obesity revealed that including the polygenic risk score to risk prediction models based on cancer treatment and lifestyle factors greatly improved performance of the model, identifying four times more high-risk survivors. We are currently working on such a polygenic risk score for dyslipidemia. In addition to further unraveling the genetic susceptibility to dyslipidemia development in survivors, we will focus on

the other metabolic syndrome components as phenotypes, metabolic syndrome as a whole, but also other relevant outcomes including GHD, sarcopenia and frailty. Apart from two GWASs among survivors, which revealed genetic polymorphisms potentially modifying obesity after CrRT exposure[65, 66], these studies are so far not available. These analyses are currently ongoing in the DCCSS-LATER cohort and in collaboration with international study groups.

Towards personalized follow-up for individual childhood cancer survivors worldwide

Research strategies to fill gaps of knowledge as discussed here, and summarized in Table 1, can continuously aid in assessing personalized risk for survivors. For metabolic sequelae it is of importance to take into account all components of metabolic syndrome, and this can be taken one step further. Several studies have observed the co-occurrence of multiple late effects, emphasizing the total burden of disease that survivors sometimes face[67-69]. Endocrinopathies after hypothalamic-pituitary damage may explain co-occurrence of metabolic syndrome and other endocrine sequelae, such as growth failure, infertility, osteoporosis and hypothyroidism. Furthermore, a detailed analysis in the SJLIFE cohort showed that metabolic syndrome components often co-occurred with pulmonary function deficits, secondary neoplasms, hearing loss and recurrent infections[68]. In future research, these mechanisms leading to a high cumulative late effects burden, why certain effects cluster, and how this affects quality of life, may further be unraveled. Another step in this regard includes, for therapies that come with a high late effects burden, attempts to adjust treatment protocols, while maintaining anti-tumor efficacy. Replacement of CrRT in ALL with improved systemic and intrathecal chemotherapy, and reduction of anthracyclines in renal tumors are successful examples of this[70-72]. In addition, innovative abdominal radiotherapy techniques, using lower and more targeted dose (highly conformal target-volume delineation, Intensity-Modulated Radiation Therapy and Volumetric Modulated Arc Therapy), were recently shown to decrease scatter to abdominal organs and are currently implemented[73, 74]. Studies have shown hopeful improvements in health outcome and life expectancy for more recently treated survivors[75-77]. On the other hand, late metabolic consequences of novel therapies such as immunotherapy remain to be investigated[78].

Survivors in The Netherlands can benefit from coordinated follow-up care that is delivered in specialized late effects clinics, according to comprehensive national guidelines[79]. The survey described in *Chapter 7*, which explored perspectives on survivor care and research priorities among health care professions from several continents, revealed agreement that not only physical care, but also psychological support is essential. Furthermore, several obstacles were identified that physicians in

other countries face in setting up structured CCS care. Collaborations, support and sharing of knowledge and resources are required to make survivor follow-up a success story worldwide.

Table 1. Summary of gaps of knowledge and future perspectives regarding metabolic syndrome in childhood cancer survivors

Goal	How
Personalized risk stratification	Use of *dosimetry based data* to express radiotherapy related risk of developing metabolic syndrome components, from the period of treatment on, in a longitudinal setting.
	Genetic predisposition to metabolic syndrome components and the syndrome as a whole. This concerns identification of *survivor-specific genetic variants* related to and interacting with cancer treatment, for which power can be enhanced by performing *genome-wide meta-analyses of multiple cohorts*. Additionally, *polygenic risk scores* based on relevant variants in the *general population* can be determined.
	Study the risk of currently applied *novel therapies* to determine the risk for more recently treated survivors
	Development of *prediction models* that calculate the combined risk of multiple factors, including therapies, genetic factors and comorbidities including growth hormone deficiency and hypogonadism
Improved diagnosis of metabolic syndrome	Continuous follow-up in *aging survivors* to study whether vascular ultrasound and adiponectin are useful diagnostic tools
	Explore the potential of the *biomarkers* adiponectin, uric acid, apoB, hsCRP and leptin by studying their value as *additional metabolic syndrome component*
Unravel pathophysiological mechanisms	Use of *dosimetry data* to further refine how organs and organ parts, including the brain and adipose tissue, are affected by abdominal and cranial irradiation
	Longitudinal follow-up from diagnosis, end of treatment towards long-term survivorship, to gain insight in the role of treatment-related determinants as well as comorbidities in the development of metabolic syndrome components
	To determine the *biological mechanism* by which anthracyclines and platinum chemotherapy may disguise overweight
	Functional *in vitro or in vivo* evidence that the variant rs114017774 influences the expression of *LRRTM4* or *CTNNA2*, and that this leads to dyslipidemia
Implementation in clinical care	*Dosimetry based* and *prediction model based surveillance* to enable early intervention for those at risk and to avoid unnecessary screening
	Development of a *surveillance guideline* for metabolic syndrome
	Surveillance including early *medical and lifestyle intervention*, and follow-up to find out *effectiveness in preventing* cardiovascular disease and diabetes mellitus

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SUMMARY

Over the past decades, cure of children with cancer has become increasingly feasible and current survival rates exceed eighty percent. However, this is in fact only partly a success story as excess multimorbidity and mortality due to cancer treatment are observed in survivors throughout adulthood. Metabolic syndrome, which is a risk factor for early death due to cardio- and cerebrovascular disease and diabetes mellitus, is more prevalent among childhood cancer survivors (CCS), even at a very young age. Therefore, survivors need to be closely monitored, but metabolic syndrome and the separate components can be underdiagnosed with the current criteria in (subgroups of) survivors. Additionally, knowledge on national prevalence as well as on clinical and genetic risk factors of metabolic syndrome and its separate components remains incomplete. This thesis project therefore aimed to identify better methods and biomarkers to increase metabolic syndrome diagnosis, and to describe prevalence and clinical and genetic determinants of metabolic syndrome and the components overweight and dyslipidemia.

Our literature review on prevalence and determinants of metabolic syndrome in CCS, identified that a history of brain tumor, cranial or abdominal radiotherapy, and alkylating chemotherapy can be considered as established variables, associated with occurrence of metabolic syndrome. Most studies so far, however, are based on small and selected cohorts, have insufficient treatment data, are questionnaire-based, or have short follow-up. Therefore, the national prevalence of metabolic syndrome and the separate components was unclear, and for several hypothesized risk factors, including overweight at diagnosis and treatment with corticosteroids, evidence was inconclusive. Additionally, more reliable metabolic syndrome (bio)markers are required, most importantly because waist circumference underestimates overweight in survivors who received abdominal radiotherapy. Consequently, this may underdiagnose metabolic syndrome. Also, a potential role for genetic susceptibility is suspected, as there can be a difference in occurrence and severity of metabolic syndrome and separate components in comparably treated survivors. However, so far, no replicated genetic polymorphisms have been found in CCS.

In our single-center cohort study among 103 survivors of nephroblastoma and neuroblastoma, we observed that metabolic syndrome was present in 14% of survivors, already at a young age, and that 33% had at least two components. Abdominal radiotherapy was significantly associated with metabolic syndrome and the components high triglycerides, low high-density-lipoprotein cholesterol and hypertension. We also noticed that almost none of the survivors had the metabolic

syndrome component "increased waist circumference", which is remarkable when taking the general population into account. This may be explained by the historic administration of abdominal radiotherapy, and it illustrates the need for additional (bio)markers to enhance metabolic syndrome diagnosis. In this study we observed that four serum biomarkers may be useful for this purpose: adiponectin, low-density-lipoprotein cholesterol, apolipoprotein B (apoB) and uric acid. We also found that vascular ultrasonography, which can detect early vascular consequences of metabolic syndrome, was not of additional benefit to improve metabolic syndrome diagnosis.

In our systematic literature review on metabolic syndrome biomarkers we identified five out of nine reviewed biomarkers as useful for the diagnosis and prediction of metabolic syndrome, i.e., uric acid, leptin, high sensitivity C-reactive protein, adiponectin and apoB. The level of evidence we found for these biomarkers was high or moderate, mainly based on general population studies. Studies among survivors are scarce and often had high risk of bias, so assessment of the use of these biomarkers in CCS is still required.

We described the methodology of the DCCSS-LATER Metabolic syndrome study on prevalence and clinical and genetic determinants of metabolic syndrome and the separate components, and on the diagnostic value of DXA scan and biomarkers. This is a nationwide Dutch cohort of the first treated (1963-2002) CCS, consisting of 2,338 long-term CCS. In this thesis we presented the first results of the overweight component. Overweight occurred in almost half of all long-term CCS. Compared to general population data, there was a significantly higher prevalence for overweight among women aged 50+ and for morbid obesity among men aged 50+. Overweight, and in particular obesity and morbid obesity, were most prevalent among survivors of central nervous system tumors. Overweight at diagnosis, cranial radiotherapy and growth hormone deficiency were associated with long-term overweight, but the historic use of corticosteroids was not. We also compared prevalence of overweight using several assessment methods. We found that waist-circumference-based methods classified a substantial number of survivors with normal BMI (i.e., incorrectly classified as healthy weight by BMI) as overweight. DXA scan was the most sensitive method. It identified overweight in an additional 30% of survivors, and even 40% in the subgroup of abdominally irradiated survivors. This was particularly the case in survivors treated with abdominal radiotherapy, total body irradiation, anthracyclines and platinum chemotherapy. In this study we also observed that high molecular weight-adiponectin did not seem to add diagnostic value in assessing overweight.

In the first genome-wide association study (GWAS) on the metabolic syndrome component dyslipidemia in three large cohorts of CCS – the CCSS Original, St

Jude Lifetime (SJLIFE) and CCSS Expansion cohorts – none of nine SNPs that were identified in discovery analysis of the entire cohort, replicated. Next, we identified rs114017774 on chromosome 2 as a potential genetic variant for dyslipidemia, specifically in cranially irradiated CCS. Meta-analysis of the CCSS Original and SJLIFE cohorts revealed a pooled odds ratio of 11.30 (95% confidence interval 5.03-25.40, $p=4.5x10^{-9}$), but this locus did not individually replicate in the CCSS Expansion or SJLIFE cohorts. This genetic variant had an interaction with cranial radiotherapy (genotypexCRT OR=14.61, 95%CI=4.59-56.55) independent of BMI. It is not reported in large general population GWASs on dyslipidemia, potentially suggesting a survivor-specific effect. Based on available sources we hypothesized that this genetic variant could potentially alter *LRRTM4* or *CTNNA2* (two genes expressed in the brain and involved in nervous system development and functioning) expression or function, making survivors more susceptible to hypothalamic damage caused by cranial radiotherapy, thereby increased dyslipidemia risk. Additional replication and functional validation are needed to further explore the relevance of this variant. Furthermore, we replicated the protective variant rs676210 in the *APOB* gene, which was identified in a previous study among survivors of acute lymphoblastic leukemia (ALL), in the CCSS Original cohort. Replication was significant in the ALL subgroup as well as the total cohort, indicating that this general population variant is relevant in survivors as well.

Surveillance standards for CCS may differ across the world, due to the (un)availability of guidelines, differences in cultural values, and discrepancies in available resources and expertise. We explored perspectives on survivor care and research priorities among clinicians from several continents and show that, regardless of country of origin, psychological support, next to several aspects of physical care, was regarded as a priority. This emphasizes the need for a well-organized approach, covering not only physical but also psychological care. Furthermore, the survey identified several obstacles that physicians in other countries face in setting up structured care for CCS.

NEDERLANDSE SAMENVATTING

De afgelopen decennia werd het in toenemende mate mogelijk kinderen met kanker te genezen, waardoor de overleving momenteel meer dan tachtig procent bedraagt. Echter, dit is slechts ten dele een succesverhaal, aangezien multimorbiditeit en mortaliteit overmatig voorkomen bij overlevers van kinderkanker (survivors). Dit is ten gevolge van de kankerbehandeling en treedt niet alleen in de eerste jaren na genezing op, maar loopt door tot de volwassen leeftijd. Metabool syndroom, een risicofactor voor vroege sterfte door hart- en vaatziekten en diabetes mellitus, komt vaker voor bij survivors, en al op jonge leeftijd. Het is daarom van belang goed toezicht te houden op het ontwikkelen hiervan, maar de diagnose van metabool syndroom en de losse componenten kan met de standaard criteria worden gemist in (subgroepen van) survivors. Daarbij komt dat de kennis op het gebied van nationale prevalentie en van klinische en genetische risicofactoren voor metabool syndroom en de losse componenten nog incompleet is. Het doel van dit proefschrift was om betere methodes en biomarkers te identificeren voor het diagnosticeren van metabool syndroom, en om de prevalentie en klinische en genetische determinanten te beschrijven van metabool syndroom en van de componenten overgewicht en dyslipidemie.

Onze literatuur review over prevalentie en determinanten van metabool syndroom in survivors toonde dat van een hersentumor, schedel- of buikbestraling en alkylerende chemotherapie in de voorgeschiedenis een duidelijke associatie is vastgesteld met het ontstaan van metabool syndroom. Niettemin zijn de meeste studies tot dusverre gebaseerd op kleine en geselecteerde cohorten, beschikken deze over onvoldoende gegevens van de kinderkankerbehandeling, zijn deze gebaseerd op enkel vragenlijsten, en is de follow-up kort. Daarom is de nationale prevalentie van metabool syndroom en van de losse componenten nog onbekend, en voor verschillende gehypothetiseerde risicofactoren, zoals overgewicht bij diagnose en behandeling met corticosteroïden, is het bewijs nog niet sluitend. Ook zijn betrouwbaardere (bio)markers voor metabool syndroom nodig, hoofdzakelijk omdat de buikomtrek een onderschatting geeft van overgewicht in survivors die behandeld zijn met abdominale radiotherapie. Derhalve kan in deze survivors de diagnose metabool syndroom worden gemist. Voorts wordt een mogelijke rol voor genetische predispositie verondersteld, omdat survivors die eenzelfde kankerbehandeling hebben ondergaan in verschillende mate te maken kunnen krijgen met metabool syndroom en de losse componenten. Vooralsnog zijn echter geen gerepliceerde genetische polymorfismen geïdentificeerd in survivors.

In onze single-center cohort studie in 103 survivors van nefroblastoom en neuroblastoom vonden we dat 14% van de survivors metabool syndroom had, al op

jonge leeftijd, en dat 33% ten minste twee metabool syndroom componenten had. Abdominale radiotherapie was significant geassocieerd met metabool syndroom en met de componenten hoge triglyceriden, laag high-density-lipoproteïne cholesterol en hypertensie. We zagen ook dat bijna geen enkele survivor de metabool syndroom component "grote buikomtrek" had, hetgeen opvallend is wanneer we de algemene populatie in ogenschouw nemen. Dit wordt mogelijk verklaard door behandeling met abdominale radiotherapie, en illustreert de behoefte aan betere (bio)markers om metabool syndroom te diagnosticeren. In deze studie observeerden we dat vier serum biomarkers nuttig kunnen zijn voor dit doeleinde: adiponectine, low-density-lipoproteïne cholesterol, apolipoproteïne B (apoB) en urinezuur. We vonden ook dat echografie van bloedvaten, wat vroege afwijkingen aan de vaten ten gevolge van metabool syndroom kan vaststellen, niet van toegevoegde waarde was voor het stellen van de diagnose metabool syndroom.

In onze systematische literatuur review over metabool syndroom biomarkers bleken vijf van de negen onderzochte biomarkers bruikbaar voor de diagnose en predictie van metabool syndroom, te weten urinezuur, leptine, high sensitivity C-reactief proteïne, adiponectine en apoB. Dit bewijs werd beoordeeld als van hoge of gemiddelde kwaliteit, en is vooral gebaseerd op studies in de algemene bevolking. Studies in survivors zijn schaars en hadden vaak een hoog risico op bias. Het is dus nog noodzakelijk om in survivors vast te stellen of deze biomarkers daadwerkelijk bruikbaar zijn.

We beschrijven in dit proefschrift de methodologie van de DCCSS-LATER Metabool syndroom studie, naar de prevalentie en klinische en genetische determinanten van metabool syndroom en van de losse componenten, en naar de diagnostische waarde van de DXA-scan en van biomarkers. Voor deze studie is het nationale cohort opgeroepen van de eerste in Nederland behandelde (tussen 1963 en 2002) survivors, hetgeen heeft geresulteerd in 2.338 deelnemende lange-termijn survivors. Dit proefschrift beschrijft de eerste resultaten van deze studie, op het gebied van de component overgewicht. Bijna de helft van alle lange-termijn survivors had overgewicht. Vergeleken met de algemene bevolking was de prevalentie van overgewicht significant hoger onder vrouwen van vijftig jaar en ouder, en was de prevalentie van morbide obesitas significant hoger onder mannen van vijftig jaar en ouder. Overgewicht, en voornamelijk obesitas en morbide obesitas, kwamen het meest voor onder survivors die een tumor van het centraal zenuwstelsel hadden gehad. Overgewicht bij kankerdiagnose, schedelbestraling en groeihormoondeficiëntie waren geassocieerd met overgewicht op de lange termijn, maar behandeling met corticosteroïden was dat niet. We hebben ook de prevalentie van overgewicht volgens verschillende methodes vergeleken. We vonden dat de methodes die gebruik maken van de buikomtrek overgewicht vaststelden in een substantieel aantal survivors met een normaal BMI (van wie het gewicht dus op basis van het BMI

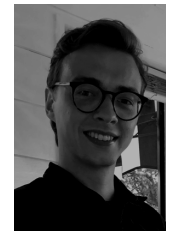
incorrect als gezond was geclassificeerd). DXA-scan was de meest gevoelige methode. Deze methode stelde overgewicht vast in 30% meer survivors, en zelfs in 40% in de subgroep van buikbestraalde survivors. Deze discrepantie werd eveneens vaker gezien in survivors die behandeld waren met totale lichaamsbestraling, anthracyclines en platinumhoudende chemotherapie. We zagen in deze studie ook dat high molecular weight-adiponectine geen toegevoegde waarde leek te hebben om overgewicht te diagnosticeren.

In de eerste genoombrede associatiestudie (GWAS) naar de metabool syndroom component dyslipidemie in drie grote survivor cohorten – de CCSS Original, St Jude Lifetime (SJLIFE) en CCSS Expansion cohorten – repliceerde geen van de negen SNPs die in de discovery analyse van het gehele CCSS Original cohort waren geïdentificeerd. Vervolgens identificeerden we rs114017774, gelegen op chromosoom 2, als een mogelijke genetische variant bijdragend aan het ontwikkelen van dyslipidemie, specifiek in schedelbestraalde survivors. Meta-analyse van de CCSS Original en SJLIFE cohorten toonde een gepoolde odds ratio van 11,30 (95% betrouwbaarheidsinterval 5,03-25,40 $p=4,5x10^{-9}$), maar dit locus repliceerde niet los in de CCSS Expansion en SJLIFE cohorten. Aanwezigheid van deze genetische variant toonde een interactie met craniale radiotherapie (genotypexCRT OR=14,61, 95%BI=4,59-56,55), welke onafhankelijk was van BMI. Deze variant wordt niet gerapporteerd in grote GWASs naar dyslipidemie in de algemene bevolking, hetgeen een survivor-specifiek effect kan suggereren. Gebaseerd op reeds beschikbare bronnen hypothetiseren we dat deze genetische variant mogelijk de expressie of functie verandert van *LRRTM4* of *CTNNA2* (twee genen die tot expressie komen in het brein en betrokken zijn bij ontwikkeling en functie van het zenuwstelsel), waarbij survivors vatbaarder zijn voor schade aan de hypothalamus ten gevolge van craniale radiotherapie, en daarbij een verhoogd risico hebben op dyslipidemie. Verdere replicatie en functionele validatie zijn noodzakelijk om de mogelijke relevantie van deze genetische variant verder te exploreren. In deze studie repliceerden we eveneens in het CCSS Original cohort de beschermende variant rs676210 in het *APOB* gen, welke eerder geassocieerd met dyslipidemie was bevonden in een studie onder survivors van acute lymfatische leukemie (ALL). Replicatie was significant in zowel de ALL subgroep als in het gehele cohort, wat indiceert dat deze variant uit de algemene bevolking tevens relevant is in survivors.

De standaarden voor follow-up van survivors kunnen wereldwijd verschillen, door het al dan niet beschikbaar zijn van richtlijnen hiervoor, door verschillen in culturele waardes, en door discrepanties in beschikbare middelen en expertise. We verkenden perspectieven op zorg voor survivors en onderzoeksprioriteiten onder zorgmedewerkers afkomstig van verschillende continenten en zagen dat, ongeacht land van oorsprong, psychologische ondersteuning, naast verschillende aspecten van

fysieke zorg, als prioriteit werd bestempeld. Dit onderschrijft de noodzaak van een goed georganiseerde aanpak, waarbij niet alleen aandacht voor somatische klachten is, maar ook voor psychologische zorg. Deze enquête stelde ook obstakels vast waar clinici in verschillende landen mee te maken hebben in hun poging gestructureerde zorg voor survivors op te zetten.

CURRICULUM VITAE



Vincent Pluimakers was born in Utrecht, The Netherlands, on February 25, 1991. He graduated from secondary school (Christelijk Gymnasium, Utrecht) in 2008. This was followed by his study at the faculty of medicine of Utrecht University and the University Medical Center Utrecht, for which he obtained his degree in 2016. After working as an internal medicine resident not in training for one year (Gelderse Vallei, Ede) he joined the research group of prof. dr. van den Heuvel-Eibrink for his PhD project at the Princess Maxima Center for Pediatric Oncology, also under supervision of dr. Neggers and dr. Janssens. The results of his research are presented in this thesis. In September 2021, he started his residency training in internal medicine in Gelre, Apeldoorn and University Medical Center Utrecht. He lives in Utrecht with his wife and their two children.

PHD PORTFOLIO

Name:	Vincent Pluimakers
PhD period:	March 2017 – December 2022
Research school:	Clinical and Translational Oncology, Utrecht University
Department:	Princess Maxima Center for Pediatric Oncology
Promotor:	Prof. dr. M.M. van den Heuvel-Eibrink
Copromotors:	Dr. S.J.C.M.M. Neggers
	Dr. G.O.R. Janssens

PhD training	Year	ECTS
Courses		
NFU Basiscursus Regelgeving en Orgnisatie voor Klinisch Onderzoekers	2017	3.0
Clinical and Translational Oncology course, Utrecht University	2017	2.0
Basic Human Genetics, Erasmus MC	2018	1.0
Genome wide association studies, Erasmus MC	2019	3.0
SNPs and human diseases, Erasmus MC	2019	3.0
Writing a scientific paper, Utrecht University	2019	4.0
Practical Biostatistics, Amsterdam UMC	2019	4.0
Statistical learning, Stanford University	2020	4.0
Best practices for writing reproducible code, Utrecht University	2021	1.5
The art of presenting science, Utrecht University	2021	4.0
Regression analysis, Leiden UMC	2021	3.0
Seminars		
Presentations at research seminars of Princess Maxima Center and Erasmus MC	2017-21	0.4
Conferences		
International Symposium on Late Complications after Childhood Cancer, Atlanta	2017	2.0
PanCare, Prague	2018	1.5
International Society of Pediatric Oncology (SIOP), Kyoto	2018	2.0
International Society of Pediatric Oncology (SIOP), Lyon (poster discussion)	2019	2.5
International Society of Pediatric Oncology (SIOP), digital	2020	1.5
American Society of Hematology (ASH), hybrid	2020	2.0
American Society of Hematology (ASH), hybrid (poster presentation)	2021	2.0
Other activities		
Supervision of bachelor honours programme student	2018-20	6.0
Article peer review for Cancer and European Journal of Endocrinology	2020	1.0

LIST OF PUBLICATIONS

THIS THESIS

Pluimakers VG, Van Waas M, Neggers SJCMM, Van den Heuvel-Eibrink MM. Metabolic syndrome as cardiovascular risk factor in childhood cancer survivors.
Critical Reviews in Oncology/Hematology, 2019 Jan;133:129-141

Pluimakers VG, Van Waas M, (et al.), Van den Heuvel-Eibrink MM, Neggers SJCMM. Metabolic syndrome detection with biomarkers in childhood cancer survivors.
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Pluimakers VG, Van Santen SS, (et al.), Van den Heuvel-Eibrink MM, Neggers SJCMM. Can biomarkers be used to improve diagnosis and prediction of metabolic syndrome in childhood cancer survivors? A systematic review.
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Pluimakers VG, Fiocco M, (et al.), Van den Heuvel-Eibrink MM, Neggers SJCMM. Metabolic syndrome parameters, determinants, and biomarkers in adult survivors of childhood cancer: protocol for the Dutch Childhood Cancer Survivor Study on Metabolic Syndrome (Dutch LATER METS).
JMIR Research Protocols, 2021 Jan;10(1):e21256

Pluimakers VG, Van Atteveld JE, (et al.), Van den Heuvel-Eibrink MM, Neggers SJCMM. Prevalence, risk factors and optimal way to determine overweight, obesity and morbid obesity, in the first Dutch cohort of 2,338 very long-term survivors of childhood cancer: a DCCSS-LATER study.
Submitted

Pluimakers VG, Broer L, (et al.), Yasui Y, Van den Heuvel-Eibrink MM. Genetic susceptibility to treatment related dyslipidemia in adult long-term survivors of childhood cancer: a report from the Childhood Cancer Survivor Study and the St Jude Lifetime cohorts.
In preparation

Bakker ME, Pluimakers VG, (et al.), Ishida Y, Maeda M. Perspectives on follow-up care and research for childhood cancer survivors: results from an international SIOP meet-the-expert questionnaire in Kyoto, 2018.
Japanese Journal of Clinical Oncology, 2021 Oct 5;51(10):1554-1560

OTHER PROJECTS

Van Atteveld JE, De Winter DTC, Pluimakers VG, (et al.), Van den Heuvel-Eibrink MM, Dutch LATER study group. Risk and determinants of low and very low bone mineral density and fractures in a national cohort of Dutch adult childhood cancer survivors (DCCSS-LATER): a cross-sectional study.
Lancet Diabetes & Endocrinology, 2023 Jan;11(1):21-32

Van Atteveld JE, De Winter DTC, Pluimakers VG, (et al.), Van den Heuvel-Eibrink MM, Dutch LATER study group. Frailty and sarcopenia within the earliest national Dutch childhood cancer survivor cohort (DCCSS-LATER): a cross-sectional study.
Lancet Healthy Longevity, 2023 Apr;4(4):e155-e165

Bolier M, Pluimakers VG, (et al.), Van den Heuvel-Eibrink MM, Neggers SJCMM. Prevalence and determinants of dyslipidemia in a national cohort of 2,338 Dutch childhood cancer survivors: a DCCS-LATER Study.
Submitted

Man WH, van Dongen TMA, Venekamp RP, Pluimakers VG, (et al.), Schilder AGM, Bogaert D. Respiratory microbiota predicts clinical disease course of acute otorrhea in children with tympanostomy tubes.
The Pediatric Infectious Disease Journal, 2019 Jun;38(6):e116-e125

DANKWOORD

Utrecht, mei 2023

Hèhè, zes jaar en twee maanden sinds de start mag ik dan toch eindelijk dit laatste onderdeel van mijn proefschrift op papier zetten. Het was een avontuur dat mij bracht in het Erasmus, het UMCU, het WKZ en het indrukwekkende Prinses Máxima Centrum dat haar deuren opende; ik had het voorrecht Atlanta, Praag en het prachtige Kyoto te bezoeken. Het was ook een mentale reis, van mezelf tegenkomen en wedergeboortes, toen ik de taaie aspecten van het onderzoek doen leerde kennen. Er was de eindfase waar ik zelf haast metabool syndroom van kreeg en waarin ik mij bij vlagen Sisyphus met zijn onmogelijke opgave waande. Ik ben blij en opgelucht dat ik nu met gepaste trots door het eindresultaat scroll en kan terugblikken op alle mooie aspecten. Wat ik allemaal heb geleerd over *late metabolic consequences of childhood cancer*. De toffe mensen waarmee ik heb mogen samenwerken. Mezelf verrijken met kennis in diepgaande cursussen. Informatie die verborgen ligt in een dataset naar het daglicht brengen, een hele dag ploeterend op een script tot het eureka moment waarop alles werkt. Al doende de medische wetenschap een heel klein beetje verder proberen te helpen. Echte weekenden hebben, en de tijd om een keer een koffie te pakken en te bedenken waar je naartoe wil met je leven.

Uiteraard is dit ook en vooral het moment om mensen te bedanken. De komende pagina's zijn voor jullie.

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Dit kan ik zeggen omdat ik mij geflankeerd weet door twee uit het juiste hout gesneden paranimfen. Jenneke, onze complementaire samenwerking van de afgelopen jaren bezegelen we uiteraard met elkaars paranimfschap. Sinds de memorabele uitputtingsslag naar het congres in Atlanta hebben we alle PhD-pieken en -dalen gedeeld. We pepten elkaar op als we om en om betwijfelden of we ons proefschrift ooit af zouden krijgen. We hadden gedeelde dus halve smart als we ons weer eens hadden laten misleiden door laaghangend fruit. Je hebt een jaloersmakende werkdrive, en als ik verzandde in een R-script hield je me bij de les met een scherp "Maar wat wil je hier nou mee zeggen?". Jordy, toen je je avontuurlijke academische hart volgde naar Australië sprak ik het voornemen uit je als paranimf te vragen als je weer in Nederland zou zijn tijdens mijn verdediging, en ik ben verheugd dat dit is uitgekomen nu we weer vlakbij elkaar wonen. Het zal wel even wennen zijn om in serieuzere context dan vroegah in rokkostuum naast elkaar te staan. Voor de verdere dankbetuiging verwijs ik je naar een paar alinea's verder.

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het werk dat je had verricht aan de stukken waar ik als jouw opvolger mee verder ging, en die nu als hoofdstuk 1 en 2 in mijn proefschrift prijken.

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Veel dank gaat uit naar iedereen die aan de LATER-studie heeft gewerkt. Wat een klus hebben we met zijn allen verricht. In willekeurige volgorde…Maaike, Felice, Gerda, Andrica, Jenneke, Inge, Esmee, Selvetta, Lisa, Shauny, Else, Tarik, Heleen, Kim, Ardine, Marry, Manita, Marjo, Suzanne, Dominique, Luciënne, Manon, Jolande, uiteraard het CB - Margriet, Nynke, Lennart en Anke - voor de coördinatie, maar ook alle planners, poli-assistentes, prikkers, functielaboranten, werkstudenten, andere promovendi, de PI's en teams uit andere ziekenhuizen. Met ons studieteam hebben we drie keer de studielogistiek uitgevogeld op de poli's. We hebben talloze brieven gepost, buizen gestickerd en dagprogramma's gemaakt. We onderzochten deelnemers op een stoel of achter een kamerscherm toen er ruimtegebrek was. Er was het eerste ECG-apparaat waarvan de zuignappen al losschoten voordat de laatste erop zat. We worstelden met vragen over parkeerkosten en eigen risico. We zwoegden op query's van het CB als er een monitor visit aankwam. Er was het pijnlijke realisatiemoment dat we materiaal hadden vernietigd dat bewaard had moeten worden. De deadlines en te halen targets hingen soms als een zwaard van Damocles boven ons hoofd. Tijdens dit alles hielden we de moed erin met elkaar en was er altijd ruimte voor een praatje en een lach en daarom hebben we er elke keer een geoliede machine van weten te maken en de klus geklaard.

Mijn kamergenoten waren ook onmisbaar. We begonnen als de Q02-matties, in een UMCU-uithoek met precaire koffievoorziening tot we gelukkig ons eigen Nespresso apparaat aanschaften, en waar we nog wel eens het hoofd moesten bieden aan een stoel gooiende chirurg met territoriumdrift. Daarna mochten we ons intrede doen in het Máxima, eerst knus op de LATER-poli en vervolgens in het penthouse in het research gebouw. Dank voor de gezelligheid op de kamer, voor de aandacht voor elkaars onderzoeks- en privézaken, voor de frequente vrijmibo's. Tijdens Covid was het samen thuiswerken met Jenneke en Joeri waardevol. Het was leuk, leerzaam en stimulerend om met een aantal van jullie - Selvetta, Jenneke, Demi en Melissa - samen te werken aan papers.

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Wouter, mijn interesse voor het doen van een promotieonderzoek werd aangewakkerd toen ik stage deed in je onderzoeksgroep. Je was de afgelopen jaren altijd enthousiast paraat als vraagbaak voor mijn R-problemen en dat was niet alleen buitengewoon prettig maar ook erg leuk om samen over te sparren. Net zo belangrijk zijn onze privé afspraken met Nynke voor een lekkere borrel en een bordspelletje.

Goede buren, wat ben ik blij dat ik de afgelopen jaren heb leren kennen wat er wordt bedoeld met deze term in het spreekwoord. Skihutfeestjes met de babyfoons op het aanrecht, Squashfligem-avondjes, koken voor elkaar, tuinborrels terwijl we toekijken

hoe onze kinderen met elkaar spelen, de praatjes op straat en bij het ophalen van pakketjes waarmee we elkaar overladen - het voelt hier echt als thuiskomen.

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Beste Thanos, we hebben in de jaren dat ik les bij je had tijdens mijn promotieonderzoek een prachtige muzikale reis afgelegd. Het was heerlijk om samen in een stuk te duiken en tot in detail te bediscussiëren wat de componist bedoeld heeft. Het vormde ook een prettige afleiding tijdens het pittigste stuk van mijn promotietraject. Ik wil zeker spoedig weer contact met je opnemen om het muziek maken te hervatten.

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Marieke en Maurits, hoewel jullie ruim de leeftijd voorbij zijn dat ik mijzelf al heel wat vond blijft het soms gek om mijn "kleine" zusje en broertje aan de slag te zien. Samenwonen, werken, een boek publiceren, een ton ophalen voor een goed doel, een huis bouwen, emigreren. Het twee-tegen-één sarren cq de hersens inslaan van weleer heeft plaatsgemaakt voor een fatsoenlijke brussen-relatie, en dat we zo nu en dan iets doen met alleen ons drieën vind ik erg leuk. Hopelijk weten we dit straks trans-Atlantisch op een manier vol te houden. Ook de al lang niet meer koude kant, Dorus en Charlotte, dient genoemd te worden. Wanneer we met zijn allen samenzijn heb ik een echt gezellig familiegevoel.

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In welke volgorde bedank je de belangrijkste mensen in je leven? Gelukkig heeft de wetenschap een oplossing gevonden voor deze kwestie: de gedeelde laatste plaats.

Lieve Bram, de echte reden dat ik zo lang over mijn proefschrift heb gedaan is natuurlijk dat ik jou erin wilde vermelden. Ik heb geen idee hoe kort of lang dit je kenmerkende eigenschappen gaan zijn, maar wat geniet ik van je knuffelbaarheid en van je vertederende gekir als ik je heen en weer schud. Ik waardeer je slaaprijke nachten en hoe je (meestal) bescheiden op je beurt wacht als je honger hebt terwijl ik bezig ben met je zus. Je herstelvermogen als ze je al te enthousiast een kopstoot verkoopt of een boek in je gezicht gooit. Ik kan niet wachten tot je de wereld verder gaat verkennen.

Lieve Nine, wat smelt ik van het dolle enthousiasme waarmee je me, bedekt in yoghurt, begroet als ik thuiskom na een werkdag. Ik geniet van hoe je net zo razend vrolijk kan worden van een schommel, dierenplaatje of iets dat blauw!-blauw!-blauw! is. Van hoe ik mijn lachen niet kan inhouden als ik je eigenlijk moet corrigeren om een streek. Van hoe liefdevol en vrij van jaloezie je bent naar Bram. Van je dansmoves die nu al beter zijn dan de mijne. Van het samen op lieveheersbeestjesmissie gaan. Ik kijk uit naar alle avonturen die nog komen.

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