BONE TOXICITY AND ACCELERATED AGING DUE TO CHILDHOOD CANCER TREATMENT

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Bottoxiciteit en vervroegde veroudering door behandeling voor kinderkanker

(met een samenvatting in het Nederlands)

Proefschrift

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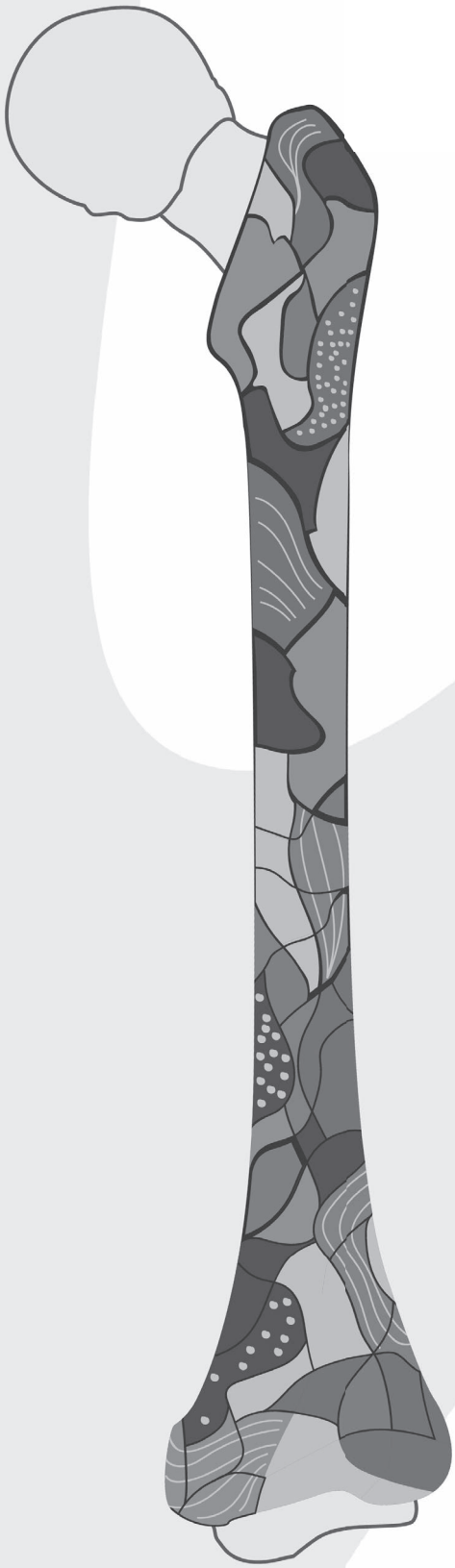
"Cancer begins and ends with people. In the midst of scientific abstraction, it is sometimes possible to forget this one basic fact...."

— **Siddhartha Mukherjee, The Emperor of All Maladies**

CONTENT



CHAPTER 1

General introduction

CHILDHOOD CANCER

Annually, about 550 to 600 children below the age of 19 years are diagnosed with cancer in the Netherlands.[1] The most common type of childhood cancer is leukemia (in particular acute lymphoblastic leukemia), which represents one quarter to one third of all childhood cancer diagnoses (Figure 1). Central nervous system tumors are the most often diagnosed solid tumors (22%).

Cancer remains the leading cause of death by disease for children.[2] However, substantial progress has been made over the past decades. Surgery and radiotherapy dominated the field of cancer therapy until chemotherapy entered clinical practice in the 1960s.[3] Since then, the survival of childhood cancer has considerably increased as a result of improved treatment regimens (including targeted stratification) and enhanced supportive care strategies (Figure 2). In the early 1970s, the 5-year survival rate for childhood cancer overall was about 40% in high-income countries,[4] while currently, this rate approximates 80%.[5] The increase in survival for children with acute lymphoblastic leukemia is even more spectacular. In 1972, the 5-year event-free survival for childhood acute lymphoblastic leukemia in the Netherlands was 4%,[6] whereas this now exceeds 90%.[7] This improved survival has led to a continuously growing population of childhood cancer survivors. It is estimated that about one in 530 young adults aged 20 to 40 years is currently a childhood cancer survivor.[2] However, even after five years of survival, the all-cause mortality rate of childhood cancer survivor is higher compared to the general population, mainly due to disease- and treatment-related mortality.[8] Apart from that, childhood cancer survivors often experience long-term morbidity as a result of the cancer, its treatment, or its consequences. Hence, as survival rates continue to increase, scientific efforts have expanded to minimize these adverse effects, and optimize quality of life.

ACUTE AND LONG-TERM TOXICITY

Childhood cancer treatment does not merely damage tumor cells, but affect healthy cells as well. As a result, children with cancer are at risk of both acute and long-term sequelae that may adversely affect their health.[9] As acute toxicity may contribute to long-term sequelae, it is important to consider these side effects as a continuum. Twenty-five years after cancer diagnosis, about 75% of survivors have at least one adverse effect, and 25% of survivors have five or more adverse effects.[10,11] These adverse effects occur in virtually all organ systems, and lead to excess mortality (independent of primary tumor progression or recurrence)[8] and impaired quality of life.[12] However, it is important to realize that most long-term

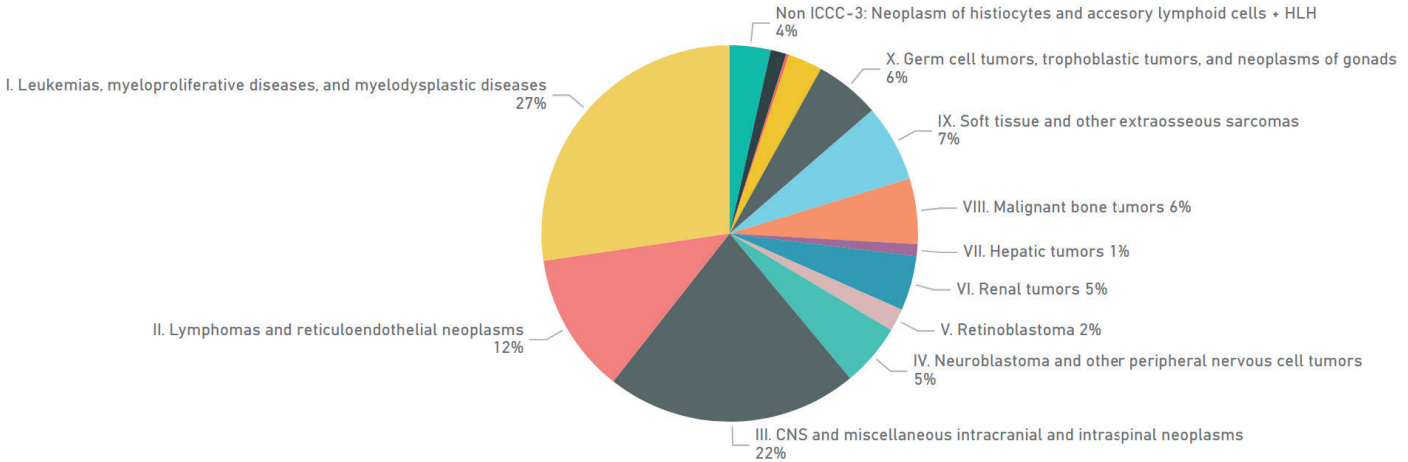


Figure 1. Distribution of childhood cancer diagnoses in the Netherlands between 2003 and 2020 according to the ICCC-3 classification. Dutch Childhood Oncology Group national registry (with permission).

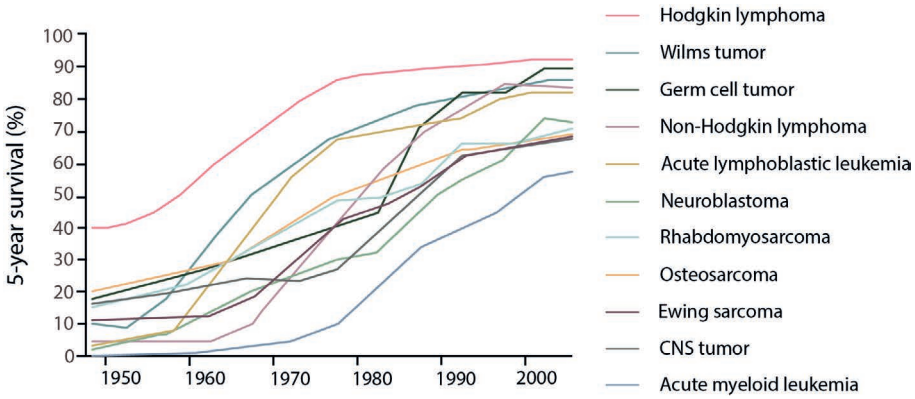


Figure 2. Survival rates by childhood cancer type between 1950 and 2000 in Germany. Figure adapted from the German childhood oncology registry (Deutsches Kinderkrebsregister Kompetenznetz Pädiatrische Onkologie/Hämatologie, kinderkrebsinfo.de).

survivor studies included the earliest treated survivors. Since then, several harmful treatments have already been successfully reduced or omitted without jeopardizing cancer outcomes.[13] For example, it has been scientifically confirmed that prophylactic cranial irradiation, which has long been standard treatment for children with acute lymphoblastic leukemia, can safely be replaced by intrathecal and systemic chemotherapy.[14] The number of children treated with radiotherapy for Hodgkin's lymphoma or Wilms tumor have also been significantly reduced over the years, and radiation fields and types have changed.[13] These adaptations have already led to a reduction in long-term toxicity in more recent childhood cancer survivors.[15] Currently, risk stratification is based on risk of treatment failure (including clinical as well as molecular cancer characteristics) as well as on initial treatment response. This allows less intensive treatment to be administered in low-risk patients, as well as more personalized and targeted cancer treatment, which has in general become the cornerstone of childhood cancer treatment.[16]

Improving the balance between optimal survival and optimal quality of life is an ongoing, continuous process. Many treatment modalities with a significant toxicity profile, such as a total body irradiation-based conditioning for allogeneic hematopoietic stem cell transplantation, remain essential for optimal survival.[17] Furthermore, current novel treatment strategies (e.g. targeted therapy) can also contribute to health problems. Therefore, it remains important to monitor toxicity in children with cancer during treatment, and to follow survivors long into adulthood and beyond, especially as certain late effects may only become apparent years after treatment.

ENDOCRINE ADVERSE EFFECTS

Central dysfunction

Endocrine disorders are among the most frequently observed conditions in childhood cancer survivors.[11] More than half of the survivors will experience at least one endocrine disorder over the course of their lives.[18] The hypothalamus and pituitary represent the center of the endocrine system (Figure 3). Hypothalamic-pituitary dysfunction may include growth hormone deficiency, central precocious puberty, luteinizing hormone/follicle-stimulating hormone deficiency, central diabetes insipidus, adrenocorticotropic hormone deficiency, or thyroid-stimulating hormone deficiency. In the context of childhood cancer, hypothalamic-pituitary dysfunction can occur as a result of tumor growth, local surgery, or radiotherapy directed to the cranium. As opposed to tumor growth or surgery, which result in immediate hypothalamic-pituitary problems, hypothalamic-pituitary dysfunction due to radiotherapy may become apparent many years after treatment.[19] The growth hormone axis is the most radiosensitive pituitary axis, which is reflected by the fact that growth hormone deficiency is the most prevalent deficiency among childhood cancer survivors.[20] There does not seem to be a "safe" dose, as growth hormone deficiency is already occasionally observed in children treated with cranial irradiation doses of 18-24 Gy for acute lymphoblastic leukemia, and even after total body irradiation with doses of 10 Gy.[21] Damage to the hypothalamic-pituitary axis affects not only the growth hormone axis, but may also affect thyroid, adrenal, as well as gonadal function.[21] These other pituitary deficiencies generally occur only after radiation doses higher than 22 or 30 Gy.[20] As such, hypothalamic-pituitary dysfunction is detrimental itself, but can drive many other (endocrine) disorders as well.

Primary dysfunction

The peripheral endocrine glands (Figure 3) can also be directly affected by childhood cancer or its treatment. Primary hypogonadism can be the result of ovarian or testicular function damage due to local tumors, abdominal/pelvic surgery, radiation directed to the ovaries or testes, or alkylating agents.[22] The thyroid gland may become both underactive and overactive after childhood cancer treatment. Neck and craniospinal irradiation, as well as ^{131}I-MIBG treatment and tyrosine kinase inhibitors may lead to hypothyroidism, whereas neck and craniospinal irradiation are (to a lesser extent) also associated with hyperthyroidism.[18] In addition, the parathyroid glands may be affected by neck irradiation causing hyper- and hypoparathyroidism.[23,24] Finally, survivors treated with abdominal or total body irradiation have an increased risk of diabetes mellitus following endocrine pancreatic insufficiency.[25]

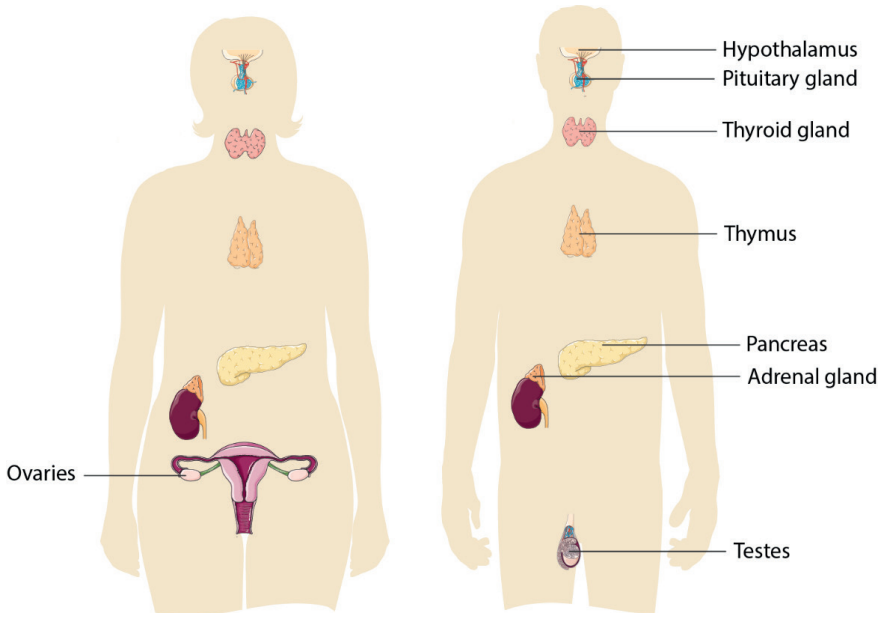


Figure 3. Overview of the endocrine system. Illustration made by JvA using Servier Medical Art, licensed under a Creative Commons Attribution 3.0 Unported License.

BONE PHYSIOLOGY

Bone anatomy and physiology

Bone is comprised of cells that are located in a matrix of organic protein and inorganic mineral.[26] Bone cells can be divided into osteoblasts, osteoclasts, and osteocytes. Osteoblasts are derived from mesenchymal stem cells (Figure 4) and synthesize the osteoid matrix and regulate its mineralization (bone formation). On the contrary, osteoclasts have the capacity to resorb bone. These cells are derived from hematopoietic precursors of the macrophage lineage. Osteocytes are terminally differentiated bone cells derived from osteoblasts and are most commonly found in bone tissue. They integrate mechanical and chemical signals from their environment to regulate both bone formation and resorption.[27] Type I collagen is the main protein of the bone matrix, whereas calcium and phosphate are the main minerals.[26]

There are two types of bone with different functions.[26] Cortical (compact) bone constitutes the shaft of long bones and the outer shell of flat bones (Figure 5). As it is formed of concentric rings of bone, it is particularly adapted to withstand bending strain. Trabecular (spongy) bone is located inside flat bones such as the

vertebrae as well as at the ends of long bones and mainly offers resistance to compressive loads. The trabecular bone compartment is the metabolically active part of bone where bone remodeling takes place, which involves the removal of mineralized bone by osteoclasts followed by the formation of bone matrix through osteoblasts.[28] Bone remodeling continues throughout life and serves to adjust bone architecture, repair microdamages in the bone matrix, and plays an important role in mineral homeostasis. Bone modeling is a distinct process that is needed for appositional growth (i.e. growth of bone in width).[29] This type of growth takes place by periosteal apposition of new bone by osteoblasts and endosteal resorption of old bone by osteoclasts. Longitudinal growth (i.e. growth of bone in length) occurs at the epiphyseal growth plate located between the epiphysis and metaphysis. This occurs by endochondral ossification, a process which creates novel trabeculae until the epiphyseal plate fuses during puberty.

Bone mineral density

Bone mass is acquired during childhood, adolescence, and young adulthood, until peak bone mass is achieved between the age of 20 to 30 (Figure 6).[30] Males generally have a higher amount of peak bone mass and attain peak bone mass later than females.[31] Dual-energy X-ray absorptiometry (DXA) is the golden standard for bone mineral density assessment.[32] The transmission of low-dose X-rays

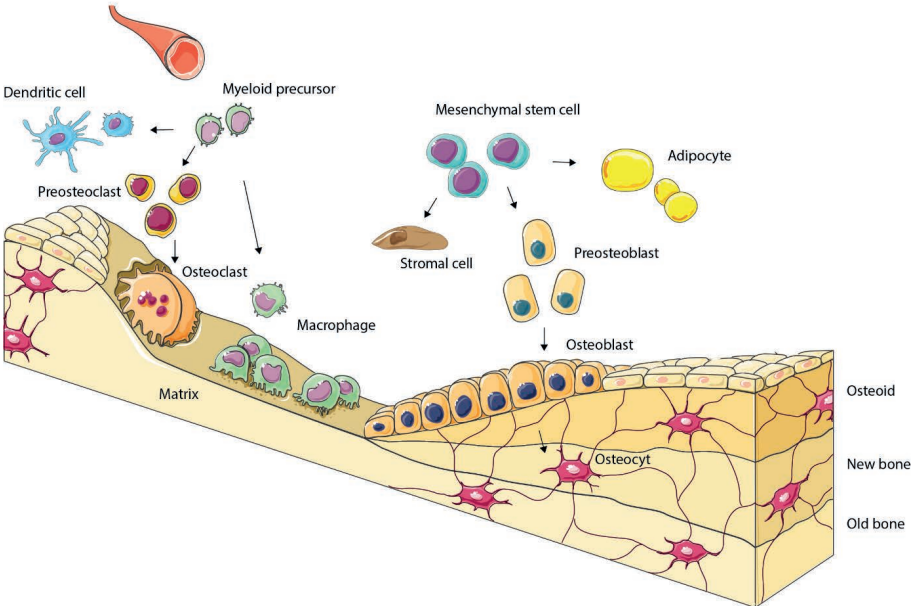


Figure 4. Schematic overview of bone cell differentiation in the bone marrow niche. Illustration made by JvA using Servier Medical Art, licensed under a Creative Commons Attribution 3.0 Unported License.

with high- and low-energy photons is used to measure the density of bones and other tissues (i.e. fat and lean mass).[33] Bone mineral density reflects the amount of calcium, phosphate, and other minerals in a certain area of bone tissue. The posterior-anterior lumbar spine (L1 to L4) and hip are typically evaluated in adults, whereas the lumbar spine and total body less head are measured in children.[32] Bone mineral density is then compared with normative values, which is expressed as a T-score and a Z-score. A T-score represents the number of standard deviations that bone mineral density differs from the young adult (20-29 years) normative female mean. This reflects the measured bone mineral density compared to normal peak bone mass. A Z-score represents the number of standard deviations that bone mineral density differs from age- and sex-matched normative means, which reflects bone mineral density compared to healthy peers.

Bone mineral density decline over time

Osteoporosis is a systematic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, which leads to diminished bio-mechanical competence of the skeleton and low-trauma or atraumatic fractures.[33]

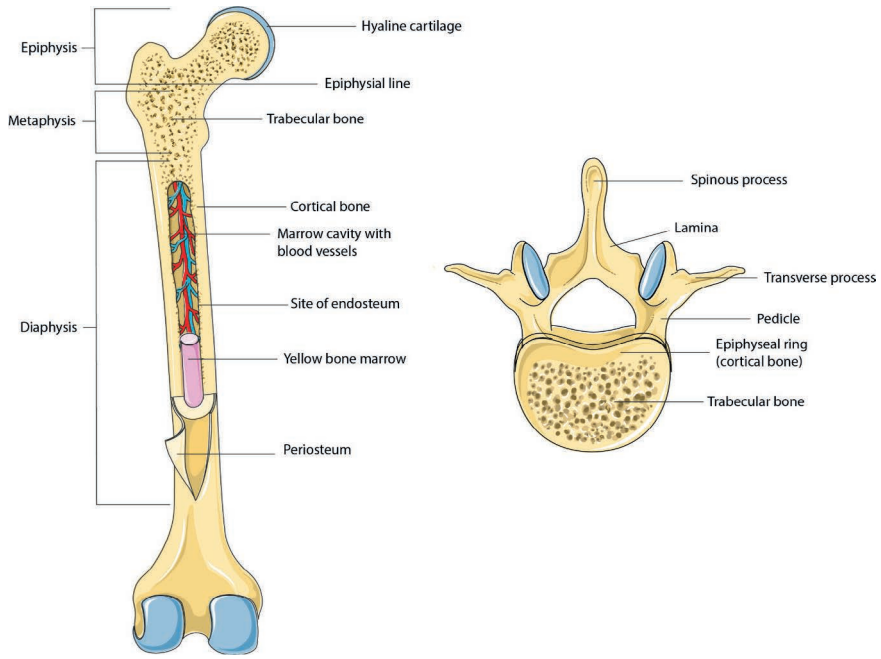


Figure 5. The anatomy of long bones and vertebrae. Illustration made by JvA using Servier Medical Art, licensed under a Creative Commons Attribution 3.0 Unported License.

In adults older than 40 years, osteoporosis is defined based on DXA-based areal bone mineral density alone according to the World Health Organization, i.e. a T-score below -2.5 standard deviations.[34] In children and adolescents however, the skeleton must be proven fragile in order to diagnose osteoporosis, as indicated by the presence of a vertebral fracture or a clinically significant fracture history (i.e. two or more long bone fractures by the age of 10 years, or three or more long bone fractures at any age up to the age of 19 years).[32] This is because the association between low bone mineral density and fractures is less evident in young individuals. In the absence of a vertebral fracture, a bone mineral density Z-score below -2 standard deviations is needed in addition to a clinically significant fracture history to diagnose osteoporosis in children and adolescents. Z-scores are used in this age group as they have not yet achieved peak bone mass. For young adults, the definition of osteoporosis is less clear. For those with delayed puberty, which often happens as a result of chronic diseases from childhood, peak bone mass is generally attained later and therefore, the pediatric definition can still be used.[35] The international Society of Clinical Densitometry proposes to keep using Z-scores to define low bone mineral density for all young adults.[32] However, the International Osteoporosis Foundation proposes to use the T-score-based definition from the World Health Organization that is used for older adults as well.[35] The presence of a vertebral compression fracture is always indicative of osteoporosis (in the absence of local disease or high-energy trauma).[32]

Attaining optimal peak bone mass is important for bone health later in life, as suboptimal peak bone mass has been associated with an increased risk of osteoporosis as well as an earlier onset of osteoporosis.[30] Genetic variation accounts for the majority of variability in peak bone mass.[36] However, lifestyle choices and (chronic) diseases, especially during the critical period of bone mass acquisition, can significantly impact the amount of peak bone mass as well (Figure 6). In addition, individuals with endocrine disorders, which frequently occur in childhood cancer survivors, generally attain peak bone mass later.[37]

BONE TOXICITY

Childhood cancer as well as its treatment and their consequences can affect normal bone physiology, leading to low bone mineral density and fractures early in life. In addition, a different bone toxicity, i.e. osteonecrosis, can occur during childhood cancer treatment.

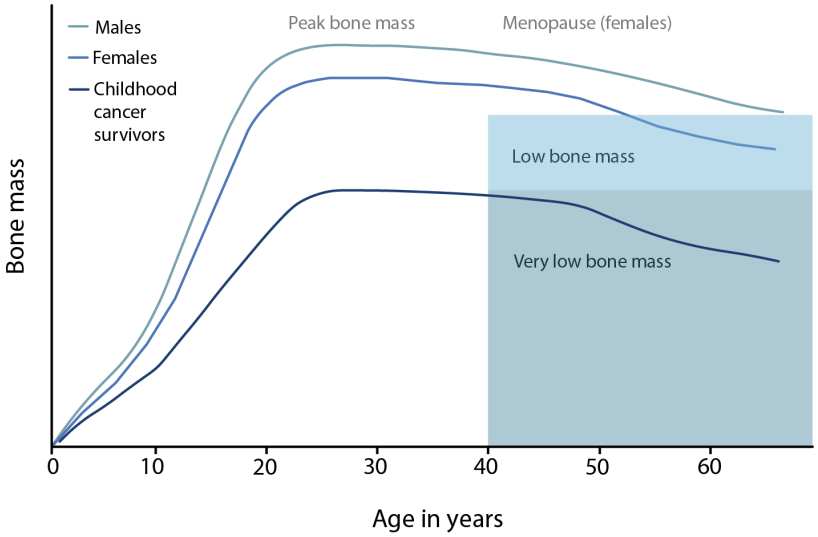


Figure 6. The course of bone mass during life.

Low bone mineral density as a consequence of childhood cancer

Childhood malignancies and their treatments are diseases that negatively affect bone health.[38] Particularly children with leukemia can already present with low bone mineral density at cancer diagnosis,[39] as leukemic cells occupy the bone marrow and increase osteoclast activity via cytokines.[40] In addition, many treatment components such as corticosteroids and (cranial) irradiation have detrimental effects on bone mineral density. Corticosteroids affect bone tissue directly by inhibiting mesenchymal stem cell differentiation into osteoblasts, as well as by increasing osteoblast apoptosis, leading to decreased bone formation.[41] At the same time, bone resorption is transiently increased through increased osteoclastogenesis. Furthermore, corticosteroids alter calcium homeostasis by reducing intestinal calcium absorption and increasing renal calcium excretion.[42] Other chemotherapeutic agents such as asparaginase and methotrexate may additionally contribute to bone mineral density decline by impairing osteoblast function. The direct effects of chemotherapeutics on bone are for example reflected by a decrease in bone mineral density and increased fracture risk during treatment for acute lymphoblastic leukemia.[43–45] However, treatment modalities for childhood cancer can impact bone mineral density years after treatment cessation as well. For instance, treatment with cranial irradiation may result in several endocrine deficiencies such as hypogonadism and growth hormone deficiency, which are known to impair bone density.[38] These deficiencies can occur soon after treatment, especially after high-dose cranial irradiation, but also many

years later after lower doses.[46] In addition, as in the general population, several indirect consequences of childhood cancer such as malnutrition, low body mass index, and inactivity may reduce bone mineral density.[30,47]

Low bone mineral density (Z-score ≤-1) is found in approximately 40%-50% of long-term childhood cancer survivors, and very low bone mineral density (Z-score ≤-2) in about 10%-20% of survivors, respectively.[48,49] These frequencies are high, and indicate an increased risk for survivors when compared with normal distribution. Prevention of impaired BMD would be ideal, for example by supplementing vitamin D during cancer treatment. However, it is unclear whether this intervention would enhance bone strength. In addition, the course of bone mineral density from cancer diagnosis through long-term survivorship is still not clearly understood. Although many studies have reported on risk factors for low bone mineral density in childhood cancer survivors, it remains unclear which specific group of childhood cancer survivors is at highest absolute risk of low bone mineral density and could benefit from bone mineral density surveillance. A simple prediction model for low bone mineral density would be of value to provide insight into this. Furthermore, internationally harmonized guidelines for bone mineral density surveillance among childhood cancer survivors are lacking.

Fractures as a consequence of childhood cancer

Bone mineral density determines about 60-70% of someone's bone strength in the general elderly population.[50] However, in children, adolescents, and young adults, the relationship between bone mineral density and fractures is less clear. For children with cancer, this has been investigated best in children with acute lymphoblastic leukemia. It has been shown that children with acute lymphoblastic leukemia have a 6-fold increased risk of fractures,[43] and that low bone mineral density is significantly associated with incident vertebral and non-vertebral fractures during and shortly after therapy.[39,44] However, in childhood cancer survivors, the proportion of survivors with a fracture over their lifetime was similar when compared with siblings,[51] and the predictive value of low bone mineral density during or after treatment for fracture risk remains unknown. Only few studies have assessed risk factors for fractures in childhood cancer survivors using multivariable models, and showed that male sex, treatment with methotrexate, and smoking significantly increased fracture risk.[51,52] In addition, one study has thus far assessed the predictors of prevalent vertebral fractures in childhood cancer survivors, which included male sex, higher cumulative corticosteroid dose, and back pain.[53] Large, national childhood cancer survivor cohorts are needed to further elucidate the risk of, and risk factors for fractures in this population.

Osteonecrosis as a consequence of childhood cancer

Besides affecting bone mineral density, childhood cancer and its treatment can also impair bone health in other ways. Osteonecrosis (previously also referred to as avascular necrosis), which literally means bone death, is a severe complication that mainly occurs during or shortly after treatment for acute lymphoblastic leukemia.[54] The exact pathophysiology is not yet clearly delineated, but multiple mechanisms are thought to contribute to its development. Intravascular microembolisms in the joint regions, increased bone marrow pressure, and direct vascular injury may (together) lead to an impaired blood flow to the bones, which induces necrosis.[55] Glucocorticoids are the main underlying factor of osteonecrosis in the context of acute lymphoblastic leukemia, in particular because they cause hyperlipidemia.[56] Consequently, lipid emboli may develop and obstruct blood vessels, resulting in ischemic necrosis.[57] In addition, corticosteroid administration (especially in combination with concurrent asparaginase), leads to a hypercoagulable state, which increases the tendency to develop emboli as well.[58] Furthermore, corticosteroids stimulate adipogenisis at the expense of osteoblastogenisis in bone marrow mesenchymal stem cells.[59] This leads to lipid accumulation in the bone marrow, resulting in increased medullary pressure. However, as osteonecrosis can already be present in acute lymphoblastic leukemia patients at first presentation, leukemia itself conceivably plays a role as well.[60]

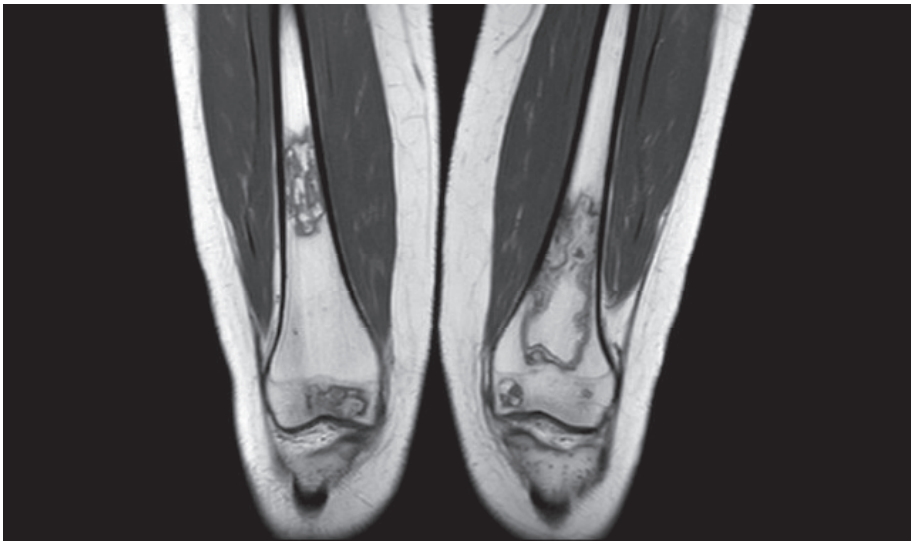


Figure 7. MRI scan showing osteonecrosis in the femoral diaphysis, metaphysis, and epiphysis.

Grade 1	Asymptomatic with findings only by MRI.
Grade 2	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions only outside joint lines in non-weight-bearing bones.
Grade 3	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions in weight-bearing bones or affecting joint lines in non-weight-bearing bones.
Grade 4	Symptomatic with deformation by imaging of one or more joints and/or substantially limiting self-care activity of daily living.

Figure 8. The Ponte di Legno toxicity working group grading of osteonecrosis.

Osteonecrosis may be symptomatic or asymptomatic. Patients with symptomatic osteonecrosis suffer from (severe) pain, immobility, limitations in activities of daily living, and sometimes even articular collapse.[61] Magnetic resonance imaging (MRI) is the golden standard to detect osteonecrosis (Figure 7), but severe osteonecrosis can be diagnosed with an X-ray as well.[62] Osteonecrosis is usually a multifocal disease, with the knees and hips being most commonly affected.[63] Several clinical and radiological staging methods to define the severity of osteonecrosis have been developed over the years. The most recent *clinical* staging method was proposed by the Ponte di Legno Toxicity Working Group (Figure 8), which is based on osteonecrosis site and severity of symptoms.[64] In the last decade, Niinimäki and colleagues have presented the first non-joint-specific *radiological* grading system, which facilitates universal application (Figure 9).[65] It uses osteonecrosis site, as well as the amount of articular surface that is involved (<30% versus ≥30%). The prognostic value of both classifications remains to be determined, but these grading systems are better applicable to the pediatric oncology setting that the previously used CTCAE criteria.

	Weight-bearing bone		**Non-weight-bearing bone**	
	Long bone	**Short bone**	**Long bone**	**Short bone**
Grade				
0	No osteonecrosis	No osteonecrosis	No osteonecrosis	No osteonecrosis
I	-	-	Diaphysis or metaphysis (0%)	Body (0%)
II	Diaphysis or metaphysis (0%)	Body (0%)	Epiphysis (<30%)	Surface (<30%)
III	Epiphysis (<30%)	Surface (<30%)	Epiphysis (≥30%)	Surface (≥30%)
IV	Epiphysis (≥30%)	Surface (≥30%)	-	-
V	Joint deformation	Joint deformation	Joint deformation	Joint deformation

Figure 9. The Niinimäki radiological classification of osteonecrosis.

Osteonecrosis occurs in about one to eight percent of children with acute lymphoblastic leukemia,[61,66–69] with age being the most important risk factor. Many studies have shown an increased risk for children older than 10 years compared to younger children.[55] Interestingly, this risk seems to decline in young adulthood, suggesting that puberty and the associated increased growth velocity plays a significant role in the vulnerability to develop osteonecrosis.[70] Although not consistently, female sex and high body mass index have also been associated with osteonecrosis.[61,71] In addition, several cardiovascular parameters, including hyperlipidemia and hypertension, seem to increase osteonecrosis risk.[57,72] Osteonecrosis has also been shown to be followed by a more pronounced decline in bone mineral density during acute lymphoblastic leukemia treatment.[63,73] These reports suggested that this may be explained by restriction from performing weight-bearing activities, but biological causality and treatment implications are so far unavailable.

As effective treatment options are limited in case symptomatic osteonecrosis develops,[74] identifying preventive measures for osteonecrosis is important. To date, only one effective strategy to prevent osteonecrosis in the context of acute lymphoblastic leukemia has been published. Alternate-week dexamethasone during delayed intensification significantly reduced osteonecrosis risk compared to continuous dexamethasone in adolescents and young adults with high-risk acute lymphoblastic leukemia treated according to the American CCG-1961 trial, despite a higher cumulative dose.[66] In the Netherlands, children with acute lymphoblastic leukemia have long been treated according to national Dutch Childhood Oncology Group (DCOG) protocols. In the DCOG ALL-9 protocol which ran from 1997 to 2004, long pulses dexamethasone were administered during post-consolidation, whereas in more recent protocols (i.e. DCOG ALL-10 and ALL-11), dexamethasone pulses were shorter. Prior to this thesis, it was unknown whether the administration of shorter pulses dexamethasone in the Dutch protocols have led to a reduction in osteonecrosis, especially since other chemotherapeutics have been intensified in these recent protocols to increase survival.

ACCELERATED AGING

Frailty and sarcopenia as a consequence of childhood cancer

In the general population, osteoporosis is mainly a health concern among elderly (in particular among postmenopausal women).[75] Interestingly, childhood cancer survivors seem to age faster overall. With normal aging, a gradual decrease in

physiological reserve occurs, but when this decrease is accelerated and homeostatic mechanisms start to fail, frailty develops (Figure 10).[76] The frailty phenotype was first described by *Fried et al.*, and is a clinical syndrome in which three or more of the following criteria have to be present: unintentional weight loss (or low muscle quantity), low muscle strength, exhaustion, slowness, and low physical activity.[77] It is best described as a state of vulnerability to poor resolution of homoeostasis after a stressor event and is a consequence of cumulative decline in many physiological systems.[76] This decline is a result of the accumulation of molecular and cellular damage during a lifetime that is influenced by many genetic, epigenetic, and environmental factors. Frailty is in elderly independently predictive of worsening mobility and disability, incident falls, hospitalization, and death.[77]

Only two large American studies have assessed frailty in childhood cancer survivors. They have shown that six to eight percent of long-term childhood cancer survivors are frail.[79,80] This prevalence was increased compared with controls. Cranial irradiation, pelvic irradiation with more than 33 Gray, and lung surgery were treatment modalities that significantly increased the risk for frailty when accounted for possible confounders.[80] In addition, frailty was shown to be associated with chronic condition onset and death,[79] which underscores the importance of this phenotype. Thus far, large national cohort studies in childhood cancer survivors, especially in Europe, have not been pursued.

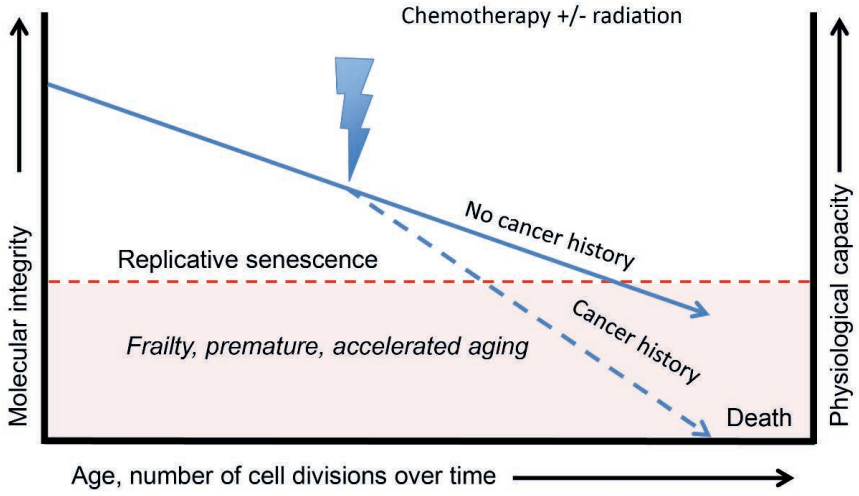


Figure 10. Decline in physiologic capacity over time in individuals with and without a cancer history. Figure from Ness et al. *Journal of Clinical Oncology,* 2018 (with permission).[78]

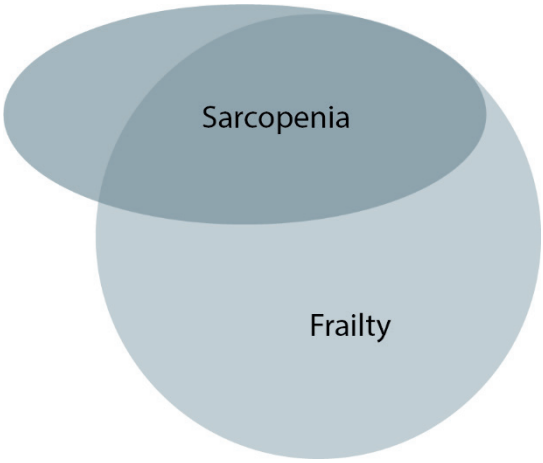


Figure 11. Diagnostic overlap between frailty and sarcopenia.

Sarcopenia is currently defined by the European Working Group on Sarcopenia in Older People (EWGSOP2) as the presence of both low muscle quantity and low muscle strength.[81] The two components of sarcopenia are both also components of the frailty phenotype. However, an individual may be sarcopenic and not frail and vice versa, depending on the number and type of frailty components that are present (Figure 11). Therefore, although sarcopenia and frailty are closely related, assessing both as separate conditions in patients is important, as they deserve separate attention and interventions.[82] Similar to frailty, sarcopenia typically occurs as an age-related process in elderly.[82] It is a progressive and generalized skeletal muscle disorder involving the accelerated loss of muscle mass as well as function, and is associated with increased adverse outcomes including falls, functional decline, and mortality. Except for one study that assessed the association between mitochondrial mutations and sarcopenia,[83] no studies have investigated risk factors for sarcopenia in childhood cancer survivors (using the EWGSOP2 definition). Therefore, the prevalence and risk factors of sarcopenia in childhood cancer survivors deserve more attention.

AIM AND OUTLINE OF THIS THESIS

The general aim of the research projects presented in this thesis is to increase knowledge on the prevalence of, and risk factors for bone toxicity and accelerated aging in children with cancer and childhood cancer survivors, to identify those childhood cancer survivors that may benefit from bone mineral density surveillance, and to identify potential interventions for these sequelae through

identification of novel modifiable risk factors. This knowledge may reduce future morbidity and mortality that results from bone toxicity and accelerated aging in children with cancer and survivors through primary prevention during cancer treatment, as well as through timely identification and early treatment during cancer survivorship.

In **chapter 2**, we describe the effect of shorter pulses dexamethasone compared with longer pulses dexamethasone during the post-consolidation phase of acute lymphoblastic leukemia treatment on the development of symptomatic osteonecrosis in three consecutive national Dutch Childhood Oncology Group protocols. In addition, we further explore risk factors for osteonecrosis and severe osteonecrosis. **Chapter 3** delineates several perspectives and implications of the recently observed association between osteonecrosis and bone mineral density decline during acute lymphoblastic leukemia treatment. In **chapter 4**, we present prediction models for low and very low bone mineral density that were developed in a large cohort of childhood cancer survivors treated at the American St. Jude Children's Research Hospital, and externally validated on a single-center Dutch cohort from the Erasmus Medical Center. In **chapter 5**, we describe evidence-based recommendations for bone mineral density surveillance among childhood, adolescent, and young adult cancer survivors made by a guideline panel of 36 experts from 10 different countries. **Chapter 6** provides a systematic review of the literature as well as consensus recommendations for vitamin D supplementation during childhood cancer treatment on bone mineral density and fractures. In **chapter 7**, we report the prevalence and risk factors of (very) low bone mineral density and fractures in the first large national multi-center cohort from the Dutch Childhood Cancer Survivor Study. In particular, we assess the association between (very) low bone mineral density and fractures, and the prevalence of, and risk factors for prevalent vertebral fractures. **Chapter 8** presents the prevalence of, and risk factors for prefrailty, frailty, and sarcopenia among Dutch childhood cancer survivors included in the Dutch Childhood Cancer Survivor Study. **Chapter 9** concludes with a general discussion of this thesis, including directions for future research.

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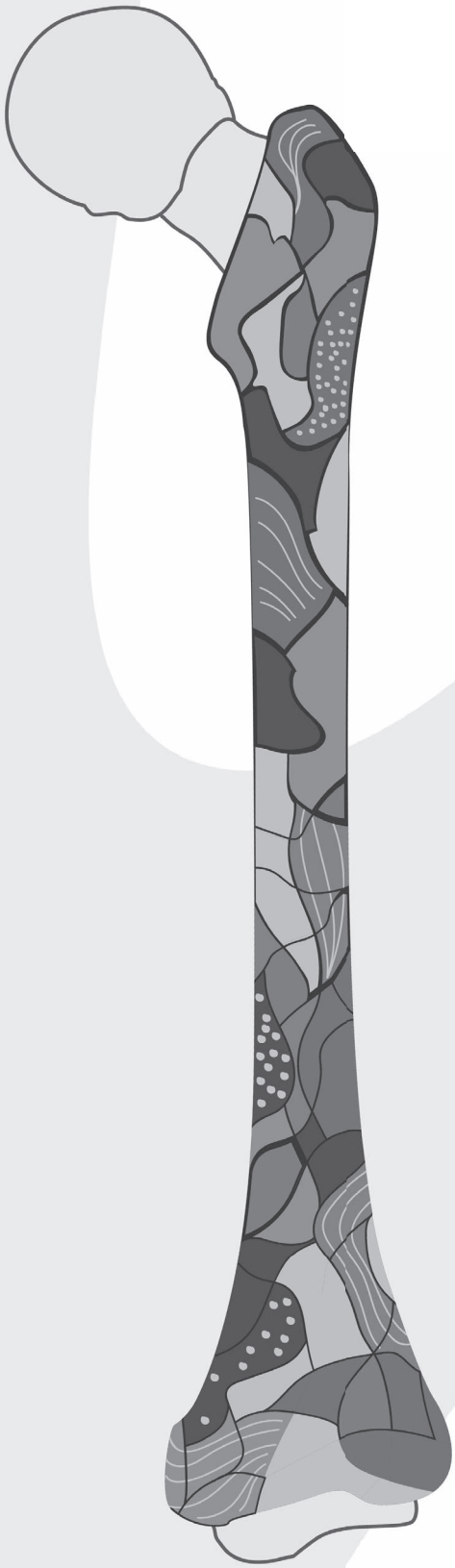

CHAPTER 2

Effect of post-consolidation regimen on symptomatic osteonecrosis in three DCOG acute lymphoblastic leukemia protocols

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ABSTRACT

The effect of shorter pulses dexamethasone in childhood acute lymphoblastic leukemia (ALL) post-consolidation treatment to reduce symptomatic osteonecrosis (sON) risk in recent asparaginase intensified pediatric ALL protocols remains unclear. We compared the cumulative incidence of sON (CIsON) in children treated with long (14-day; ALL-9) and short (5-day) pulses dexamethasone (asparaginase intensified ALL-10/11 medium risk group [MRG]). Children aged 1-18 years treated for ALL (n=1470) between 01/1997-03/2015 in the Dutch Childhood Oncology Group (DCOG) ALL-9 (n=795) or ALL-10/11 MRG (n=675) protocol were included. The CIsON was estimated using competing risk models; the association between risk factors and sON was investigated using Cox regression models. Characteristics of patients with irreversible ON were assessed. No statistically significant difference between the CIsON for both regimens was found (p=0.54). The 3-year CIsON since start post-consolidation was 4.9% (95%CI=3.4-6.5%) in ALL-9 and 5.4% (95%CI=3.6-7.1%) in ALL-10/11 MRG. Age was the only risk factor for sON (cause specific hazard ratio HR_{cs}=1.40 (95%CI=1.32-1.50), p<0.001). The 3-year CIsON since ALL diagnosis was 1.2% (95%CI=0-2.3%) in children aged 1-9 years, 14.3% (95%CI=10.0-18.5%) aged 10-14 years, and 31.4% (95%CI=30.9-31.9%) aged 15-18 years. Irreversible ON (median follow-up 4.5 years) occurred in n=5/13 (38%), n=17/33 (52%) and n=18/26 (69%) children aged 1-9, 10-14 and 15-18 years, respectively. We suggest that the protective effect of shorter pulses dexamethasone on sON may be attenuated by recent intensification of asparaginase, highlighting the relevance of therapeutic context when interpreting results of treatment-related toxicity. Especially children aged 15-18 years developed symptomatic, in particular irreversible osteonecrosis.

INTRODUCTION

Symptomatic osteonecrosis (sON) is a serious side effect of childhood acute lymphoblastic leukemia (ALL) treatment, occurring in 1 to 8% of patients. Among teenagers and adolescents, frequencies ranging from 10 to 20% have been described.[1–6] Symptomatic osteonecrosis is suggested to be caused by a disruption of the blood supply to especially the epiphysis of weight-bearing bones by either intra- or extraluminal obliteration of the blood vessels, for example by microthrombi or hyperlipidemia, which occurs concomitantly with the direct adverse effect of anti-leukemic treatment on bone remodeling.[7,8] Osteonecrosis may resolve completely with conservative treatment or may result in debilitating long-term sequelae such as severe pain, functional limitations and articular collapse, ultimately leading to joint replacement at an early age.[1,9]

Several non-treatment-related risk factors of symptomatic osteonecrosis during childhood ALL therapy have been described. Especially children older than 10 years at ALL diagnosis are at risk for developing osteonecrosis.[9–13] In addition, some studies showed a higher risk for patients with a high body mass index (BMI), female sex and Caucasian race.[1,10–12,14] Although these risk factors for symptomatic ON are appreciated, identifying patients at risk of severe, irreversible ON remains challenging.

Corticosteroids have been used for the treatment of childhood ALL since the very early beginning of leukemia treatment, and although effective to this aim, they largely contribute to the development of sON.[2] Predniso(lo)ne and dexamethasone are used in induction, intensification and maintenance treatment phases.[3] Higher cumulative doses of corticosteroids may be associated with an elevated risk of osteonecrosis.[2] However, shorter corticosteroid pulses showed to decrease the risk of osteonecrosis despite a higher cumulative dose in a large randomized controlled trial from the Children's Oncology Group (CCG-1961).[5] This strategy has been widely adopted in other pediatric ALL treatment protocols.[15,16]

Asparaginase, a key component of ALL treatment as well, has shown to increase the risk of ON especially when administered concurrently with corticosteroids.[8,17] Asparaginase treatment has been intensified in contemporary ALL protocols, which contributed to increased survival rates that now exceed 90%.[18] However, the benefit of shorter pulses dexamethasone on sON development in the context of recent asparaginase intensified regimens remains unclear.

In the Netherlands, children with ALL are treated according to the national Dutch Childhood Oncology Group (DCOG) protocols. The DCOG ALL-9 protocol contained long pulses dexamethasone without asparaginase, whereas the most recent DCOG ALL-10 and ALL-11 medium risk group (MRG) protocols contained shorter pulses dexamethasone plus asparaginase during post-consolidation therapy. The primary aim of this study was to compare the cumulative incidence of sON (CIsON) between these groups. The secondary aim was to investigate the associations between risk factors and sON and to assess the characteristics of patients with severe, irreversible ON.

METHODS

Patients

Children aged 1-18 years with newly diagnosed ALL between 01/1997-03/2015 treated according to the DCOG ALL-9 or ALL-10/11 MRG protocol were eligible for this study. Children with ALL below 1 year of age were treated according to the Interfant protocol and patients with presence of the t(9;22) translocation (Philadelphia chromosome) or the corresponding fusion gene BCR/ABL in the leukemic cells were treated according to the DCOG EsPhALL protocol from December 2005 onwards and therefore not included in this study. Patients treated according to the ALL-10 and ALL-11 standard risk and high risk groups were not included because the treatment for these groups did not contain post-consolidation dexamethasone pulses. Patients with Down syndrome were excluded due to unequal distribution across treatment protocols. For our primary aim, only the subset of patients who reached the start of post-consolidation therapy per protocol was analyzed (landmark analysis), since dexamethasone pulses started from this time point onwards. Consent from patients and/or legal guides for data collection had been previously obtained.

Steroid and asparaginase regimens

Details of the DCOG ALL-9[19], ALL-10[20] and ALL-11 treatment protocols are shown in Supplementary Table 1. Patients were treated with dexamethasone during induction and with long pulses dexamethasone (14 days 6 mg/m^2/day every seven weeks, cumulative dose non-high risk group, 1,370 mg/m^2; high risk group, 1,244 mg/m^2) without asparaginase during post-consolidation in ALL-9, while patients in ALL-10/11 MRG were treated with prednisone during induction and with short pulses of dexamethasone (five days 6 mg/m^2/day every three weeks, cumulative dose, 1,115 mg/m^2) combined with 30 weeks PEG-asparaginase during post-consolidation.

Data collection

In the national DCOG database (medical ethical committee number 187.154/1999/212 [ALL-9], 2004-203 [ALL-10] and 2012-287 [ALL-11]), occurrence and core characteristics of sON were prospectively collected. Clinicians had to report clinical symptoms of osteonecrosis and date of radiographic confirmation at diagnosis, after 32 weeks of treatment, at treatment cessation and one year after treatment cessation in ALL-9 and at the start of each consecutive treatment block until one year after treatment cessation in ALL-10/11. In addition, we retrospectively assessed detailed clinical information of these children from medical records. Characteristics of patients with sON treated according to the ALL-9 protocol have been previously described.[1] Data from the ALL-10 and ALL-11 protocols were combined for all analyses because treatment factors known to be associated with osteonecrosis did not substantially differ between these protocols.

Risk factors

Potential risk factors for sON included type of post-consolidation treatment regimen (ALL-10/11 MRG versus ALL-9), sex (male versus female), age (years) and BMI (standard deviation score [SDS]) at ALL diagnosis. BMI SDS was calculated with the LMS method by Cole & Green using Dutch BMI reference values.[21] Less than 0.5% of patients had missing BMI SDS values.

Definition of osteonecrosis and severe osteonecrosis

Symptomatic osteonecrosis was defined as persistent pain in joints and/or limbs (not resulting from vincristine neuropathy) developed during or in the first year after treatment for ALL and confirmed by MRI (and/or X-ray). All these MRI scans were interpreted by musculoskeletal radiologists in one of the seven pediatric oncology centers in the Netherlands. Because osteonecrosis was a relatively unknown condition during the ALL-9 period, all MRI scans of patients diagnosed with osteonecrosis were revised by a single experienced pediatric musculoskeletal radiologist (ML) to confirm the diagnosis. In 10 patients, diagnosis of sON was based on symptoms and classic abnormalities on X-rays through revision by the same pediatric radiologist. Severe ON was defined as Ponte di Legno Toxicity Working Group grade 4 (i.e. deformation by imaging of one or more joints and/or substantially limiting self-care activity of daily living) in ALL-10/11 MRG.[22] Irreversible ON was defined as joint replacement or irreversible symptoms at last follow-up for all study participants.

Statistical considerations

Patient characteristics of ALL-9 and ALL-10/11 were compared by employing chi-square tests (binary variables), student t-tests (normally distributed continuous variables) and Mood's median tests (skewed continuous variables). The CIsON since start post-consolidation therapy was estimated for patients treated in ALL-10/11 MRG versus ALL-9 using competing risk models with stem cell transplantation, second malignancy, relapse and death as competing event.[23] The CIsON since ALL diagnosis was estimated for different age categories. Fine and Gray's test was used to assess the difference between the cumulative incidence. A univariable and multivariable Cox proportional hazard regression model was used to estimate the effect of risk factors on sON. Analyses were performed using IBM SPSS Statistics version 25. Mstate package[24] in the R-15 software environment was used to estimate the CIsON.[25]

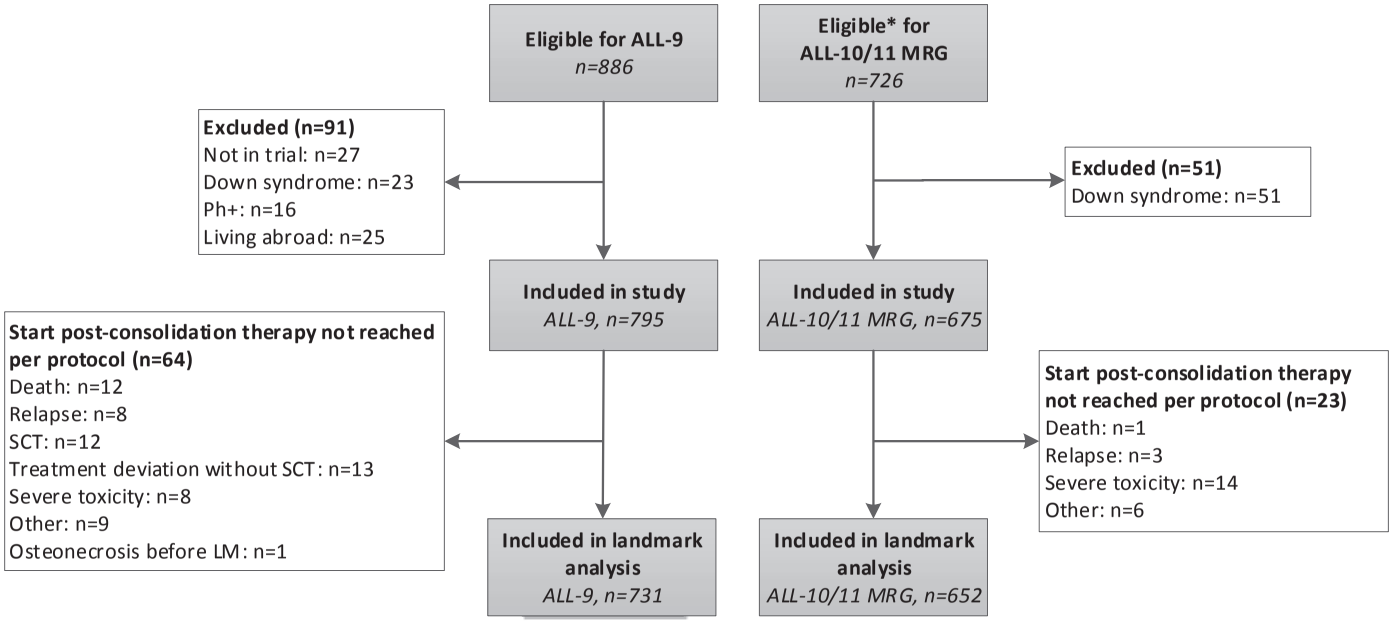
RESULTS

Patients

Of 1612 patients eligible for ALL-9 (n=886) and ALL-10/11 MRG (n=726), 1470 were included in this study (Figure 1). Thirteen hundred eighty-four of these patients reached the start of post-consolidation therapy per protocol and were included in the landmark analysis. Reasons for protocol deviations in 86 children were as follows: competing event (n=36), protocol deviation due to refractory disease (n=13), severe toxicity (n=22) or other reasons (n=15); one child had already developed sON before post-consolidation therapy. No statistically significant difference for baseline characteristics of patients treated in the ALL-9 and ALL-10/11 MRG protocol was found (Table 1).

Occurrence of symptomatic osteonecrosis

Seventy-nine of 1470 patients developed sON during or within one year after completion of ALL therapy. At first presentation of sON, 15 patients (19%) experienced symptoms at a single site, whereas 81% had multifocal symptoms. Weight-bearing joints were affected in all patients (knee 61%; hip 53%; ankle/foot; 18%). Symptoms in upper extremities were additionally reported and radiologically confirmed in 7 patients (9%).



Abbreviations: ALL=acute lymphoblastic leukemia; MRG=medium risk group; Ph+= Philadelphia chromosome positive. *Patients not in trial, Ph+ patients and patients living abroad were not eligible for these protocols.

Figure 1. Flow diagram of study participants.

The effect of post-consolidation treatment regimen on the development of sON

Thirty-six of 731 children in ALL-9 and 38 of 652 children in ALL-10/11 MRG who were included in the landmark analysis developed sON, respectively. No statistically significant difference between the CIsON since start postconsolidation therapy for the two groups was found (p=0.54, Figure 2); at 3 years since start of post-consolidation therapy, the CIsON was 4.9% (95% CI=3.4-6.5%) and 5.4% (95% CI=3.6-7.1%) for patients treated in ALL-9 and ALL-10/11 MRG, respectively. In addition, the CIsON since ALL diagnosis was estimated, which showed no statistically significant difference (p=0.80, Supplementary Figure 1). At 3 years since ALL diagnosis, the CIsON was 5.0% (95% CI=3.5-6.6%) and 5.6% (95% CI=3.9-7.4%) for patients treated in ALL-9 and ALL-10/11 MRG, respectively.

Risk factors for symptomatic osteonecrosis

Cause-specific hazard ratio (HRCS) estimates from a univariable and multivariable Cox proportional hazard model for sON since start of post-consolidation therapy are shown in Table 2. In a multivariable analysis including type of postconsolidation treatment regimen, age at ALL diagnosis, sex and BMI SDS, age was the only significant independent risk factor (HRCS=1.40; 95% CI=1.32-1.50;$p<0.001$). Type of post-consolidation treatment regimen was not associated with sON (HRCS=0.69; 95% CI=0.43-1.11; p=0.12).

The association between age and the development of sON and severe ON

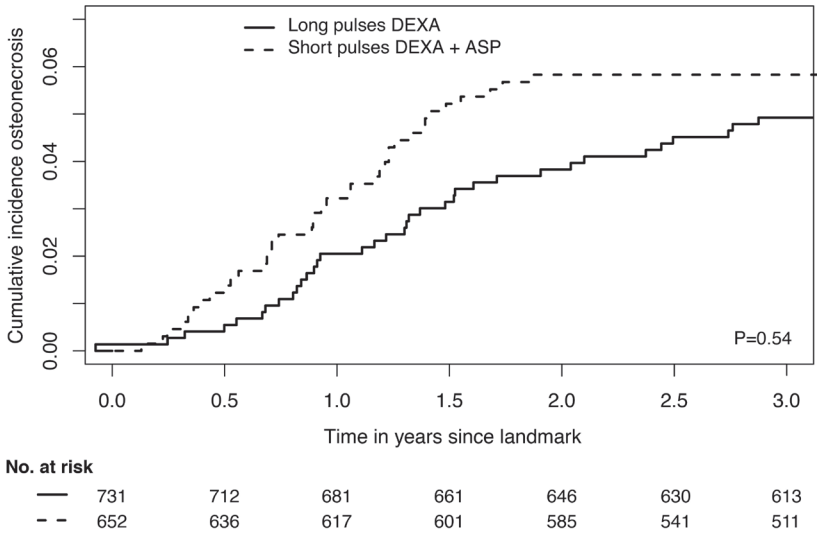
The median age at ALL diagnosis of children who developed sON was 13 years (IQR=5; range 1—17 years) and of those without osteonecrosis, 4 years (IQR=5;range 1—18 years). A statistically significant difference between the CIsON for different age categories at ALL diagnosis was observed ($p<0.001$, Figure 3). At 3 years since ALL diagnosis, the CIsON was 1.2% (95% CI=0-2.3%), 14.3% (95% CI=10.0-18.5%) and 31.4% (95% CI=30.9-31.9%) for children aged 1 to 9 years, 10 to 14 years and 15 to 18 years, respectively.

		Long pulses DEXA (ALL-9) *N=795*		Short pulses DEXA + ASP (ALL-10/11 MRG) *N=675*		
		Number	**%**	**Number**	**%**	**P-value[1]**
Sex						0.097
	Male	485	61.0	383	56.7	
	Female	310	39.0	292	43.3	
Age (yrs)						0.239
	Median	4		5		
	IQR	6		7		
	Range	1—17		1—18		
Height (cm)						0.107
	Median	112		116		
	IQR	39		46		
	Range	72—195		72—196		
Weight (kg)						0.063
	Median	19.8		21.0		
	IQR	15.8		21.6		
	Range	8.9—103.0		6.8—94.8		
BMI (SDS)						0.102
	Median	-0.32		-0.24		
	IQR	1.4		1.3		
	Range	-4.2—7.6		-4.8—3.3		

Abbreviations: ASP=asparaginase; BMI=body mass index; DEXA=dexamethasone; GCs=glucocorticoids; HR= high risk; IQR=interquartile range; MR=medium risk; NA=not available; NHR= non-high risk
[1]Chi-square p-value for categorical variables, student T-test p-value for normally distributed continuous variables and Mood's median test p-values for non-normally distributed continuous variables.

Table 1. Baseline characteristics of patients treated with long pulses dexamethasone (ALL-9) and patients treated with short pulses dexamethasone (asparaginase intensified ALL-10/11 MRG) during post-consolidation therapy.

Fifteen of 38 children with symptomatic osteonecrosis treated in ALL-10/11 MRG experienced severe osteonecrosis. Severe osteonecrosis occurred in none of the 6 children with sON aged 1-9 years, in 5 of the 15 (33%) children with sON aged 10-14 years, and in 10 of the 17 (59%) children with sON aged 15-18 years. In the entire cohort, symptoms of osteonecrosis completely resolved with conservative treatment in 32 patients (41%) at 0.1-9.0 years (median 4.5 years) of follow-up after diagnosis of sON (in 7 patients, symptoms at follow-up were unknown). Irreversible ON occurred in 5 of the 13 (38%) children with sON aged 1-9 years, in 17 of the 33 (52%) children with sON aged 10-14 years, and in 18 of the 26 (69%) children with sON aged 15-18 years.



Abbreviations: ASP=asparaginase; DEXA=dexamethasone; sON=symptomatic osteonecrosis

Figure 2. Cumulative incidence of symptomatic osteonecrosis for patients treated with long pulses dexamethasone (n=731) and patients treated with short pulses dexamethasone plus asparaginase (n=652) since start post-consolidation therapy (landmark).

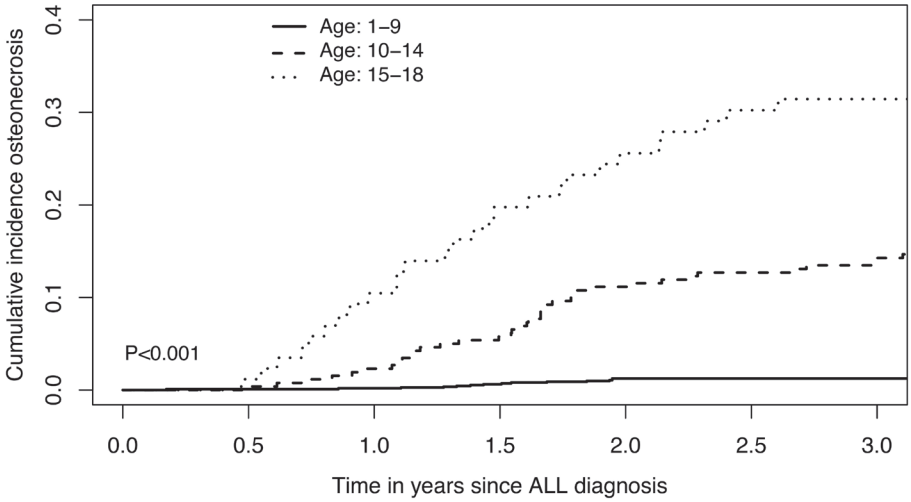
Management of sON

When osteonecrosis occurred during therapy, anti-cancer treatment was modified in 54 patients (68%). Treatment with dexamethasone was permanently discontinued in 45 patients (57%), decreased in 6 patients (8%), and changed to prednisone in 3 patients (4%). Patients were conservatively treated with physical therapy (57 patients, 72%), weight-bearing restrictions (43 patients, 54%), and/or bisphosphonates (15 patients, 19%). Surgical interventions such as drilling, excision

	Univariable model *n=1383*			**Multivariable model** *n=1383*		
	HR_{cs}	**95% CI**	**P-value**	**HR_{cs}**	**95% CI**	**P-value**
Post-consolidation regimen (ALL-10/11 MRG vs. ALL-9)	1.21	0.76-1.92	0.416	0.69	0.43-1.11	0.123
Age (yrs)	1.38	1.30-1.46	<0.001	1.40	1.32-1.49	<0.001
Sex (male vs. female)	1.18	0.75-1.87	0.479	1.53	0.97-2.44	0.070
BMI (SDS)	1.01	0.82-1.24	0.942	0.90	0.73-1.10	0.289

Abbreviations: BMI=body mass index; CI=confidence interval; DEXA=dexamethasone; HR=hazard ratio; SDS=standard deviation score.

Table 2. Cause-specific hazard ratio (HR_{CS}) estimates along with their 95% confidence intervals (CI) for the risk of symptomatic osteonecrosis since start post-consolidation therapy from a univariable and multivariable Cox proportional hazard regression model.



No. at risk

——	1124	1108	1083	1059	1040	1032	983
– –	260	251	229	210	191	179	164
· · · ·	86	84	70	61	53	47	39

Abbreviations: ALL=acute lymphoblastic leukemia; sON=symptomatic osteonecrosis

Figure 3. Cumulative incidence of symptomatic osteonecrosis for children aged 1 to 9 years (n=1124), 10 to 14 years (n=260) and 15 to 18 years (n=86) since ALL diagnosis.

and grafting of the osteonecrosis and/or osteotomy were performed in 11 patients (14%). Ultimately, a joint replacement was performed in 12 patients (15%); four of these patients had previously had another type of surgical intervention. Of the 15 patients with severe osteonecrosis, 10 (67%) required joint replacement, three (20%) reported chronic pain and two (13%) had no symptoms at 0.1-9.0 years (median 4.5 years) of follow-up.

DISCUSSION

In this study, no statistically significant difference in the CIsON since start of post-consolidation therapy for children treated with short pulses dexamethasone (ALL-10/11 MRG) versus long pulses dexamethasone (ALL-9) was found. Furthermore, type of post-consolidation treatment regimen was not associated with symptomatic osteonecrosis in univariable and multivariable Cox proportional hazard regression analyses.

Based on the findings of the CCG-1961 trial[5], we hypothesized that patients treated with short pulses dexamethasone in ALL-10/11 MRG would have a lower

CIsON compared to those treated with long pulses dexamethasone in ALL-9.[26] This hypothesis was consistent with a recent preclinical study which showed that asparaginase added to a discontinuous dexamethasone regimen did not increase sON occurrence in mice.[27]

We realized however, that to increase the survival of children with ALL over the past decades, intensification of asparaginase has played an important role. Although the combined administration of dexamethasone and asparaginase in our protocols does not allow to prove the relative contribution of asparaginase to the development of sON, we think it is conceivable that intensification of ALL treatment components other than dexamethasone regimens such as intensified asparaginase administration may explain our findings. There is evidence that asparaginase is associated with sON development, especially when it is administered concurrently with dexamethasone.[8,17] We have previously shown that in patients with sON, a hypercoagulable state may result from a lower dexamethasone-related increase of anticoagulants in combination with a subsequent decline of these anticoagulants after introduction of asparaginase.[8] Furthermore, asparaginase increases plasma concentration of dexamethasone, and in particular PEG-asparaginase may increase triglyceride levels (associated with osteonecrosis) especially in combination with dexamethasone according to a recently published study in 925 children with ALL.[28–30] In a controlled pre-clinical model, mice receiving asparaginase plus continuous dexamethasone experienced osteonecrosis more often than those receiving dexamethasone alone.[31] Discontinuous dexamethasone reduced the risk of sON compared to continuous dexamethasone in the CCG-1961 trial more in patients who received intensified treatment compared to those receiving standard treatment, also suggesting that other treatment components may play a role in the effect of dexamethasone pulses duration on sON development.[5] In the NOPHO ALL2008 study, patients randomized to intermittent PEG-asparaginase (3 doses at 6-week intervals, no concurrent PEG-asparaginase and dexamethasone) seemed to developed sON less often than patients assigned to continuous PEG-asparaginase (10 doses at 2-week intervals, concurrent PEG-asparaginase and dexamethasone) during post-consolidation therapy, although this difference was not statistically significant.[32] Other explanations for our finding could be that the long pulses dexamethasone in the DCOG ALL-9 protocol were already shorter than the continuous dexamethasone in the CCG-1961 trial (14 days versus 20 days, respectively) and that the dexamethasone pulses were administered throughout maintenance in the DCOG protocols compared to during delayed intensification only in the CCG-1961 trial.

Our results highlight the relevance of therapeutic context when interpreting results of treatment-related toxicity. Further research addressing the effect of dexamethasone and asparaginase schedules on the occurrence of sON is needed. This is of interest since older children who are at highest risk of (severe) sON are also most likely to have an unfavorable leukemia outcome, which raises serious cautiousness towards ALL treatment reduction because of toxicity.[20] Furthermore, adequate treatment of sON remains an issue: overall success of treatment such as non-weight-bearing exercise, physical therapy and bisphosphonates is limited since about 50% of patients reported persistent symptoms after five years of follow-up, sometimes leading to chronic physical disabilities.[1,12] Hence, prevention of sON by treatment scheduling modification seems preferable since it could possibly lead to a decrease in osteonecrosis associated morbidity without jeopardizing leukemia outcome.

The association between age and the risk of symptomatic osteonecrosis has been thoroughly studied.[9–13] Adolescents are disproportionally affected by this toxicity relative to younger children and adults.[4] We here confirmed findings from large studies (Children's Oncology Group, Berlin-Frankfurt-Münster study group).[3,5] Furthermore, when we investigated the effect of age, the 3-year cumulative incidence of sON was significantly different in children aged 1-9 years (1.2%), 10-14 years (14.3%), and 15-18 years (31.4%). This means that among children older than 10 years, an age group that most studies have focused on[2,4,6,12,17], children aged 15-18 seem to develop sON most often, even relative to children aged 10-14 years. These results were also demonstrated in the CCG-1961 trial. This is especially important since our study for the first time shows that in these 15-18 year old patients, the sON is more serious and in most of these children even irreversible. Of all patients affected by severe ON, 67% ultimately required joint replacement and 20% still experienced chronic pain at follow-up, indicating the clinical relevance of this complication of osteonecrosis. Our finding is in line with previous studies that showed an increased risk of severe ON and hip replacement among older children.[5,14] More studies are needed to better understand the occurrence of severe and progressive ON. However, acknowledging and creating awareness that patients aged 15-18 years represent a generally small subcategory of patients with ALL that most frequently experience sON and its complicated course is important.

The current study was based on 20 years of prospective registration of symptomatic osteonecrosis in national ALL treatment protocols. However, the results of our study must be interpreted in light of several limitations. We did not

perform a randomized controlled trial, so differences between the cohorts other than those adjusted for may exist. We think limiting our analysis to the MRG in ALL-10/11 was justified, but could have introduced bias. However, an overall analysis comparing the CIsON of ALL-9 to that of the entire ALL-10/11 cohort showed similar results, indicating that the groups were representative. Although we attempted to rule out the differences in induction therapy by employing a landmark analysis, this protocol variation, as well as differences in asparaginase formulation, should be appreciated. Furthermore, all patients in ALL-10/11 received both short pulses dexamethasone and asparaginase, so assessing the effect of each treatment component separately was not possible.

We conclude that no statistically significant difference in the cumulative incidence of symptomatic osteonecrosis for children treated with short pulses dexamethasone plus asparaginase versus long pulses dexamethasone alone during ALL post-consolidation therapy was found. We therefore postulate that the protective effect of shorter pulses dexamethasone on sON occurrence may be attenuated by intensification (and type) of other treatment components such as asparaginase in recent pediatric ALL protocols. This highlights the relevance of therapeutic context when interpreting results of treatment-related toxicity. Among children older than 10 years, the age group that most studies have focused on, especially children aged 15-18 years developed symptomatic, in particular severe, irreversible osteonecrosis and may benefit from close monitoring.

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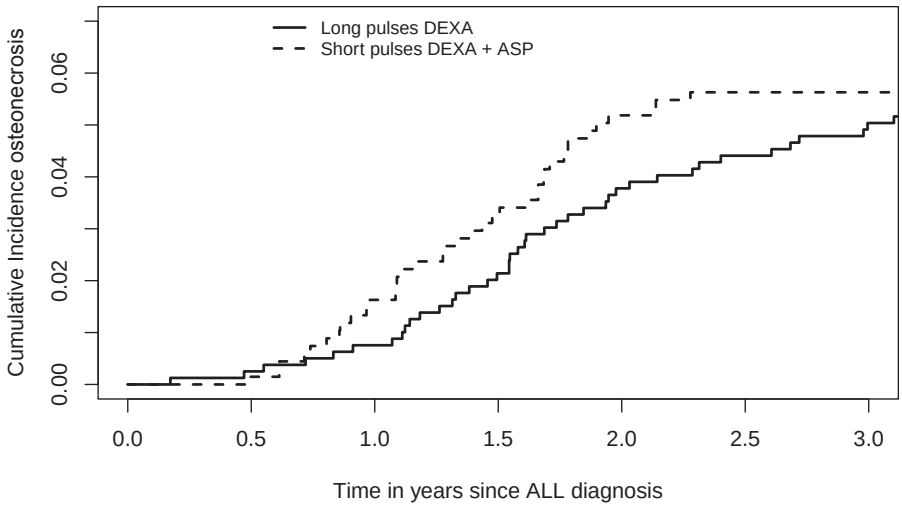
ALL-9[19] *(1997—2004)*		
Inclusion criteria	- Newly diagnosed patients with T-lineage or precursor-B lineage ALL - Diagnosis ALL confirmed by DCOG laboratory - Age between ≥1 and <19 years - No treatment with systemic corticosteroids and/or cytostatics in a 4-week interval prior to diagnosis	
	NHR	HR
Stratification criteria	- WBC <50x10^9/L at dx - No CNS involvement or testis involvement or mediastinal enlargement at dx - No presence of the t(4;11)(q11;q23) or t(9;22) translocation or the corresponding fusion genes MLL/AF4 or BCR/ABL in the leukemia cells at dx - No T-lineage ALL	- All other patients
Induction/consolidation		
DEXA	6 mg/m^2/day for 28 days (3x5 day taper)	6 mg/m^2/day for 28 days (induction; 3x5 day taper) 6 mg/m^2/day for 7 days every 3 wks (intensification I)
L-ASP	4x6,000 IU/m^2	4x6,000 IU/m^2 (induction) 9x10,000 IU/m^2 (intensification I)
Post-consolidation		
DEXA	6 mg/m^2/day for 14 days every 7 wks; 98 wks	6 mg/m^2/day for 14 days every 7 wks; 77 wks
L-ASP	NA	NA
No. DEXA pulses + ASP	0	0
Cumulative dose		
DEXA	1,370 mg/m^2	1,244 mg/m^2
L-ASP	24,000 IU/m^2	114,000 IU/m^2
ALL-10[20] *(2004—2012)*		
Inclusion criteria	- According to ALL-9 inclusion criteria - From December 2005 onwards: no presence of Ph-positive ALL (documented presence of t(9;22)(q34;q11) and/or of the BCR/ABL fusion transcript)	
	MR	
Stratification criteria	- Cytomorphological CR at day 33 - MRD-positivity at TP1 and/or at TP2, but MRD level at day 79 <10^{-3} - No presence of the t(4;11)(q11;q23) translocation or the corresponding fusion gene MLL/AF4 in the leukemia cells at dx (From July 2012 onwards: In case of IKZF1 deletion 1 year of additional maintenance therapy)	
Induction/consolidation		
PRED	60 mg/m^2/day for 28 days (3x3 day taper)	
L-ASP	8x5,000 IU/m^2	

Post-consolidation	
DEXA	6 mg/m^2/day for 5 days every 3 wks; 84 wks
PEG-ASP	15x2,500 IU/m^2
No. DEXA pulses + ASP	10
Cumulative dose	
GCs[1]	1,115 mg/m^2
ASP	40,000 IU/m^2 L-ASP 37,500 IU/m^2 PEG-ASP
***ALL-11** (2012—onwards)*	
Inclusion criteria	According to ALL-10
	MR
Stratification criteria	According to ALL-10
Induction/consolidation	
PRED	60 mg/m^2/day for 28 days (3x3 day taper)
PEG-ASP	3x1,500 IU/m^2 *If MRD+ at TP1, eligible for randomization: A=standard PEG-ASP (14x individualized dose during intensification) and B=experimental, early PEG-ASP (14x individualized dose during protocol 1B/M and intensification)*
Post-consolidation	
DEXA	6 mg/m^2/day for 5 days every 3 wks; 84 wks
PEG-ASP	14x individualized dose (no randomization or randomization A) or 8x individualized dose (randomization B)
No. DEXA pulses + ASP	10 (no randomization or randomization A) or 6 (randomization B)
Cumulative dose	
GCs[1]	1,115 mg/m^2
PEG-ASP	NA (individualized dose)

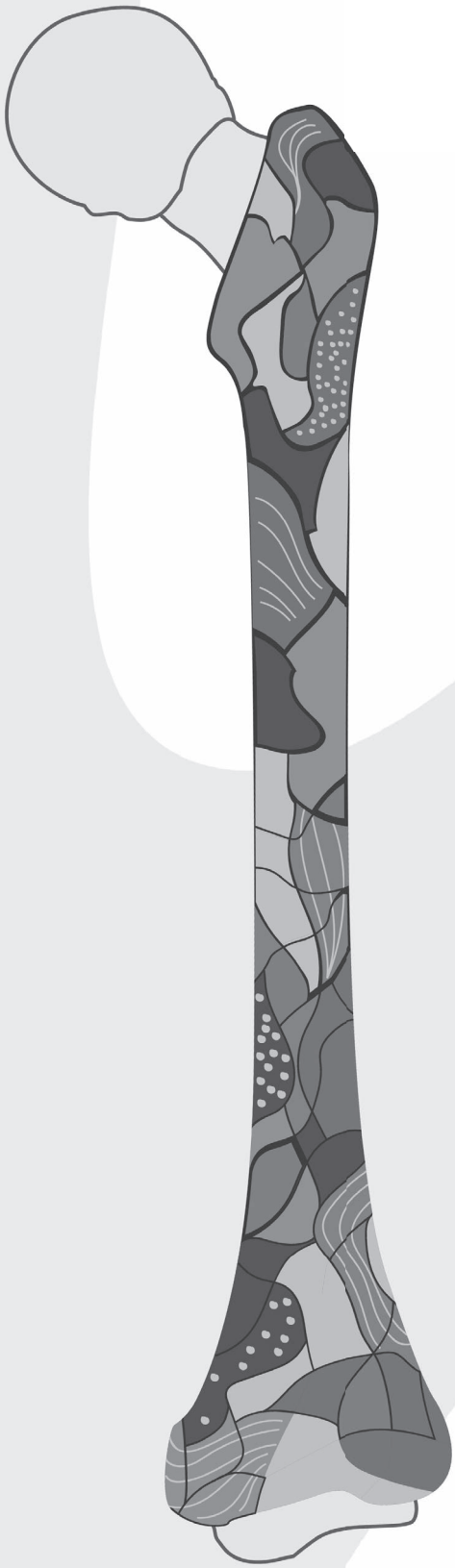
Abbreviations: ALL=acute lymphoblastic leukemia; ASP=asparaginase; CR=complete remission; DCOG=Dutch Childhood Oncology Group; DEXA=dexamethasone; dx=diagnosis; CNS=central nervous system; GCs=glucocorticoids; HR=high risk; MR=medium risk; MRD=minimal residual disease; NA=not applicable; NHR=non-high risk; PRED=Prednisone; TP=time point; WBC=white blood cell count
[1]Dexamethasone equivalent

Supplementary Table 1. Overview of chemotherapeutic agents, previously reported to be associated with osteonecrosis, in DCOG ALL-9, ALL-10 and ALL-11.



Abbreviations: ALL=acute lymphoblastic leukemia, ASP=asparaginase; DEXA=dexamethasone

Supplementary Figure 1. Cumulative incidence of symptomatic osteonecrosis for patients treated with long pulses dexamethasone (n=795) and patients treated with short pulses dexamethasone plus asparaginase (n=675) since ALL diagnosis.



CHAPTER 3

Recent perspectives on the association between osteonecrosis and bone mineral density decline in childhood acute lymphoblastic leukemia

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ABSTRACT

The attention to treatment-related toxicity has increased since the survival of children with acute lymphoblastic leukemia (ALL) has improved significantly over the past few decades. Intensive ALL treatment schedules including corticosteroids and asparaginase have been shown to give rise to skeletal abnormalities such as osteonecrosis and low bone mineral density (BMD), which may lead to debilitating sequelae in survivors. Although osteonecrosis and low BMD are different entities with suggested separate pathophysiological mechanisms, recent studies indicate that osteonecrosis is associated with accelerated BMD decline. Common underlying mechanisms for osteonecrosis and BMD decline are considered, such as an enhanced sensitivity to corticosteroids in children that suffer from both osteonecrosis and low BMD. In addition, restriction of weight-bearing activities, which is generally advised in patients with osteonecrosis, could aggravate BMD decline. This induces a clinical dilemma, since bone stimulation is important to maintain BMD but alternative interventions for osteonecrosis are limited. Furthermore, this recent finding of accelerated BMD decline in children with osteonecrosis emphasizes the need to develop effective preventive measures for osteonecrosis, which may include targeting BMD decline.

REVIEW

Introduction

The increased survival of children with acute lymphoblastic leukemia (ALL) has led to a growing awareness of treatment-related toxicity.[1] Osteonecrosis and low bone mineral density (BMD) are two common skeletal toxicities of ALL and its treatment, that may lead to important morbidity both during therapy and years thereafter.[2] In the past decade, our understanding of their pathophysiology and risk factors has increased, but effective preventive measures are still largely unavailable.

Although osteonecrosis and low BMD are different conditions, there is emerging evidence that these toxicities are associated and possibly even causally related.[3,4] However, limited knowledge is available about the mechanism of this relationship, which is pivotal when developing preventive strategies for symptomatic osteonecrosis and clinically important low BMD (i.e. BMD Z-score ≤-2, especially in the presence of low-trauma fractures). This review examines the relationship between osteonecrosis and BMD decline, common risk factors for both, and implications for treatment and preventive strategies.

Osteonecrosis

In patients with osteonecrosis, the blood supply to the bones is insufficient to meet their demands, causing bone death.[5] This impaired blood supply may be caused by intravascular emboli (for example resulting from a hypercoagulable state in patients treated for ALL[5]), increased bone marrow pressure, and/or direct blood vessel damage.[6,7] Clinically important symptomatic osteonecrosis occurs in about 1 to 8% of all patients treated for ALL.[6] Adolescents are most commonly affected (10 to 20%),[6] and we recently showed in a large cohort of 1470 children with ALL, that the cumulative incidence of symptomatic osteonecrosis and the frequency of severe osteonecrosis (Ponte di Legno Toxicity Working Group [PTWG] grade 4) are highest among patients aged 15 to 18 years (31.4%,

PTWG grade 1	Asymptomatic with findings only by MRI.
PTWG grade 2	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions only outside joint lines in non-weight-bearing bones.
PTWG grade 3	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions in weight-bearing bones or affecting joint lines in non-weight-bearing bones.
PTWG grade 4	Symptomatic with deformation by imaging of one or more joints and/or substantially limiting self-care activity of daily living.

Abbreviations: MRI=magnetic resonance imaging; PTWG=Ponte di Legno toxicity working group

Table 1. Grading of osteonecrosis associated with treatment of childhood acute lymphoblastic leukemia according to the PTWG.

59% of those had severe osteonecrosis).[8] In other hematological malignancies, this problem has been less extensively studied.

Symptoms are most commonly present in weight-bearing joints and range from slight limitations in the range of motion to severe pain and joint destruction.[9,10] Several classification systems for osteonecrosis exist, both clinical (based on symptoms), radiological (based on magnetic resonance imaging [MRI] scans), and combined classifications. To facilitate comparisons of frequencies and severities across ALL treatment protocols, the PTWG has established a consensus definition and grading of osteonecrosis (Table 1), which is based on the severity of clinical symptoms and MRI abnormalities (involvement of weight-bearing bones, joint lines, or joint deformation).[11] The Niinimäki classification allows radiological classification of osteonecrotic lesions at multiple sites.[12] The severity is based on the localization of the lesions (weight-bearing versus non-weight-bearing bones and epiphysis versus diaphysis/metaphysis), as well as the area of articular surface involvement (<30% versus ≥30%) and presence of joint deformation.

Symptomatic osteonecrosis most often occurs during the maintenance phase of ALL treatment[9], but can already be present at ALL diagnosis[13] and in rare cases even years after treatment cessation.[14] The main treatment-related risk factor is exposure to corticosteroids, especially when administered concurrently with asparaginase.[8,15] Patients who underwent hematopoietic stem cell transplantation (HSCT) are also at increased risk of developing symptomatic osteonecrosis.[10]

Low bone mineral density and fractures

Children treated for ALL are at increased risk of BMD decline and consequent bone fractures.[16,17] A large national Dutch study showed that BMD of the lumbar spine was below normative values in children at ALL diagnosis, and remained lower during treatment.[17] The 3-year cumulative incidence of symptomatic fractures was 17.8% in this cohort. Significantly lower BMD was observed in children with fractures versus those without. In a previous study including a subset of these patients, the fracture incidence was compared with that of healthy controls, which showed that the fracture rate in patients with ALL was 6 times higher.[18] In a well-characterized pan-Canadian cohort, the cumulative incidence of fractures was 36% (32.5% for vertebral fractures and 23.0% for non-vertebral fractures) from ALL diagnosis until 6 years of follow-up.[16] The peak annual incidence of vertebral fractures occurred at 12 months, and of non-vertebral fractures at 24 months. Most importantly, every 1 standard deviation reduction in BMD Z-score at ALL diagnosis increased the risk of vertebral and non-vertebral fractures by 89% and 70%, respectively.

At the same time, the skeleton has significant regenerative capacity, reflected by reshaping of vertebral bodies following vertebral fractures, as well as an increase in BMD after ALL treatment discontinuation in most children.[16,17,19] It is not entirely clear whether low BMD during ALL treatment impacts bone health in the (very) long term, since longitudinal studies from the time of diagnosis with more than 10 years of follow-up are lacking. However, it is conceivable that a proportion of children with persistently low BMD may be at life-long increased risk of fractures and associated pain, vertebral deformity, and functional morbidity.[20,21] Hence, prediction of this subset of survivors is important. We recently published a prediction model for low BMD (Z-score ≤-1) and very low BMD (Z-score ≤-2) based on easily measured patient and treatment characteristics (including sex, attained age, height, weight, current smoking status, and previous cranial irradiation and abdominal irradiation), which correctly identified BMD status in most white adult survivors of childhood cancer.[22]

Association between osteonecrosis and BMD

In 2015, the association between symptomatic osteonecrosis and BMD decline during and shortly after ALL therapy was established for the first time.[4] We showed that at ALL diagnosis, lumbar spine and total body BMD were not significantly different in patients who subsequently developed symptomatic osteonecrosis versus those who did not. However, BMD decline over time was more pronounced in children with symptomatic osteonecrosis compared to children without symptomatic osteonecrosis, which started right after ALL diagnosis but became more substantial following osteonecrosis diagnosis. At cessation of treatment, this also led to a significantly lower BMD in patients with symptomatic osteonecrosis. Hence, we revealed that additional BMD decline in patients with symptomatic osteonecrosis started only after osteonecrosis occurrence. This study suggests that advised weight-bearing restrictions to avoid aggravation of osteonecrosis may accelerate BMD decline.

The results of a recently published study by *Inaba et al.*[3] confirmed the association between osteonecrosis and BMD decline in 334 children with ALL who were prospectively screened for osteonecrosis by MRI. They found that knee osteonecrosis (59.3%) occurred more often than hip osteonecrosis (12.0%). Interestingly, they showed that the development of very low BMD (BMD Z-score ≤ –2.0) was associated with osteonecrosis of the knee, but not with osteonecrosis of the hip. The decrease in BMD Z-score was especially pronounced in patients with ≥30% epiphyseal osteonecrosis involvement. Inaba and colleagues did not separately analyze the course of BMD decline (before or after osteonecrosis

occurrence) or the effect of osteonecrosis on BMD decline in patients with symptomatic versus asymptomatic osteonecrosis. This would be relevant because an observation that BMD decline is most evident after symptomatic osteonecrosis occurrence would support the idea that immobilization for osteonecrosis aggravates BMD decline. Since lumbar spine BMD alone was measured in the study by Inaba and colleagues, and not hip BMD (a site that is frequently affected by osteonecrosis), it is less conceivable that the reported general BMD decline was induced by destruction of the bone due to localized osteonecrotic lesions.

Hence, it remains unclear whether the most commonly used management for symptomatic osteonecrosis, i.e. weight-bearing restrictions, may aggravate the skeletal pathology in children with ALL. This is especially important since, although the application of these restrictions seems rational, the efficacy as a singular intervention to improve osteonecrosis symptoms has never been proven.[23] Alternatively, several common underlying mechanisms in children with osteonecrosis and low BMD might be considered, as discussed next.

	ON	BMD↓
Patient characteristics		
Sex	± (female)	**?** (male)
Older age at ALL diagnosis	**+**	±
BMI	± (higher)	**+** (lower)
Caucasian race	**+**	**+**
Disease factors		
Leukemic disease	±	**+**
Treatment factors		
Corticosteroids	**+**	**+**
Asparaginase (+ GCs)	**+**	**+**
Methotrexate	**?**	**?**
Radiotherapy	**+**	**+**
HSCT	**+**	**+**
Treatment induced metabolic changes		
Marrow adipose tissue	**+**	**+**
Hyperlipidemia	**+**	**?**

+	Independent risk factor in childhood ALL
±	Conflicting evidence for this risk factor in childhood ALL
?	Associated with the outcome in other populations

Abbreviations: ALL=acute lymphoblastic leukemia; BMD=bone mineral density; BMI=body mass index; GCs=glucocorticoids;
HSCT=hematopoietic stem cell transplantation; ON=osteonecrosis; RT=radiotherapy

Table 2. Common risk factors for osteonecrosis and low BMD in the context of pediatric ALL.

Common risk factors for osteonecrosis and low bone mineral density

Osteonecrosis and low BMD are considered different entities with a different pathogenesis; however, the co-occurrence of these side effects suggests that common factors may play a role in their pathophysiology (Table 2). Osteonecrosis (although uncommon) and low BMD can already be present at leukemia diagnosis, indicating that the leukemic disease itself (for example due to enhanced bone resorption via cytokine release by lymphoblasts[24]) could play a role in the development of both complications.[13,17] Furthermore, the main treatment-related risk factor (i.e. corticosteroid exposure) is shared, so a common pathophysiology through enhanced sensitivity to corticosteroids may be considered.[25,26] Asparaginase increases dexamethasone plasma levels, and may thus potentiate the detrimental effects of corticosteroids on the bone when administered concurrently.[27,28] In addition, especially PEG-asparaginase leads to hyperlipidemia, which is associated with osteonecrosis in children with ALL.[29] The association between hyperlipidemia and low BMD has not been established in this population, but interestingly, studies in the general adult population do show such an adverse effect.[30,31] High-dose methotrexate may additionally modify the risk of osteonecrosis and BMD decline due to inhibition of bone formation and mineralization,[6,32,33] although it has not been identified as an independent risk factor for either toxicity in children with cancer. HSCT has been associated with an increased risk of symptomatic osteonecrosis, independently of corticosteroid dose,[10] and low BMD has been frequently described after HSCT, even in groups in which the majority had not been treated with corticosteroids.[2,34] In addition, direct radiation to the bone can lead to osteo(radio)necrosis due to damage of the microvasculature within and surrounding the bone, and cranial/craniospinal irradiation and total body irradiation have been associated with low BMD in survivors of childhood ALL.[21,35,36] Lastly, components of ALL treatment impair BMD by inducing mesenchymal stem cells to differentiate into adipocytes rather than osteoblasts, amongst other mechanisms.[37] Fatty infiltration into the bone marrow is also assumed to be associated with osteonecrosis development, although the main contributing factor is thought to be decreased blood flow to the bone due to micro thrombi which induces osteocyte death.[6]

In addition, several common non-treatment-related risk factors for osteonecrosis and low BMD have been identified. Older age is the most important risk factor for osteonecrosis in children with ALL,[6] and has also been shown to increase the risk of BMD decline.[17,38] The role of sex in developing osteonecrosis has not been consistently shown, however, some studies in children with ALL have

indicated that females are at increased risk for osteonecrosis.[9,39] A large study in 845 adult survivors of childhood ALL has shown that males are at increased risk of developing low BMD (Z-score ≤-1), although this association has not been shown during ALL therapy.[21] The effect of body mass index (BMI) on the risk of osteonecrosis is also opposite to its effect on low BMD: a *high* BMI has in some studies been associated with osteonecrosis (probably through the same mechanism as hyperlipidemia since those conditions are highly correlated[40]),[41] whereas *low* BMI or body weight has been associated with low BMD.[17] Finally, Caucasian children have been shown to be at increased risk for both osteonecrosis and low BMD.[42–44]

Variation in the occurrence of these serious side effects among similarly treated patients suggests a role for genetic susceptibility. Carriership of single nucleotide polymorphisms (SNPs) in, for example, the MTHFR, MTRR, and VDR genes has been associated with low BMD at ALL diagnosis and during ALL treatment,[45,46] and SNPs near glutamate receptor genes, in the ACP1 gene, and in the VDR Fok I start site have been associated with the development of osteonecrosis.[28,43,47] Although genetic variations in the VDR gene led to an increased risk of both lower BMD and osteonecrosis, the exact SNPs were different.

Interventions to overcome osteonecrosis and bone mineral density decline

Because of the possibility that BMD decline is accelerated by osteonecrosis occurrence, the development of adequate interventions for osteonecrosis becomes even more important, since this could prevent BMD decline to a certain extent. However, concomitant osteonecrosis and low BMD in pediatric ALL pose a challenging clinical dilemma. Weight-bearing restrictions to ameliorate osteonecrosis symptoms may be carefully considered to prevent the acceleration of bone density decline, as other treatment options for osteonecrosis and evidence for their effectiveness are limited. For this purpose, non-weight-bearing exercises such as swimming and cycling, under the supervision of a specialized physiotherapist, may be encouraged as this perhaps enhances bone formation and stimulates muscle strength while limiting weight-bearing of the joints.[4] Bisphosphonates have been evaluated for pain reduction and prevention of articular collapse in patients with symptomatic osteonecrosis. Although small case-series have indeed suggested that bisphosphonates lead to pain relief and improved mobility, they failed to show a decline in the progression of joint destruction.[48] Whether early administration of bisphosphonates to children with asymptomatic osteonecrosis has the potential to mitigate disease

progression remains unknown. Except for acute phase reactions, no adverse events of bisphosphonate therapy were reported. However, the safety and efficacy of bisphosphonate administration during leukemia treatment needs to be demonstrated in preclinical studies and randomized controlled trials. Until then, their prescription should be restricted to compassionate grounds in selected patients. In case of low BMD, bisphosphonates are reserved for children with severe osteoporosis (BMD Z-score ≤-2 in the presence of clinically relevant fractures) and low potential for BMD restitution.[26]

In more advanced stage osteonecrosis (PTWG grade 3), several non-conservative treatment options to prevent articular collapse have been reported, such as core decompression (with or without mesenchymal stem cells), bone grafting and osteotomies.[49] Unfortunately, evidence that these interventions prevent further joint damage remains limited, and a recent study in 85 children and young adults with osteonecrosis during ALL treatment suggested that core decompression does not delay or improve the rates of femoral head survival.[50] Furthermore, the timing of such interventions is difficult given the variable natural history of the progression of osteonecrosis. Preferably, these interventions should take place before any joint damage (before PTWG grade 4), although intervening too early in patients who would never experience joint collapse should be avoided, as about 60% of patients with symptomatic osteonecrosis show reversible symptoms.[9] The identification of patients at high risk for severe osteonecrosis could guide this decision, but so far, only older age (about 15 to 21 years),[8,39,51] multiple joints affected at diagnosis of osteonecrosis,[51] and the presence of bone marrow edema on MRI in some studies[52] have been shown to increase the risk of progressive osteonecrosis.

Given the limited availability and efficacy of treatment options for osteonecrosis, some patients eventually require joint replacement,[49] which is an undesirable outcome as these patients are young and prostheses have a lifespan of approximately 15 to 25 years.[53] Thus, strategies to identify patients at risk of severe, progressive osteonecrosis at an early stage, as well as strategies to prevent osteonecrosis, are preferable and should be pursued.

Emerging preventive measures for osteonecrosis and bone mineral density decline

Several of these strategies have recently been explored or are currently under investigation. In 2012, *Mattano and colleagues* from the Children's Oncology Group proved the first and only preventive measure for osteonecrosis thus

far by showing that the administration of shorter pulses of dexamethasone reduced the risk of osteonecrosis in patients aged 10 to 21 years despite a higher cumulative dose and without compromising leukemia outcomes.[39] This simple scheduling modification was adopted in many ALL protocols.[54] However, our group showed that the protective effect of these shorter pulses on osteonecrosis development may be dependent on the therapeutic context and especially that the administration of concurrent intensified asparaginase (administered in current ALL protocols) could attenuate the benefit.[8] The ongoing British Osteonecrosis Study (BONES) will assess risk factors and specific radiological features that predict progression in those with (symptomatic) osteonecrosis (ClinicalTrials.gov Identifier: NCT02598401).[55] Since in this study patients with ALL aged 10 to 24 are prospectively screened for osteonecrosis by MRI and for BMD decline by dual energy X-ray absorptiometry, it could potentially confirm whether BMD decline precedes and/or follows osteonecrosis. Furthermore, in assessing the association between osteonecrosis and BMD decline, stratification of patients with asymptomatic and symptomatic osteonecrosis could identify whether osteonecrosis itself (suggesting common risk factors), or interventions for symptomatic osteonecrosis (i.e. weight-bearing restrictions), are associated with BMD decline.

Janke et al. from St. Jude Children's Research Hospital showed that hypertension might be a modifiable risk factor for osteonecrosis[7] and initiated an ongoing randomized controlled trial investigating the effect of an antihypertensive drug (lisinopril) on osteonecrosis development (ClinicalTrials.gov Identifier: NCT04401267). Furthermore, recent publications on the role of hyperlipidemia in the development of osteonecrosis[28,29,56] and other toxicities[57] prompted the design of a randomized controlled trial assessing the effect of omega-3 supplements on lipid levels and osteonecrosis occurrence in children and young adults with ALL treated according to the NOPHO (and now the ALLTogether) protocol, which is currently recruiting (ClinicalTrials.gov Identifier: NCT04209244). The results of these trials are expected within the next few years and may hopefully identify an effective way to prevent osteonecrosis, and thereby potentially low BMD as well.

Targeting BMD decline during ALL therapy directly is also an area of ongoing research. Adequate dietary calcium (200-1,100 mg/day) and vitamin D (at least 400 IU/day) is important,[58] and vitamin D and calcium supplementation has been shown to increase BMD in otherwise healthy children and adults with low vitamin D levels (25OHD levels <20 ng/ml).[59,60] Whether vitamin D and calcium supplementation has a significant effect on estimates of bone strength in

children with cancer at higher 25OHD thresholds remains unknown. *Mogil et al.* showed that low-magnitude, high-frequency mechanical stimulation significantly improved tibial trabecular bone content in a per-protocol analysis of childhood cancer survivors completing at least 70% of prescribed sessions.[61] The effect of this intervention on BMD parameters during ALL therapy is currently underway as part of the St. Jude Total Therapy XVII trial (ClinicalTrials.gov Identifier: NCT03117751).

Conclusion

The recently established association between osteonecrosis and accelerated BMD decline suggests that common factors may play a role in their pathophysiology. Furthermore, BMD declines complicate considerations about the treatment of osteonecrosis; weight-bearing restrictions should be carefully considered and supervised by an experienced physical therapist to minimize potential consequent BMD decline. The results of ongoing randomized controlled trials investigating the preventive effect of several agents on osteonecrosis and BMD decline are eagerly awaited.

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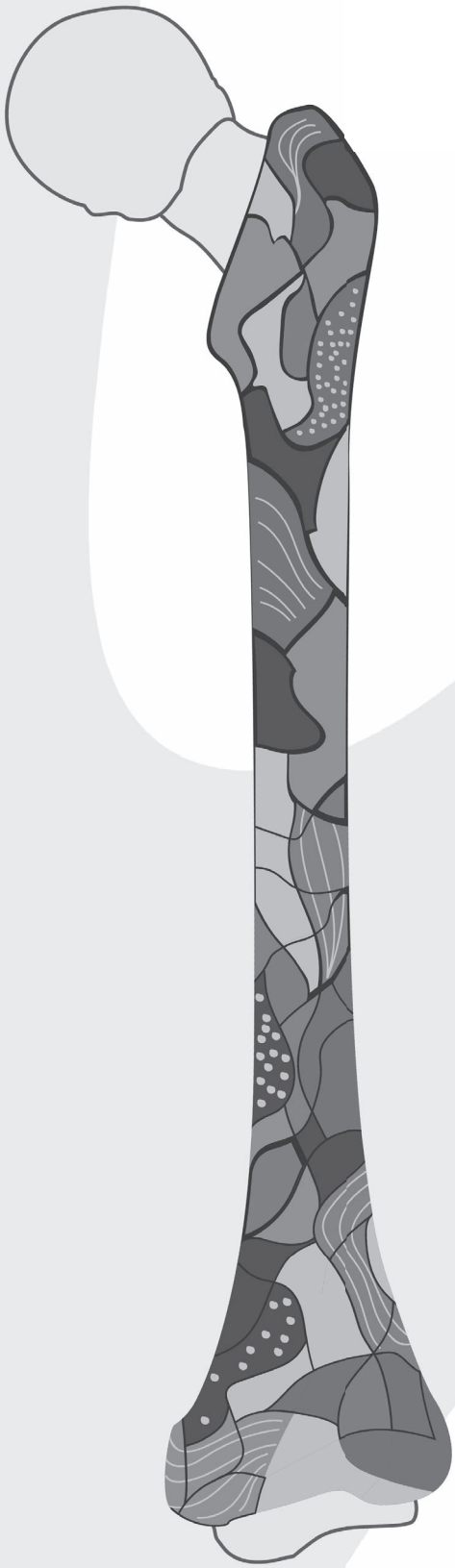

CHAPTER 4

Prediction of low and very low bone mineral density among adult survivors of childhood cancer

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ABSTRACT

Purpose: To develop and validate prediction models for low and very low bone mineral density (BMD) based on clinical and treatment characteristics that identify adult survivors of childhood cancer who require screening by dual-energy X-ray absorptiometry (DXA).

Methods: Caucasian survivors (N=2032, median attained age, 29.3 [range:18.1-40.9] years) enrolled in the St. Jude Lifetime Cohort (SJLIFE, development) and survivors treated at the Erasmus Medical Center (validation), the Netherlands (N=403, median age, 24.2 [range:18.0-40.9] years) were evaluated with DXA to determine lumbar spine (BMD_{LS}) and total body (BMD_{TB}) BMD. Low and very low BMD were defined as BMD_{LS} and/or BMD_{TB} Z-score ≤-1 or ≤-2, respectively. Multivariable logistic regression was used to build prediction models; performance was assessed using receiver operating characteristic curves. Diagnostic values were calculated at different probabilities.

Results: Low BMD was prevalent among 51% and 45% of SJLIFE and Dutch participants, and very low BMD among 20% and 10%, respectively. The model for low BMD included male sex (odds ratio [OR]=3.07), height (OR=0.95), weight (OR=0.98), attained age (OR=0.97), current smoking (OR=1.48) and cranial irradiation (OR=2.11). Areas under the curves (AUCs) were 0.72 (95%-CI=0.70-0.75) in SJLIFE and 0.69 (95%-CI=0.64-0.75) in the Dutch cohort. The sum of the sensitivity (69.0%), and specificity (64.0%) was maximal at the predicted probability of 0.50. The model for very low BMD included male sex (OR=3.28), height (OR=0.95), weight (OR=0.97), attained age (OR=0.98), cranial (OR=2.07) and abdominal (OR=1.61) irradiation, yielding AUCs of 0.76 (95%-CI=0.73-0.78; SJLIFE cohort) and 0.75 (95%-CI=0.67-0.83; Dutch cohort).

Conclusion: Validated prediction models for low and very low BMD, using easily measured patient and treatment characteristics, correctly identified BMD status in most adult Caucasian survivors through age 40 years.

INTRODUCTION

The survival of childhood cancer has improved to more than 80% over the last several decades.[1] As a result of the increasing population of long-term survivors, recognition of late effects among survivors including low bone mineral density (BMD) and subsequent risk of fractures has increased.[2,3] The prevalence of low BMD, generally defined as a BMD Z-score below -1, varies from 20 to 50% among survivors of acute lymphoblastic leukemia (ALL)[4–6], and from 40 to 60% among survivors of non-hematological cancers.[7–9] The prevalence of very low BMD (Z-score below -2) ranges from 13 to 25% among pediatric cancer survivors.[4,7,9]

Adult survivors of childhood cancer are at risk of low BMD as a result of disturbances in bone metabolism during childhood or adolescence, which may inhibit attainment of peak bone mass.[10] These disturbances may develop as a consequence of the malignancy itself,[11] the side effects of the cancer experience, such as altered dietary intake and reduced physical activity during and after cancer treatment,[12–14] or because normal bone mineral accretion is affected by corticosteroids and chemotherapeutic agents.[6,15] Additionally, BMD may be adversely affected as a result of gonadal failure following exposure to pelvic radiation or alkylating agents, or because of hypothalamic pituitary endocrinopathies following central nervous system irradiation.[6,16] Lastly, genetic susceptibility to developing BMD deficits may play a role.[17]

Low BMD is of concern as it may increase the risk of osteoporosis and fragility fractures.[18] Multiple body sites can be used to measure BMD, including the hip, lumbar spine and total body. Studies in non-cancer populations have shown that depending on the site and severity, fractures can result in pain, temporary or permanent loss of function, and may require hospitalization, rehabilitation and after-hospital care, leading to a reduction in Disability Adjusted Life Years.[19]

The most widely validated technique to assess BMD is dual-energy X-ray absorptiometry (DXA).[20] However, DXA screening is not routinely recommended for all survivors because of concerns related to procedural financial costs and radiation exposure. Thus, it is important to identify sub-groups of survivors at high-risk of having low BMD who may benefit most from DXA screening, as survivors with low BMD may benefit from targeted interventions directed at improving bone health.[21–23]

Although many studies have identified risk factors for low BMD among survivors of childhood cancer,[6,24–26] no prediction model has been developed and the

optimal surveillance strategy for survivors at risk of low BMD has not been established. The aim of this study was to develop and validate clinically applicable prediction models that identify young adult survivors of childhood cancer at risk of low and very low BMD based on individual patient characteristics and past cancer treatment.

PATIENTS AND METHODS

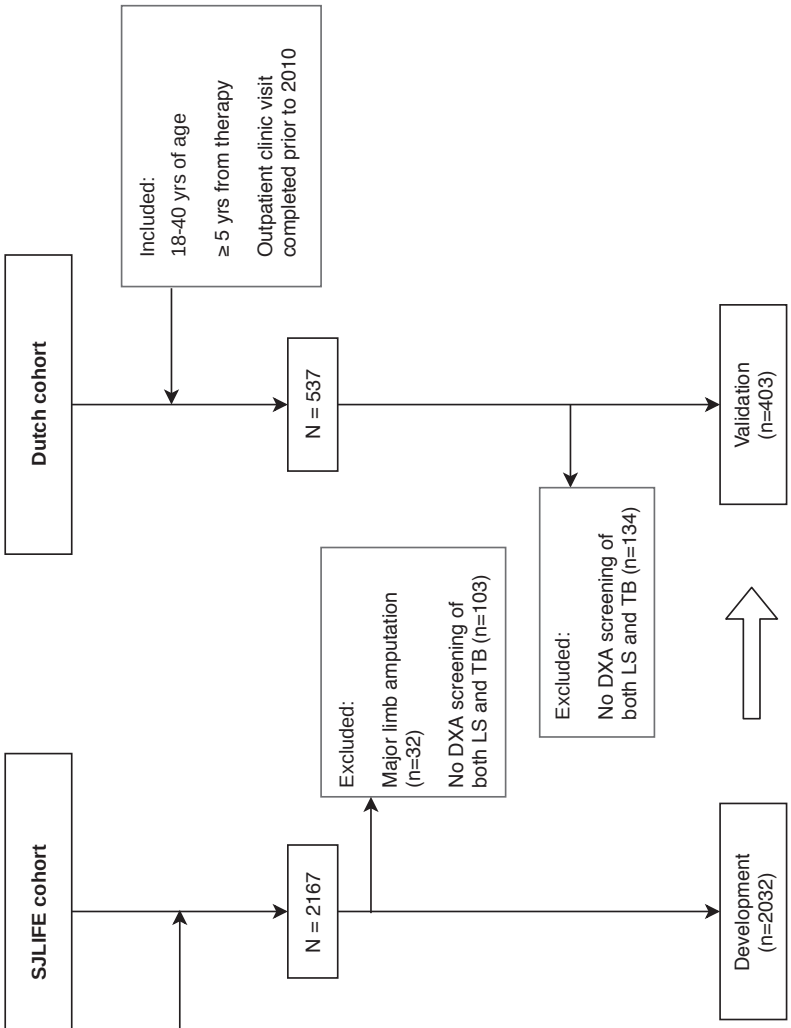
Study population

St. Jude Lifetime cohort (development model)

The development cohort consisted of participants in the St. Jude Lifetime Cohort Study (SJLIFE), a retrospective cohort study with ongoing prospective follow-up that includes periodic clinical assessments.[27,28] To be eligible for this analysis, SJLIFE participants had to be between 18 and 40 years of age, ≥10 years from diagnosis, treated for childhood cancer at St. Jude Children's Research Hospital (Memphis, TN, United States), have undergone DXA of both the lumbar spine (LS) and total body (TB) prior to June 30, 2016 (n=2032) and reported their ethnicity as Caucasian (Figure 1). All participants underwent a core battery of testing that included DXA screening. This study was approved and conducted according to the standards of the Institutional Review Board. Informed consent was obtained from all participants.

Dutch survivors (model validation)

The Dutch survivors included a single center cohort of 544 survivors of childhood cancer who visited the Long-Term Effects Registry (LATER) outpatient clinic between 2003 and 2008. All patients had been treated for childhood cancer at the Erasmus Medical Center-Sophia Children's Hospital (Rotterdam, the Netherlands) between 1965 and 2003 and had been finished with cancer treatment for at least 5 years. Among the 544 survivors attending the LATER clinic, 403 (74.1%) who were between 18 and 40 years of age and who had been referred for DXA of the LS and TB according to the Dutch Children's Oncology Group Long-term follow-up guidelines, or at the discretion of the treating physician, were included in this study (Figure 1). Survivors who underwent DXA screening tended to be older at both primary cancer diagnosis and at follow-up, and more likely to be treated with corticosteroids compared to those who did not undergo screening.[6] Race and ethnicity of participants was not recorded. Informed consent was obtained from participants to use LATER clinic measurements for research purposes.



Abbreviations: SLIFE=St. Jude Lifetime Cohort Study; DXA=dual X-ray absorptiometry; LS=lumbar spine; TB=total body

Figure 1. Flow diagram of study participants.

BMD measurement

BMD of the lumbar spine (L1—L4) (BMD_{LS}) and total body (BMD_{TB}) were assessed using DXA (Hologic 4500 QDR fan array scanner among SJLIFE participants and Lunar Prodigy or Lunar DPX-L, Madison, WI, USA among Dutch participants). Z-scores reflecting the number of standard deviations that a BMD value of a survivor differs from the mean BMD of a healthy reference population were used to correct for age and sex. Low BMD was defined as BMD_{LS} and/or BMD_{TB} Z-score below -1.0. Very low BMD was defined as BMD_{LS} and/or BMD_{TB} Z-score below -2.0. Z-scores were used as endpoints instead of T-scores as our analyses focused on a young adult population.[29]

Predictors

Possible predictors of BMD status were selected based on previous reports showing an association with low BMD in childhood cancer survivors[7,15,16,25,30–34] or the general population[35], and on the ease of which variables could be obtained in a late effects clinic or primary care setting. Patient characteristics included sex, age at diagnosis, and current smoking status (yes/no), as well as height (cm), weight (kg), and attained age at DXA examination. Treatment factors included previous treatment (yes/no) with corticosteroids, methotrexate, alkylating agents, cranial irradiation or abdominal irradiation (both including total body irradiation). There were no missing data in the SJLIFE development cohort. In the Dutch validation cohort, each missing datum was replaced with its median value per gender (15.9% of the participants were missing height and weight; 15.6% smoking status).

Statistical analysis

The prediction models were developed following the TRIPOD criteria.[36] In the development cohort, univariable logistic regression was used to identify factors associated with an increased risk of low BMD. Factors associated with an increased risk of low BMD in the univariable analyses at $p<0.20$ were included in multivariable analyses.

We created a prediction model for low BMD using backward multivariable logistic regression. All predictors that were associated with low BMD with $p<0.05$ for the likelihood ratio test were included in the final model. *A priori,* we chose to include sex in the model because the decline in bone mass that occurs with aging differs by sex. We also included height because DXA provides a 2-dimensional assessment of a 3-dimensional structure, consequently resulting in lower BMD Z-scores in

short individuals.[6,7,16,31] During model development, cumulative drug doses for methotrexate and alkylating agents, radiation dose to the hypothalamus-pituitary axis, and BMI, were considered but were not found to improve discrimination between survivors with and without low BMD above that of models in which treatments were dichotomized or where height and weight replaced BMI. The strength of the associations between the predictors and BMD was reported using beta-coefficients, odds ratios (OR) and 95% confidence intervals (95% CI). Model performance was assessed using estimates of discrimination and calibration. Discrimination was evaluated by generating a receiver operating characteristic (ROC) curve estimating the area under the curve (AUC), while calibration was evaluated using the Homer-Lemeshow goodness of fit statistic. Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of the model were calculated at different cut-points of predicted probabilities. The ability of the prediction model developed in the SJLIFE cohort to discriminate between participants with or without low BMD was assessed by calculating the AUC's of the same model in the Dutch validation cohort. Sensitivity analyses using only Dutch survivors with complete data were also performed.

A model for very low BMD was built and validated using the same methodology. An online calculator to determine the predicted probability of low and very low BMD for an individual survivor is available at https://riskcalculator-bonemineraldensity-childhoodcancer.azurewebsites.net/

RESULTS

Cohort characteristics

Survivors participating in SJLIFE tended to be shorter (mean, 168.8 [± standard deviation 10.24] vs. 173.5 [±9.10], $p<0.001$), heavier (79.5 [±21.12] vs. 71.0 [±13.1], $p<0001$), and older (median attained age, 29.2 [± interquartile range 9.5] vs. 24.2 [±9.2], $p<0.001$) than survivors in the Dutch cohort (Table 1). Moreover, SJLIFE participants were more likely to be treated with an alkylating agent (56.5% vs. 50.6%, $p=0.03$), cranial (33.9% vs. 22.5%, $p<0.001$) or abdominal (21.7% vs. 6.5%, $p<0.001$) radiation or to smoke (24.4% vs. 17.9%, $p=0.005$), and less likely to have received methotrexate (53.9% vs. 60.5%, $p=0.01$) or glucocorticoids (53.9% vs. 70.0%, $p<0.001$). Median time between cancer diagnosis and DXA examination was 21.6 (range: 10.4 to 40.6) years for the SJLIFE cohort and 15.1 (range: 5.1 to 39.8) years for the Dutch survivors. Characteristics of Dutch survivors with complete values are presented in Supplementary Table 1.

		SJLIFE cohort (N=2032)		Dutch cohort (n=403)		
		Mean (SD; range)	N (%)	Mean (SD; range)	N (%)	P-value[1]
Child hood cancer diagnosis	Acute lymphoblastic leukemia		741 (36.5)		184 (45.7)	<0.001
	Other leukemia		86 (4.2)		18 (4.5)	
	Hodgkin lymphoma		205 (10.1)		46 (11.4)	
	Non-Hodgkin lymphoma		150 (7.4)		50 (12.4)	
	CNS tumor		265 (13.0)		23 (5.7)	
	Renal tumor		122 (6.0)		48 (11.9)	
	Neuroblastoma		98 (4.8)		16 (4.0)	
	Soft tissue sarcoma		113 (5.6)		8 (2.0)	
	Bone tumor		78 (3.8)		10 (2.5)	
	Other		174 (8.6)		0 (0.0)	
Sex	Male		1075 (52.9)		237 (58.8)	0.03
	Female		957 (47.1)		166 (41.2)	
Height (cm)		168.8 (10.24; 128.6-204.4)		173.5 (9.10; 145.5-196.0)		<0.001
Weight (kg)		79.5 (21.12; 31.7-171.0)		71.0 (13.1; 36.7-126.0)		<0.001
Age at diagnosis (yrs)		6.1 (9.1; 0-22.7)[2]		6.5 (8.3; 0-16.8)[2]		0.97
Attained age (yrs)		29.3 (9.5 ; 18.1-40.9)[2]		24.2 (9.2; 18.0-40.9)[2]		<0.001
Current smoker	Yes		496 (24.4)		72 (17.9)	0.005
	No		1536 (75.6)		331 (82.1)	
Subsequent diagnosis[3]	Yes		198 (9.7)		48 (11.9)	0.19
	No		1834 (90.3)		355 (88.1)	

		SJLIFE cohort (N=2032)		Dutch cohort (n=403)		
		Mean (SD; range)	N (%)	Mean (SD; range)	N (%)	P-value[1]
Alkylating Agent	Yes		1149 (56.6)		204 (50.6)	0.03
	No		883 (43.5)		199 (49.4)	
Metho-trexate	Yes		1095 (53.9)		244 (60.5)	0.01
	No		937 (46.1)		159 (39.5)	
Gluco-corticoid	Yes		1095 (53.9)		282 (70.0)	<0.001
	No		937 (46.1)		121 (30.0)	
Cranial RT[4]	Yes		688 (33.9)		91 (22.6)	<0.001
	No		1344 (66.1)		312 (77.4)	
Abdominal RT[5]	Yes		441 (21.7)		26 (6.5)	<0.001
	No		1591 (78.3)		377 (93.5)	

Abbreviations: SJLIFE=St. Jude Lifetime Cohort; SE=standard error; N=number of patients; CNS=central nervous system; IQR=interquartile range; TBI=total body irradiation

[1]Chi-square p-value for dichotomous covariates, two-sample independent *t*-test p-value for normally distributed continuous covariates and Mann-Whitney U Test p-value for non-normally distributed continuous covariates; [2]Median (IQR; range) (non-normally distributed); [3]Non-melanoma skin cancer was excluded; [4]Including TBI, excluding scatter radiation; [5]Including pelvic radiation and TBI, excluding scatter radiation

Table 1. Characteristics of study participants.

	β (SE)	OR	95% CI
β_0	10.91		
Sex	1.12 (0.14)	3.07	2.35—4.02
Height	-0.05 (0.01)	0.95	0.93—0.96
Weight	-0.02 (<0.01)	0.98	0.97—0.98
Attained age	-0.03 (0.01)	0.97	0.96—0.99
Current smoker	0.39 (0.11)	1.48	1.19—1.85
Cranial irradiation	0.75 (0.11)	2.11	1.69—2.63
SJLIFE cohort AUC total (95% CI)	0.72 (0.70—0.75)		
Dutch cohort AUC total (95% CI)	0.69 (0.64—0.75)		

Abbreviations: SJLIFE=St. Jude Lifetime Cohort; β=regression coefficient; SE=standard error; OR=odds ratio; CI=confidence interval; β_0=intercept; AUC=area under the curve

Table 2. Multivariable logistic regression model for low BMD among adult childhood cancer survivors.

Low BMD was observed in 51.5% of the SJLIFE cohort and in 44.7% of the Dutch cohort. In the SJLIFE cohort, the prevalence of low BMD_{LS} was 25.1% (mean Z-score [±SD]= -0.16 [±1.24]) while low BMD_{TB} occurred in 48.0% of patients (-0.94 [±1.25]) (Supplementary Figure 1). Among Dutch survivors, low BMD_{LS} occurred in 27.3% of patients (-0.32 [±1.04]), while low BMD_{TB} occurred in 37.0% of patients (-0.51 [±1.10]). Very low BMD occurred among 20.2% and 10.2% of participants in the SJLIFE and Dutch cohorts, respectively.

Prediction model for low BMD among adult survivors

Results of univariable analyses in the SJLIFE development cohort are provided in Supplementary Table 2. Backward multivariable logistic regression analysis identified male sex (OR=3.07, 95% CI=2.35 to 4.02), shorter height (OR=0.95, 95% CI=0.93 to 0.96), lower weight (OR=0.98, 95% CI=0.97 to 0.98), younger attained age (OR=0.97, 95% CI=0.96 to 0.99), current smoking (OR=1.48, 95% CI=1.19 to 1.85) and cranial irradiation (OR=2.11, 95% CI=1.69 to 2.63) as predictors for low BMD (Table 2). The AUC of this model was 0.72 (95% CI=0.70 to 0.75; Figure 2A). The Hosmer and Lemeshow Goodness-of-Fit test showed non-significant results in all steps of backward logistic regression, providing a chi-square p-value of 0.466 in the final step.

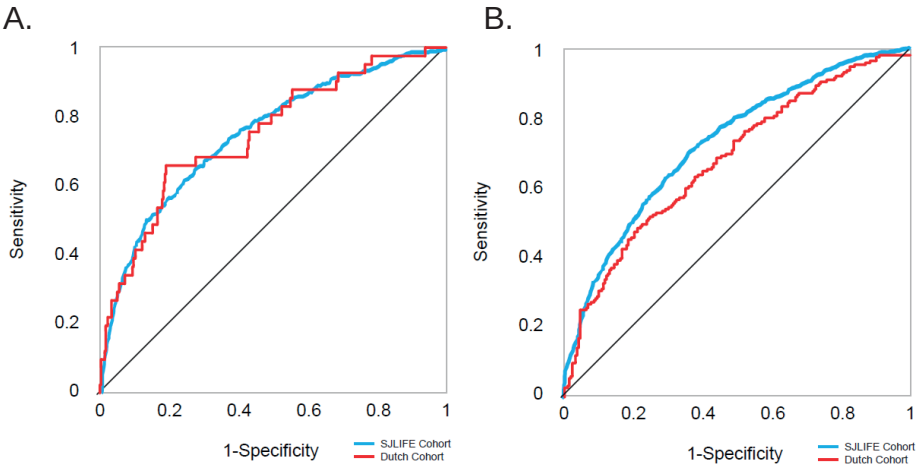


Figure 2. Receiver operating characteristic curves of the prediction models for low BMD (A) and very low BMD (B) in the SJLIFE development cohort and Dutch validation cohort.

The diagnostic values of the model are presented in Supplementary Table 3. At relatively low cut-points of predicted probability, for example, 0.20, sensitivity was high (98.3%) whereas specificity was low (9.6%). At a cut-point of 0.50, the sum of sensitivity (69.0%) and specificity (64.0%) was highest, with 53.0% of the cohort predicted to have low BMD. The positive predictive value (PPV) was 67.0% and the negative predictive value (NPV) was 66.1%. When this model was tested in the Dutch survivors, an AUC of 0.69 (95% CI=0.64 to 0.75) was observed (Table 2, Figure 2A). Sensitivity was 50.6%, specificity was 77.6%, PPV was 64.3% and NPV was 65.8% at a cut-point of 0.5 among Dutch survivors. Sensitivity analyses limited to Dutch survivors with complete data generated similar findings (AUC=0.71, 95% CI=0.66 to 0.77).

Prediction model for very low BMD among adult survivors

Univariable analyses of associations between patient and treatment factors and very low BMD are shown in Supplementary Table 2. Male sex (OR=3.28, 95% CI=2.37 to 4.54), shorter height (OR=0.95, 95% CI=0.93 to 0.96), lower weight (OR=0.97, 95% CI=0.96 to 0.98), younger attained age (OR=0.98, 95% CI=0.96 to 1.00), cranial irradiation (OR=2.07, 95% CI=1.59 to 2.68) and abdominal irradiation (OR=1.61, 95% CI=1.23 to 2.11) were included in the model for very low BMD, yielding an AUC of 0.76 (95% CI=0.73 to 0.78). In the validation cohort, the AUC of the model was 0.75 (95% CI=0.67 to 0.83) (Table 3, Figure 2B, Supplementary Table 4). Among Dutch survivors with complete data (sensitivity analyses), the AUC was 0.80, 95% CI=0.71 to 0.88).

	β (SE)	OR	95% CI
β_0	9.98		
Sex	1.19 (0.17)	3.28	2.37—4.54
Height	-0.06 (0.01)	0.95	0.93—0.96
Weight	-0.03 (<0.01)	0.97	0.96—0.98
Attained age	-0.02 (0.01)	0.98	0.96—1.00
Cranial irradiation	0.73 (0.13)	2.07	1.59—2.68
Abdominal irradiation	0.48 (0.14)	1.61	1.23—2.11
SJLIFE cohort AUC total (95% CI)		0.76 (0.73—0.78)	
Dutch cohort AUC total (95% CI)		0.75 (0.67—0.83)	

Abbreviations: SJLIFE=St. Jude Lifetime Cohort; β=regression coefficient; SE=standard error; OR=odds ratio; CI=confidence interval; β_0=intercept; AUC=area under the curve

Table 3. Multivariable logistic regression model for very low BMD among adult childhood cancer survivors.

DISCUSSION

We developed and validated prediction models that can be used to identify young adult survivors of childhood cancer with a high probability of having low and very low BMD. Although a high prevalence of low BMD among survivors has been described in several studies,[9,26] evidence-based guidance for surveillance of low BMD among survivors by DXA is limited. Screening guidelines for at-risk individuals have primarily been based on expert opinion and it remains unclear which individual survivor will benefit most from DXA examination as part of the late effects surveillance program.[37–40] The calculated AUC of the prediction model that we developed for low BMD was 0.72 in the development cohort, and for very low BMD 0.76. In the validation cohort, the AUCs for low and very low BMD were 0.69 and 0.75, respectively. This discriminatory power is similar to prediction models of fracture (FRAX™) in non-cancer populations (AUC 0.60 to 0.72)[41–43] and for other late effects such as cardiomyopathy and stroke among childhood cancer survivors (AUC 0.63 to 0.74).[44,45] According to their AUCs, these models will provide a fair to good discrimination between adult survivors with normal, low or very low BMD.[46] The availability of an online calculator will facilitate the clinical use of the models (Appendix 1).

Based on results from the current study, we recommend DXA examination in a survivor at a predicted probability of low BMD of ≥50%. At this cut-point the sum of sensitivity and specificity was highest in both cohorts (development cohort, 133;

validation cohort, 128). We preferred a balance between sensitivity and specificity because although low BMD is common among survivors and can cause significant morbidity, it is generally not life-threatening and screening by DXA involves exposure to low dose radiation. We chose to focus our primary analysis on low BMD instead of very low BMD, because in the general population most fractures occur among individuals with modest deficits in bone density,[47] and because research supports that survivors experience significant comorbidities such as sarcopenia and peripheral neuropathies, which may further elevate their fracture risk.[48] For a survivor predicted to be of low risk based on this model, DXA examination may be deferred until mid-adulthood, for instance, at 40 years of age or older. We estimate that in services that follow current long-term follow-up guidelines (Children's Oncology Group, United Kingdom Children's Cancer Study Group, Scottish Intercollegiate Guidelines Network and Dutch Children's Oncology Group), which recommend DXA screening either for all survivors at least once, or for high-risk subgroups (e.g., survivors who receive cranial irradiation), that the number of DXA scans performed will either decrease slightly or stay the same based on our recommendations. In services where implementation of our screening models may increase the number of scans performed, the increased financial costs and clinical burden should be weighed against the benefit of detecting a greater number of survivors with BMD deficits.

Currently, most adult survivors of childhood cancer are younger than 50 years of age. Among survivors, the association between low BMD and fractures is not well-established and the validity of general BMD and fracture prediction tools have not been assessed.[49] Although our models were designed to predict low BMD and not fracture, they incorporate some of the same factors (that is, sex, height, weight, attained age and smoking status) included in the widely-used WHO fracture risk assessment tool FRAX™, which was designed to predict the 10-year probability of osteoporotic fracture among the elderly.[50] However, unlike the FRAX™ tool, we found that younger survivors and males had a higher likelihood of having low BMD. A higher risk of low BMD among younger survivors may be explained by the fact that peak bone mass in the general population is not reached until an individual is in their mid-twenties,[51] and while the acquisition of bone mass can be delayed by cancer treatments, improvements in BMD can be observed among survivors many years after therapy.[30] This delay in bone maturation may occur more frequently among male survivors as males tend to attain their peak bone mass later than females in non-cancer populations.[51] Furthermore, the FRAX™ tool was developed for individuals older than 40 years, particularly postmenopausal women, while in our development cohort the median age of survivors was 29 years.

Identification of low BMD among childhood cancer survivors is important as several therapeutic options to remediate deficits exist. For those survivors with low BMD, long-term follow-up guidelines for survivors recommend remediation of hormonal insufficiencies, optimization of calcium and vitamin D levels through diet or supplementation, weight-bearing exercise, and consideration of pharmacologic intervention with bisphosphonates for survivors with fragility fractures or severe refractory BMD deficits.[37–40] However, many of these recommendations are based on studies of older non-cancer populations, and among childhood cancer survivors, the efficacy of these interventions has varied across studies or have not been examined.[21–23, 52]

There are several important considerations when interpreting our results. Firstly, selection bias may have occurred as participants included in the Dutch cohort received a DXA based on physician referral, which may have led to a higher prevalence of BMD deficits and an unequal representation of cancer diagnosis subgroups. Secondly, data for certain risk factors known to be associated with low BMD or fracture, such as personal and family history of fractures, presence of hormonal insufficiencies, weight-bearing exercise and alcohol consumption,[14,50,53] were not available for all study participants and therefore, could not be assessed. Inclusion of these data, as well as biochemical or genetic markers may improve the discriminatory power of future models.[25,54,55] Third, as neither race nor ethnicity were recorded for Dutch participants, the ethnic backgrounds of participants may have varied between cohorts, nonetheless, prediction models performed similarly across cohorts. Finally, our models were developed in Caucasian survivors, hence, they require validation in survivors of other races and ethnicities.

We created and validated prediction models for low and very low BMD of the LS and TB among adult survivors of childhood cancer through age 40 years based on easily obtainable predictors, including sex, height, weight, attained age, smoking status and prior exposure to cranial and abdominal irradiation. Because these models identify most survivors with low BMD correctly, we consider their use a reasonable tool for personalized diagnostics and surveillance. For patients with confirmed deficits, targeted treatment directed at improving bone health and preventing fractures among this vulnerable population can be provided.

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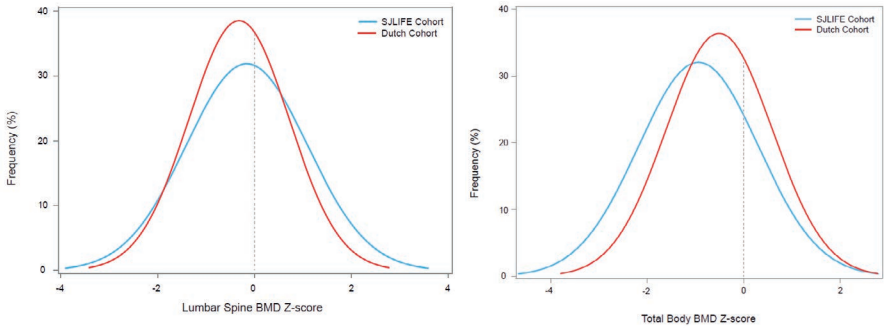
		Dutch cohort (n=339)	
		Mean (SD; range)	**N (%)**
Childhood cancer diagnosis	Acute lymphoblastic leukemia		149 (44.0)
	Other leukemia		16 (4.7)
	Hodgkin lymphoma		36 (10.6)
	Non-Hodgkin lymphoma		39 (11.5)
	CNS tumor		23 (6.8)
	Renal tumor		47 (13.9)
	Neuroblastoma		14 (4.1)
	Soft tissue sarcoma		6 (1.8)
	Bone tumor		9 (2.7)
	Other		0 (0.0)
Sex	Male		199 (587)
	Female		140 (41.3)
Height (cm)		173.4 (9.65; 145.5—196.0)	
Weight (kg)		71.2 (14.2; 36.7—126.0)	
Age at diagnosis (yrs)		6.4 (10.0; 0—16.8)[2]	
Attained age (yrs)		25.0 (10.0; 18.0—40.9)[2]	
Current smoker	Yes		72 (21.2)
	No		267 (78.8)
Subsequent diagnosis[3]	Yes		42 (12.4)
	No		297 (87.6)
Alkylating Agent	Yes		170 (50.1)
	No		169 (49.9)
Methotrexate	Yes		197 (58.1)
	No		142 (41.9)
Glucocorticoid	Yes		227 (67.0)
	No		112 (33.0)
Cranial RT[4]	Yes		84 (24.8)
	No		255 (75.2)
Abdominal RT[5]	Yes		26 (7.7)
	No		313 (92.3)

Abbreviations: SE=standard error; N=number of patients; CNS =central nervous system; IQR=interquartile range; TBI=total body irradiation

[1]Chi-square p-value for dichotomous covariates, two-sample independent t-test p-value for normally distributed continuous covariates and Mann-Whitney U Test p-value for non-normally distributed continuous covariates; [2]Median (IQR; range) (non-normally distributed); [3]Non-melanoma skin cancer was excluded; [4]Including TBI, excluding scatter radiation; [5]Including pelvic radiation and TBI, excluding scatter radiation

Supplementary Table 1. Characteristics of Dutch survivors with complete values.

A. B.



Supplementary Figure 1. Distribution of lumbar spine BMD Z-scores (A) and total body BMD Z-scores (B) in the SJLIFE development cohort and Dutch validation cohort.

	Low BMD				Very low BMD			
	Frequency of low BMD (%)[1]	**OR (95% CI)**	**β (SE)**	**p-value**	**Frequency of very low BMD (%)**	**OR (95% CI)**	**β (SE)**	**p-value**
Sex		1.11 (0.94—1.33)	0.11 (0.09)	0.23		1.13 (0.91—1.41)	0.12 (0.11)	0.26
Male	52.7				21.1			
Female	50.1				19.1			
Height (cm)		0.96 (0.95—0.97)	-0.04 (<0.01)	<0.001		0.95 (0.94—0.96)	-0.06 (0.01)	<0.001
1st tertile	62.1				30.2			
2nd tertile	50.5				18.1			
3rd tertile	41.9				12.2			
Weight (kg)		0.97 (0.97—0.98)	-0.03 (<0.01)	<0.001		0.96 (0.96—0.97)	-0.04 (<0.01)	<0.001
1st tertile	64.3				32.2			
2nd tertile	54.5				18.1			
3rd tertile	35.6				10.2			
Age at diagnosis (yrs)		0.96 (0.94—0.97)	-0.04 (0.01)	<0.001		0.96 (0.94—0.98)	-0.04 (0.01)	0.001
1st tertile	52.8				21.2			
2nd tertile	57.9				23.6			
3rd tertile	43.7				15.7			

	Low BMD				Very low BMD			
	Frequency of low BMD (%)[1]	**OR (95% CI)**	**β (SE)**	**p-value**	**Frequency of very low BMD (%)**	**OR (95% CI)**	**β (SE)**	**p-value**
Attained age (yrs)		0.97 (0.96—0.99)	-0.03 (0.01)	0.001		0.98 (0.96—0.99)	-0.02 (0.01)	0.009
1st tertile	58.6				25.1			
2nd tertile	48.4				17.9			
3rd tertile	47.4				17.6			
Current smoker		1.42 (1.16—1.74)	0.35 (0.10)	0.001		1.07 (0.83—1.37)	0.06 (0.13)	0.61
Yes	58.1				21.0			
No	49.4				19.9			
Alkylating Agent		1.23 (1.03—1.46)	0.20 (0.09)	0.022		1.29 (1.03—1.61)	0.25 (0.11)	0.025
Yes	53.7				21.9			
No	48.6				17.9			
Methotrexate		0.87 (0.73—1.04)	-0.14 (0.09)	0.12		0.74 (0.59—0.92)	-0.31 (0.11)	0.006
Yes	49.9				17.9			
No	53.4				22.8			
Glucocorti-coid		0.85 (0.71—1.01)	-0.16 (0.09)	0.066		0.75 (0.60—0.93)	-0.29 (0.11)	0.008
Yes	49.6				18.0			
No	53.7				22.7			
Cranial irradiation		2.31 (1.91—2.79)	0.84 (0.10)	<0.001		2.68 (2.15—3.34)	0.99 (0.11)	<0.001
Yes	65.0				31.3			
No	44.6				14.5			
Abdominal irradiation		1.58 (1.28—1.96)	0.46 (0.11)	<0.001		2.60 (2.05—3.30)	0.96 (0.12)	<0.001
Yes	60.3				33.8			
No	49.0				16.4			

Abbreviations: β = regression coefficient; SE = standard error; OR = odds ratio; CI = confidence interval; NA = not available; ICR = interquartile range; DXA = dual energy X-ray absorptiometry
[1]For continuous variables, frequency of low and very low BMD is reported by tertile

Supplementary Table 2. Univariable logistic regression analysis for low BMD and very low BMD in the SJLIFE development cohort.

Cut-point predicted probability	Frequency of predicted low BMD (%)	Sensitivity (%)	Specificity (%)	∑	PPV (%)	NPV (%)
0.10	99.3	99.8	1.3	101	51.8	86.7
0.20	94.4	98.3	9.6	108	53.6	84.1
0.30	85.1	93.6	23.9	118	56.6	77.9
0.40	70.6	83.6	43.2	127	60.9	71.2
0.50	53.0	69.0	64.0	133	67.0	66.1
0.60	34.6	48.9	80.6	130	72.8	59.8
0.70	18.9	29.3	92.0	121	79.5	55.1
0.80	7.9	12.9	97.5	110	84.4	51.3
0.90	1.6	2.8	99.7	103	90.6	49.1

Abbreviations: ∑=sum of sensitivity and specificity; PPV=positive predictive value; NPV=negative predictive value

Supplementary Table 3. Diagnostic values of the prediction model for low BMD at different cut-points for predicted probability.

Cut-point predicted probability	Frequency of predicted low BMD (%)	Sensitivity (%)	Specificity (%)	∑	PPV (%)	NPV (%)
0.10	69.9	89.9	0.0	90	25.9	93.1
0.20	37.1	66.1	35.1	101	35.9	89.1
0.30	21.1	50.2	70.2	120	48.0	87.3
0.40	11.4	32.4	86.3	119	57.3	84.6
0.50	6.5	20.7	93.0	114	63.9	82.9
0.60	3.7	13.2	97.0	110	71.0	81.8
0.70	1.8	7.1.	98.6	106	78.4	80.9
0.80	<1	2.2	99.5	102	81.8	80.2
0.90	<1	0.2	99.5	100	0.50	79.9

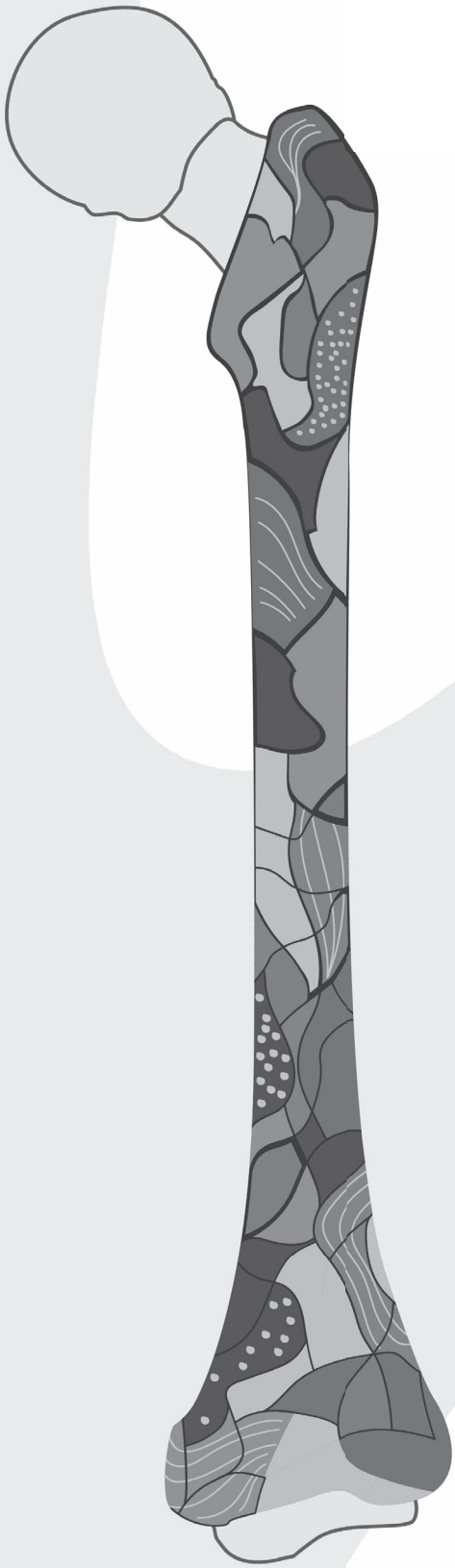
Abbreviations: ∑=sum of sensitivity and specificity; PPV=positive predictive value; NPV=negative predictive value

Supplementary Table 4. Diagnostic values of the prediction model for very low BMD at different cut-points for predicted probability.

Appendix 1. Results of the prediction models for a fictitious survivor.

The use of the models in clinical practice can be shown using the following fictitious case descriptions:

1. *A 32 year-old non-smoking female survivor with a height of 172cm and a weight of 68kg, diagnosed with a Wilms tumor at the age of three, who was treated with chemotherapy and surgery, visits the outpatient late effects clinic. Entry of her clinical characteristics into the online calculator showed a probability of low BMD of 30% and of very low BMD of 7%. This individual would not be recommended to undergo DXA screening as their predicted probability of low BMD is below the optimal cut-point of 50%.*
2. *A 28 year-old smoking 175cm tall, 62kg heavy male survivor visits the outpatient late effects clinic next. He was diagnosed with pre-B ALL CNS-2 at the age of three, treated according to local ALL treatment protocols of that time including prophylactic cranial irradiation. According to our models, his probability of low and very low BMD is 82% and 37%, respectively. In contrast to the previous survivor, he would be recommended to undergo DXA screening.*



CHAPTER 5

Bone mineral density surveillance for childhood, adolescent, and young adult cancer survivors: evidence-based recommendations from the International Late Effects of Childhood Cancer Guideline Harmonization Group

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SUMMARY

Childhood, adolescent, and young adult cancer survivors are at increased risk of reduced bone mineral density. Clinical practice surveillance guidelines are important for timely diagnosis and treatment of these survivors, which could improve bone mineral density parameters and prevent fragility fractures. Discordances across current late effects guidelines necessitated international harmonisation of recommendations for bone mineral density surveillance. The International Late Effects of Childhood Cancer Guideline Harmonization Group therefore established a panel of 36 experts from ten countries, representing a range of relevant medical specialties. The evidence of risk factors for very low and low bone mineral density and fractures, surveillance modality, timing of bone mineral density surveillance, and treatment of very low and low bone mineral density were evaluated and critically appraised, and harmonised recommendations for childhood, adolescent, and young adult cancer survivors were formulated. We graded the recommendations based on the quality of evidence and balance between potential benefits and harms. Bone mineral density surveillance is recommended for survivors treated with cranial or craniospinal radiotherapy and is reasonable for survivors treated with total body irradiation. Due to insufficient evidence, no recommendation can be formulated for or against bone mineral density surveillance for survivors treated with corticosteroids. This surveillance decision should be made by the survivor and health-care provider together, after careful consideration of the potential harms and benefits and additional risk factors. We recommend to carry out bone mineral density surveillance using dual- energy X-ray absorptiometry at entry into long-term follow-up, and if normal (Z-score >–1), repeat when the survivor is aged 25 years. Between these measurements and thereafter, surveillance should be done as clinically indicated. These recommendations facilitate evidence-based care for childhood, adolescent, and young adult cancer survivors internationally.

INTRODUCTION

The survival of children, adolescents, and young adults with cancer has greatly improved over recent decades, with current 5-year overall survival rates approximating 80% in high-income countries.[1,2] However, childhood, adolescent, and young adult cancer survivors often experience long-term side effects.[3,4] Previous studies suggest an increased proportion of survivors with low (Z-score ≤–1) and very low (Z-score ≤–2) bone mineral density compared with the general population[5,6] as well as an increased fracture rate.[7,8] Bone mineral density deficits could occur due to the cancer itself, its treatment, or consequences such as endocrine defects (eg, hypogonadism, growth hormone deficiency, malnutrition or consequences such as endocrine defects (eg, hypogonadism, growth hormone deficiency), malnutrition or malabsorption, and sedentary lifestyles.[9–11] These factors can lead to impaired bone accrual, resulting in a lower peak bone mass, usually achieved in people aged between 20 and 30 years.[12,13] Because peak bone mass predicts osteoporosis in adulthood and affects the age of osteoporosis onset,[14] it is hypothesised that as the current childhood, adolescent, and young adult cancer survivor population ages, more survivors might experience fragility fractures at relatively young ages.[15] These fragility fractures could cause substantial morbidity such as reduced mobility, chronic pain, and difficulty with performing activities of daily living.[16]

General population studies have shown that early detection and treatment of very low and low peak bone mass acquisition might overcome suboptimal peak bone density acquisition and prevent fractures.[17,18] Therefore, several North American and European groups have implemented bone mineral density surveillance in their clinical practice survivorship guidelines.[19–22] However, these guidelines have not systematically analysed the literature. Thus, definitions of high-risk groups, timing of surveillance, and treatment recommendations vary considerably, which impedes effective international implementation and adherence. To overcome such limitations, the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG) was established.[23] This collaborative endeavor aimed to establish a common vision and integrated strategy for surveillance of chronic health problems in childhood, adolescent, and young adult cancer survivors. This IGHG report summarises available evidence and provides the first harmonised recommendations for bone mineral density surveillance among childhood, adolescent, and young adult cancer survivors.

METHODS

Guideline panel

The guideline panel was composed of 36 experts from ten countries, representing several pediatric oncology and other related societies, as well as a broad range of medical specialties (appendix 1 pp 2–3). Three dedicated working groups addressed the following topics (appendix 1, p 4): 1) who needs bone mineral density surveillance?; 2a) what surveillance modality should be used?; 2b) when should surveillance be initiated and at what frequency should it be done?; and 3) what should be done when abnormalities are identified? Details on the IGHG methods have been previously reported (appendix 1, p 5).[23]

Scope and definitions

This guideline provides bone mineral density surveillance recommendations for childhood, adolescent, and young adult cancer survivors diagnosed with cancer up to 25 years of age, who are at least 2 years after completion of treatment, regardless of current age. Definitions of the main potential osteotoxic treatments and outcomes can be found in appendix 1 (p 6). We analysed risk factors for very low bone mineral density (including studies with a Z-score ≤–2 as outcome), low bone mineral density (Z-score ≤–1 and ≤–2), lower bone mineral density Z-score (continuous), and for fractures (all types). A Z-score indicates the number of standard deviations that bone mineral density differs from age-matched and sex-matched normative values, whereas a T-score compares bone mineral density with the healthy young adult mean (peak bone mass). Z-scores (and not T-scores) were used as all included studies were done in childhood, adolescent, and young adults, who might not have yet achieved peak bone mass.[24,25] Although we mainly focused on risk factors for very low bone mineral density, we also included studies using a bone mineral density Z-score threshold equal to or less than –1. This threshold was considered relevant in the context of childhood, adolescent and young adult bone mineral density surveillance, because Z-scores equal to or less than –1 but higher than –2 might predispose to developing very low bone mineral density as survivors age, and because adult studies showed a two to three times increased fracture risk for every one standard deviation reduction in bone mineral density.[26] All skeletal sites were analysed together, because risks of very low or low bone mineral density for different skeletal sites have similar implications for bone mineral density surveillance.

Search strategy and selection criteria

Initially, the panel evaluated concordances and discordances between the online available Children's Oncology Group, Dutch Childhood Oncology Group, UK Children's Cancer and Leukaemia Group, and Scottish Intercollegiate Guideline Network survivorship guidelines.[19–22] Clinical questions were then formulated for all discordant areas. For concordant areas, clinical questions were drafted if there was any uncertainty about sufficient literature support or if more details were needed.

We searched English literature published between Jan 1, 1990, and May 12, 2021, using PubMed and the MEDLINE databases to identify relevant evidence (appendix 1, p 13). Two independent reviewers determined whether the identified articles met pre-determined inclusion criteria (appendix 1, p 15). Subsequently, all guideline members were contacted to determine if additional evidence was available. Cross-references identified during the review procedure that had not been initially identified were added if relevant.

We summarised information from included studies using evidence tables and generated a summary of the total body of evidence per clinical question. The quality of the evidence was graded using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) method (appendix 1, pp 16–20).27 If evidence in childhood, adolescent, and young adult cancer survivors was not available or insufficient to answer a clinical question, we searched for recommendations in clinical practice guidelines on osteoporosis or low bone mineral density in closely related populations (appendix 1, p 14). These recommendations were extrapolated to support expert opinion after careful consideration.

Translating evidence into recommendations

We used the Grading of Recommendations Assessment, Development, and Evaluation method's evidence-to-decision framework to formulate recommendations.[28] Our harmonised recommendations for bone mineral density surveillance were based on evidence, expert opinions, cost–benefit conside-rations, the balance between potential benefits and harms of surveillance, and the need to maintain flexibility of application across different health-care systems. According to the IGHG method, only treatment-related risk groups were considered for bone mineral density surveillance. Decisions were made through group discussion and consensus. The strength of the recommendations were graded according to published evidence-based methods (appendix 1, p 21).[27,29]

Finally, two independent experts in the field (CS and J-MK) and six survivor representatives (DC, LG, MB, TC, MS, and ZT) had the opportunity to provide input on the harmonised recommendations.

RESULTS

Appendix 1 (pp 7–8) shows the evaluation of concordant and discordant areas between the existing survivorship guidelines. Because the panel felt that both concordant and discordant areas required systematic, in-depth review of the evidence, clinical questions were drafted for all areas (appendix 1, pp 9–12). 74 studies in childhood, adolescent, and young adult cancer survivors (figure) and three clinical practice guidelines in related populations (two childhood cancer guidelines and one general paediatric guideline; appendix 1, pp 22–26) were included. Evidence tables and summary of findings tables are presented in appendix 1 (pp 27–218, 219–365).

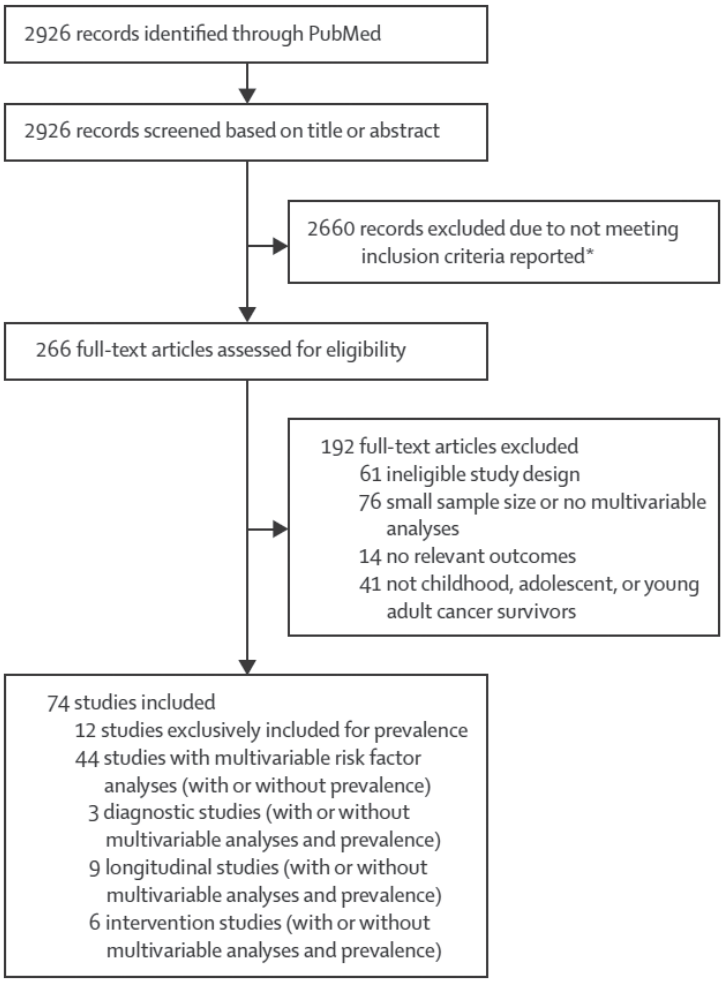
Risk of reduced bone mineral density and fractures

Childhood, adolescent, and young adult cancer survivors are at increased risk of low bone mineral density (moderate-quality evidence),[6,7,9–11,30–70] very low bone mineral density (moderate-quality evidence),[6,7,9,10,30–37,39–44,47–55,58–62,64,66–71] and lower bone mineral density Z-scores (moderate- quality evidence) after a follow-up period ranging from 2·7 to 27·2 years.[30,31,33,38,39,41,43,44,50,52–55,58,60,61,64,66,67,69–79] We found an increased risk of fractures in survivors versus controls (very low-quality evidence) after a follow-up ranging from 9·1 to 22·7 years.[7,8,80] See appendix 1 (pp 219–37) for details on prevalence of very low and low bone mineral density and incidence of fractures. Clinical fractures are significantly associated with low bone mineral density in survivors (low-quality evidence),[58,68] but not with lower versus higher bone mineral density Z-score as a continuum (moderate-quality evidence).[81] It is unknown whether very low or low bone mineral density during therapy or previous fractures lead to an increased risk of reduced bone mineral density and fractures at 2 or more years after treatment cessation (no studies).

Risk factors for very low bone mineral density

Identified risk factors associated with very low bone mineral density include cranial or craniospinal radio- therapy (high-quality evidence),[6,54] abdominal or pelvic irradiation (moderate-quality evidence),[6] hypogonadism (moderate-quality evidence),[10,54] growth hormone deficiency (low-quality evidence),[10,54,82,83] low BMI (high-quality evidence),[6,54,69] and male sex (moderate-quality evidence).[6,41,54]

For the remaining potential risk factors, no increased risk of very low bone mineral density was found, or no or only very low-quality evidence was available (table 1, table 2).



*Full details listed in the appendix (p 15).

Figure. PRISMA flow chart of selected studies.

Who needs bone mineral density surveillance?

Risk and risk factors for low BMD, very low BMD, lower BMD Z-score, and fractures in CAYA cancer survivors diagnosed up to 25 years of age

	Very low BMD (Z-score ≤-2)	Low BMD (Z-score ≤-1 and ≤-2)	Lower BMD Z-score (continuous)	Fractures (all types)
Risk				
Risk	↑⊕⊕⊕⊖ MODERATE [6,7,9,10,30–37,39–44,47–55,58–62,64,66–71]	↑⊕⊕⊕⊖ MODERATE [6,7,9–11,30–70]	↑⊕⊕⊕⊖ MODERATE [30,31,33,38,39,41,43,44,50,52–55,58,60,61,64,66,67,69–72]	↑⊕⊖⊖⊖ VERY LOW [7,8,80]
Risk after low BMD/fracture	No studies	No studies	No studies	No studies
Host factors				
Male sex	↑⊕⊕⊕⊖ MODERATE [6,41,54]	↑⊕⊕⊕⊕ HIGH [6,9,64,65,68,32,40–42,45,51,54,56]	↑⊕⊕⊖⊖ LOW [9,35,41,43,44,49,53,67,74,76]	↑⊕⊕⊕⊖ MODERATE [41,58,81]
Age at diagnosis	=⊕⊕⊕⊕ HIGH [6,54,69]	=⊕⊕⊖⊖ LOW [6,9,54,56,64,68,69]	⇳ ⊕⊖⊖⊖ VERY LOW [9,35,44,53,67,70,72]	=⊕⊕⊖⊖ LOW [80,81]
White race	↑⊕⊖⊖⊖ VERY LOW [41]	↑⊕⊕⊕⊖ MODERATE [40,41,51,65,68]	↑⊕⊕⊕⊖ MODERATE [41,43]	↑⊕⊖⊖⊖ VERY LOW [80]
Low BMI/weight/lean mass	↑⊕⊕⊕⊕ HIGH [6,54,69]	↑⊕⊕⊕⊕ HIGH [6,9,40,42,51,54,56,64,68,69]	↑⊕⊕⊕⊖ MODERATE [9,33,35,36,43,52,53,72,74,126]	No studies
Certain SNPs	=⊕⊖⊖⊖ VERY LOW [66]	=⊕⊖⊖⊖ VERY LOW [66]	↑⊕⊕⊖⊖ LOW [9,73,74,78]	↑⊕⊖⊖⊖ VERY LOW [127]
Family history of OP/#	No studies	No studies	No studies	No studies
Treatment factors				
Corticosteroids (y/n)	=⊕⊕⊕⊖ MODERATE [6,54]	↑⊕⊕⊕⊖ MODERATE [6,9,51,54,68]	↑⊕⊖⊖⊖ VERY LOW [9,38,44]	=⊕⊖⊖⊖ VERY LOW [80,127]
Higher corticosteroid dose	↑⊕⊖⊖⊖ VERY LOW [69]	↑⊕⊕⊕⊖ MODERATE [32,46,69]	↑⊕⊕⊕⊖ MODERATE [41,67,70,72,74]	↑⊕⊕⊖⊖ LOW [81]
DEXA vs. PRED	No studies	No studies	=⊕⊖⊖⊖ VERY LOW [79]	No studies
Methotrexate (y/n)	=⊕⊕⊕⊖ MODERATE [6,54]	=⊕⊕⊕⊖ MODERATE [6,9,54,68]	=⊕⊕⊖⊖ LOW [9,38]	↑⊕⊖⊖⊖ VERY LOW [80,81]
Higher methotrexate dose	No studies	=⊕⊕⊖⊖ LOW [32]	=⊕⊖⊖⊖ VERY LOW [70]	↑⊕⊖⊖⊖ VERY LOW [127]
Ifosfamide (y/n)	=⊕⊕⊕⊖ MODERATE [6]	=⊕⊕⊕⊖ MODERATE [6,9]	=⊕⊕⊖⊖ LOW [9]	=⊕⊖⊖⊖ VERY LOW [80]
Higher ifosfamide dose	No studies	No studies	No studies	No studies

Who needs bone mineral density surveillance?

Risk and risk factors for low BMD, very low BMD, lower BMD Z-score, and fractures in CAYA cancer survivors diagnosed up to 25 years of age

	Very low BMD (Z-score ≤-2)	Low BMD (Z-score ≤-1 and ≤-2)	Lower BMD Z-score (continuous)	Fractures (all types)
Cyclophosphamide (y/n)	=⊕⊕⊕⊖ MODERATE[6]	=⊕⊕⊕⊖ MODERATE[6,9,68]	=⊕⊕⊖⊖ LOW[9]	=⊕⊖⊖⊖ VERY LOW[80]
Higher cyclo dose	No studies	No studies	=⊕⊕⊖⊖ LOW[68,70]	No studies
Cisplatin (y/n)	No studies	No studies	No studies	No studies
Higher cisplatin dose	No studies	No studies	No studies	No studies
6-MP (y/n)	No studies	No studies	No studies	No studies
Higher 6-MP dose	No studies	No studies	=⊕⊖⊖⊖ VERY LOW[70]	No studies
Cyclosporine (y/n)	No studies	No studies	No studies	No studies
Higher cyclosporine dose	No studies	No studies	No studies	No studies
TKIs (y/n)	No studies	No studies	No studies	No studies
TKI dose	No studies	No studies	No studies	No studies
Tacrolimus (y/n)	No studies	No studies	No studies	No studies
Higher tacrolimus dose	No studies	No studies	No studies	No studies
C(S)RT (y/n)	↑⊕⊕⊕⊕ HIGH[6,54]	↑⊕⊕⊕⊕ HIGH[6,9,32,38,51,54,64,68]	↑⊕⊕⊖⊖ LOW[9,33,38,50,52,67,72,79]	=⊕⊕⊖⊖ LOW[81]
Higher C(S)RT dose	No studies	↑⊕⊕⊖⊖ LOW[32]	No studies	=⊕⊖⊖⊖ VERY LOW[127]
HSCT (y/n)	=⊕⊕⊖⊖ LOW[54]	=⊕⊕⊖⊖ LOW[9,54]	↑⊕⊖⊖⊖ VERY LOW[9,44]	No studies
TBI (y/n)	No studies	↑⊕⊕⊕⊕ HIGH[9,45,54,64,65]	↑⊕⊕⊖⊖ LOW[9,44,67,75]	No studies
Higher TBI dose	No studies	No studies	No studies	No studies
Abdominal/pelvic RT (y/n)	↑⊕⊕⊕⊖ MODERATE[6]	↑⊕⊕⊖⊖ LOW[6,9]	=⊕⊕⊖⊖ LOW[9]	=⊕⊖⊖⊖ VERY LOW[80]
Higher abd./pelvic RT dose	No studies	No studies	No studies	No studies

Who needs bone mineral density surveillance?

Risk and risk factors for low BMD, very low BMD, lower BMD Z-score, and fractures in CAYA cancer survivors diagnosed up to 25 years of age

	Very low BMD (Z-score ≤-2)	Low BMD (Z-score ≤-1 and ≤-2)	Lower BMD Z-score (continuous)	Fractures (all types)
Medical conditions				
GHD (y/n)	↑⊕⊕⊖⊖ LOW[10,54,82,83]	↑⊕⊕⊕⊖ MODERATE[10,54,61,68,82,83]	=⊕⊕⊖⊖ LOW[67,75]	No studies
Hypogonadism (y/n)	↑⊕⊕⊕⊖ MODERATE[10,54]	↑⊕⊕⊖⊖ LOW[10,38,51,54,61,68,82,84]	↑⊕⊖⊖⊖ VERY LOW[38,67]	No studies
Vitamin D deficiency (y/n)	No studies	No studies	No studies	No studies
Hyperthyroidism (y/n)	No studies	No studies	No studies	No studies
Endocrine dysfunction* (y/n)	No studies	↑⊕⊖⊖⊖ VERY LOW[62]	No studies	No studies
Health behaviors				
Inadequate vit. D intake (y/n)	No studies	=⊕⊕⊕⊖ MODERATE[51]	No studies	No studies
Vitamin D deficiency (y/n)	No studies	↑⊕⊕⊖⊖ LOW[65]	No studies	No studies
Inadequate Ca intake (y/n)	No studies	=⊕⊕⊕⊖ MODERATE[51]	↑⊕⊖⊖⊖ VERY LOW[33]	No studies
Inadequate vit. B intake (y/n)	No studies	No studies	No studies	No studies
Lack of exercise (y/n)	No studies	↑⊕⊕⊕⊖ MODERATE[11,40,51,56]	↑⊕⊕⊖⊖ LOW[39,63]	=⊕⊕⊖⊖ LOW[11,80]
Current/prior smoking (y/n)	=⊕⊕⊕⊖ MODERATE[6]	↑⊕⊕⊕⊖ MODERATE[6,9,56,61]	=⊕⊕⊖⊖ LOW[9]	↑⊕⊖⊖⊖ VERY LOW[80]
Alcohol consumption (y/n)	No studies	⇕⊕⊖⊖⊖ VERY LOW[61]	No studies	No studies
Carbonated beverages (y/n)	No studies	No studies	No studies	No studies

*GHD, hypogonadism or thyroid dysfunction. ↑ indicates an increased risk, = indicates no significant effect, and ⇕ indicates conflicting evidence.
Abbreviations: BMD=bone mineral density; BMI=body mass index; CAYA=childhood, adolescent, and young adult; CRT=cranial irradiation; CSRT=craniospinal irradiation; DEXA=dexamethasone; GHD=growth hormone deficiency; HSCT=hematopoietic stem cell transplantation; OP=osteoporosis; PRED=prednisone; RT=radiotherapy; SNP=single nucleotide polymorphism; TBI=total body irradiation; TKI=tyrosine kinase inhibitors; y/n=yes/no; 6-MP=6-mercaptopurine; #=fracture.

Table 1. Conclusions and quality of the evidence for the risk and risk factors for bone mineral density deficits in childhood, adolescent, and young adult cancer survivors diagnosed up to 25 years of age.

What surveillance modality should be used?		
Diagnostic value to detect (very) low BMD in CAYA cancer survivors diagnosed up to 25 years of age		
Variable	**Outcome**	**Quality of evidence**
Diagnostic value of *QCT* vs. DXA	Unknown	No studies
Correlation between *QCT and DXA derived BM(A)D and BMD Z-scores*	Significant (r 0.33-0.64)	⊕⊕⊖⊖ LOW[41,85]
Diagnostic value of *QUS* vs. DXA	Moderate	⊕⊖⊖⊖ VERY LOW[86]
Diagnostic value of *QUS* vs. QCT	Unknown	No studies
Diagnostic value of *pQCT* vs. QCT	Unknown	No studies
Added value of *QUS* to QCT and DXA in predicting fractures	Unknown	No studies
Location of BMD measurement (lumbar spine, total body and/or hip) that should be evaluated	Unknown	No studies
When should surveillance be initiated and at what frequency should it be performed?		
Risk over time of (very) low BMD in CAYA cancer survivors diagnosed up to 25 years of age		
Variable	**Outcome**	**Quality of evidence**
Course of BMD Z-scores over time from 2 years until at least 10 years since end of cancer treatment	Increase	⊕⊕⊕⊖ MODERATE [32,40,49,64,71,87–90]
Latency time of low BMD and fractures	Unknown	No studies
Risk of fractures for *low BMD* vs. normal BMD	Increased	⊕⊕⊖⊖ LOW[58,68]
Risk of fractures for *lower BMD* vs. higher BMD	Not significant	⊕⊕⊖⊖ LOW[81]
What should be done when abnormalities are identified?		
Use of medical interventions to improve BMD in CAYA cancer survivors diagnosed up to 25 years of age		
Variable	**Outcome**	**Quality of evidence**
Effect of *growth hormone replacement therapy* in GH deficient survivors	Significant	⊕⊖⊖⊖ VERY LOW[91–93]
Effect of *calcium and vitamin D supplementation*	Not significant	⊕⊖⊖⊖ VERY LOW[43]
Effect of *weight-bearing physical exercise*	Not significant	⊕⊖⊖⊖ VERY LOW[94]
Effect of *twice daily treatment with a vibrating plate*	Not significant (intention-to-treat analysis) Significant (per-protocol analysis)	⊕⊖⊖⊖ VERY LOW[95]
Effect of *bisphosphonates*	Unknown	No studies
Effect of *PTH*	Unknown	No studies
Effect of *Denosumab*	Unknown	No studies
Effect of *vitamin B12 supplementation*	Unknown	No studies
Effect of *sex hormone replacement therapy*	Unknown	No studies

Abbreviations: BMD=bone mineral density; CAYA=childhood, adolescent, and young adult; DXA=dual-energy X-ray absorptiometry; GH=growth hormone; PTH=parathyroid hormone; pQCT=peripheral quantitative computed tomography; QCT=quantitative computed tomography; QUS=quantitative ultrasound.

Table 2. Conclusions and quality of the evidence for surveillance modality, timing of bone mineral density surveillance, and treatment of bone mineral density deficits in childhood, adolescent, and young adult cancer survivors diagnosed up to 25 years of age.

Treatment-related risk factors for low bone mineral density

The main treatment-related risk factors for low bone mineral density include cranial or craniospinal radiotherapy (high-quality evidence),[6,9,32,38,51,54,64,68] total body irradiation (high-quality evidence),[9,45,54,64,65] and corticosteroids (administered as anticancer treatment; moderate-quality evidence).[6,9,51,54,68] Survivors treated with higher doses of cranial or craniospinal radiotherapy (low-quality evidence)[32] and corticosteroids (moderate-quality evidence)[32,46,69] are at greater risk of low bone mineral density. A dose threshold for increased risk of low bone mineral density could not be determined from the available literature for cranial or craniospinal radiotherapy or corticosteroids. The effect of higher total body irradiation doses is unclear, as no studies investigated this. Furthermore, it is unknown whether administering dexamethasone leads to higher risk of low bone mineral density than administering prednisone (no studies). However, very low-quality evidence for a greater risk of dexamethasone versus prednisone on lower bone mineral density Z-scores as a continuum was found.[79] Survivors treated with abdominal or pelvic irradiation[6,9] also have increased risk of low bone mineral density (low-quality evidence). No significant associations between low bone mineral density and treatment with methotrexate (moderate-quality evidence),[6,9,54,68] ifosfamide (moderate-quality evidence),[6,9] cyclophosphamide (moderate-quality evidence),[6,9,68] and haematopoietic stem cell transplantation without total body irradiation (low-quality evidence)[9,54] were found. Finally, the panel identified no studies that assessed the independent effect of cisplatin, 6-mercaptopurine, cyclosporine, tyrosine kinase inhibitors, or tacrolimus on low bone mineral density in childhood, adolescent, and young adult cancer survivors.

Other risk factors for low bone mineral density

Survivors with growth hormone deficiency (moderate-quality evidence)[10,54,61,68,82,83] or hypogonadism (low-quality evidence)[10,38,51,54,61,68,82,84] have increased risk of low bone mineral density. In more than half of the included studies, either some of the survivors received hormone replacement therapy or the percentage of treated survivors was not reported (appendix 1, pp 321–23 and 326–28). Survivor characteristics and health behaviours associated with low bone mineral density include low BMI (high-quality evidence),[6,9,40,42,51,54,56,64,68,69] male sex (high-quality evidence),[6,9,32,40–42,45,51,54,56,64,65,68] White race (moderate-quality evidence),[40,41,51,65,68] little physical activity (moderate-quality evidence),[11,40,51,56] and current or previous smoking (moderate-quality evidence).[6,9,56,61] We found conflicting evidence for the association between alcohol consumption[61] and low bone mineral density. No significant effect of age at cancer diagnosis[6,9,54,56,64,68,69] or inadequate dietary

vitamin D and calcium intake (moderate-quality evidence, one study)[51] on the risk of low bone mineral density was observed. However, we found an increased risk of low bone mineral density for survivors with biochemical vitamin D deficiency (25-hydroxyvitamin D levels <20 ng/mL; low-quality evidence).[65] None of the included studies assessed the risk of low bone mineral density in relation to biochemical vitamin B deficiency, hyperthyroidism, or consumption of carbonated beverages.

Risk factors for fractures

Risk factors for fractures in childhood, adolescent, and young adult cancer survivors include male sex (moderate-quality evidence)[41,58,81] and higher doses corticosteroids (low-quality evidence;[81] table 1). When evidence about other risk factors for fractures was available, it was of very low-quality or no increased risk was identified.

Diagnostic value of bone mineral density surveillance modalities

No studies investigating the diagnostic value of quantitative CT compared with dual-energy X-ray absorptiometry (DXA) in childhood, adolescent, and young adult cancer survivors were identified. However, two studies showed a significant correlation between quantitative CT and DXA derived bone mineral density parameters in this population (low-quality evidence).[41,85] Very low-quality evidence indicated that the diagnostic value of quantitative ultrasound compared to DXA was moderate.[86] No studies addressing the diagnostic value of quantitative ultrasound and peripheral quantitative CT versus quantitative CT, as well as the added value of quantitative ultrasound to quantitative CT and DXA in predicting fractures in survivors, were identified. Also, the optimal site of bone mineral density measurement has not been evaluated (no studies).

Risk of low or very low bone mineral density over time

The average time from diagnosis to the time of very low or low bone mineral density development in survivors is unknown (no studies). Moderate-quality evidence suggests that bone mineral density Z-scores increase from two years until at least ten years from completion of cancer treatment in childhood, adolescent, and young adult cancer survivors.[32,40,49,64,71,87–90]

Interventions to maintain or increase bone mineral density

Scarce evidence for efficacy of interventions to remediate very low or low bone mineral density in childhood, adolescent, and young adult cancer survivors was identified. A significant effect of growth hormone replacement therapy on bone mineral density in survivors with growth hormone deficiency was reported (very low-quality evidence).[91–93] However, no significant effect of weight-bearing physical exercise (low-quality evidence)[94] or calcium and vitamin D supplementation (very low-quality evidence)[43] on bone mineral density was found. In addition, one study showed no significant effect of twice daily treatment with a vibrating plate on total body bone mineral density Z-score in survivors in an intention-to-treat analysis, although there was significant improvement of tibial trabecular bone content among participants completing at least 70% of prescribed sessions.[95] The effects of bisphosphonates, parathyroid hormone, denosumab, vitamin B12 supplementation, and sex steroid replacement therapy were not studied in any of the included reports.

Potential benefits and harms of bone mineral density surveillance

The potential benefits and harms of bone mineral density surveillance are shown in panel 1. Most importantly, it is unclear whether early treatment of bone mineral density deficits leads to better skeletal health (ie, no further progression of bone mineral density deficits and prevention of fractures) in childhood, adolescent, and young adult cancer survivors, which is a prerequisite for bone mineral density surveillance. However, in healthy children, weight-bearing physical exercise, and calcium and vitamin D supplementation (in case of deficit) have been shown to maintain or enhance bone mineral density.[96,97] Diagnosing very low and low bone mineral density at an early stage among at-risk survivors could provide rationale for targeted healthy lifestyle recommendations. Furthermore, in older adults from the general population, treatment of very low bone mineral density prevents fractures and consequent morbidity, decreased quality of life, and hospitalisation.[98]

Who needs bone mineral density surveillance?

The panel strongly recommends that childhood, adolescent, and young adult cancer survivors and their healthcare providers should be aware of the risk of very low or low bone mineral density and pay specific attention to possible consequences (eg, acute and chronic back pain, low-trauma vertebral fractures and non- vertebral fractures, and loss of height due to vertebral fractures) after treatment with cranial or craniospinal radiotherapy (high-quality evidence for

very low bone mineral density), total body irradiation (high-quality evidence for low bone mineral density), or corticosteroids (moderate-quality evidence for low bone mineral density; panel 2).

Potential benefits

- DXA can accurately detect low (Z-score ≤-1) and very low (Z-score ≤-2) BMD (gold standard) (*evidence-based guidelines*[24,109,110]).
- Optimization of bone health could prevent further BMD Z-score decline (*evidence-based guidelines*[109,110]*):*
  - Supplementation of calcium and vitamin D
  - Avoidance of negative lifestyles such as smoking and alcohol use
  - Participation in recommended (weight-bearing) physical activity
  - Evaluation of endocrine defects such as hypogonadism, and hormone replacement therapy
- Referral to a medical bone health specialist may be beneficial for further evaluation, interpretation, treatment, and follow-up. Further treatment with osteoporosis medication (e.g. bisphosphonates) may be warranted in survivors with severe bone fragility and low potential for BMD restitution and vertebral body reshaping (*expert opinion*).
- In older adults from the general population, treatment of low and very low BMD prevents fractures and consequent morbidity, decreased quality of life, and hospitalization,[98] although in CAYA cancer survivors, evidence is lacking to support these benefits (*evidence-based guidelines*[109,110]).
- Diagnosing reduced BMD at an early stage could provide rationale for more targeted healthy lifestyle recommendations, and certain interventions could be initiated at the time they may be most effective (before the end of puberty).[96,115] This may reduce the need for treatment with bone-modifying agents such as bisphosphonates that may cause considerable side-effects (*expert opinion*).
- CAYA cancer survivors who do not have low or very low BMD when they undergo surveillance may benefit from being reassured that their fracture risk is less likely to be increased (*expert opinion*), although they should appreciate that BMD is only one parameter of bone strength.
- The prevalence of frailty (accelerated aging) is increased in CAYA cancer survivors compared to siblings,[124] and therefore, they could be at higher risk of falls (and consequent fractures). For this reason, identifying (very) low BMD in this group at an early stage could be of particular importance. Falls prevention methods could be recommended for these survivors (*expert opinion*).

Potential harms

- It is unclear whether early treatment of low or very low BMD leads to better health (i.e. no further progression, prevention of fractures) in CAYA cancer survivors (*no studies*).
- BMD surveillance may increase the risk of misdiagnosis, overdiagnosis, and overtreatment (i.e. incorrect diagnosis of (very) low BMD, or identification of (very) low BMD that would never lead to a fracture, possibly resulting in unnecessary treatment with, for example, bisphosphonates, which may lead to side-effects) (*expert opinion*).
- BMD surveillance by DXA may lead to potential harms from radiation exposure (especially in the context of cumulative radiation dose after treatment for CAYA cancer), although the dose of one DXA scan is considered negligible (less than one chest X-ray or a short flight[125]) (*expert opinion*).
- BMD surveillance may cause stress and anxiety (although the stress and anxiety generally resolve post screening) and the self-perception of being a patient versus healthy (*expert opinion*).
- CAYA cancer survivors might be falsely reassured that they do not have an increased fracture risk when their BMD is normal (*expert opinion*).
- DXA may be costly.

Abbreviations: BMD=bone mineral density; CAYA=childhood, adolescent and young adult; DXA=dual-energy X-ray absorptiometry.

Panel 1. Potential benefits and harms of bone mineral density surveillance for childhood, adolescent, and young adult cancer survivors.

General recommendation

CAYA cancer survivors and their healthcare providers should be aware of the risk of low (Z-score ≤-1 and >-2) and very low (Z-score ≤-2) bone mineral density, and pay specific attention to possible consequences (e.g. acute and chronic back pain, vertebral and non-vertebral low-trauma fractures, and loss of height due to vertebral fractures) after treatment with:
- Cranial or craniospinal radiotherapy (high-quality evidence for very low BMD)
- Total body irradiation (high-quality evidence for low BMD, unknown effect for very low BMD)
- Corticosteroids as anti-cancer treatment (moderate-quality evidence for low BMD, no significant effect for very low BMD)

Other risk factors for low and very low bone mineral density in CAYA cancer survivors include[1]:
- Hypogonadism (moderate-quality evidence for very low BMD; BMD assessment is recommended according to standard endocrine care, which is best done by a medical bone health specialist[2])
- Growth hormone deficiency (moderate-quality evidence for low BMD; BMD assessment is recommended according to standard endocrine care, which is best done by a medical bone health specialist[2])
- Low BMI or underweight (high-quality evidence for very low BMD)
- Male sex (moderate-quality evidence for very low BMD)
- White race (moderate-quality evidence for low BMD)
- Lack of physical activity[3] (moderate-quality evidence for low BMD)
- Current or prior smoking (moderate-quality evidence for low BMD)

Who needs bone mineral density surveillance?

Bone mineral density surveillance is recommended for CAYA cancer survivors treated with cranial or craniospinal radiotherapy (high-quality evidence for very low BMD).

Bone mineral density surveillance is reasonable for CAYA cancer survivors treated with TBI (high-quality evidence for low BMD).

Due to insufficient evidence[4], no recommendation can be formulated for or against BMD surveillance for CAYA cancer survivors treated with corticosteroids as anti-cancer treatment. The surveillance decision should be made by the CAYA cancer survivor and healthcare provider together, after careful consideration of the potential harms and benefits (see Survivor Information Brochure) and additional risk factors.

What surveillance modality should be used?

A DXA scan of the lumbar spine (posterior-anterior L1-L4), total body less head (in children and adolescents), and total hip (in adolescents and adults) are recommended for surveillance of bone mineral density (evidence-based guidelines).

QCT is not recommended for surveillance of bone mineral density (evidence-based guidelines and expert opinion).

When should surveillance be initiated and at what frequency should it be performed?

BMD surveillance is recommended at entry into LTFU (between two to five years following completion of therapy), and if normal (Z-score >-1), it is recommended to repeat surveillance at 25 years of age when peak bone mass should be achieved. Between these two measurements and thereafter, BMD surveillance should be performed as clinically indicated based on BMD and ongoing risk assessment (expert opinion).

What should be done when abnormalities are identified?

In CAYA cancer survivors with a BMD Z-score ≤-2, referral to (or consultation of) a medical bone health specialist[2] is recommended for further (endocrine) evaluation, interpretation of BMD findings, treatment, and follow-up (expert opinion).

In CAYA cancer survivors with a BMD Z-score ≤-1 and >-2, it is recommended to:
- Evaluate for the presence of endocrine defects (hypogonadism, GHD etc.), and consult a medical bone health specialist[2] for further evaluation and interpretation of BMD findings as clinically indicated (very low-quality evidence and evidence-based guidelines)
- Repeat DXA after 2 years, and thereafter as clinically indicated based on BMD change (i.e. in case of BMD decline more than the DXA machine's least significant change) and ongoing risk assessment (expert opinion)

In all at-risk CAYA cancer survivors[5], regardless of their BMD Z-score, it is recommended to counsel about lifestyle habits that are important to maintain or improve bone health:
- Engage in regular physical activity[3], especially weight-bearing and fall prevention activities (evidence-based guidelines and expert opinion)
- Abstain from smoking (moderate-quality evidence for low BMD and evidence-based guidelines)
- Limit or avoid alcohol intake (evidence-based guidelines)
- Consume adequate dietary vitamin D (at least 400 IU/day) and calcium (at least 500 mg/day) irrespective of vitamin D status, and advise vitamin D supplementation in survivors with 25OHD levels <20 ng/ml[6] (plus calcium if the recommended amount of dietary calcium is not met) as per local or national guidelines (evidence-based guidelines and expert opinion)
- Advise nutritional supplementation for CAYA cancer survivors with low BMI or underweight (expert opinion)

It is reasonable to refer at-risk CAYA cancer survivors[5] with a history of low-trauma vertebral and non-vertebral fractures (from entry into LTFU onwards) to a medical bone health specialist[2] for further evaluation and treatment (expert opinion).

Abbreviations: BMD=bone mineral density; BMI=body mass index; CAYA=childhood, adolescent and young adult; DXA=dual energy X-ray absorptiometry; LTFU=long-term follow-up; PBM=peak bone mass; TBI=total body irradiation.
[1]As in the general population (except for sex; female sex in the general population); [2]A medical bone health specialist is defined as any specialist who is caring for BMD deficits in CAYA cancer survivors, such as an endocrinologist (most settings), internist, pediatrician, rheumatologist, family physician, or general practitioner, depending on country and setting; [3]The WHO global recommendation on physical activity for health for adults is 150 minutes of moderate-intensity activity (or equivalent) per week, measured as a composite of physical activity undertaken across multiple domains: for work (paid and unpaid, including domestic work); for travel (walking and cycling); and for recreation (including sports). For adolescents, the recommendation is 60 minutes of moderate- to vigorous-intensity activity daily; [4]Insufficient evidence to determine if early detection of low BMD after treatment with corticosteroids reduces morbidity in CAYA cancer survivors, and whether the risk of very low BMD is increased in the long-term; [5]Survivors treated with C(S)RT (high-quality evidence), TBI (high-quality evidence), or corticosteroids (moderate-quality evidence); [6]Target 25OHD levels should be >20 ng/ml.

Green representing a strong recommendation to do with a low degree of uncertainty; Yellow representing a moderate recommendation to do with a higher degree of uncertainty; Red representing a recommendation not to do.

Panel 2. Harmonised recommendations for bone mineral density surveillance for childhood, adolescent, and young adult cancer survivors.

Overall, the balance of desirable and undesirable anticipated effects of surveillance varies depending on the risk of very low or low bone mineral density (appendix 1, pp 366–73). In childhood, adolescent, and young adult cancer survivors treated with cranial or craniospinal radiotherapy (high-quality evidence for very low bone mineral density), the panel was convinced that the potential benefits of bone mineral density surveillance clearly outweigh the potential harms. For these survivors, we strongly recommend bone mineral density surveillance. Mechanisms through which cranial or craniospinal radiotherapy could cause very low bone mineral density include hypogonadism, growth hormone deficiency, low BMI or lean mass, and obesity leading to diabetes.[10,99] These mechanisms might also apply to survivors treated with total body irradiation, but evidence is more scarce (high-quality evidence for low bone mineral density, but no studies for very low bone mineral density). Therefore, we made a moderate recommendation for

bone mineral density surveillance after treatment with total body irradiation. A dose threshold for cranial or craniospinal radiotherapy and total body irradiation could not be determined, but the risk of very low or low bone mineral density is likely associated with the risk of hypothalamic-pituitary axis, ovarian or testicular injury after radiotherapy, and with age of radiotherapy administration. In addition, radiotherapy might affect bones directly and increase fracture risk, but this might not directly result from low bone mineral density but from a perturbation in bone modelling and remodelling.[100]

It is well-known that corticosteroids (administered as anticancer treatment) have a detrimental effect on bone (especially trabecular bone) during its administration, resulting in an increased risk of low-trauma vertebral fracture and non- vertebral fracture.[101] However, for survivors with and without bone issues during therapy, the effect of corticosteroids on bone mineral density more than 2 years after the last exposure is unclear, as longitudinal studies from corticosteroid initiation until many years after its termination are not available. We found an increased risk of low bone mineral density after corticosteroid treatment (moderate-quality evidence), but not of very low bone mineral density (moderate-quality evidence) and fractures (very low-quality evidence). An increased risk of low bone mineral density (moderate-quality evidence) and fractures (low-quality evidence) after higher doses corticosteroids was observed. The included longitudinal studies (mostly involving survivors treated with steroids) showed that bone mineral density Z-scores increased after treatment cessation.[32,40,49,87–90] This observation is supported by literature from corticosteroid-treated adult populations, which shows that after corticosteroid termination, bone mineral density increases and clinical fracture risk declines.[102,103] However, the age of corticosteroid administration might play a role in this respect, as corticosteroid exposure during puberty, an important period for bone mass acquisition, could affect bone mineral density more severely and permanently than during the prepubertal period.[104,105] In addition, the fracture risk associated with corticosteroids in other populations might be due to mechanisms not definitively assessed by bone mineral density measurements, such as altered bone structure and increased risk of falls due to muscle weakness.[106] Ultimately, a large proportion of childhood, adolescent, and young adult cancer survivors have been treated with (higher doses) corticosteroids, and it is unclear if the benefits of bone mineral density surveillance outweigh the potential harms for this substantive group. Therefore, no recommendation can be formulated for or against bone mineral density surveillance. This surveillance decision should be made by the survivor and health-care provider together, after careful consideration of the potential harms and benefits (panel 1 and appendix 2) and additional risk factors (panel 2).

Abdominal or pelvic irradiation was not included in the surveillance recommendations because the moderate-quality evidence for an increased risk of very low bone mineral density was based on only one study, and because it is uncertain whether the risk of very low or low bone mineral density for survivors treated with abdominal or pelvic irradiation who did not develop hypogonadism is increased.

In childhood, adolescent, and young adult cancer survivors with hypogonadism or growth hormone deficiency, bone mineral density measurements should be done as part of standard endocrine care, which is best done by a medical bone health specialist in the context of hypogonadism or growth hormone deficiency management. A medical bone health specialist might include specialists such as an endocrinologist (most settings), internist, paediatrician, rheumatologist, or general practitioner, depending on country and setting. We refer to the IGHG guidelines on premature ovarian insufficiency[107] and male gonadotoxicity[108] for survivors at risk of hypogonadism.

What surveillance modality should be used?

The included clinical practice guidelines in related populations all advise DXA (lumbar spine and total body less head or total hip, depending on age) for bone mineral density surveillance.[24,109,110] The International Society of Clinical Densitometry 2019 paediatric position states that the lumbar spine and total body less head are the preferred sites for bone mineral density measurements.[25] In addition, although hip measurements are generally not preferred in younger children due to variability in skeletal development and limited reference values, they can be useful in adolescents at-risk for bone fragility who would benefit from continuity of measurements through transition into adulthood, as is the case for childhood, adolescent, and young adult cancer survivors. One of the included guidelines recommends to avoid quantitative CT for clinical application based on higher radiation doses applied compared to DXA (radiation exposure from peripheral quantitative CT is much lower, although slightly higher than DXA),[110] and the International Society of Clinical Densitometry considers both quantitative modalities primarily research techniques.[24] Although the panel appreciates the value of volumetric bone mineral density measured by peripheral quantitative CT and quantitative CT, clear quantitative CT-derived bone mineral density thresholds associated with fractures have not yet been established. Furthermore, the panel has concerns about the availability of both quantitative modalities in most settings (expert opinion). We found that the diagnostic value of quantitative ultrasound compared with DXA is moderate in survivors (very-low quality evidence). However, quantitative ultrasound is not endorsed

generally in diagnosing reduced bone mineral density in childhood, adolescent, and young adults, and appropriate normative data are scarce (expert opinion). Based on these considerations, we recommend a DXA scan of the lumbar spine (posterior-anterior Lumbar 1–4), total body less head (in children and adolescents) and total hip (in adolescents and adults) for bone mineral density surveillance (strong recommendation). Quantitative CT is not recommended for bone mineral density surveillance (evidence-based guidelines and expert opinion). No recommendation for or against using peripheral quantitative CT and quantitative ultrasound for bone mineral density surveillance was formulated.

When should surveillance be initiated and at what frequency should it be done?

Bone mineral density Z-scores seem to increase in survivors from entry into long-term follow-up onwards. However, longitudinal studies that include more than 2 years off therapy investigating the average time to development of very low and low bone mineral density to inform the timing of bone mineral density surveillance were absent. In general, screening for a condition is only justified when results have treatment implications.[111] Bisphosphonates can effectively treat osteoporosis and reduce fracture risk in children and adults.[98,112] However, because bisphosphonates have several known and theoretical side-effects in children,[113] their use is presently only considered in individuals with severe bone fragility (fragility fractures with or without very low bone mineral density), and reduced potential for bone mineral density restitution and vertebral body reshaping following vertebral fracture.[104,114] Prophylactic bisphosphonate therapy (ie, treating a low bone mineral density Z-score in the absence of fractures) is not currently recommended.[110,114] This means that bisphosphonates are not indicated when bone mineral density deficits are detected through primary surveillance in this age group. Initiating bisphosphonate treatment solely based upon very low bone mineral density is also controversial in childhood, adolescent, and young adult cancer survivors that have achieved peak bone mass (around the age of 25 years), because of low absolute fracture risk, low bisphosphonate efficacy if active bone loss is absent, and potentially long treatment duration, but might be considered in older survivors under specific circumstances. Peak bone mass is predictive of bone status in later adulthood and therefore represents an important landmark in time.[14] However, the interval between entering long-term follow- up and attaining the age of 25 years might be long for many survivors, which represents a period of potential increased risk of very low or low bone mineral density and fractures. In addition, it is important to optimise peak bone mass acquisition, and interventions such as exercise and hormone replacement

therapy are most effective during puberty.[96,115] Hence, bone mineral density surveillance is recommended at entry into long-term follow-up (between 2 to 5 years following completion of therapy) and if normal (Z-score >-1), it is recommended to repeat surveillance at 25 years of age when peak bone mass should be achieved (expert opinion). In the small proportion of survivors for whom these timepoints overlap or are in proximity, one DXA scan is sufficient. Between these two measurements and thereafter, bone mineral density surveillance should be done as clinically indicated based on bone mineral density and ongoing risk assessment. Furthermore, bone mineral density trajectories are more informative than a single, cross- sectional measurement.

What should be done when abnormalities are identified?

The two included clinical practice guidelines for bone mineral density treatment state that bisphosphonate therapy should be reserved for patients with overt bone fragility.[109,110] Furthermore, these guidelines conclude that adequate dietary calcium and vitamin D intake is important for optimal bone health, and that supplementation is warranted in case of deficit. The presence of endocrinopathies such as hypogonadism and growth hormone deficiency should be evaluated and corrected. In addition, these guidelines recommend adequate weight-bearing physical activity, and one guideline underscores that negative lifestyles such as smoking and any alcohol use should be avoided.[110]

The panel drafted the following recommendations based on published evidence-based guidelines and expert opinion. In childhood, adolescent, and young adult cancer survivors with a bone mineral density Z-score of less than or equal to –2, referral to (or consultation of) a medical bone health specialist is recommended for further (endocrine) evaluation, interpretation of bone mineral density findings, treatment, and follow-up (expert opinion). In survivors with a bone mineral density Z-score of less than or equal to –1 and higher than –2, it is recommended to: 1) evaluate for the presence of endocrine defects (eg, hypogonadism and growth hormone deficiency), and consult a medical bone health specialist for further evaluation as clinically indicated (very low-quality evidence and evidence-based guidelines); and 2) repeat DXA after 2 years, and thereafter as clinically indicated based on bone mineral density change (ie, in case of bone mineral density decline more than the DXA machine's least significant change)[25] and ongoing risk assessment (expert opinion). In all at-risk childhood, adolescent, and young adult cancer survivors (ie, those treated with cranial or craniospinal radiotherapy, total body irradiation, or corticosteroids), regardless of their bone mineral density Z-score, it is recommended to counsel about the following lifestyle habits that

are important to maintain or improve bone health: 1) engage in regular physical activity,[116] especially weight-bearing and falls prevention activities (evidence-based guidelines and expert opinion); 2) abstain from smoking (moderate-quality evidence and evidence-based guidelines) and limit or avoid alcohol intake (evidence-based guidelines); 3) consume adequate dietary vitamin D (at least 400 IU/day) and calcium (at least 500 mg/day) irrespective of vitamin D status, and advise vitamin D supplementation in survivors with 25-hydroxyvitamin D levels <20 ng/mL (plus calcium if the recommended amount of dietary calcium is not met) as per local or national guidelines (evidence-based guidelines and expert opinion); and 4) advise nutritional supplementation for survivors with low BMI or underweight (expert opinion).

Finally, there were insufficient studies that assessed fracture outcomes to draw formal fracture-based surveillance recommendations. However, low-trauma vertebral fractures are the clinical signature of osteoporosis, and low-trauma non-vertebral fractures could also indicate a diagnosis of osteoporosis.[24] Therefore, it is reasonable to refer at-risk childhood, adolescent, and young adult cancer survivors with a history of low-trauma vertebral fracture and non-vertebral fracture (from entry into long- term follow-up onwards) to a medical bone health specialist for further examination and treatment.

DISCUSSION

This study provides a comprehensive bone mineral density surveillance strategy for childhood, adolescent, and young adult cancer survivors, which could enhance early identification and adequate treatment and follow-up of survivors with very low or low bone mineral density in a variety of long-term follow-up settings, with the goal to prevent clinically relevant fractures and their consequences. In addition, the guideline panel identified gaps in the current literature that could guide future research to improve bone mineral density surveillance and fracture prevention strategies in survivors (panel 3).

Very low or low bone mineral density in survivors results from a complex, multifactorial process. We identified cranial or craniospinal radiotherapy, abdominal or pelvic irradiation, total body irradiation, and corticosteroids as the main treatment-related risk factors for very low or low bone mineral density. The primary mechanism through which these treatment modalities impact bone mineral density varies; however, they can all lead to primary or secondary hypogonadism.[117] Hypogonadism is a well-established cause of osteoporosis in

the general population,[118,119] and was independently associated with very low and low bone mineral density in this review. Therefore, we hypothesise that hypogonadism is also a key driver of very low and low bone mineral density in childhood, adolescent and young adult cancer survivors. Interestingly, moderate-quality evidence suggests no increased risk of very low or low bone mineral density after treatment with ifosfamide or cyclophosphamide, two alkylating agents that can induce hypogonadism, especially at higher doses.[107] We propose that the number of survivors with hypogonadism after ifosfamide or cyclophosphamide at any dose might have been too small (or received sex steroid replacement therapy) in available studies to detect a significant association with very low or low bone mineral density.

- Effect of different types of abdominal/pelvic irradiation (independent of hypogonadism) on the risk of low and very low BMD in female CAYA cancer survivors.
- Effect of corticosteroids on the risk of low and very low BMD in CAYA cancer survivors with increasing follow-up time.
- Effect of the age of corticosteroid treatment on the risk of low and very low BMD.
- Independent effect of TBI and HSCT on the risk of low and very low BMD in CAYA cancer survivors.
- Safe corticosteroid dose with regard to the risk of low and very low BMD in CAYA cancer survivors, and if so, what is this dose.
- Safe C(S)RT dose with regard to the risk of low and very low BMD in CAYA cancer survivors, and if so, what is this dose.
- Safe TBI dose with regard to the risk of low and very low BMD in CAYA cancer survivors, and if so, what is this dose.
- Risk and risk factors of low and very low BMD in CAYA cancer survivors older than 40 years.
- Sex- and pubertal stage-based differences in risk factors for low and very low BMD in CAYA cancer survivors.
- Risk and risk factors of low and very low BMD in CAYA cancer survivors treated for bone or soft tissue sarcomas.
- Risk and risk factors of incident low-trauma vertebral and non-vertebral fractures in CAYA cancer survivors, including treatment-related risk factors and other risk factors such as very low BMD, history of fractures, and maternal hip fracture etc. (included in the FRAX® fracture risk profile for older adults), the most frequent sites of fractures, and the disability and impact on quality of life resulting from low-trauma fractures.
- Further improvement and validation of prediction models (including demographic, lifestyle, and treatment factors) for low and very low BMD, and development of a prediction model for low-trauma fractures in CAYA cancer survivors.
- Risk and risk factors of impaired bone structure and its association (± BMD) with low-trauma fractures in CAYA cancer survivors.
- BMD trajectory and latency time of low-trauma fractures from cancer diagnosis into very long-term follow-up.
- Association between QCT, pQCT and QUS measurements and fracture risk in CAYA cancer survivors.

Abbreviations: BMD=bone mineral density; CAYA=childhood, adolescent, and young adult; C(S)RT=cranio(spinal) radiotherapy; HSCT=hematopoietic stem cell transplantation; (p) QCT=(peripheral) quantitative computed tomography; TBI=total body irradiation.

Panel 3. Gaps in knowledge of bone mineral density deficits and fractures in childhood, adolescent, and young adult cancer survivors and directions for future research.

As in the general population, host and lifestyle factors (eg, White race, low BMI, and smoking) contribute to the risk of very low or low bone mineral density in childhood, adolescent and young adult cancer survivors. This makes it difficult to designate a distinct group of survivors at highest absolute risk of very low or low bone mineral density solely based on prior cancer treatment. In this guideline, we recommend bone mineral density surveillance for those survivors with an excess risk of very low bone mineral density based on their previous cancer treatment (ie, those treated with cranial or craniospinal radiotherapy or total body irradiation). Another approach is to incorporate treatment-related, demographic, and lifestyle factors into a prediction model for the absolute risk of very low or low bone mineral density, which has been done for White adult childhood cancer survivors.[6] However, this model needs to be further validated in survivors younger than 18 and older than 40 years of age, as well as in childhood cancer survivors of non-White ethnicity, before its use can generally be recommended in childhood, adolescent, and young adult cancer survivors.

We recommend using DXA as the optimal modality for bone mineral density surveillance in childhood, adolescent, and young adult cancer survivors. However, in growing children, more so than in adults, several limitations should be considered when interpreting areal bone mineral density Z-scores generated by DXA. First, areal bone mineral density might be underestimated in shorter individuals (for example as a result of growth hormone deficiency or delayed pubertal development) due to the confounding effect of bone size.[120] To correctly interpret bone mineral density findings in these individuals, the bone mineral density and bone mineral apparent density (g/cm^3) can be estimated to reduce this confounding effect,[121,122] and a medical bone health specialist can be consulted to further assist in bone mineral apparent density interpretation. Second, although the association between lumbar spine bone mineral density and vertebral fracture remains consistent, Z-scores vary significantly in children depending upon the reference database that is used.[123] This is further complicated by the fact that a Z-score of –1·5 for example might be normal for one individual, but abnormal for another, and that fragility fractures can occur at a bone mineral density Z-score better than –2.[123] Monitoring bone mineral density trajectory by doing serial DXA scans (recommended in this guideline) could overcome some of these limitations.

An important aspect of the IGHG guideline process is the identification of gaps in the knowledge and development of directions for future research. According to our findings, future studies should investigate the risk of reduced bone

mineral density after several treatment modalities and disease types (such as bone and soft tissue sarcomas) in more detail, and further develop and validate prediction models for very low bone mineral density and low-trauma fractures (panel 3). One important development in recent years is that bone research has expanded to consider not only bone mineral density, but also bone structure, as an important indicator of bone strength. For example, it is now understood that low-trauma vertebral fractures are a key sign of osteoporosis in the cancer setting, but that such fractures are frequently asymptomatic and thereby go undetected in the absence of vertebral fracture imaging. Although a longitudinal study of incident low-trauma vertebral fracture and non- vertebral fracture has been carried out up to 6 years after childhood acute lymphoblastic leukaemia diagnosis,[101] studies beyond this duration and including other cancer settings are absent. Therefore, additional longitudinal studies are needed to identify the risk and risk factors of incident low-trauma fractures, the most frequent sites of fractures, as well as the bone mineral density trajectory with age.

Several limitations of the available literature are important in interpreting our bone mineral density surveillance recommendations. For instance, much literature is available on the risk of bone mineral density Z-scores equal to or less than –1, but less is known about the risk of bone mineral density Z-scores equal to or less than –2 and low trauma fractures, which are more clinically relevant outcomes. Furthermore, only few of the available studies discussed the bone health of patients with sarcomas, and the follow-up times differed significantly across included studies. In addition, although all studies did multivariable analyses, some did not adjust for all essential confounders (eg, sex, BMI, age, and Tanner stage). These limitations hinder comparison of results between studies. Finally, recommendations regarding diagnostic modality, optimal timing of bone mineral density surveillance, and interventions for survivors with reduced bone mineral density were extrapolated from non-cancer populations and clinical expertise due to lack of evidence in childhood, adolescent and young adult cancer survivors.

CONCLUSION

This IGHG guideline provides harmonised recommendations for bone mineral density surveillance that might improve health outcomes by facilitating more consistent long-term follow-up care for childhood, adolescent and young adult cancer survivors. In addition, it promotes strategically planned research that will inform future guideline updates.

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APPENDIX 1

Appendix 1 of this study is not included in this thesis due to its extensive nature. It can be found online in: Lancet Diabetes Endocrinol. 2021 Sep;9(9):622-637. doi: 10.1016/S2213-8587(21)00173-X. Epub 2021 Jul 30.

APPENDIX 2

Appendix 2. A Survivor Information Brochure.

Why should I be aware of the risk of low bone mineral density (weak bones)?

- Bone mineral density is an important determinant of bone strength. This means that if you have low bone mineral density (weak bones), you probably break your bones more easily.
- Having weak bones around the age of 25 (when your bones should be the heaviest) predicts for osteoporosis and bone fractures later in life.
- As a survivor of childhood, adolescent or young adult cancer you may have a higher risk of developing weak bones compared to people of similar age in the general population.
- If your brain and spinal cord were exposed to radiation as part of your treatment for a childhood, adolescent, or young adult cancer (cranial[spinal] irradiation), or if you were treated with total body irradiation, you have an increased risk of developing weak bones.
- If you were treated with corticosteroids (as anti-cancer treatment) you may have an increased risk of weak bones as well. However, it is unclear if corticosteroids can lead to weak bones in the long term.
- While some people treated with cranial(spinal) irradiation, total body irradiation, and/or corticosteroids will develop weak bones at a young age, most will not.
- However, among those who develop weak bones, detecting it early can possibly prevent bone fractures and may therefore reduce consequences such as pain, surgery, and temporary immobilization.
- It is possible to detect weak bones early by having bone mineral density screening, but bone mineral density screening has advantages and disadvantages.
- This information sheet can be used to help you and your healthcare provider decide if having bone mineral density screening is the right choice for you.

What is bone mineral density screening?

- Bone mineral density screening is performed with a bone scan that uses low dose X-rays to see how strong your bones are.

What are the potential advantages of having bone mineral density screening?

- You may feel reassured if you have normal bone mineral density at this time. However, weak bones may still develop in the future, and your fracture risk may still be increased due to other reasons.
- Early detection would allow doctors to monitor the bone mineral density course over time. In addition, early detection would allow referral to a specialized bone doctor who can further evaluate your bone health, which may both help to determine if/when treatment is needed.
- You may be more likely to have weak bones detected at an earlier timepoint when certain interventions may be most effective (before the end of puberty), and as a result, bone fractures may be prevented.

What are the potential disadvantages of having bone mineral density screening?

- You may experience anxiety and stress about having bone mineral density screening and what the test results will show.
- You may feel more like a patient rather than a healthy survivor if you decide to have bone mineral density screening.
- You may be incorrectly diagnosed with weak bones (misdiagnosis), or diagnosed with weak bones that never would have caused fractures (overdiagnosis), although your doctor carefully considers treatment.
- We do not know if early treatment of weak bones leads to better health (no further weakening of the bones or prevention of fractures) in childhood, adolescent and young adult cancer survivors. However, in the general population, we know that this is the case.
- The diagnosis of weak bones may affect your ability to obtain healthcare and/or life insurance.

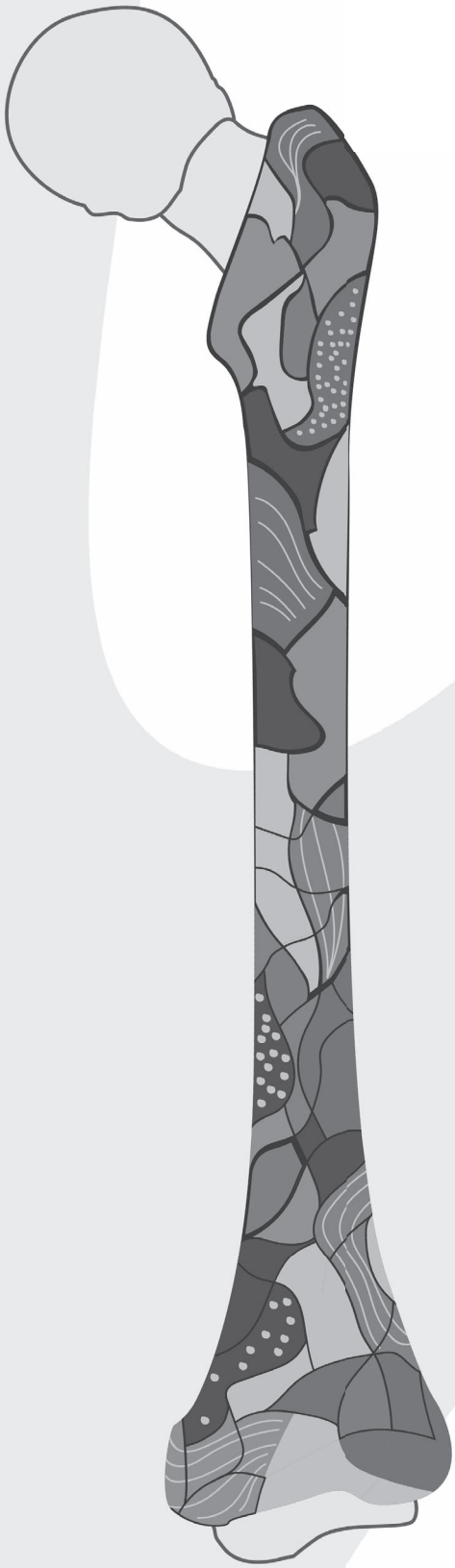
What are the potential disadvantages associated with this bone scan?

- This bone scan is associated with potential harms from radiation exposure (especially in the context of cumulative radiation dose after cancer treatment), although the dose of one scan is considered negligible (less than one chest X-ray or a short flight).
- This bone scan may be costly and may not be covered by your health insurance. However, your healthcare provider could write a letter of medical necessity to explain that you are at increased risk of weak bones and why you may benefit from a bone scan.

What are the international screening recommendations?

- If you were treated with radiotherapy to your brain or spinal cord, total body irradiation, and/or corticosteroids, it is important that you are aware of the risk of weak bones, and pay specific attention to their possible consequences (acute back pain, [spinal] fractures, and loss of height due to spinal fractures).
- If you were treated with radiotherapy to your brain or spinal cord, bone mineral density screening is recommended at entry into long-term follow-up (beginning two or more years following completion of therapy) and at 25 years of age.
- If you were treated with total body irradiation, bone mineral density screening is reasonable at entry into long-term follow-up and at 25 years of age.
- If you were treated with corticosteroids as anti-cancer treatment, we cannot recommend for or against routine bone mineral density screening because we do not know if your health outcomes will be better if we detect weak bones early. It is important that you make the decision whether or not to screen together with your healthcare providers, oncology and survivorship team, and individual support networks. Careful consideration of the potential advantages and disadvantages is advised.

Thank you for taking the time to read this information sheet. If you have any questions regarding the information included in this brochure or if you require emotional support and advice regarding your thoughts and feelings, please contact your healthcare provider for advice and support.



CHAPTER 6

Risk and determinants of reduced bone mineral density and fractures in a national cohort of Dutch childhood cancer survivors (n=2,003): a DCCSS-LATER Study

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ABSTRACT

Background: We aimed to assess risk factors for (very) low bone mineral density (BMD), as well as the risk and risk factors of (vertebral) fractures in a national cohort of Dutch childhood cancer survivors.

Methods: 2,003 survivors aged 18-45 years at invitation were included (mean age at participation 33.1±7.2 years). We assessed BMD by dual-energy X-ray absorptiometry (DXA, n=1,548). Fractures that occurred >5 years after diagnosis were assessed by medical history (n=1,892) and compared with Swedish normative data. Vertebral fractures were evaluated by vertebral fracture assessment (n=249). Associations between demographic, treatment-related, endocrine, as well as lifestyle-related factors and reduced BMD and (vertebral) fractures were evaluated using logistic regression analysis.

Findings: The standardized incidence ratio of any first fracture was 3.53 for male and 5.35 for female survivors. Vertebral fractures were prevalent in 13.3% of evaluable survivors. For low (Z-score ≤-1) or very low (Z-score ≤-2) BMD, male sex, underweight, shorter follow-up time, total body irradiation, cranial irradiation, carboplatin, alkylating agents, hypogonadism, growth hormone deficiency (GHD), hyperthyroidism, low physical activity, severe vitamin D deficiency, vitamin B12 deficiency, and folic acid deficiency were statistically significant. Male sex, obesity, previous/current smoking, and very low lumbar spine BMD were significantly associated with reported clinical fractures, and older attained age, platinum compounds, GHD, and low physical activity with vertebral fractures.

Interpretation: Childhood cancer survivors are at increased risk of any first fracture. Very low lumbar spine BMD was significantly associated with fractures. Several modifiable risk factors for reduced BMD and vertebral fractures were identified.

INTRODUCTION

The survival of childhood cancer continues to increase as a result of improved treatment regimens and supportive care strategies.[1] However, the majority of childhood cancer survivors suffer from late adverse effects, including skeletal sequelae such as low bone mineral density (BMD) and fractures.[2,3] These fractures are of concern as they may not only lead to temporary pain and immobilization, but also to chronic morbidity and mortality.[4]

In the general elderly population, reduced BMD increases fracture risk, but in younger individuals this relationship is less well established.[5] The occurrence of fragility fractures (especially vertebral fractures) is therefore important in the diagnosis of osteoporosis and considerations regarding treatment in this age group.[5] Previous studies have shown that vertebral fractures are common during and shortly after treatment for childhood acute lymphoblastic leukemia (ALL).[6,7] However, the prevalence of and risk factors for vertebral fractures after other childhood cancer types are unknown, and evidence regarding risk factors associated with non-vertebral fractures in survivors is limited.[8,9]

The International Guideline Harmonization Group (IGHG) recently identified low-quality evidence for the association between low BMD and fractures in survivors.[10] Nevertheless, supported by evidence from adult literature, the authors deemed to have sufficient rationale to recommend BMD surveillance for high-risk survivors (i.e. those treated with cranial/craniospinal or total body irradiation). In addition, directions for future research in this field were delineated, including identification of risk factors for very low BMD (Z-score ≤-2) as well as vertebral and non-vertebral low-trauma fractures. Filling these knowledge gaps could improve surveillance strategies and provide insights in underlying mechanisms and potential interventions to prevent bone fragility in survivors.

The aims of this study were to: 1) assess the risk and risk factors of low and very low BMD and different types of fractures in a national cohort of adult childhood cancer survivors; 2) investigate the relationship between (very) low BMD and fractures in this cohort; and to 3) determine the prevalence and potential risk factors for vertebral fractures (detected by vertebral fracture assessment [VFA]) in a subset of these survivors.

METHODS

Patients

This cross-sectional study is part of the Dutch Childhood Cancer Survivor Study (DCCSS) LATER cohort.[11] The LATER cohort consists of 6,165 individuals who survived at least five years after cancer diagnosis, were diagnosed before the age of 19 years, and treated in one of the seven Dutch pediatric oncology centers between 1963 and 2002. Of this cohort, 2,169 survivors were ineligible due to various reasons (i.e. death, lost to follow-up, living abroad, refusal for participation or registration in any research, attained age <18 or >45 years, or ineligible according to the treating physician) (Figure 1). A total of 3,996 survivors were eligible and invited for this study, which was approved by the Institutional Review Board of the Amsterdam University Medical Center, the Netherlands (no. 2011/116). Informed consent was obtained from all participating survivors.

Bone mineral density

Dual-Energy X-ray absorptiometry (DXA; Hologic Discovery A and Horizon A, Marlborough, MA, USA) was used to measure lumbar spine (LS BMD; L1-L4) and total body BMD (TB BMD) in six late-effect clinics, and total hip BMD (TH BMD) in three clinics. As all survivors were aged 18-50 years, BMD values were expressed as Z-scores, which represent the number of standard deviations that BMD differs from age- and sex-matched reference data provided by the DXA manufacturer. We defined low BMD as a Z-score ≤-1 and very low BMD as a Z-score ≤-2, using BMD thresholds and terminology to describe reduced bone mass as recommended by the International Society for Clinical Densitometry.[12] If osteosynthesis or other foreign (metal) material was present, BMD results for that particular site were excluded.

Fractures

We took a survivors' medical history to characterize fractures that occurred at least five years after cancer diagnosis. Fracture history included information on the number of fractures, fracture site(s), and year of fracture(s). We categorized reported clinical fractures into any fracture, long bone fracture (i.e. lower arm, upper arm, lower leg, upper leg, or hip fracture), and fragility fracture (i.e. vertebral, lower arm, upper arm, or hip fracture).[9,13] Vertebral fractures were evaluated in a subset of survivors included at the University Medical Center Groningen, of which VFA's (by DXA) were available. We chose to use the term vertebral fracture, although a vertebral deformity assessed by VFA may not

always represent a vertebral fracture.[14] We evaluated the presence, severity, and morphology of vertebral fractures of the thoracolumbar spine (T2-L4) using the Genant semiquantitative method.[15] Grade 1 was considered a mild vertebral fracture (20-25% reduction of anterior, middle, or posterior vertebral height), grade 2 a moderate vertebral fracture (25-40% reduction), and grade 3 a severe vertebral fracture (>40% reduction).

Potential risk factors

Demographic as well as disease- and therapy-related data for primary tumors, recurrences and subsequent malignancies were derived from medical records. These included chemotherapy regimens and total cumulative doses, radiotherapy fields and (fractionated) dose, and hematopoietic stem cell transplantation (HSCT). We determined cumulative corticosteroid doses based on previous treatment protocols and calculated the prednisone equivalent dose.[16] If the treatment protocol was missing, we estimated this based on disease type and treatment decade. Chemotherapy and radiotherapy dose thresholds were chosen based on clinical relevance or previous reports in the literature. Additional data were collected during a late-effects clinic visit between May 2016 and February 2020. Body mass index (BMI) was derived from height and weight measures (height/weight^2). We also registered whether survivors had ever been diagnosed with endocrine disorders (i.e. growth hormone deficiency [GHD] or hypogonadism). In addition, survivors completed various questionnaires, including questionnaires regarding individual lifestyle behaviors. Furthermore, peripheral blood samples were taken after an overnight fast. We assessed serum thyroid stimulating hormone (TSH), free thyroxine (FT4), insulin-like growth factor 1 (IGF-1), 25-hydroxyvitamin D (25OHD), folic acid, vitamin B12, and homocysteine levels. Definitions of the potential risk factors are shown in Supplementary Table 1.

Statistical analysis

Patient demographics and disease- and treatment-related characteristics were summarized. The characteristics of study participants were compared to those of non-participants and the underlying cohort using a Chi-Square test. In addition, characteristics of specifically those participants with a DXA scan or VFA were compared to the characteristics of non-participants plus participants without a DXA scan or VFA and the underlying cohort. The incidence of any first fracture that occurred between 1987 and 2014 was compared with sex- and age- adjusted normative fracture incidence data from a Swedish national registry, as Dutch

normative data were not available. We calculated a standardized incidence ratio (SIR) using the statistical package popEpi.[17] The SIR reflects the proportion of observed fractures and expected fractures corrected for person-years at risk. Risk factors for low and very low BMD and different types of fractures (yes vs. no) were first assessed using univariable logistic regression analysis. We performed a Fisher's exact test for risk factors with less than five observations in each cell. Potential risk factors were included in multivariable models based on the univariable results (i.e. a p-value <0.2) and on results of previous reported studies (i.e. sex, attained age, and BMI). Separate multivariable models for demographic and treatment-related risk factors and for endocrine and lifestyle-related risk factors were estimated. For prevalent vertebral fractures (yes vs. no), the effect of risk factors was only assessed using univariable models due to the small sample size. All analyses were performed in R version 4.0.3 (Vienna, Austria).[18]

RESULTS

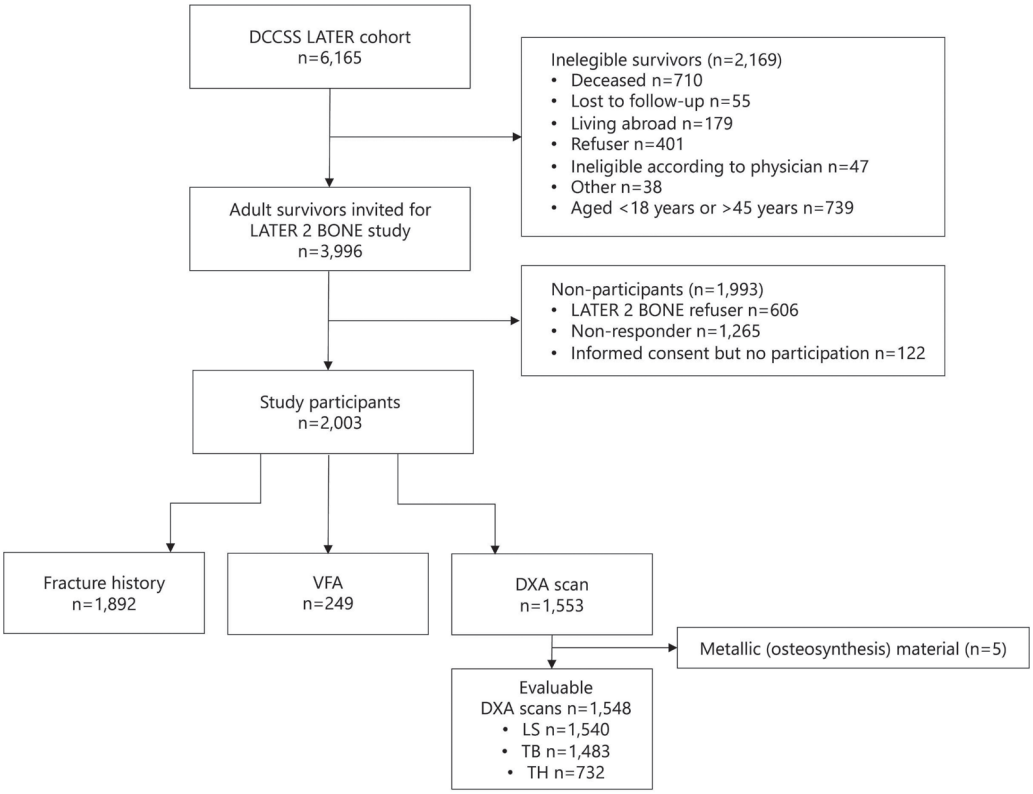
A total of 3,996 survivors were eligible, and 2,003 (50.1%) participated in this study (Figure 1). Mean age was 33.1 (standard deviation ±7.2) years, and median time since cancer diagnosis was 25.3 (interquartile range 20.3-31.3) years. We observed significant differences in sex, primary cancer diagnosis, frequency of previously received radiotherapy, chemotherapy, and HSCT between participants and non-participants (Table 1). However, the participating cohort was representative regarding age at cancer diagnosis, age at study invitation, follow-up time, and surgery frequency. We obtained a total of 1,553 DXA scans (77.5%). Survivors with a DXA scan were representative regarding age at cancer diagnosis, age at study invitation, follow-up time, and frequency of previous treatment with radiotherapy (Table 1). Five survivors had metal (osteosynthesis) material at all measured skeletal sites, leaving 1,548 DXA scans available for evaluation. For 1,892 survivors (94.5%), we had a history of fractures, and a VFA was performed in a representative cohort of 249 survivors (12.4%) (Supplementary Table 2). The year of fracture was missing for 170 fractures (17.9% of all fractures) and 164 fractures (17.2%) seemed to have occurred before 5 years after diagnosis according to the year of fracture (Supplementary Table 3). These fractures were retained in most analyses, but excluded in SIR calculations.

Prevalence of reduced BMD and fractures

Low BMD at any site occurred in 36.1% (95%CI=33.7-38.6%) of childhood cancer survivors, and very low BMD in 9.6% (95%CI=8.2-11.2%). The percentage of low

LS, TB, and TH BMD was 27.9%, 21.2% and 17.2%, respectively, whereas very low BMD at these sites was observed in 7.2%, 4.5%, and 1.8% of survivors. Low BMD was most often present in survivors of myeloid and other leukemia and central nervous system (CNS) tumors, and very low BMD among survivors of CNS tumors (Supplementary Figure 1).

Supplementary Table 4 shows that 32.8% (95%CI=30.7-34.9%), 17.8% (95%CI=16.1-19.6%), and 13.3% (95%CI=11.8-14.9%) of survivors had experienced any fracture, a long bone fracture, or a fragility fracture since five years after diagnosis, respectively. Fractures were most often localized in the lower arm (28.4% of all fractures). The SIR of any first fracture was 3.53 (95%CI=3.06-4.06) for male and 5.35 (95%CI=4.46-6.52) for female survivors. The SIR per age group was relatively



Abbreviations: DCCSS LATER=Dutch Childhood Cancer Survivor LATER Study; DXA=dual-energy X-ray absorptiometry; LS=lumbar spine; TB=total body; TH=total hip; VFA=vertebral fracture assessment.

Figure 1. Flowchart of study participants.

Characteristics	Participants (n=2,003)	Participants with DXA (n=1,553)	Non-participants (n=1,993)	Non-participants and participants without DXA (n=2,443)	Underlying cohort (n=6,165)	P-value participants vs. non-participants[¥]	P-value participants vs. underlying cohort[¥]
Sex						**<0.001; <0.001**	**0.004**; 0.070
Male	1,037 (51.8)	819 (52.7)	1,217 (61.1)	1,435 (58.7)	3,433 (55.7)		
Female	966 (48.2)	734 (47.3)	776 (38.9)	1,008 (41.3)	2,731 (44.3)		
Transgender	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.02)		
Primary childhood cancer (ICCC)						**<0.001; <0.001**	**<0.001; <0.001**
Leukemias, myeloprofiferative diseases and myelodysplastic diseases	748 (37.3)	609 (39.2)	696 (34.9)	835 (34.2)	2,094 (34.0)		
Lymphomas and reticulo endothelial neoplasms	373 (18.6)	282 (18.2)	349 (17.5)	440 (18.0)	1,062 (17.2)		
CNS and miscellaneous intracranial and intraspinal neoplasms	192 (9.6)	139 (9.0)	298 (15.0)	351 (14.4)	844 (13.7)		
Neuroblastoma and other peripheral nervous cell tumors	119 (5.9)	97 (6.2)	94 (4.7)	116 (4.7)	324 (5.3)		
Retinoblastoma	10 (0.5)	6 (0.4)	13 (0.7)	17 (0.7)	33 (0.5)		
Renal tumors	237 (11.8)	175 (11.3)	200 (10.0)	262 (10.7)	596 (9.7)		
Hepatic tumors	18 (0.9)	14 (0.9)	28 (1.4)	32 (1.3)	52 (0.8)		
Bone tumors	90 (4.5)	71 (4.6)	84 (4.2)	103 (4.2)	370 (6.0)		
Soft tissue and other extraosseous sarcomas	134 (6.7)	107 (6.9)	129 (6.5)	156 (6.4)	450 (7.3)		
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	60 (3.0)	37 (2.4)	78 (3.9)	101 (4.1)	232 (3.8)		
Other malignant epithelial neoplasms and malignant melanomas	20 (1.0)	15 (1.0)	23 (1.2)	28 (1.1)	102 (1.7)		

Characteristics	Participants (n=2,003)	Participants with DXA (n=1,553)	Non-participants (n=1,993)	Non-participants and participants without DXA (n=2,443)	Underlying cohort (n=6,165)	P-value participants vs. non-participants[¥]	P-value participants vs. underlying cohort[¥]
Other and unspecified malignant neoplasms	2 (0.1)	1 (0.1)	1 (0.1)	2 (0.1)	6 (0.1)		
Age at diagnosis (yr)*						0.99; 0.52	**<0.001; <0.001**
0-4	998 (49.8)	786 (50.6)	994 (50.0)	1,206 (49.4)	2,727 (45.3)		
5-9	553 (27.6)	435 (28.0)	551 (27.7)	669 (27.4)	1,628 (27.1)		
10-14	366 (18.3)	273 (17.6)	359 (18.0)	452 (18.5)	1,285 (21.4)		
15-17	86 (4.3)	59 (3.8)	85 (4.3)	112 (4.6)	376 (6.3)		
Age at invitation[#]						0.53; 0.19	**<0.001; <0.001**
<18	NA	NA	NA	NA	49 (1.2)		
18-29	771 (38.5)	616 (39.7)	522 (37.6)	677 (36.9)	1,313 (32.9)		
30-39	871 (43.5)	663 (42.7)	629 (45.3)	837 (45.6)	1,511 (37.9)		
≥40	361 (18.0)	274 (17.6)	236 (17.0)	323 (17.6)	1,118 (28.0)		
Follow-up time since cancer diagnosis[¶]						0.21; 0.74	**<0.001; <0.001**
10-20	466 (23.3)	362 (23.3)	432 (21.7)	536 (21.9)	981 (20.4)		
20-30	916 (45.7)	719 (46.3)	956 (48.0)	1,153 (47.2)	1,931 (40.1)		
30-40	544 (27.2)	417 (26.9)	546 (27.4)	673 (27.5)	1,393 (29.0)		
40-50	77 (3.8)	55 (3.5)	59 (3.0)	81 (3.3)	460 (9.6)		
50-60	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	46 (1.0)		
Radiotherapy[a]							
Any radiotherapy	676 (33.7)	493 (31.8)	566 (28.4)	749 (30.7)	2,527 (41.2)	**<0.001;** 0.48	**<0.001; <0.001**
Cranial[¥]	320 (16.0)	227 (14.7)	180 (13.0)	273 (14.9)		**0.015;** 0.85	
Abdomen/pelvis	148 (7.4)	107 (6.9)	63 (4.6)	104 (5.7)		**<0.001;** 0.13	
Total body	83 (4.2)	59 (3.8)	28 (2.0)	52 (2.8)		**<0.001;** 0.11	

Characteristics	Participants (n=2,003)	Participants with DXA (n=1,553)	Non-participants (n=1,993)	Non-participants and participants without DXA (n=2,443)	Underlying cohort (n=6,165)	P-value participants vs. non-participants[¥]	P-value participants vs. underlying cohort[¥]
Chemotherapy[a‡]							
Any chemotherapy	1,784 (89.1)	1,385 (89.2)	1,603 (80.4)	2,002 (82.0)	5,005 (81.7)	**<0.001; <0.001**	**<0.001; <0.001**
Alkylating agents	1,015 (53.3)	798 (53.8)	581 (43.9)	798 (45.8)		**<0.001; <0.001**	
Anthracyclines	1,067 (53.8)	830 (54.0)	628 (45.7)	865 (47.5)		**<0.001; <0.001**	
Platinum compounds	298 (14.9)	221 (14.2)	168 (12.1)	244 (13.3)		**0.021;** 0.42	
Vinca-alkaloids	1,589 (79.4)	1244 (80.2)	1,015 (73.2)	1,360 (74.1)		**<0.001; <0.001**	
Methotrexate	939 (46.9)	756 (48.7)	588 (42.4)	771 (42.0)		**0.010; <0.001**	
Glucocorticoïds	1,165 (58.2)	917 (59.0)	738 (53.2)	986 (53.7)		**0.004; 0.002**	
Hematopoietic stem cell transplantation[a]						**<0.001;** 0.09	0.23; 0.67
Autologous	54 (2.7)	40 (2.6)	33 (1.7)	47 (1.9)	155 (2.6)		
Allogenic	95 (4.7)	68 (4.4)	54 (2.7)	81 (3.3)	231 (3.9)		
Surgery[a‡]							
Any surgery	965 (48.2)	733 (47.3)	1,003 (50.3)	1,235 (50.8)	3,185 (52.2)	0.14; **0.031**	**0.002; <0.001**
Amputation	42 (2.1)	29 (1.9)	29 (2.1)	42 (2.3)		0.99; 0.40	

Abbreviations: CNS=central nervous system; ICCC=International Classification for childhood cancer; NA=not applicable (survivors < 18 yrs or > 45 yrs excluded); yr=year

[¥]First p-value for participants versus non-participants or underlying cohort, and second p-value for participants with a DXA scan versus non-participants and participants without DXA or underlying cohort

[a]For primary cancer and recurrences

*Not reported for survivors refusing registration

[#]Not reported for survivors refusing participation

[¶]Not reported for survivors refusing registration and those who are ineligible due to reasons such as death, lost to follow-up, or living abroad

[‡]Subgroup data not reported for survivors refusing participation

[¥]Including cranial irradiation for brain tumors and craniospinal irradiation

Table 1. Baseline characteristics of the study cohort.

stable for males, whereas for females, the SIR was highest for those who were at risk between age 5-10 (SIR=7.11) and 30-40 years (SIR=7.47) (Supplementary Table 5). Survivors of hepatic tumor, bone tumor, and myeloid leukemia experienced long bone and fragility fractures most often (Supplementary Figure 1). Sixty-three fractures (6.6% of all fractures) occurred in survivors of bone tumor, of which 36.5% occurred at the primary tumor site.

Among the survivors with a VFA, 43 prevalent vertebral fractures were observed in 33 survivors (13.3%, 95%CI=9.3-18.1%). In 93.9% of these cases, a vertebral fracture had not been mentioned by the survivor during fracture history. Most vertebral fractures were a grade 1 fracture (67.4%) and had a crush morphology (72.1%) (Supplementary Figure 2). Vertebral fractures were most prevalent in survivors of neuroblastoma, bone tumor, and CNS tumors (Supplementary Figure 3).

Factors associated with low and very low BMD

Risk factors for low and very low BMD using univariable logistic regression analysis are shown in Supplementary Table 6 and 7.

In multivariable analysis including demographic and treatment-related risk factors, male sex (odds ratio (OR)=2.15), underweight (OR=4.00), and high carboplatin dose (≥2000 mg/m2; OR=2.07) were significantly associated with low BMD at any site (Supplementary Table 8). Moreover, a significant dose effect relationship for cranial radiation therapy (CRT) was found (>0-<10 Gy; OR=2.40, ≥20-<40 Gy; OR= 2.54, and ≥40 Gy; OR=3.91). A shorter follow-up time was significantly associated with low LS BMD (OR=0.96), and prior exposure to total body irradiation (TBI) with low TB BMD (>0-<10 Gy; OR=2.82 and ≥10 Gy; OR=3.51) as well as with low TH BMD (>0-<10 Gy; OR=2.47 and ≥10 Gy; OR=4.61). We also found a significant association between high dose of alkylating agents (≥8,000 g/m^2; OR=2.31) and low TH BMD.

Male sex (OR=2.68), underweight (OR=6.71), and CRT (OR=2.92) significantly increased the risk of very low BMD at any site (Table 2). A significant association was found between prior exposure to TBI (OR=3.83) and very low TB BMD. Survivors who received a prednisone equivalent dose ≥10,000 mg/m2 had an increased odds of very low BMD at any site (OR=1.48, 95%CI=0.66-3.31), very low LS BMD (OR=2.22, 95%CI=0.90-5.47), and very low TB BMD (OR=1.33, 95%CI=0.45-3.92), but this was not statistically significant.

	Very low BMD at any site		Very low lumbar spine BMD		Very low total body BMD	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Sex (male)	2.68 (1.81-3.98)	**<0.001**	3.77 (2.31-6.14)	**<0.001**	1.81 (1.04-3.14)	**0.034**
Attained age (per year)	1.00 (0.97-1.03)	0.99	1.02 (0.97-1.07)	0.36	0.97 (0.93-1.01)	0.14
BMI*					0.96 (0.90-1.02)	0.18
Underweight	6.71 (3.63-12.43)	**<0.001**	7.77 (4.04-14.97)	**<0.001**	-	
Normal	Ref	Ref	Ref	Ref	-	
Overweight/ obese	0.47 (0.31-0.71)	**<0.001**	0.36 (0.22-0.60)	**<0.001**	-	
Follow-up time (per year)	-	**-**	0.97 (0.92-1.02)	0.23	-	-
TBI	1.76 (0.82-3.75)	0.15	0.78 (0.32-1.95)	0.60	3.83 (1.41-10.45)	**0.009**
CRT#	2.92 (1.87-4.59)	**<0.001**	2.03 (1.18-3.50)	**0.011**	5.34 (2.96-9.63)	**<0.001**
Carboplatin	1.57 (0.84-2.95)	0.16	1.46 (0.70-3.04)	0.31	1.96 (0.88-4.36)	0.10
Corticosteroid dose (mg/m²)					-	-
0	Ref	Ref	Ref	Ref	Ref	Ref
>0 - <10,000	0.95 (0.64-1.43)	0.82	1.04 (0.65-1.66)	0.87	0.93 (0.51-1.71)	0.82
≥10,000	1.48 (0.66-3.31)	0.34	2.22 (0.90-5.47)	0.08	1.33 (0.45-3.92)	0.61

Abbreviations: BMD=bone mineral density; BMI=body mass index; CI=confidence interval; CRT=cranial irradiation; OR=odds ratio; Ref=reference; TBI=total body irradiation
*Adjusted for amputation. Very low total body BMD: BMI analyzed as continuous variable due to limited power.
#Including cranial irradiation for brain tumors and craniospinal irradiation

Table 2. Demographic and treatment-related risk factors for very low BMD (Z-score ≤-2) using multivariable logistic regression analysis.

In the multivariable model with endocrine and lifestyle-related risk factors, hypogonadism (OR=2.78), low physical activity (OR=1.63), severe vitamin D deficiency (OR=1.82), and folic acid deficiency (OR=1.44) were significantly associated with low BMD at any site (Supplementary Table 9). The interaction term sex*hypogonadism was added to our models to assess sex differences in the effect of hypogonadism on low BMD, but numbers were too low to establish robust models. In addition, survivors with GHD (OR=2.75) or hyperthyroidism (OR=4.03) had an increased risk of low LS BMD.

GHD (OR=4.40) and severe vitamin D deficiency (OR=1.84) were significantly associated with very low BMD at any site, and low physical activity (OR=1.93) and vitamin B12 deficiency (OR=3.84) with very low TB BMD (Table 3). Only 13.0% of the survivors with at least one of the assessed vitamin deficiencies had multiple deficiencies. Numbers for very low TH BMD were too low to perform multivariable analysis.

Factors associated with reported clinical fractures

Supplementary Table 10 shows risk factors for any fracture, long bone fracture, and fragility fracture using univariable logistic regression models.

In multivariable analysis, male sex (OR=1.48), former (OR=1.90) and current (OR=1.65) smoking, and very low LS BMD (OR=1.79) were significantly associated with fractures at any site (Table 4). Male sex (OR=1.37), obesity (OR=1.82), and very low LS BMD (OR=2.19) significantly increased the odds of long bone fractures. Obesity (OR=1.66) and very low LS BMD (OR=2.08) were also significantly associated with occurrence of fragility fractures. A sensitivity analysis which only included fractures that certainly occurred more than five years after cancer diagnosis according to year of fracture showed similar risk factors for any fracture (Supplementary Table 11). In these models for long bone and fragility fractures, the effect of very low LS BMD increased (OR=2.57 and OR=2.63, respectively) and remained the only significant risk factor for both outcomes.

	Very low BMD at any site		**Very low lumbar spine BMD**		**Very low total body BMD**	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Hypogonadism	1.45 (0.67-3.13)	0.35	0.69 (0.23-2.04)	0.50	2.33 (0.95-5.71)	0.06
GHD	4.40 (2.26-8.54)	**<0.001**	2.41 (1.02-5.72)	**0.045**	4.68 (2.11-10.40)	**<0.001**
Hyperthyroidism	1.70 (0.56-5.12)	0.35	-	-	-	-
Smoking						
Never	-	-	Ref	Ref	-	-
Former	-	-	1.50 (0.79-2.88)	0.22	-	-
Current	-	-	1.66 (0.93-2.94)	0.08	-	-
Low physical activity	1.52 (0.95-2.45)	0.08	1.72 (0.95-3.09)	0.07	1.93 (1.05-3.53)	**0.033**
Severe vitamin D deficiency	1.84 (1.12-3.02)	**0.016**	1.79 (1.00-3.21)	0.05	1.99 (1.03-3.85)	**0.041**
Vitamin B12 deficiency	1.80 (0.77-4.24)	0.18	-	-	3.84 (1.52-9.69)	**0.004**
Folic acid deficiency	-	-	-	-	1.24 (0.63-2.44)	0.54

Abbreviations: BMD=bone mineral density; CI=confidence interval; GHD=growth hormone deficiency; OR=odds ratio; Ref=reference
Model adjusted for sex, attained age, and BMI (=body mass index adjusted for amputation; continuous)

Table 3. Endocrine and lifestyle-related risk factors for very low BMD (Z-score ≤-2) using multivariable logistic regression analysis.

	Any fracture		Long bone fracture		Fragility fracture	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Sex (male)	1.48 (1.14-1.91)	**0.003**	1.37 (1.02-1.85)	**0.037**	1.08 (0.77-1.52)	0.67
BMI*						
Underweight	0.85 (0.41-1.78)	0.67	0.94 (0.40-2.19)	0.89	1.13 (0.46-2.79)	0.79
Normal	Ref	Ref	Ref	Ref	Ref	Ref
Overweight	1.07 (0.81-1.41)	0.64	1.30 (0.93-1.80)	0.12	1.39 (0.95-2.02)	0.09
Obese	1.07 (0.72-1.60)	0.73	1.82 (1.19-2.79)	**0.006**	1.66 (1.01-2.73)	**0.046**
Attained age (per year)	0.99 (0.97-1.01)	0.28	0.99 (0.96-1.01)	0.26	0.98 (0.96-1.01)	0.26
Age at diagnosis (per year)	0.98 (0.96-1.01)	0.29	0.98 (0.94-1.01)	0.16	0.98 (0.94-1.02)	0.31
HSCT#	-	-	1.38 (0.79-2.42)	0.25	1.59 (0.86-2.94)	0.14
Smoking						
Never	Ref	Ref	Ref	Ref	Ref	Ref
Former	1.90 (1.35-2.68)	**<0.001**	1.37 (0.93-2.02)	0.11	1.51 (0.98-2.33)	0.06
Current	1.65 (1.19-2.29)	**0.003**	0.93 (0.63-1.37)	0.72	0.84 (0.53-1.33)	0.45
Heavy drinking	-	-	-	-	1.75 (0.77-3.96)	0.18
Low physical activity	0.86 (0.61-1.21)	0.41	-	-	-	-
Severe vitamin D deficiency	-	-	-	-	1.31 (0.82-2.09)	0.27
Very low LS BMD	1.79 (1.12-2.86)	**0.016**	2.19 (1.34-3.58)	**0.002**	2.08 (1.19-3.64)	**0.010**

Abbreviations: BMD=bone mineral density; BMI=body mass index; CI=confidence interval; LS=lumbar spine; OR=odds ratio; Ref=reference
*Adjusted for amputation
#With myeloablative conditioning

Table 4. Risk factors for reported clinical fractures using multivariable logistic regression analysis.

Factors associated with observed prevalent vertebral fractures

Older attained age (OR=1.06 per year), platinum compounds (OR=2.78), GHD (OR=3.33), and low physical activity (OR=2.44) were significantly associated with vertebral fractures in univariable analysis (Supplementary Table 12). In addition, we observed a significantly higher vertebral fracture prevalence in survivors treated with spinal radiotherapy. However, all of these survivors also had GHD. CRT (OR=1.74, 95%CI=0.69-4.36), low BMD (OR=1.79, 95%CI=0.86-3.77), very low BMD (OR=1.86, 95%CI=0.70-4.99), and severe vitamin D deficiency (OR=1.88, 95%CI=0.77-4.54) also increased the odds of prevalent vertebral fractures, but this did not reach statistical significance.

DISCUSSION

In our national cohort of childhood cancer survivors, we found a substantial increase in fracture risk for male and female five-year survivors of all ages. This is the first study that compared fracture incidence in very long-term survivors with normative data using sex- and age-adjusted person-years at risk. One previous questionnaire-based study that compared fracture frequencies between childhood cancer survivors and siblings during their lifetime found no increased risk for survivors,[8] whereas two other studies found an increased hazard of hospitalization due to fractures for survivors.[3,19] We found that 13.3% of survivors (mean age 33 years) had a prevalent vertebral fracture, which may indicate osteoporosis.[20] This frequency markedly exceeds the prevalence of about 3% observed in adults below 60 years of age from the general population (in Norway and China).[21,22] A Canadian study including survivors of childhood ALL showed a higher prevalence of vertebral fractures (23%).[6] Moreover, the fact that the fracture rate was highest in female survivors aged 30-40 years, and that older age was a risk factor for vertebral fracture in this study, suggests that skeletal morbidity could become even more prominent as survivors age.

We showed that very low LS BMD was significantly associated with any type of reported clinical fractures, as well as with long bone and fragility fractures in childhood cancer survivors. Although this association was anticipated based on literature in elderly, it had been less clearly established in young adult populations, including childhood cancer survivors. So far, only one study in survivors of hematologic malignancies has shown a significant association between BMD and fractures estimated with a univariable model.[9] The association between very low LS BMD and reported clinical fractures is a pivotal finding, and it may support the hypothesis that BMD surveillance for high-risk survivors can prevent fractures when adequate interventions are initiated in case of reduced BMD.[10] Notably, a one standard deviation reduction in BMD was also associated with vertebral fracture (OR=1.79, 95%CI=0.86-3.77), which is similar to observations in the general elderly population.[20]

Our results support BMD surveillance for survivors treated with CRT or TBI as recently presented by the IGHG.[10] In addition, we found that low BMD was associated with higher CRT doses, although we could not observe a safe CRT dose (i.e. a dose that was not significantly associated with low BMD). The observed effects of CRT, and to a lesser extent of TBI, are conceivably related to the presence of hypothalamic-pituitary deficiencies, including central hypogonadism and GHD, which we also identified as risk factors for reduced BMD. However, TBI may also

damage other endocrine glands and bones directly. Due to small sample size, it was not possible to assess whether associations between CRT or TBI and BMD were different for survivors with and without endocrine disorders. We identified an association between high dose alkylating agents (≥8,000 g/m^2) and low TH BMD, which may be due to the fact that alkylating agents are known to induce primary hypogonadism, especially at higher doses.[23] Unfortunately, gonadal hormone status was not available at the time of the analysis. Furthermore, high-dose carboplatin (≥2,000 g/m^2) significantly increased the risk of low BMD in this study, which is consistent with carboplatin-induced trabecular bone loss as observed in healthy mice.[24]

Our findings suggest that more intensive surveillance and adequate interventions for the assessed endocrine disorders (as recently proposed by the IGHG[25]) and vitamin deficiencies may be needed. In addition to hypogonadism and GHD, we showed that hyperthyroidism was a risk factor for low LS BMD. Although the contribution of hyperthyroidism to higher risk of reduced BMD has not been previously assessed in survivors, it is a known risk factor in the general adult population, as excess thyroid hormones stimulates bone resorption and consequently decreases BMD.[26] Only one study has previously assessed the effect of vitamin deficiencies on BMD deficits in survivors, and found that survivors with vitamin D deficiency (25OHD levels <20 nmol/L) had a more than three-fold increased risk of reduced BMD.[27] Our results suggest that the effects of vitamin deficiencies on BMD are not limited to vitamin D deficiency, since deficiencies in vitamin B12 and folic acid were also associated with reduced BMD, which is consistent with observations in the general elderly population.[28,29] The exact underlying mechanisms for the association between vitamin B12 and folic acid deficiencies and low BMD are not fully elucidated.[29]

We also identified several modifiable risk factors for vertebral fractures (i.e. GHD, low physical activity, and possibly severe vitamin D deficiency). Larger studies are needed to validate these results. The fact that the vast majority of vertebral fractures were asymptomatic underscores the importance of vertebral imaging, especially in survivors with very low BMD without a history of other fractures (and with back pain).[5]

There are several limitations that may be taken into consideration when interpreting our results. The observed differences between the characteristics of participants and non-participants could indicate selection bias, although we only had data from non-responders (and not from refusers) and absolute

differences were small. Furthermore, the exact year that fractures occurred was missing or the fracture seemed to have occurred before five years after diagnosis (according to the year of fracture) for a substantial number of fractures. These first fractures, as well as all subsequent fractures, could not be compared with the Swedish normative data. This, and the fact that we used Swedish normative data (known to have a higher fracture incidence compared with the Netherlands)[30] may have led to an underestimation of the SIR of fracture in survivors. As this was a cross-sectional study, we could only assess associations between current risk factors and reported clinical fractures, and not their effect on future incident fractures. In addition, we had no information available regarding the level of trauma that preceded the fractures, and could therefore not distinguish low-trauma fractures. However, we attempted to approach this type of fractures by separately analyzing long bone fractures and fractures in skeletal sites commonly associated with osteoporosis (i.e. fragility fractures). We only had a VFA available for a subgroup of survivors, limiting the possibility to detect independent risk factors for vertebral fractures.

We conclude that long-term childhood cancer survivors are at increased risk of clinical vertebral and non-vertebral fractures. Moreover, as the vast majority of vertebral fractures identified by VFA were asymptomatic, this likely represents an underestimation of the true magnitude of skeletal morbidity among survivors. Reduced BMD (especially very low LS BMD) was shown to be a strong indicator of the increased fracture risk, which underscores the importance of active BMD surveillance for high-risk survivors. High-dose carboplatin was identified as a new treatment-related risk factor for low BMD, whereas corticosteroid (dose) was not significantly associated with reduced BMD or fractures. Our data suggest that more intensive surveillance for endocrine disorders may be advised, as timely interventions for survivors with endocrine disorders (including hyperthyroidism), as well as supplementation of vitamin D, vitamin B12, and folic acid deficiencies may improve bone health.

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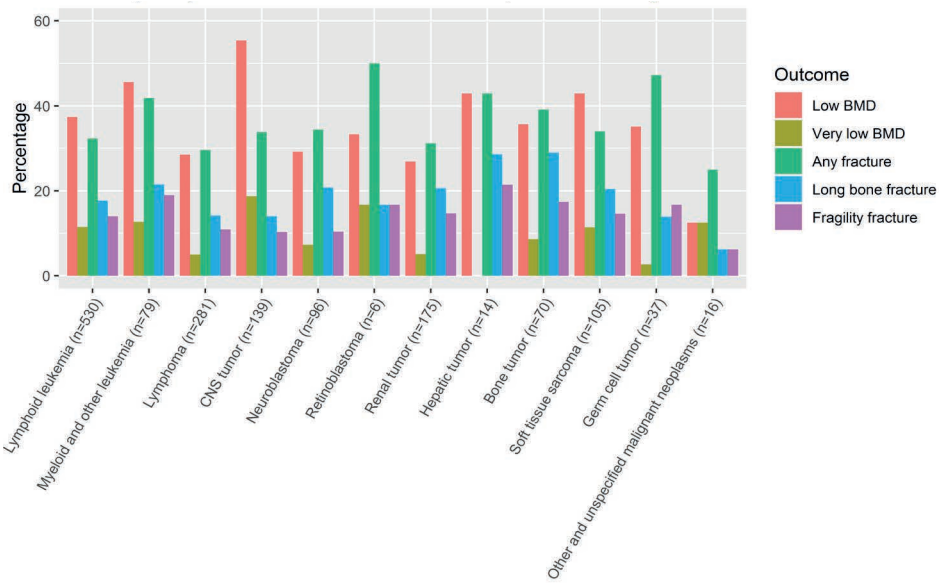
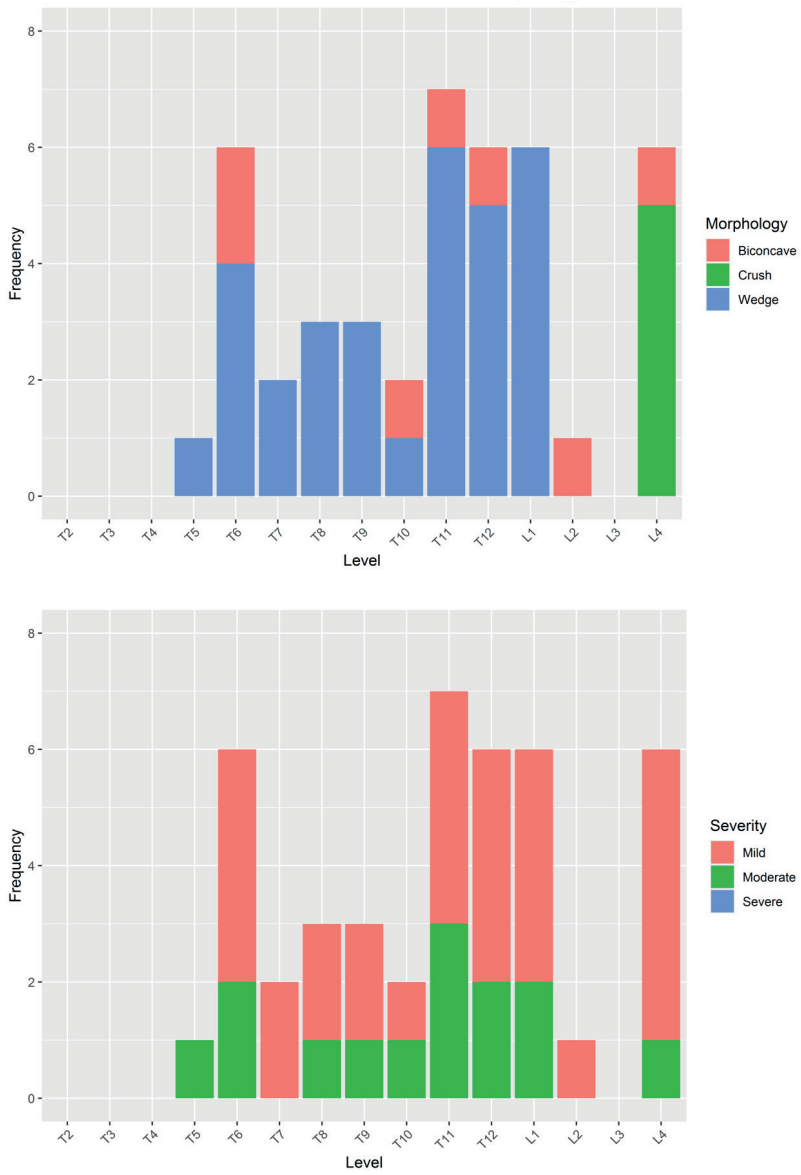


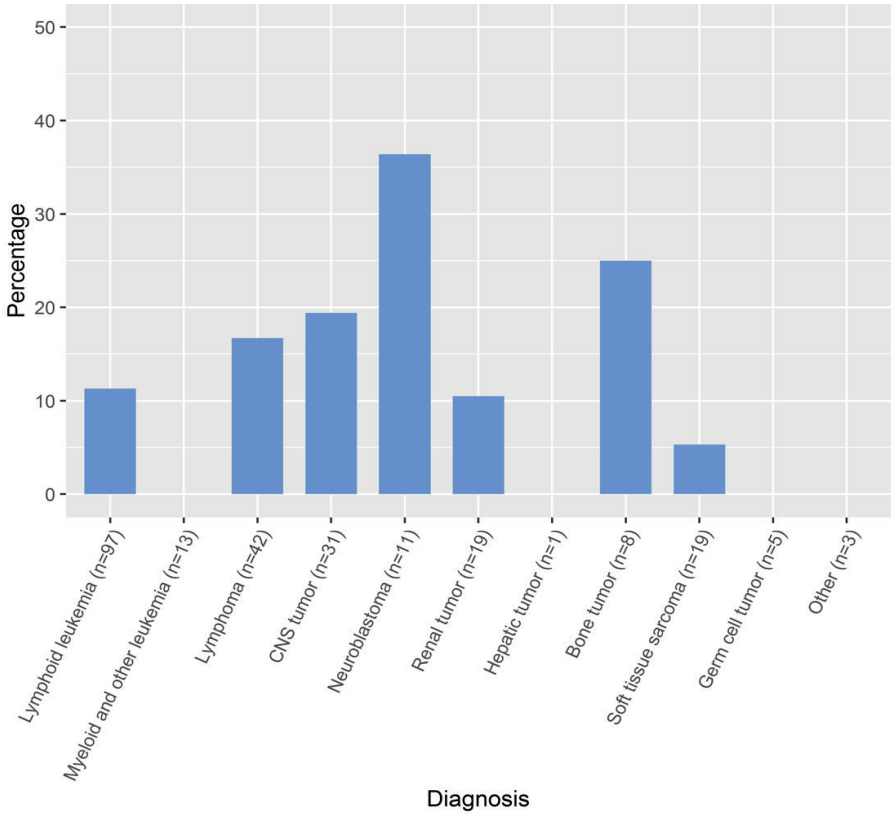
Abbreviations: BMD=bone mineral density; CNS=central nervous tumor

Supplementary Figure 1. Frequency of reduced BMD and reported clinical fractures per cancer diagnosis in survivors with a DXA scan (n=1,548).



Abbreviations: T=thoracic; L=lumbar
Mild vertebral fracture: 20 to 25% reduction in anterior, middle, or posterior vertebral height. Moderate vertebral fracture: >25% to 40% reduction in anterior, middle, or posterior vertebral height. Severe vertebral fracture: >40% reduction in anterior, middle, or posterior vertebral height.
Biconcave vertebral fracture: reduction in middle vertebral height. Crush fracture: reduction in posterior vertebral height. Wedge fracture: reduction in anterior vertebral height.

Supplementary Figure 2. The distribution of observed prevalent vertebral fracture severity and morphology per vertebral level.



Abbreviations: CNS=central nervous tumor

Supplementary Figure 3. The frequency of observed prevalent vertebral fractures per cancer diagnosis.

Covariate	Definition	Source
Body mass index	BMI was derived from height and weight measures (height/weight^2) and adjusted for amputation using estimated total body weight percentages of the amputated limb	BMI calculator for amputees; amputee coalition
Body mass index category	Underweight: BMI <18.5 kg/m^2 Normal: BMI ≥18.5 and <25 kg/m^2 Overweight: BMI ≥25-<30 kg/m^2 Obese: BMI ≥30 kg/m2	WHO
Heavy drinking	Males: >14 alcoholic consumptions per week (self-report) Females: >7 alcoholic consumptions per week (self-report)	NIAAA
Dietary calcium intake	A detailed questionnaire on dietary calcium intake was used to assess calcium intake (mg) per week. Values below 3500 mg calcium per week were considered as inadequate calcium intake (50% of recommended daily amount).	NEVO-online, RIVM Mayo Clinic, Nutrition and healthy eating
Physical activity	Physical activity (including commuting, household, work or school, and leisure-time activities) was measured using the SQUASH questionnaire. The number of minutes spent on moderate-to-vigorous physical activity per week was compared with that in age- and sex-matched Dutch young adults from the general population (Lifelines cohort). Values below the 20th percentile were considered low.	Wendel-Vos et al.[1] Lifelines cohort[2]
Hypogonadism	Ever diagnosed with hypogonadism, assessed using medical charts	NA
Growth hormone deficiency	Ever diagnosed with GHD, assessed using medical charts, or low IGF-1 levels according to age with a plausible reason to have GHD (e.g. treated with CRT). IGF-1 levels were assessed using the IDS-iSYS assay.	Manufacturer and expert opinion
Hyperthyroidism	FT4 levels >24.3 and TSH levels <0.56. FT4 and TSH levels were assessed using the Fujirebio Lumipulse G assay. Of note: FT4 was calibrated to the RMP. The threshold for hyperthyroidism for FT4 was derived from de Grande et al.[3] TSH was calibrated according to the IFCC harmonization recommendation. The threshold for hyperthyroidism for TSH was derived from Thienpont et al.[4]	De Grande et al. 2017[3] Thienpont et al. 2017[4]
Hypothyroidism	FT4 levels <11 pmol/L or TSH levels >10 mU/L. FT4 and TSH levels were assessed using the Fujirebio Lumipulse G assay.	Manufacturer and expert opinion
Vitamin D deficiency	25OHD levels <50 nmol/L. 25OHD levels were assessed using the Fujirebio Lumipulse G assay.	Manufacturer
Severe vitamin D deficiency	25OHD levels <30 nmol/L. 25OHD levels were assessed using the Fujirebio Lumipulse G assay.	Manufacturer
Elevated homocysteine	Homocysteine levels >19 µmol/L. Homocysteine levels were assessed using the Cobas 6000 c501 assay.	Manufacturer
Vitamin B12 deficiency	Vitamin B12 levels <150 pmol/L or vitamin B12 levels ≥ 150 and <220 pmol/L with elevated homocysteine levels. Homocysteine and vitamin B12 levels were assessed using the Cobas 6000 c501 and c601 assay, respectively.	UpToDate
Folic acid deficiency	Folic acid levels <6.8 nmol/L. Folic acid levels were assessed using the Cobas 6000 c601 assay.	WHO

Abbreviations: 25OHD=25-hydroxyvitamin D; BMI=body mass index; CRT=cranial irradiation; GHD=growth hormone deficiency; FT4=free thyroxine; NA=not applicable; NIAAA=National Institute on Alcohol Abuse and Alcoholism; SQUASH= Short QUestionnaire to ASsess Health enhancing physical activity; TSH=thyroid stimulating hormone; WHO=World Health Organization

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Supplementary Table 1. Definitions of covariates used in the analyses.

Characteristics	Participants (n=249)	Non-participants (n=3,747)	Underlying cohort (n=6,165)	P-value participants vs. non-participants	P-value participants vs. underlying cohort
Sex				0.85	0.99
Male	139 (55.8)	2,115 (56.4)	3,433 (55.7)		
Female	110 (44.2)	1,632 (43.6)	2,731 (44.3)		
Transgender	0 (0.0)	0 (0.0)	1 (0.02)		
Primary childhood cancer (ICCC)				0.29	0.20
Leukemias, myeloprofiferative diseases and myelodysplastic diseases	110 (44.2)	1334 (35.6)	2,094 (34.0)		
Lymphomas and reticulo endothelial neoplasms	42 (16.9)	680 (18.1)	1,062 (17.2)		
CNS and miscellaneous intracranial and intraspinal neoplasms	31 (12.4)	459 (12.2)	844 (13.7)		
Neuroblastoma and other peripheral nervous cell tumors	11 (4.4)	202 (5.4)	324 (5.3)		
Retinoblastoma	0 (0.0)	23 (0.6)	33 (0.5)		
Renal tumors	19 (7.6)	418 (11.2)	596 (9.7)		
Hepatic tumors	1 (0.4)	45 (1.2)	52 (0.8)		
Bone tumors	8 (3.2)	166 (4.4)	370 (6.0)		
Soft tissue and other extraosseous sarcomas	19 (7.6)	244 (6.5)	450 (7.3)		
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	5 (2.0)	133 (3.5)	232 (3.8)		
Other malignant epithelial neoplasms and malignant melanomas	3 (1.2)	40 (1.1)	102 (1.7)		
Other and unspecified malignant neoplasms	0 (0.0)	3 (0.1)	6 (0.1)		
Age at diagnosis (yr)*				0.96	0.46
0-4	120 (48.2)	1872 (50.0)	2,727 (45.3)		
5-9	71 (28.5)	1033 (27.6)	1,628 (27.1)		
10-14	47 (18.9)	678 (18.1)	1,285 (21.4)		
15-17	11 (4.4)	160 (4.3)	376 (6.3)		
Age at invitation#				0.37	**0.001**
<18	NA	NA	49 (1.2)		
18-29	104 (41.8)	1189 (37.9)	1,313 (32.9)		
30-39	100 (40.2)	1400 (44.6)	1,511 (37.9)		
≥40	45 (18.1)	552 (17.6)	1,118 (28.0)		

Characteristics	Participants (n=249)	Non-participants (n=3,747)	Underlying cohort (n=6,165)	P-value participants vs. non-participants	P-value participants vs. underlying cohort
Follow-up time since childhood cancer diagnosis[¶]				0.21	**0.006**
10-20	66 (26.5)	832 (22.2)	981 (20.4)		
20-30	108 (43.4)	1764 (47.1)	1,931 (40.1)		
30-40	63 (25.3)	1027 (27.4)	1,393 (29.0)		
40-50	12 (4.8)	124 (3.3)	460 (9.6)		
50-60	0 (0.0)	0 (0.0)	46 (1.0)		
Radiotherapy[a,i]					
Any radiotherapy	80 (32.1)	1162 (31.1)	2,527 (41.2)	0.72	**0.004**
Cranial[¥]	36 (14.5)	284 (16.3)		0.47	
Abdomen/pelvis	24 (9.6)	124 (7.1)		0.16	
Total body	9 (3.6)	74 (4.2)		0.64	
Chemotherapy[a,i]					
Any chemotherapy	220 (88.4)	3,167 (84.6)	5,005 (81.7)	0.11	**0.007**
Alkylating agents	139 (57.2)	876 (52.7)		0.19	
Anthracyclines	129 (52.0)	938 (54.0)		0.55	
Platinum compounds	22 (8.8)	275 (15.7)		**0.004**	
Vinca-alkaloids	201 (80.7)	1,388 (79.2)		0.58	
Methotrexate	135 (54.2)	804 (45.9)		**0.014**	
Glucocorticoïds	151 (60.6)	1014 (57.8)		**0.040**	
Hematopoietic stem cell transplantation[a]				0.97	0.95
Autologous	6 (2.4)	81 (2.2)	155 (2.6)		
Allogenic	9 (3.6)	140 (3.8)	231 (3.9)		
Surgery[a,i]					
Any surgery	109 (44.1)	1,859 (49.8)	3,185 (52.2)	0.08	**0.01**
Amputation	2 (0.8)	40 (2.3)		0.16	

Abbreviations: CNS=central nervous system; ICCC=International Classification for childhood cancer; NA=not applicable (survivors < 18 yrs or > 45 yrs excluded); yr=year
[a]For primary cancer and recurrences
*Not reported for survivors refusing registration
[#]Not reported for survivors refusing participation
[¶]Not reported for survivors refusing registration and those who are ineligible due to reasons such as death, lost to follow-up, or living abroad
[i]Subgroup data not reported for survivors refusing participation
[¥]Including cranial irradiation for brain tumors and craniospinal irradiation

Supplementary Table 2. Baseline characteristics of participants with a VFA (DCCSS LATER cohort).

	1st fracture	2nd fracture	3rd fracture	4th fracture	5th fracture	6th fracture	7th fracture	8th fracture
Total no. of fractures	620	194	88	28	12	6	3	1
Missing year of fracture	93	40	19	6	7	3	2	0
Fracture occurred before 5 years after dx*	129	29	5	1	0	0	0	0

Abbreviations: dx=cancer diagnosis; no.=number
*According to year of fracture

Supplementary Table 3. Overview of missing data for survivors with reported clinical fractures.

Skeletal site	No. of survivors (%) n=1,892	No. of fractures (%) n=952
Upper arm	22 (1.2)	25 (2.6)
Lower arm	218 (11.5)	270 (28.4)
Upper leg	18 (1.0)	20 (2.1)
Lower leg	114 (6.0)	134 (14.1)
Hip	7 (0.4)	7 (0.7)
Vertebrae	10 (0.5)	10 (1.1)
Other	376 (19.9)	484 (50.8)
Unknown	2 (0.1)	2 (0.2)
Skeletal site	**No. of survivors (%) n=1,892**	
Any fracture	620 (32.8)	
Long bone fracture*	336 (17.8)	
Fragility fracture#	252 (13.3)	

Abbreviations: No.=number
*Including fractures of the hip, upper arm, lower arm, upper leg, and lower leg
#Including fractures of the hip, upper arm, lower arm, and vertebrae

Supplementary Table 4. Frequency of reported clinical fractures per skeletal site.

Age at risk	SIR of any first fracture (95%CI)
Males	
5-10 years	3.88 (2.06-6.63)
10-20 years	3.97 (3.30-4.76)
20-30 years	2.76 (2.05-3.64)
30-40 years	3.85 (2.24-6.16)
Total (all ages)	3.53 (3.06-4.06)
Females	
5-10 years	7.11 (4.21-11.23)
10-20 years	5.16 (3.98-6.71)
20-30 years	4.39 (2.94-6.33)
30-40 years	7.47 (4.27-12.11)
Total (all ages)	5.35 (4.46-6.52)

Abbreviations: CI=confidence interval; SIR=standardized incidence ratio

Supplementary Table 5. Standardized incidence ratio of any reported first fracture by sex and age.

	Low BMD at any site			Low lumbar spine BMD	
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**	**OR (95% CI)**
Demographics					
Sex		1.99 (1.61-2.57)	**<0.001**		2.89 (2.28-3.67)
Male	355 (43.5)			305 (37.5)	
Female	204 (27.9)			125 (17.2)	
Attained age (per year)		0.99 (0.98-1.00)	0.16		0.98 (0.96-0.99)
First tertile	195 (36.7)			159 (29.9)	
Second tertile	202 (39.5)			159 (31.2)	
Third tertile	161 (32.1)			111 (22.3)	
BMI*					
Underweight	41 (73.2)	4.14 (2.25-7.59)	**<0.001**	35 (62.5)	3.46 (1.97-6.05)
Normal	333 (39.8)	Ref	Ref	271 (32.5)	Ref
Overweight/obese	183 (28.3)	0.60 (0.48-0.74)	**<0.001**	123 (19.1)	0.49 (0.38-0.62)
Age at dx (per year)		1.02 (0.99-1.04)	0.21		1.03 (1.00-1.05)
First tertile	178 (33.9)			128 (24.7)	
Second tertile	191 (36.9)			152 (29.3)	
Third tertile	190 (37.6)			150 (29.8)	
Follow-up time (per year)		0.98 (0.97-1.00)	**0.019**		0.97 (0.95-0.98)
First tertile	168 (39.8)			172 (33.1)	
Second tertile	183 (35.5)			146 (28.4)	
Third tertile	208 (32.9)			112 (22.2)	
Treatment factors					
HSCT#		2.02 (1.33-3.07)	**0.001**		1.58 (1.02-2.43)
Yes	49 (52.1)			35 (37.2)	
No	507 (35.1)			393 (27.3)	
TBI		2.31 (1.37-4.00)	**0.002**		1.67 (0.98-2.86)
Yes	33 (55.9)			23 (39.0)	
No	525 (35.5)			406 (27.6)	
TBI dose (Gy)					
0	525 (35.5)	Ref	Ref	406 (27.6)	Ref
>0-<10	20 (57.1)	2.42 (1.23-4.77)	**0.011**	15 (42.9)	1.97 (1.00-3.88)
≥10	13 (54.2)	2.15 (0.96-4.83)	0.06	8 (33.3)	1.31 (0.56-3.09)
CRT†		2.36 (1.77-3.14)	**<0.001**		1.66 (1.3-2.23)
Yes	122 (54.0)	1.99 (1.61-2.57)		84 (37.3)	
No	437 (33.2)			346 (26.5)	
CRT dose (Gy)					
0	437 (33.2)	Ref	Ref	346 (26.5)	Ref
>0-<20	20 (43.5)	1.55 (0.85-2.80)	0.15	10 (21.7)	0.77 (0.38-1.57)
≥20-<40	34 (44.7)	1.63 (1.02-2.59)	**0.041**	21 (28.0)	1.08 (0.64-1.82)

	Low total body BMD			Low total hip BMD		
P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
<0.001		1.11 (0.87-1.42)	0.34		1.35 (0.91-1.96)	0.13
	174 (22.1)			76 (19.1)		
	140 (20.1)			50 (14.9)		
0.0064		1.00 (0.98-1.01)	0.68		1.00 (0.97-1.03)	0.88
	114 (22.0)			42 (16.8)		
	106 (21.5)			46 (18.3)		
	94 (20.0)			38 (16.6)		
<0.001	27 (54.0)	4.40 (2.46-7.87)	**<0.001**	17 (56.7)	4.42 (2.07-9.46)	**<0.001**
Ref	170 (21.1)	Ref	Ref	89 (22.8)	Ref	Ref
<0.001	116 (18.7)	0.86 (0.66-1.12)	0.27	19 (6.2)	0.22 (0.13-0.37)	**<0.001**
0.051		1.00 (0.97-1.03)	0.81		1.02 (0.97-1.06)	0.47
	108 (21.3)			39 (16.8)		
	106 (20.9)			41 (15.7)		
	100 (21.3)			46 (19.2)		
<0.001		1.00 (0.98-1.02)	0.70		0.99 (1.97-1.02)	0.66
	118 (23.3)			45 (18.1)		
	96 (19.4)			41 (16.3)		
	100 (20.8)			40 (17.2)		
0.040		2.41 (1.53-3.79)	**<0.001**		3.91 (2.25-6.80)	**<0.001**
	33 (37.9)			100 (15.1)		
	281 (20.2)			25 (41.0)		
0.059		2.79 (1.61-4.83)	**<0.001**		4.17 (2.18-8.00)	**<0.001**
	23 (41.8)			18 (43.9)		
	291 (20.5)			108 (15.8)		
Ref	291 (20.5)	Ref	Ref	108 (15.8)	Ref	**Ref**
0.051	13 (39.4)	2.52 (1.24-5.13)	**0.011**	11 (36.7)	3.08 (1.43-6.67)	**0.004**
0.53	10 (45.5)	3.23 (1.38-7.55)	**0.007**	7 (63.6)	9.33 (2.69-32.43)	**<0.001**
<0.001		2.78 (2.05-3.77)	**<0.001**		1.75 (1.10-2.79)	**0.018**
	86 (38.2)			30 (24.8)		
	228 (18.2)			96 (15.8)		
Ref	228 (18.2)	Ref	Ref	96 (15.8)	NA	NA
0.48	14 (30.4)	1.96 (1.03-3.74)	**0.04**	2 (6.9)	NA	NA
0.77	30 (40.0)	2.99 (1.85-4.86)	**<0.001**	8 (22.9)	NA	NA

	Low BMD at any site			Low lumbar spine BMD	
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**	**OR (95% CI)**
≥40	65 (65.7)	3.84 (2.50-5.91)	**<0.001**	51 (51.5)	2.95 (1.95-4.46)
CRT dose (per 10Gy)					
First tertile	-	-	-	-	-
Second tertile	-	-	-	-	-
Third tertile	-	-	-	-	-
Abdominal/pelvic RT		0.83 (0.54-1.27)	0.39		0.87 (1.05-1.38)
Yes	34 (32.8)			26 (25.5)	
No	524 (36.6)			403 (28.2)	
Abdominal/pelvic RT dose (Gy)					
0	524 (36.6)	Ref	Ref	403 (28.2)	Ref
>0-<20	10 (34.5)	0.91 (0.42-1.98)	0.81	6 (20.7)	0.66 (0.27-1.64)
≥20	24 (31.6)	0.80 (0.49-1.31)	0.38	20 (27.4)	0.96 (0.57-1.63)
Platinum compounds		1.36 (1.01-1.81)	**0.040**		1.37 (1.01-1.87)
Yes	93 (42.2)			74 (33.6)	
No	465 (35.1)			355 (26.9)	
Cisplatin		0.97 (0.65-1.43)	0.86		1.13 (0.75-1.70)
Yes	41 (35.3)			35 (30.2)	
No	517 (36.2)			394 (27.7)	
Cisplatin dose (mg/m²)		0.96 (0.88-1.05)	0.34		1.00 (0.90-1.09)
First tertile	173 (35.2)			131 (26.7)	
Second tertile	187 (35.3)			160 (30.0)	
Third tertile	198 (37.8)			138 (26.5)	
Carboplatin		1.86 (1.28-2.71)	**<0.001**		1.60 (1.08-2.36)
Yes	59 (50.0)			44 (37.3)	
No	499 (34.9)			385 (27.1)	
Carboplatin dose (mg/m²)					
0	499 (34.9)	Ref	Ref	385 (27.1)	Ref
>0-<2,000	22 (44.0)	1.46 (0.83-2.58)	0.19	18 (36.0)	1.51 (0.84-2.73)
≥2,000	37 (57.8)	2.55 (1.54-4.24)	**<0.001**	26 (40.6)	1.84 (1.10-3.07)
Alkylating agents		1.23 (0.99-1.53)	0.056		1.14 (0.91-1.44)
Yes	301 (37.9)			228 (28.8)	
No	226 (33.1)			177 (26.1)	
Alkylating dose (CED, g/m²)					
0	226 (33.1)	Ref	Ref	177 (26.1)	Ref
>0-<8,000	192 (35.2)	1.10 (0.87-1.39)	0.42	147 (27.0)	1.04 (0.81-1.35)
≥8,000	109 (43.6)	1.56 (1.16-2.10)	**0.003**	81 (32.7)	1.37 (1.00-1.88)

	Low total body BMD			Low total hip BMD		
P-value¶	**No. (%)**	**OR (95% CI)**	**P-value¶**	**No. (%)**	**OR (95% CI)**	**P-value¶**
<0.001	41 (41.4)	3.17 (2.08-4.86)	**<0.001**	20 (37.7)	NA	NA
					1.22 (1.10-1.35)	**<0.001**
-	-	-	-	22 (28.2)		
-	-	-	-	61 (18.0)		
-	-	-	-	43 (14.0)		
0.55		0.91 (0.54-1.52)	0.71		0.93 (0.46-1.88)	0.83
	19 (19.8)			10 (16.4)		
	295 (21.4)			116 (17.5)		
Ref	295 (21.4)	Ref	Ref	116 (17.5)	NA	NA
0.38	7 (25.0)	1.2 (0.51-2.91)	0.65	4 (25.0)	NA	NA
0.88	12 (17.6)	0.79 (0.4-1.49)	0.46	6 (13.3)	NA	NA
0.041		1.54 (1.10-2.16)	**0.013**		1.46 (0.86-2.48)	0.16
	55 (27.9)			21 (22.3)		
	258 (20.1)			105 (16.5)		
0.57		0.92 (0.54-1.54)	0.74		1.52 (0.81-2.86)	0.19
	19 (19.8)			14 (23.3)		
	294 (21.2)			112 (16.7)		
0.82		0.94 (0.83-1.07)	0.37		1.00 (0.87-1.15)	1.00
	115 (24.1)			14 (33.3)		
	75 (15.1)			64 (16.4)		
	123 (24.3)			48 (16.1)		
0.019		2.23 (1.49-3.34)	**<0.001**		1.50 (0.72-3.12)	0.28
	41 (35.6)			10 (23.3)		
	272 (19.9)			116 (16.8)		
Ref	272 (19.9)	Ref	Ref	116 (16.8)	NA	NA
0.17	15 (31.9)	1.89 (1.01-3.53)	**0.048**	1 (7.8)	NA	NA
0.020	26 (40.6)	2.75 (1.64-4.61)	**<0.001**	9 (30.0)	NA	NA
0.27		1.15 (0.89-1.49)	0.28		1.66 (1.09-2.52)	**0.017**
	164 (21.7)			79 (19.8)		
	128 (19.4)			39 (13.0)		
Ref	128 (19.4)	Ref	Ref	39 (13.0)	Ref	Ref
0.74	102 (19.5)	1.01 (0.75-1.34)	0.97	45 (16.8)	1.36 (0.85-2.16)	0.20
0.051	62 (26.7)	1.52 (1.07-2.15)	**0.019**	34 (26.0)	2.35 (1.41-3.94)	**0.001**

	Low BMD at any site			Low lumbar spine BMD	
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**	**OR (95% CI)**
Corticosteroids		0.89 (0.72-1.09)	0.27		0.88 (0.70-1.10)
Yes	294 (34.9)			225 (26.8)	
No	265 (37.6)			205 (29.3)	
Corticosteroid dose (mg/m²)					
0	265 (37.6)	Ref	Ref	205 (29.3)	Ref
>0-<10,000	265 (34.1)	0.86 (0.69-1.06)	0.16	203 (26.2)	0.86 (0.68-1.07)
≥10,000	29 (43.9)	1.30 (0.78-2.17)	0.31	22 (33.3)	1.20 (0.70-2.06)
Vinca alkaloids		0.92 (0.71-1.20)	0.55		0.88 (0.67-1.16)
Yes	433 (35.6)			338 (27.4)	
No	115 (37.6)			91 (30.0)	
Methotrexate		0.97 (0.79-1.19)	0.76		0.98 (0.79-1.23)
Yes	270 (35.7)			209 (27.7)	
No	288 (36.5)			220 (28.0)	
Endocrine disorders					
Hypogonadism		3.66 (2.17-6.19)	**<0.001**		2.02 (1.22-3.34)
Yes	43 (66.2)			28 (43.1)	
No	516 (34.8)			402 (27.3)	
Growth hormone deficiency		2.32 (1.48-3.65)	**<0.001**		2.21 (1.40-3.49)
Yes	45 (55.6)			36 (45.0)	
No	512 (35.0)			393 (27.0)	
Hyperthyroidism		2.99 (1.40-6.38)	**0.005**		3.26 (1.56-6.84)
Yes	18 (62.1)			16 (55.2)	
No	527 (35.4)			406 (27.4)	
Hypothyroidism		NA	1.00		NA
Yes	2 (28.6)			2 (28.6)	
No	543 (35.9)			420 (27.9)	
Lifestyle-related factors					
Smoking					
Never	324 (35.8)	Ref	Ref	246 (27.3)	Ref
Former	65 (30.7)	0.79 (0.57-1.09)	0.16	51 (24.3)	0.85 (0.60-1.21)
Current	93 (38.0)	1.10 (0.82-1.47)	0.54	72 (29.5)	1.11 (0.82-1.52)
Heavy drinking		0.83 (0.43-1.61)	0.58		1.25 (0.64-2.45)
Yes	13 (31.0)			13 (31.7)	
No	468 (35.1)			359 (27.0)	
Low dietary calcium intake		1.05 (0.82-1.33)	0.72		0.93 (0.72-1.21)
Yes	150 (37.4)			108 (27.0)	
No	347 (36.4)			269 (28.4)	

	Low total body BMD			Low total hip BMD		
P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
0.26		0.80 (0.62-1.03)	0.08		0.64 (0.44-0.94)	**0.025**
	161 (19.5)			60 (14.4)		
	153 (23.3)			66 (20.8)		
Ref	153 (23.3)	Ref	Ref		0.64 (0.44-0.94)	**0.025**
0.18	140 (18.3)	0.74	**0.023**	60 (14.4)		
0.50	21 (33.9)	1.69	0.06	66 (20.8)		
0.35		1.10 (0.79-1.51)	0.58		1.13 (0.70-1.82)	0.63
	256 (21.4)			101 (17.6)		
	57 (19.9)			25 (15.9)		
0.88		0.88 (0.69-1.13)	0.33		0.87 (0.59-1.27)	0.47
	147 (21.2)			61 (16.2)		
	57 (19.9)			65 (18.3)		
0.006		3.30 (1.98-5.48)	**<0.001**		4.05 (2.19-7.48)	**<0.001**
	29 (45.3)			20 (42.6)		
	285 (20.1)			106 (15.5)		
<0.001		2.72 (1.71-4.35)	**<0.001**		1.81 (0.99-3.32)	**0.05**
	32 (40.5)			16 (26.2)		
	280 (20.0)			110 (16.4)		
0.002		2.23 (1.01-4.92)	**0.047**		NA	0.54
	10 (37.0)			4 (21.1)		
	298 (20.9)			117 (16.7)		
1.00		NA	1.00		NA	1.00
	1 (14.3)			1 (14.3)		
	307 (21.2)			120 (16.8)		
Ref		Ref	Ref	78 (18.2)	Ref	Ref
0.37		0.66 (0.43-1.00)	**0.049**	21 (15.8)	0.63 (0.50-1.42)	0.16
0.50		0.99 (0.70-1.40)	0.96	13 (12.4)	0.84 (0.34-1.19)	0.52
0.51		0.55 (0.21-1.41)	0.21		0.27 (0.04-2.04)	0.21
	5 (12.5)			1 (5.3)		
	264 (20.7)			111 (17.1)		
0.93		1.10 (0.83-1.47)	0.50		1.20 (0.78-1.83)	0.41
	88 (23.1)			38 (18.9)		
	196 (21.4)			80 (16.3)		

	Low BMD at any site			Low lumbar spine BMD	
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**	**OR (95% CI)**
Low physical activity		1.73 (1.29-2.31)	**<0.001**		1.19 (0.87-1.63)
Yes	104 (47.9)			67 (31.2)	
No	398 (34.8)			314 (27.6)	
Vitamin D deficiency		1.35 (1.09-1.68)	**0.006**		1.31 (1.04-1.65)
Yes	226 (40.3)			175 (31.4)	
No	452 (33.7)			247 (25.9)	
Severe vitamin D deficiency		2.13 (1.55-2.91)	**<0.001**		1.86 (1.34-2.57)
Yes	93 (52.0)			71 (39.9)	
No	481 (34.0)			351 (26.3)	
Elevated homocysteine levels		1.40 (0.92-2.15)	0.12		1.36 (0.87-2.14)
Yes	40 (43.5)			31 (34.1)	
No	505 (35.4)			390 (27.5)	
Vitamin B12 deficiency		1.62 (0.92-2.83)	0.093		1.31 (0.72-2.37)
Yes	24 (47.1)			17 (33.3)	
No	521 (35.5)			404 (27.7)	
Folic acid deficiency		1.30 (0.98-1.74)	0.073		1.26 (0.93-1.72)
Yes	93 (41.2)			72 (32.0)	
No	452 (34.9)			349 (27.1)	

Abbreviations: BMD=bone mineral density; BMI=body mass index; CED=cyclophosphamide equivalent dose; CI=confidence interval; CRT=cranial irradiation; Gy=gray; NA=not applicable (due to patient numbers <5); No.=number; OR=odds ratio; RT=radiotherapy; Ref=reference; HSCT=stem cell transplantation; TBI=total body irradiation

¶Logistic regression p-value for variables with more than five observations in each cell. For variables with less than five observations in each cell, a Fisher exact p-value was calculated.

*Adjusted for amputation

#With myeloablative conditioning

‡Including cranial irradiation for brain tumors and craniospinal irradiation

Supplementary Table 6. Risk factors for low BMD (Z-score ≤-1) using univariable logistic regression analysis.

	Low total body BMD			Low total hip BMD		
P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
0.28		2.61 (1.89-3.60)	**<0.001**		1.47 (0.89-2.44)	0.13
	77 (37.7)			24 (22.4)		
	207 (18.9)			97 (16.4)		
0.021		1.09 (0.84-1.42)	0.50		1.31 (0.88-1.94)	0.19
	119 (22.1)			52 (19.2)		
	189 (20.6)			69 (15.4)		
<0.001		1.69 (1.18-2.42)	**0.004**		1.40 (0.82-2.41)	0.22
	50 (29.8)			20 (21.3)		
	258 (20.1)			101 (16.1)		
0.18		1.57 (0.97-2.54)	0.069		1.36 (0.64-2.93)	0.43
	25 (29.1)			9 (21.4)		
	284 (20.7)			113 (16.7)		
0.38		2.03 (1.11-3.70)	**0.021**		2.34 (1.08-5.07)	**0.032**
	17 (34.7)			10 (31.3)		
	292 (20.8)			112 (16.3)		
0.13		1.33 (0.95-1.85)	0.097		0.73 (0.42-1.26)	0.26
	56 (25.5)			17 (13.5)		
	253 (20.5)			105 (17.7)		

	Very low BMD at any site			
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**
Demographics				
Sex		2.57 (1.77-3.74)	**<0.001**	
Male	108 (13.2)			87 (10.7)
Female	41 (5.6)			24 (3.3)
Attained age (per year)		1.00 (0.98-1.02)	0.94	
First tertile	51 (9.6)			40 (7.5)
Second tertile	28 (9.4)			39 (7.7)
Third tertile	50 (10.0)			32 (6.4)
BMI*				
Underweight	23 (41.1)	5.93 (3.33-10.56)	**<0.001**	20 (35.7)
Normal	88 (10.5)	Ref	Ref	68 (8.2)
Overweight/obese	37 (5.7)	0.52 (0.35-0.77)	**0.001**	22 (3.4)
Age at dx (per year)		1.01 (0.97-1.05)	0.62	
First tertile	44 (8.4)			30 (5.8)
Second tertile	54 (10.4)			42 (8.1)
Third tertile	51 (10.1)			39 (7.7)
Follow-up time (per year)		0.99 (0.97-1.02)	0.61	
First tertile	50 (9.6)			41 (7.9)
Second tertile	51 (9.9)			37 (7.2)
Third tertile	48 (9.4)			33 (6.5)
Treatment factors				
HSCT#		2.96 (1.76-4.97)	**<0.001**	
Yes	21 (22.3)			14 (14.9)
No	128 (8.9)			97 (6.7)
TBI		3.42 (1.86-6.31)	**<0.001**	
Yes	15 (25.4)			9 (15.3)
No	134 (9.1)			102 (6.9)
TBI dose (Gy)		1.13 (1.06-1.20)	**<0.001**	
First tertile	42 (8.7)			31 (6.5)
Second tertile	52 (10.1)			37 (7.2)
Third tertile	55 (10.2)			43 (8.0)
CRT†		2.28 (1.54-3.40)	**<0.001**	
Yes	39 (17.3)			23 (10.2)
No	110 (8.4)			88 (6.7)
CRT dose (per 10Gy)		1.25 (1.15-1.36)	**<0.001**	
First tertile	42 (8.5)			34 (6.9)
Second tertile	63 (12.3)			43 (8.4)
Third tertile	44 (8.3)			34 (6.5)
Abdominal/pelvic RT		0.45 (0.18-1.12)	0.085	
Yes	5 (4.8)			3 (2.9)
No	144 (10.1)			108 (7.6)

Very low lumbar spine BMD			Very low total body BMD	
OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
3.51 (2.21-5.58)	**<0.001**		2.00 (1.18-3.39)	0.34
		46 (5.9)		
		21 (3.0)		
0.99 (0.96-1.02)	0.52		0.99 (0.96-1.03)	0.68
		23 (4.4)		
		23 (4.7)		
		21 (4.5)		
6.25 (3.43-11.39)	**<0.001**	7 (14.0)	3.39 (1.43-8.04)	**<0.001**
Ref	Ref	37 (4.6)	Ref	Ref
0.40 (0.24-0.78)	**<0.001**	22 (3.5)	0.77 (0.45-1.31)	0.27
1.02 (0.98-1.06)	0.36		0.97 (0.92-1.03)	0.81
		28 (5.5)		
		20 (3.9)		
		19 (4.0)		
0.98 (0.95-1.01)	0.18		1.00 (0.97-1.04)	0.70
		22 (4.3)		
		25 (5.1)		
		20 (4.2)		
2.42 (1.32-4.43)	**0.004**		3.44 (1.73-6.84)	**<0.001**
		11 (12.6)		
		56 (4.0)		
2.42 (1.16-5.05)	**0.019**		3.92 (1.77-8.68)	**<0.001**
		8 (14.5)		
		59 (4.2)		
1.09 (1.01-1.18)	**0.019**		1.14 (1.05-1.24)	**0.001**
		23 (5.0)		
		26 (5.3)		
		18 (3.5)		
1.58 (0.97-2.56)	0.064		4.13 (2.48-6.87)	**<0.001**
		27 (12.0)		
		40 (3.2)		
1.16 (1.05-1.28)	**0.005**		1.37 (1.24-1.52)	**<0.001**
		17 (3.6)		
		34 (6.8)		
		16 (3.2)		
NA	0.11		NA	0.31
		2 (2.1)		
		65 (4.7)		

	Very low BMD at any site			
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**
Platinum compounds		1.60 (1.04-2.46)	**0.031**	
Yes	30 (13.6)			21 (9.5)
No	119 (9.0)			90 (6.8)
Cisplatin		1.32 (0.73-2.37)	0.36	
Yes	14 (12.1)			9 (7.8)
No	135 (9.4)			102 (7.2)
Cisplatin dose (mg/m²)		1.01 (0.88-1.16)	0.86	
First tertile	48 (9.8)			37 (7.6)
Second tertile	52 (9.8)			39 (7.4)
Third tertile	49 (9.4)			35 (6.7)
Carboplatin		1.92 (1.14-3.23)	**0.015**	
Yes	19 (16.1)			13 (11.0)
No	130 (9.1)			98 (6.9)
Carboplatin dose (mg/m²)				
0	130 (9.1)	Ref	Ref	98 (6.9)
>0-<2,000	8 (16.0)	1.90 (0.87-4.14)	0.11	6 (12.0)
≥2,000	11 (17.2)	2.07 (1.06-4.07)	**0.034**	7 (10.9)
Alkylating agents		1.20 (0.84-1.72)	0.37	
Yes	77 (9.7)			55 (6.9)
No	56 (8.2)			43 (6.4)
Alkylating dose (CED, g/m²)				
0	56 (8.2)	Ref	Ref	43 (6.4)
>0-<8,000	52 (9.5)	1.18 (0.80-1.75)	0.41	40 (7.3)
≥8,000	25 (10.0)	1.24 (0.76-2.04)	0.39	15 (6.0)
Corticosteroids		1.00 (0.71-1.40)	0.98	
Yes	81 (9.6)			63 (7.5)
No	68 (9.6)			48 (6.9)
Corticosteroid dose (mg/m²)				
0	68 (9.6)	Ref	Ref	48 (6.9)
>0-<10,000	68 (8.8)	0.90 (0.63-1.28)	0.55	53 (6.8)
≥10,000	13 (19.7)	2.30 (1.19-4.43)	**0.013**	10 (15.2)
Vinca alkaloids		1.32 (0.84-2.08)	0.24	
Yes	125 (10.1)			92 (7.5)
No	24 (7.8)			19 (6.3)
Methotrexate		1.13 (0.81-1.59)	0.48	
Yes	77 (10.2)			58 (7.7)
No	72 (9.1)			53 (6.8)
Endocrine disorders				
Hypogonadism		3.31 (1.83-5.99)	**<0.001**	
Yes	16 (24.6)			8 (12.3)
No	133 (9.0)			103 (7.0)
Growth hormone deficiency		4.51 (2.70-7.51)	**<0.001**	

Very low lumbar spine BMD			Very low total body BMD	
OR (95% CI)	**P-value¶**	**No. (%)**	**OR (95% CI)**	**P-value¶**
1.44 (0.86-2.37)	0.15		2.53 (1.44-4.45)	**0.001**
		18 (9.1)		
		49 (3.8)		
1.09 (0.54-2.21)	0.82		2.37 (1.13-4.94)	**0.022**
		9 (9.4)		
		58 (4.2)		
1.03 (0.88-1.19)	0.74		1.09 (0.91-1.32)	0.35
		26 (5.4)		
		19 (3.8)		
		22 (4.4)		
1.67 (0.91-3.08)	0.10		2.78 (1.44-5.35)	**0.002**
		12 (10.4)		
		55 (4.0)		
Ref	Ref	55 (4.0)	Ref	Ref
1.84 (0.77-4.42)	0.17	6 (12.8)	3.49 (1.42-8.56)	**0.006**
1.66 (0.74-3.73)	0.22	6 (9.4)	2.47 (1.02-5.96)	**0.045**
1.10 (0.73-1.66)	0.66		1.29 (0.76-2.19)	0.35
		35 (4.6)		
		24 (3.6)		
Ref	Ref	24 (3.6)	Ref	Ref
1.17 (0.75-1.82)	0.50	22 (4.2)	1.16 (0.64-2.10)	0.62
0.95 (0.52-1.74)	0.87	13 (5.6)	1.58 (0.79-3.15)	0.20
1.10 (0.74-1.62)	0.64		0.87 (0.53-1.42)	0.57
		35 (4.2)		
		32 (4.9)		
Ref	Ref	32 (4.9)	Ref	Ref
1.00 (0.66-1.49)	0.98	28 (3.7)	0.75 (0.44-1.25)	0.27
2.42 (1.16-5.05)	**0.018**	7 (11.3)	2.49 (1.05-5.90)	**0.038**
1.20 (0.72-2.01)	0.48		1.57 (0.77-3.21)	0.22
		58 (4.9)		
		9 (3.2)		
1.15 (0.78-1.69)	0.48		1.20 (0.73-1.96)	0.47
		36 (4.9)		
		31 (4.1)		
1.87 (0.87-4.02)	0.11		5.05 (2.50-10.20)	**<0.001**
		11 (17.2)		
		56 (3.9)		
2.42 (1.27-4.62)	**0.007**		6.08 (3.25-11.37)	**<0.001**

	Very low BMD at any site			
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**
Yes	24 (29.6)			12 (15.0)
No	125 (8.5)			99 (6.8)
Hyperthyroidism		2.50 (1.00-6.23)	0.05	
Yes	6 (20.7)			3 (10.3)
No	141 (9.5)			107 (7.2)
Hypothyroidism		NA	0.14	
Yes	2 (28.6)			2 (28.6)
No	145 (9.6)			108 (7.2)
Lifestyle-related factors				
Smoking				
Never	85 (9.4)	Ref	Ref	57 (6.3)
Former	19 (9.0)	0.95 (0.60-1.60)	0.84	18 (8.6)
Current	28 (11.4)	1.24 (0.79-1.96)	0.35	23 (9.4)
Heavy drinking		NA	0.79	
Yes	4 (9.5)			3 (7.3)
No	122 (9.2)			91 (6.9)
Low dietary calcium intake		0.88 (0.59-1.32)	0.54	
Yes	36 (8.9)			25 (6.3)
No	96 (10.1)			70 (7.4)
Low physical activity		1.53 (0.98-2.37)	0.06	
Yes	29 (13.4)			21 (9.8)
No	105 (9.2)			75 (6.6)
Vitamin D deficiency		1.06 (0.74-1.50)	0.76	
Yes	56 (10.0)			46 (8.3)
No	91 (9.5)			64 (6.7)
Severe vitamin D deficiency		2.32 (1.51-2.32)	**<0.001**	
Yes	32 (17.9)			24 (13.5)
No	115 (8.6)			86 (6.5)
Elevated homocysteine levels		1.16 (0.59-2.28)	0.67	
Yes	10 (10.9)			5 (5.5)
No	136 (9.5)			104 (7.3)
Vitamin B12 deficiency		2.08 (0.99-4.37)	0.05	
Yes	9 (17.6)			5 (9.8)
No	137 (9.3)			104 (7.1)
Folic acid deficiency		1.27 (0.81-1.99)	0.30	
Yes	26 (11.5)			20 (8.9)
No	120 (9.3)			89 (6.9)

Abbreviations: BMD=bone mineral density; BMI=body mass index; CED=cyclophosphamide equivalent dose; CI=confidence interval; CRT=cranial irradiation; Gy=gray; NA=not applicable (due to patient numbers <5); No.=number; OR=odds ratio; RT=radiotherapy; Ref=reference; HSCT=stem cell transplantation; TBI=total body irradiation

¶Logistic regression p-value for variables with more than five observations in each cell. For variables with less than five observations in each cell, a Fisher exact p-value was calculated.

Very low lumbar spine BMD			Very low total body BMD	
OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
		15 (19.0)		
		52 (3.7)		
NA	0.46		NA	0.35
		2 (7.4)		
		64 (4.5)		
NA	0.09		NA	1.00
		0 (0.0)		
		66 (4.6)		
Ref	Ref	40 (4.6)	Ref	Ref
1.39 (0.80-2.41)	0.25	6 (3.1)	0.64 (0.27-1.53)	0.32
1.54 (0.93-2.55)	0.10	12 (5.0)	1.09 (0.56-2.11)	0.80
NA	0.76		NA	0.68
		2 (5.0)		
		53 (4.2)		
0.84 (0.52-1.34)	0.46		1.09 (0.63-1.90)	0.76
		19 (5.0)		
		42 (4.6)		
1.54 (0.92-2.55)	0.10		2.26 (1.28-4.00)	**0.005**
		18 (8.8)		
		45 (4.1)		
1.25 (0.84-1.86)	0.26		0.97 (0.58-1.63)	0.92
		24 (4.5)		
		42 (4.6)		
2.26 (1.39-3.66)	**<0.001**		2.60 (1.45-4.69)	**0.001**
		16 (9.5)		
		50 (3.9)		
0.74 (0.29-1.85)	0.52		2.32 (1.07-5.03)	**0.033**
		8 (9.3)		
		58 (4.2)		
1.42 (0.55-3.65)	0.47		4.54 (2.04-10.12)	**<0.001**
		8 (16.3)		
		58 (4.1)		
1.31 (0.79-2.18)	0.29		1.70 (0.94-3.08)	0.08
		15 (6.8)		
		51 (4.1)		

*Adjusted for amputation
#With myeloablative conditioning
ǂIncluding cranial irradiation for brain tumors and craniospinal irradiation

Supplementary Table 7. Risk factors for very low BMD (Z-score ≤-2) using univariable logistic regression analysis.

	Low BMD at any site		Low lumbar spine BMD		Low total body BMD		Low total hip BMD	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex (male)	2.15 (1.71-2.72)	**<0.001**	3.28 (2.52-4.26)	**<0.001**	1.15 (0.87-1.51)	0.32	1.13 (0.72-1.76)	0.60
Attained age (per year)	1.01 (0.98-1.04)	0.58	1.01 (0.98-1.04)	0.41	0.99 (0.96-1.01)	0.17	1.02 (0.98-1.05)	0.37
BMI*								
Underweight	4.00 (2.06-7.80)	**<0.001**	3.38 (1.78-6.40)	**<0.001**	3.86 (2.01-7.43)	**<0.001**	4.78 (2.03-11.29)	**<0.001**
Normal	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Overweight/obese	0.55 (0.43-0.70)	**<0.001**	0.45 (0.34-0.59)	**<0.001**	0.85 (0.64-1.14)	0.29	0.18 (0.10-0.32)	**<0.001**
Follow-up time (per year)	0.97 (0.94-1.00)	0.05	0.96 (0.92-0.99)	**0.005**	-	-	-	-
TBI dose (Gy)								
0	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>0-<10	2.06 (0.92-4.60)	0.08	1.40 (0.62-3.20)	0.42	2.82 (1.16-6.83)	**0.022**	2.47 (0.97-6.31)	0.06
≥10	1.49 (0.56-3.98)	0.42	0.78 (0.28-2.14)	0.63	3.51 (1.24-9.90)	**0.018**	4.61 (1.18-17.93)	**0.028**
CRT dose (Gy)#							1.03 (1.02-1.05)	**<0.001**
0	Ref	Ref	Ref	Ref	Ref	Ref	-	
>0-<20	2.40 (1.27-4.55)	**0.007**	1.27 (0.59-2.73)	0.55	2.64 (1.31-5.30)	**0.006**	-	
≥20-<40	2.54 (1.50-4.30)	**<0.001**	1.92 (1.06-3.46)	**0.03**	4.22 (2.39-7.42)	**<0.001**	-	
≥40	3.91 (2.41-6.34)	**<0.001**	3.18 (1.96-5.16)	**<0.001**	2.81 (1.72-4.58)	**<0.001**	-	
Platinum compounds	-	-	-	-	-	-	1.09 (0.57-2.07)	0.79
Carboplatin dose (mg/m²)								
0	Ref	Ref	Ref	Ref	Ref	Ref	-	-
>0-<2000	0.76 (0.38-1.49)	0.42	0.91 (0.45-1.84)	0.80	0.91 (0.41-2.02)	0.81	-	-
≥2000	2.07 (1.18-3.64)	**0.011**	1.42 (0.79-2.53)	0.24	2.12 (1.18-3.82)	**0.012**	-	-
Alkylating dose (CED, g/m²)								
0	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
>0 <8,000	1.00 (0.77-1.30)	1.00	0.98 (0.67-1.19)	0.44	1.01 (0.72-1.42)	0.95	1.19 (0.70-2.03)	0.51
≥8,000	1.29 (0.93-1.80)	0.13	1.08 (0.76-1.55)	0.66	1.28 (0.88-1.88)	0.20	2.31 (1.29-4.13)	**0.005**

	Low BMD at any site		Low lumbar spine BMD		Low total body BMD		Low total hip BMD	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Corticosteroid dose (mg/m²)								
0	-	-	-	-	Ref	Ref	-	-
>0 -<10,000	-	-	-	-	0.85 (0.60-1.21)	0.36	-	-
≥10,000	-	-	-	-	1.07 (0.52-2.18)	0.86	-	-

Abbreviations: BMD=bone mineral density; BMI=body mass index; CED=cyclophosphamide equivalent dose; CI=confidence interval; CRT=cranial irradiation; Gy=gray; OR=odds ratio; Ref=reference; TBI=total body irradiation
#Including cranial irradiation for brain tumors and craniospinal irradiation

Supplementary Table 8. Demographic and treatment-related risk factors for low BMD (Z-score ≤-1) using multivariable logistic regression analysis.

	Low BMD at any site		Low lumbar spine BMD		Low total body BMD		Low total hip BMD	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
Hypogonadism	2.78 (1.48-5.20)	**0.001**	0.97 (0.51-1.84)	0.92	2.50 (1.35-4.62)	**0.004**	3.44 (1.52-7.77)	**0.003**
Growth hormone deficiency	1.63 (0.92-2.88)	0.09	2.75 (1.51-5.01)	**<0.001**	1.78 (1.00-3.19)	0.05	1.61 (0.68-3.80)	0.28
Hyperthyroidism	2.39 (0.96-5.94)	0.06	4.03 (1.71-9.48)	**0.001**	1.34 (0.53-3.36)	0.54	-	-
Low physical activity	1.63 (1.18-2.25)	**0.003**	-	-	2.26 (1.61-3.18)	**<0.001**	1.35 (0.76-2.38)	0.31
Vitamin D deficiency	-	-	-	-	-	-	1.12 (0.71-1.78)	0.61
Severe vitamin D deficiency	1.82 (1.27-2.61)	**0.001**	1.49 (1.03-2.14)	**0.032**	1.58 (1.06-2.35)	**0.023**	-	-
Vitamin B12 deficiency	1.60 (0.84-3.04)	0.15	-	-	1.92 (0.98-3.75)	0.06	2.25 (0.93-5.44)	0.07
Folic acid deficiency	1.44 (1.02-2.03)	**0.039**	1.45 (1.03-2.05)	**0.035**	1.22 (0.83-1.79)	0.31	-	-

Abbreviations: BMD=bone mineral density; CI=confidence interval; OR=odds ratio
Model adjusted for sex, attained age, and BMI (=body mass index adjusted for amputation; continuous)

Supplementary Table 9. Endocrine and lifestyle-related risk factors for low BMD (Z-score ≤-1) using multivariable logistic regression analysis.

	Any fracture			Long bone fracture			Fragility fracture		
	No. (%)	**OR (95% CI)**	**P-value¶**	**No. (%)**	**OR (95% CI)**	**P-value¶**	**No. (%)**	**OR (95% CI)**	**P-value¶**
Demographics									
Sex		1.57 (1.29-1.91)	**<0.001**		1.35 (1.07-1.72)	**0.013**		1.19 (0.91-1.55)	0.21
Male	368 (37.5)			195 (19.9)			140 (14.3)		
Female	252 (27.7)			141 (15.5)			112 (12.3)		
Attained age (per year)		0.99 (0.98-1.00)	0.17		0.99 (0.97-1.00)	0.075		0.98 (0.97-1.00)	0.091
First tertile	226 (35.5)			127 (20.0)			100 (15.7)		
Second tertile	188 (29.9)			107 (17.0)			76 (12.1)		
Third tertile	206 (32.9)			102 (16.3)			76 (12.1)		
BMI*									
Underweight	19 (28.4)	0.83 (0.48-1.44)	0.54	11 (16.4)	0.99 (0.51-1.93)	0.98	11 (16.4)	1.41 (0.72-2.77)	0.32
Normal	330 (32.3)	Ref	Ref	169 (16.5)	Ref	Ref	125 (12.2)	Ref	Ref
Overweight	186 (33.1)	1.04 (0.83-1.29)	0.73	102 (18.1)	1.12 (0.85-1.47)	0.41	74 (13.2)	1.09 (0.80-1.48)	0.59
Obese	79 (34.3)	1.10 (0.81-1.49)	0.54	51 (22.2)	1.44 (1.01-2.05)	**0.043**	40 (17.4)	1.51 (1.03-2.23)	**0.037**
Age at dx (per year)		0.98 (0.96-1.00)	0.092		0.97 (0.94-1.00)	**0.022**		0.97 (0.94-1.00)	0.068
First tertile	206 (32.5)			120 (18.9)			88 (13.9)		
Second tertile	223 (35.4)			123 (19.5)			95 (15.1)		
Third tertile	191 (30.4)			93 (14.8)			69 (11.0)		
Follow-up time (per year)		1.00 (0.98-1.01)	0.70		1.00 (0.98-1.01)	0.59		0.99 (0.97-1.01)	0.46
First tertile	212 (33.6)			119 (18.9)			94 (14.9)		
Second tertile	198 (31.4)			108 (17.1)			75 (11.9)		
Third tertile	210 (33.3)			109 (17.3)			83 (13.2)		
Treatment factors									
HSCT#		1.13 (0.77-1.66)	0.52		1.44 (0.93-2.22)	0.10		1.70 (1.07-2.70)	**0.024**
Yes	44 (35.5)			29 (23.4)			25 (20.2)		
No	574 (32.7)			307 (17.5)			227 (12.9)		

	Any fracture			Long bone fracture			Fragility fracture		
	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
TBI		1.01 (0.62-1.63)	0.97		1.38 (0.80-2.36)	0.24		1.42 (0.78-2.56)	0.25
Yes	26 (32.9)			18 (22.8)			14 (17.7)		
No	590 (32.7)			318 (17.6)			238 (13.2)		
TBI dose (Gy)									
0	590 (32.7)	Ref	Ref	318 (17.6)	Ref	Ref	238 (13.2)	Ref	Ref
>0-<10	18 (40.9)	1.42 (0.77-2.62)	0.26	11 (25.0)	1.56 (0.78-3.11)	0.21	8 (18.2)	1.46 (0.67-3.18)	0.34
≥10	8 (23.5)	0.63 (0.28-1.41)	0.26	7 (20.6)	1.21 (0.52-2.80)	0.66	6 (17.6)	1.41 (0.58-3.44)	0.45
CRT[†]		0.84 (0.65-1.11)	0.22		0.84 (0.60-1.17)	0.30		0.97 (0.67-1.39)	0.86
Yes	89 (29.8)			47 (15.7)			39 (13.0)		
No	530 (33.4)			289 (18.2)			213 (13.4)		
CRT dose (Gy)									
0	530 (33.4)	Ref	Ref	289 (18.2)	Ref	Ref	213 (13.4)	Ref	Ref
>0-<20	18 (34.6)	1.05 (0.59-1.89)	0.86	11 (21.2)	1.20 (0.61-2.37)	0.59	10 (19.2)	1.53 (0.76-3.10)	0.23
≥20-<40	26 (25.5)	0.68 (0.43-1.08)	0.10	13 (12.7)	0.66 (0.36-1.19)	0.17	11 (10.8)	0.78 (0.41-1.48)	0.45
≥40	41 (29.1)	0.82 (0.56-1.19)	0.30	23 (16.3)	0.87 (0.55-1.39)	0.57	18 (12.8)	0.94 (0.56-1.58)	0.82
Abdominal/pelvic RT		0.81 (0.56-1.19)	0.28		1.03 (0.66-1.59)	0.92		0.99 (0.60-1.64)	0.97
Yes	41 (28.7)			26 (18.2)			19 (13.3)		
No	575 (33.1)			310 (17.8)			233 (13.4)		
Platinum compounds		1.01 (0.77-1.32)	0.93		0.87 (0.62-1.23)	0.43		0.83 (0.56-1.23)	0.36
Yes	94 (33.0)			46 (16.1)			33 (11.6)		
No	525 (32.7)			290 (18.1)			218 (13.6)		
Cisplatin		1.09 (0.78-1.53)	0.61		1.10 (0.73-1.66)	0.64		1.09 (0.69-1.73)	0.72
Yes	56 (34.6)			31 (19.1)			23 (14.2)		
No	563 (32.6)			305 (17.7)			228 (13.2)		
Carboplatin		0.97 (0.68-1.40)	0.88		0.76 (0.47-1.23)	0.27		0.73 (0.42-1.27)	0.27
Yes	47 (32.2)			21 (14.4)			15 (10.3)		
No	572 (32.8)			315 (18.1)			236 (13.5)		

	Any fracture			Long bone fracture			Fragility fracture		
	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
Alkylating agents		0.89 (0.74-1.09)	0.27		0.81 (0.64-1.03)	0.087		0.90 (0.67-1.18)	0.45
Yes	313 (32.0)			162 (16.6)			127 (13.0)		
No	284 (34.5)			162 (19.7)			117 (14.2)		
Corticosteroids		0.90 (0.75-1.10)	0.31		0.86 (0.69-1.11)	0.26		0.98 (0.75-1.27)	0.86
Yes	315 (31.7)			167 (16.8)			131 (13.2)		
No	305 (33.9)			169 (18.8)			121 (13.5)		
Vinca alkaloids		0.71 (0.56-0.89)	**0.004**		0.87 (0.67-1.18)	0.41		0.95 (0.68-1.31)	0.75
Yes	469 (31.2)			262 (17.4)			198 (13.2)		
No	150 (39.0)			74 (19.2)			53 (13.8)		
Anthracyclines		1.04 (0.85-1.26)	0.72		1.02 (0.81-1.30)	0.86		1.05 (0.80-1.37)	0.73
Yes	341 (33.2)			185 (18.0)			140 (13.6)		
No	275 (32.4)			150 (17.7)			111 (13.1)		
Methotrexate		1.00 (0.82-1.21)	0.96		0.96 (0.76-1.22)	0.74		1.13 (0.86-1.47)	0.39
Yes	292 (32.7)			156 (17.5)			125 (14.0)		
No	327 (32.8)			180 (18.1)			126 (12.6)		
Endocrine disorders									
Hypogonadism		0.69 (0.44-1.09)	0.11		0.85 (0.49-1.48)	0.57		0.86 (0.46-1.60)	0.64
Yes	26 (25.5)			16 (15.7)			12 (11.8)		
No	594 (33.2)			320 (17.9)			240 (13.4)		
Growth hormone deficiency		0.81 (0.53-1.24)	0.33		0.98 (0.59-1.63)	0.94		0.80 (0.43-1.48)	0.48
Yes	31 (28.4)			19 (17.4)			12 (11.0)		
No	586 (33.0)			315 (17.7)			238 (13.4)		
Hyperthyroidism		0.66 0.31-1.42)	0.29		NA	0.38		NA	0.22
Yes	9 (24.3)			4 (10.8)			2 (5.4)		
No	593 (32.6)			326 (17.9)			246 (13.5)		

	Any fracture			Long bone fracture			Fragility fracture		
	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
Hypothyroidism		NA	0.76		NA	0.70		NA	1.00
Yes	3 (25.0)			1 (8.3)			1 (8.3)		
No	599 (32.5)			329 (17.9)			247 (13.4)		
Lifestyle-related factors									
Smoking									
Never	332 (29.6)	Ref	Ref	190 (16.9)	Ref	Ref	142 (12.7)	Ref	Ref
Former	105 (40.7)	1.63 (1.23-2.16)	**0.001**	55 (21.3)	1.33 (0.95-1.86)	0.10	42 (16.3)	1.34 (0.92-1.95)	0.13
Current	99 (36.8)	1.38 (1.05-1.83)	**0.022**	46 (17.1)	1.01 (0.71-1.44)	0.95	33 (12.3)	0.96 (0.64-1.44)	0.86
Heavy drinking		1.08 (0.59-1.95)	0.81		1.35 (0.68-2.66)	0.39		1.95 (0.98-3.87)	0.056
Yes	17 (34.0)			11 (22.0)			11 (22.0)		
No	525 (32.4)			281 (17.3)			205 (12.6)		
Low dietary calcium intake		1.06 (0.85-1.33)	0.60		1.30 (0.99-1.70)	0.06		1.27 (0.94-1.71)	0.12
Yes	164 (33.6)			102 (20.9)			76 (15.6)		
No	363 (32.3)			190 (16.9)			143 (12.7)		
Low physical activity		0.74 (0.56-0.99)	**0.045**		0.99 (0.70-1.40)	0.96		1.06 (0.72-1.54)	0.78
Yes	74 (27.6)			48 (17.9)			38 (14.2)		
No	459 (33.9)			244 (18.0)			183 (13.5)		
Vitamin D deficiency		0.89 (0.73-1.09)	0.28		0.92 (0.72-1.18)	0.51		1.07 (0.82-1.41)	0.61
Yes	216 (30.9)			119 (17.0)			97 (13.9)		
No	386 (33.4)			211 (18.3)			151 (13.1)		
Severe vitamin D deficiency		0.84 (0.62-1.13)	0.25		1.09 (0.76-1.55)	0.64		1.46 (1.01-2.11)	**0.044**
Yes	68 (29.2)			44 (18.9)			41 (17.6)		
No	534 (32.9)			286 (17.6)			207 (12.8)		

	Any fracture			Long bone fracture			Fragility fracture		
	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
Elevated homocysteine levels		1.41 (0.95-2.09)	0.09		0.66 (0.37-1.17)	0.15		0.70 (0.37-1.33)	0.28
Yes	44 (40.0)			14 (12.7)			11 (10.0)		
No	560 (32.1)			317 (18.2)			238 (13.6)		
Vitamin B12 deficiency		1.16 (0.70-1.93)	0.56		0.80 (0.41-1.59)	0.53		1.00 (0.49-2.05)	1.00
Yes	24 (35.8)			10 (14.9)			9 (13.4)		
No	580 (32.4)			321 (18.0)			240 (13.4)		
Folic acid deficiency		1.17 (0.89-1.53)	0.26		1.05 (0.76-1.46)	0.77		1.15 (0.80-1.65)	0.45
Yes	98 (35.5)			51 (18.5)			41 (14.9)		
No	506 (32.0)			280 (17.7)			208 (13.2)		
Low BMD (Z-score ≤-1)									
Any site		1.17 (0.94-1.46)	0.16		1.36 (1.04-1.77)	**0.025**		1.36 (1.01-1.84)	**0.042**
Yes	195 (35.6)			115 (21.0)			87 (15.9)		
No	311 (32.0)			159 (16.4)			118 (12.2)		
Lumbar spine		1.30 (1.03-1.64)	**0.029**		1.47 (1.11-1.94)	**0.007**		1.39 (1.01-1.90)	**0.042**
Yes	158 (37.7)			94 (22.4)			69 (16.5)		
No	347 (31.8)			180 (16.5)			136 (12.5)		
Total body		0.89 (0.68-1.16)	0.39		1.07 (0.77-1.48)	0.70		1.33 (0.93-1.89)	0.12
Yes	96 (31.0)			56 (18.1)			48 (15.5)		
No	384 (33.5)			196 (17.1)			139 (12.1)		
Total hip		0.93 (0.61-1.40)	0.72		1.08 (0.66-1.76)	0.77		0.96 (0.55-1.68)	0.89
Yes	40 (32.0)			24 (19.2)			17 (13.6)		
No	201 (33.7)			108 (18.1)			84 (14.1)		
Very low BMD (Z-score ≤-2)									
Any site		1.25 (0.88-1.78)	0.22		1.45 (0.96-2.18)	0.076		1.54 (0.98-2.40)	0.059
Yes	55 (37.9)			34 (23.4)			27 (18.6)		
No	451 (32.8)			240 (17.5)			178 (13.0)		

	Any fracture			Long bone fracture			Fragility fracture		
	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶	No. (%)	OR (95% CI)	P-value¶
Lumbar spine		1.69 (1.13-2.51)	**0.010**		1.85 (1.19-2.89)	**0.006**		1.84 (1.13-2.99)	**0.014**
Yes	48 (44.9)			30 (28.0)			23 (21.5)		
No	457 (32.5)			244 (17.4)			182 (13.0)		
Total body		0.88 (0.51-1.50)	0.64		1.30 (0.71-2.39)	0.39		1.38 (0.71-2.68)	0.35
Yes	20 (30.3)			14 (21.2)			11 (16.7)		
No	460 (33.1)			238 (17.1)			176 (12.7)		

Abbreviations: BDM=bone mineral density; BMI=body mass index; CI=confidence interval; CRT=cranial irradiation; Gy=gray; No.=number; OR=odds ratio; RT=radiotherapy; HSCT=stem cell transplantation; TBI=total body irradiation

¶Logistic regression p-value for variables with more than five observations in each cell. For variables with less than five observations in each cell, a Fisher exact p-value was calculated.

*Adjusted for amputation

#With myeloablative conditioning

ıIncluding cranial irradiation for brain tumors and craniospinal irradiation

Supplementary Table 10. Risk factors for reported clinical fractures using univariable logistic regression analysis.

	Any fracture		Long bone fracture		Fragility fracture	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Sex (male)	1.42 (1.07-1.89)	**0.016**	1.32 (0.94-1.87)	0.11	0.95 (0.64-1.42)	0.82
BMI*						
Underweight	0.61 (0.25-1.46)	0.27	0.93 (0.36-2.42)	0.88	1.24 (0.46-3.28)	0.67
Normal	Ref	Ref	Ref	Ref	Ref	Ref
Overweight	0.98 (0.72-1.33)	0.87	1.12 (0.77-1.64)	0.56	1.18 (0.76-1.83)	0.46
Obese	0.88 (0.56-1.38)	0.57	1.44 (0.87-2.38)	0.16	1.20 (0.65-2.21)	0.56
Attained age (per year)	0.99 (0.96-1.01)	0.19	0.99 (0.96-1.02)	0.39	0.99 (0.96-1.02)	0.42
Age at diagnosis (per year)	0.97 (0.94-1.00)	0.059	0.96 (0.93-1.00)	0.082	0.96 (0.91-1.00)	0.077
HSTC#	-	-	1.01 (0.51-2.01)	0.97	1.19 (0.56-2.54)	0.65
Smoking						
Never	Ref	Ref	Ref	Ref	Ref	Ref
Former	2.06 (1.43-2.99)	**<0.001**	1.34 (0.86-2.10)	0.20	1.46 (0.88-2.42)	0.15
Current	2.01 (1.42-2.84)	**<0.001**	1.09 (0.71-1.68)	0.71	0.99 (0.59-1.68)	0.98
Heavy drinking	-	-	-	-	1.42 (0.53-3.77)	0.49
Low physical activity	0.75 (0.50-1.12)	0.16	-	-	-	-
Severe vitamin D deficiency	-	-	-	-	1.06 (0.60-1.88)	0.85
Very low LS BMD	1.77 (1.08-2.91)	**0.025**	2.57 (1.51-4.36)	**<0.001**	2.63 (1.43-4.81)	**0.002**

Abbreviations: BMD=bone mineral density; BMI=body mass index; CI=confidence interval; LS=lumbar spine; OR=odds ratio; Ref=reference
*Adjusted for amputation
#With myeloablative conditioning

Supplementary Table 11. Independent risk factors for reported clinical fractures that certainly occurred more than five years after cancer diagnosis based on year of fracture using multivariable logistic regression analysis.

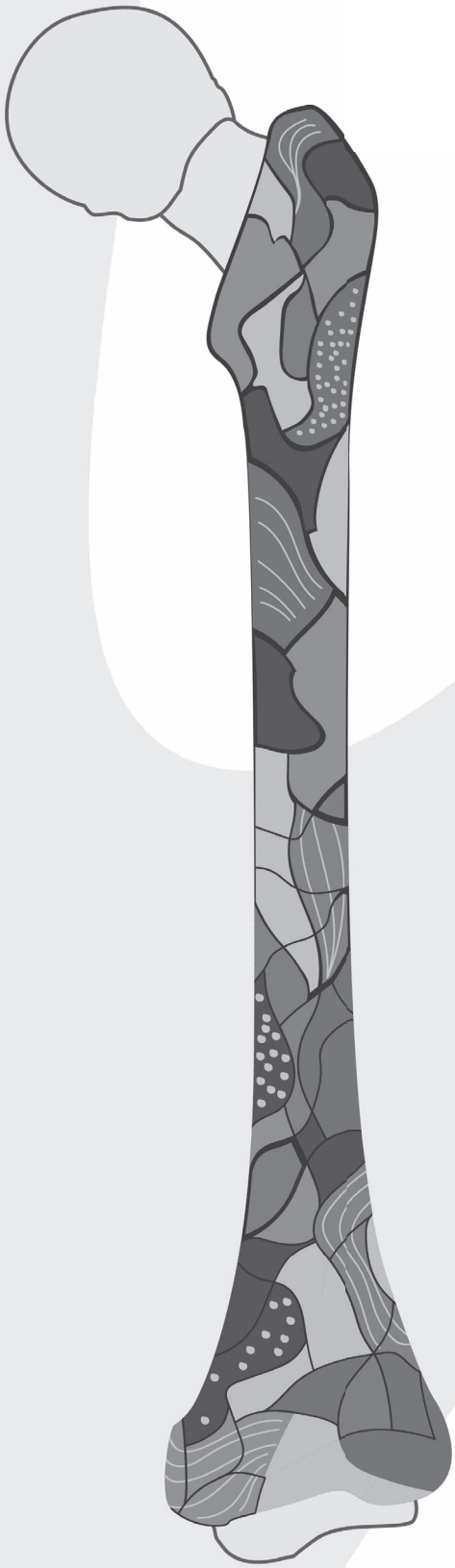
	Vertebral fracture		
	No. (%)	**OR (95% CI)**	**P-value¶**
Demographics			
Sex		0.82 (0.39-1.71)	0.59
Male	17 (12.2)		
Female	16 (14.5)		
Attained age (per year)		1.06 (1.01-1.12)	**0.027**
First tertile	10 (12.0)		
Second tertile	6 (7.2)		
Third tertile	17 (20.5)		
BMI* (per kg/m²)		1.04 (0.95-1.13)	0.40
First tertile	7 (8.4)		
Second tertile	12 (14.5)		
Third tertile	14 (17.1)		
Age at diagnosis (per year)		1.03 (0.95-1.12)	0.46
First tertile	10 (12.0)		
Second tertile	9 (10.8)		
Third tertile	14 (16.9)		
Follow-up time (per year)		1.04 (0.99-1.10)	0.081
First tertile	9 (10.8)		
Second tertile	9 (10.8)		
Third tertile	15 (18.1)		
Treatment factors			
HSCT#		NA	0.23
Yes	0 (0.0)		
No	33 (14.0)		
TBI		NA	0.61
Yes	0 (0.0)		
No	33 (13.8)		
CRT†		1.74 (0.69-4.36)	0.24
Yes	7 (19.4)		
No	26 (12.2)		
Spinal RT		NA	**0.030**
Yes	4 (40.0)		
No	29 (12.1)		
Abdominal/pelvic RT		NA	0.54
Yes	4 (16.7)		
No	29 (12.9)		
Platinum compounds		2.78 (1.00-7.71)	**0.0498**
Yes	6 (27.3)		
No	27 (11.9)		

	Vertebral fracture		
	No. (%)	**OR (95% CI)**	**P-value¶**
Alkylating agents		1.11 (0.52-2.36)	0.79
Yes	19 (13.7)		
No	13 (12.5)		
Corticosteroids		1.34 (0.64-2.80)	0.43
Yes	17 (8.8)		
No	16 (8.5)		
Vinca-alkyloids		1.09 (0.42-2.80)	0.86
Yes	27 (13.4)		
No	6 (12.5)		
Anthracyclines		0.64 (0.31-1.34)	0.24
Yes	11 (10.9)		
No	19 (16.0)		
Methotrexate		0.77 (0.37-1.60)	0.48
Yes	16 (11.9)		
No	17 (14.9)		
Endocrine disorders			
Hypogonadism		NA	0.075
Yes	3 (37.5)		
No	30 (12.4)		
Growth hormone deficiency		3.33 (1.08-10.29)	**0.037**
Yes	5 (31.3)		
No	28 (12.0)		
Hyperthyroidism		NA	0.51
Yes	1 (20.0)		
No	31 (13.0)		
Hypothyroidism		NA	1.00
Yes	0 (0.0)		
No	32 (13.2)		
Low BMD		1.79 (0.86-3.77)	0.12
Yes	19 (17.0)		
No	14 (10.2)		
Very low BMD		1.86 (0.70-4.99)	0.22
Yes	6 (20.7)		
No	27 (12.3)		
Lifestyle-related factors			
Smoking		0.54 (0.23-1.27)	0.16
Current/former	8 (8.8)		
Never	21 (15.2)		
Heavy drinking		NA	0.41

	Vertebral fracture		
	No. (%)	**OR (95% CI)**	**P-value¶**
Yes	1 (25.0)		
No	27 (12.1)		
Low dietary calcium intake		1.13 (0.51-2.52)	0.78
Yes	10 (14.9)		
No	23 (13.5)		
Low physical activity		2.44 (1.03-5.78)	**0.043**
Yes	9 (24.3)		
No	24 (11.7)		
Vitamin D deficiency		1.05 (0.50-2.23)	0.89
Yes	14 (13.5)		
No	18 (12.9)		
Severe vitamin D deficiency		1.88 (0.77-4.54)	0.16
Yes	8 (20.0)		
No	24 (11.8)		
Elevated homocysteine levels		NA	1.00
Yes	2 (10.0)		
No	30 (13.3)		
Vitamin B12 deficiency		NA	0.66
Yes	2 (16.7)		
No	30 (12.9)		
Folic acid deficiency		NA	0.12
Yes	2 (5.0)		
No	30 (14.6)		

Abbreviations: BMD=bone mineral density; BMI=body mass index; CI=confidence interval; CRT=cranial irradiation; OR=odds ratio; RT=radiotherapy; TBI=total body irradiation
¶Logistic regression p-value for variables with more than five observations in each cell. For variables with less than five observations in each cell, a Fisher exact p-value was calculated
*Adjusted for amputation
#Including cranial irradiation for brain tumors and craniospinal irradiation

Supplementary Table 12. Risk factors for observed prevalent vertebral fractures in 249 survivors using univariable analysis.



CHAPTER 7

Vitamin D supplementation for children with cancer: a systematic review and consensus recommendations

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ABSTRACT

Background: Prevalent vitamin D deficiency (VDD) and low bone mineral density (BMD) have led to vitamin D supplementation for children with cancer, regardless vitamin D status. However, it remains unsettled whether this enhances bone strength. We sought to address this issue by carrying out a systemic review.

Methods: We conducted a literature search using PubMed, Embase and Cochrane databases. Studies including children up to five years after cancer therapy were assessed for the association between 25-hydroxyvitamin D (25OHD) levels and BMD Z-scores or fractures, and the effect of vitamin D supplementation on BMD or fractures. Evidence quality was assessed using the GRADE methodology.

Results: Nineteen studies (16 observational, 3 interventional, mainly involving children with hematologic malignancies) were included. One study which analyzed 25OHD as a threshold variable (≤10 ng/ml) found a significant association between 25OHD levels and BMD Z-scores, while 25OHD as a continuous variable was not significantly associated with BMD Z-scores in 14 observational studies. We found no significant association between lower 25OHD levels and fractures (2 studies), nor between vitamin D (and calcium) supplementation and BMD or fracture frequency (3 studies) (very low quality evidence).

Conclusion: There is lack of evidence for an effect of vitamin D (and calcium) supplementation on BMD or fractures in children with cancer. Further research is needed; until then, we recommend dietary vitamin D/calcium intake in keeping with standard national guidelines, and periodic 25OHD monitoring to detect levels <20 ng/ml. Vitamin D/calcium supplementation is recommended in children with low levels, to maintain levels ≥20 ng/ml year-long.

INTRODUCTION

Improved treatment strategies have substantially increased survival rates for childhood cancer over the past decades. The five-year survival rate is currently greater than 80% and the majority of children are cured.[1] However, this improved survival comes at a cost, as it is often accompanied by treatment-related morbidity.[2] One of these side-effects is low bone mineral density (BMD). Low BMD may already be present at cancer diagnosis, for example due to the malignancy itself,[3,4] but is also common among survivors of childhood cancer due to cancer treatment or its consequences.[5–7] Low BMD is associated with an increased risk of fractures in children with cancer[8,9] and in childhood cancer survivors.[10] These fractures may lead to significant morbidity, hospitalization, and decreased quality of life.[11]

In the general pediatric population, BMD and fractures are influenced by multiple factors, such as sex, age, and weight.[12] In addition, low BMD and fractures can partly be attributed to vitamin D deficiency (VDD).[13,14] Vitamin D (derived from ultraviolet radiation or dietary intake) is converted in the liver to 25-hydroxyvitamin D (25OHD), and is further hydroxylated in the kidney to the active metabolite 1,25-dihydroxyvitamin D (1,25$[OH]_2$D). Low 25OHD levels decrease calcium and phosphate absorption and lead to an acute compensatory rise in parathyroid hormone (PTH), resulting in bone resorption to release calcium. Persistent VDD results in excessive bone resorption, generalized BMD decline, and bone mineralization defects. However, there remains some controversy around optimal and deficient serum 25OHD levels, mainly due to the large variability of 25OHD levels across commonly used assays and different races.[15–17] Generally, serum 25OHD levels lower than 12 ng/ml (30 nmol/L) are associated with deficiency, but levels between 12 to 20 ng/ml (30 to 50 nmol/L) are already considered inadequate for bone strength in children.[14,18]

VDD occurs mainly due to decreased sunlight exposure, inadequate dietary intake, malabsorption, or liver and renal diseases.[19] Children with cancer are therefore theoretically at risk for VDD, and some studies have shown that VDD is indeed more prevalent among children with hematologic malignancies compared to healthy children.[20–22] The high prevalence of VDD and low BMD has led clinicians to often advise vitamin D supplements to children with cancer. In non-cancer populations, vitamin D and calcium supplementation may increase BMD in children[23] and adults[24] with low vitamin D levels, and can prevent fractures in adults.[19,25] In children with cancer, however, multiple disease- and treatment-related risk factors for developing low BMD, such as cranial irradiation

and glucocorticoids, have been described (in addition to the risk factors in the general population).[26,27] The relative contribution of these risk factors to low BMD, as well as their potential confounding effect on the association between VDD and BMD, are unclear. Therefore, it remains unsettled whether vitamin D supplementation in all children with cancer, regardless their vitamin D status, enhances bone strength. The aim of this systematic review was to assess the influence of VDD on the risk of low BMD and fractures, as well as the effect of vitamin D supplementation on BMD and fractures in children with cancer up to five years after the completion of therapy.

METHODS

This systematic review was prepared according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[28]

Search strategy and selection

We conducted a systematic literature search in PubMed, Embase and Cochrane databases until August 2019. Search terms included children with cancer, survivors of childhood cancer, vitamin D serum concentration, and low BMD or fractures, and all related synonyms (Supplemental Table 1). After removal of duplicates, the title and abstract of the retrieved records were screened to identify articles that would potentially match our predetermined inclusion criteria: (1) the study population consisted of children with cancer until five years after treatment cessation, with at least 95% of the population diagnosed at ≤18 years of age; (2) the study assessed the relationship between serum 25OHD levels and BMD Z-scores (measured by dual-energy X-ray absorptiometry [DXA], quantitative computed tomography [QCT] or quantitative ultrasound [QUS]), or the relationship between 25OHD levels and fractures, or the effect of vitamin D supplementation (all forms) on BMD (raw value or Z-score) change and/or fracture frequency; (3) the study did not exclusively or mainly report on BMD after hematopoietic stem cell transplantation; (4) the study was not a case-report or case-series (n<10) and was written in English; and (5) the study was original research. We only included studies measuring 25OHD (and not 1,25$[OH]_2$D), as serum 25OHD levels are considered the best clinical indicator of vitamin D status (in patients with normal kidney function).[29,30] We excluded studies in childhood cancer survivors starting more than five years after treatment cessation because we aimed to assess the rationale and effect of vitamin D supplementation on bone health during cancer treatment. Before exclusion of reviews, the reference list was screened for relevant articles. Subsequently, full-text articles were obtained

and assessed according to the inclusion criteria. When multiple articles reported on the same cohort, we included the article that reported the most relevant data to our research questions. Finally, we performed a cross-reference check on all included articles using Web of Science. Article screening was independently executed by two reviewers, JEvA and IEV, whereas disagreements were resolved by consensus or consultation of a third reviewer (SJCMMN).

Data extraction

We retrieved data on the sample size, sex distribution, age at baseline, country, study design, childhood cancer diagnosis, BMD imaging modality and skeletal site, and follow-up duration from all included studies.

For observational studies, we additionally retrieved data on VDD threshold, the percentage of children receiving vitamin D supplementation, and the prescribed dose. As outcome measures, the difference between the percentage of children with low (areal and/or volumetric) BMD (aBMD and/or vBMD Z-score ≤-1 or ≤-2) or fractures by vitamin D status (VDD yes vs. no), risk estimates for low BMD or fractures by vitamin D status, mean or median 25OHD levels, mean or median aBMD and vBMD Z-scores at each timepoint, the percentage of children with any fracture in the whole study population, and the association between (change in) 25OHD levels and aBMD and vBMD Z-scores and fractures were extracted if reported in the study.

For interventional studies, if available, we additionally retrieved data on supplementation, the percentage of children with low aBMD and/or vBMD per skeletal site or fractures, risk estimates for low BMD and fractures, and the mean difference of BMD values (g/cm^2, mg/cm^3, or Z-score) between baseline and follow-up in the intervention and control group. Also, the p-value of the effect of the intervention on BMD and fractures was extracted.

Critical appraisal

The same two independent reviewers (JvA and IEV) assessed the validity of the included articles with the Quality in Prognostic Studies (QUIPS) tool for observational studies and the Cochrane risk of bias tool for interventional studies.[31,32] The quality of the total body of evidence was assessed using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology.[33] Discrepancies in the grading were resolved by consensus or consultation of a third reviewer (SJCMMN).

Consensus recommendations

Our panel consisted of experts in the field of pediatric oncology and endocrinology, in particular bone health and disease, representing 4 different countries and two different continents. Recommendations were drafted based on the evidence, expert opinion, as well as other considerations such as costs and applicability across different health-care systems. Unanimous agreement was reached for all recommendations by a digital consensus meeting on the 13th of October 2020 in combination with rigorous pre- and post-meeting revisions.

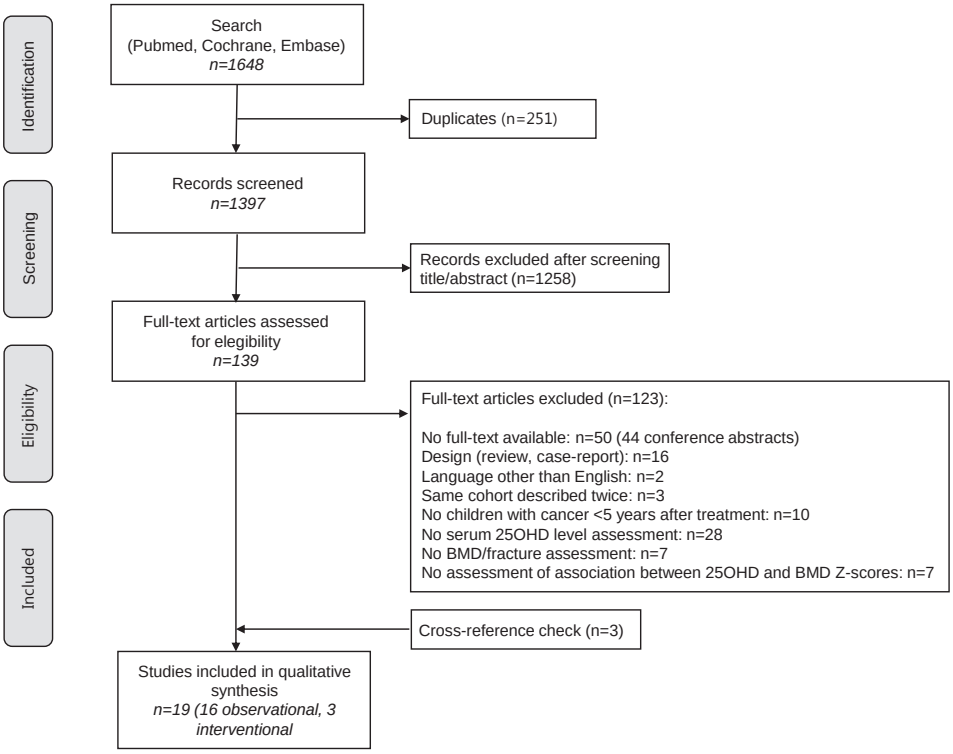
RESULTS

Search results

The search in PubMed, Embase and Cochrane yielded 320, 1219 and 109 records, respectively. After duplicate removal, 1397 titles and abstracts were screened and subsequently, 139 full-text articles were reviewed (Figure 1). Sixteen articles were eligible for analysis; a cross-reference check retrieved three additional articles. A total of 19 articles, including 16 observational studies[21,34–49] and three interventional studies,[50–52] were included in this review.

Study characteristics

Of the 16 observational studies, 11 studies[21,36–40,42,43,46,48,49] (69%) were conducted in children with a hematologic malignancy, two studies[34,41] (13%) in children with solid tumors, and three studies[35,45,47] (19%) in children with any childhood cancer diagnosis (Table 1). Nine studies[36–38,40,41,43,47–49] (56%) had a cross-sectional and seven studies[21,34,35,39,42,45,46] (44%) a longitudinal design. Sample sizes of the studies varied considerably from 20 to 171 patients. Median or mean age at baseline of the study population ranged from 3.9 to 15.0 years. The serum 25OHD threshold for VDD was not consistent among the studies; 25OHD levels less than 20 ng/ml were most frequently used (55% of studies that defined a threshold). Four studies (36%) used a threshold of 12 ng/ml or lower.[36,38,42,49] aBMD Z-scores of the lumbar spine (LS), total body (TB), total body less head (TBLH), total hip (TH) and/or femoral neck (FN) were ascertained by DXA in 15 studies[21,34–39,41–43,45–49] (94%) and vBMD Z-scores of the femur by QCT in one study[40] (6%). In addition, one study calculated height-adjusted (i.e. apparent vBMD) Z-scores.[36] The frequency of symptomatic fractures (all types, diagnosed due to pain) was reported in six studies[34,36,37,39,40,42] (38%), of which two studies[34,39] (13%) assessed the association between serum 25OHD levels and fractures.



Abbreviations: BMD=bone mineral density; 25OHD=25-hydroxyvitamin D

Figure 1. PRISMA flow diagram of study selection.

All three interventional studies (two open-label RCTs[50,52] and one quasi-experimental study[51]) were performed in children with acute lymphoblastic leukemia (ALL) (Table 2). Sample sizes ranged from 16 to 115 children. Age at start of the intervention ranged from 3.7 to 15.2 years and the duration of the intervention ranged from 6.7 to 12 months. Vitamin D was supplemented in combination with calcium during the first phases of ALL treatment in all children in two studies[50,51] (67%) and in children with 25OHD levels <30 ng/ml in one study[52] (33%). The formulation (vitamin D_3 versus the active form of vitamin D, $1,25[OH]_2D$) and vitamin D supplement doses (400-600 IU/day versus 10,000 IU every 2 months oral vitamin D3 versus 10-20 IU/day $1,25[OH]_2D$) varied. aBMD (g/cm^2 or Z-score) of the LS, TB, TBLH, and/or TH was measured by DXA in two studies[50,51] (67%), and vBMD (mg/cm^3) of the LS and femur was measured by QCT in one study[52] (33%). All three studies compared the frequency of symptomatic fractures in the intervention and control group.

Author (year)	No. of patiënts	Sex (male)	Age at baseline (years)	Country[1]	Design	Childhood cancer diagnosis	25OHD VDD threshold[2]	Vit D suppl. (%, dose)	BMD modality and site; fractures	Timepoints (months)
Hematologic malignancies										
Boot 1999	32	21	Mean: 7.9	NL	L	ALL	<12 ng/ml	NR	Modality: DXA Site: LS, TB Fractures: +	Dx, 6, 12, 24, 12 after Rx
Bordbar 2016	60	39	Mean: 9.9	IR	C	ALL	<20 ng/ml	100% 200 IU/d	Modality: DXA Site: LS, FN Fractures: NR	6 after Dx
El-Ziny 2005	43	23	Mean: 7.0	EG	L	Acute leukemia	NR	NR	Modality: DXA Site: LS Fractures: NR	Dx, 3, 12
El-Ziny 2007	20	11	Mean: 8.9	EG	L	Malignant lymphoma	NR	NR	Modality: DXA Site: LS Fractures: NR	Dx, 3, 12
Gunes 2010	70	41	Mean: 10.6	TR	C	ALL	NR	NR	Modality: DXA Site: LS Fractures: NR	45.5 after Rx
Halton 1995	40	27	Median: 3.9	CA	C	ALL	<10 ng/ml	NR	Modality: DXA Site: LS Fractures: NR	Dx
Jain 2017	65	52	Median: 15.0	IN	C	ALL	<10 ng/ml	NR	Modality: DXA Site: LS, TB Fractures: +	52 after Rx
Kadan-Lottick 2001	75	NA	Mean: 6.8	US	C	ALL	NR	NR	Modality: DXA Site: TB Fractures: +	30 after Rx
Kelly 2009	41	25	Median: 10	CO	C	ALL	<9 ng/ml	NR	Modality: DXA Site: TB Fractures: NR	During or after completion of Rx
Marinovic 2005	37	20	Median: 7.9	FR	L	ALL	NR	NR	Modality: DXA Site: LS, TB Fractures: +	26 after Rx, +12

Author (year)	No. of patiënts	Sex (male)	Age at baseline (years)	Country[1]	Design	Childhood cancer diagnosis	25OHD VDD threshold[2]	Vit D suppl. (%, dose)	BMD modality and site; fractures	Timepoints (months)
Mostoufi-Moab 2012	50	19	Median: 7.9	US	C	ALL	<20 ng/ml	40% 400 IU/d	Modality: QCT Site: tibia Fractures: +	10 after Rx, +12
Solid tumors										
Bilariki 2010	52	30	Median: 12.1	FR	L	Solid tumor	<20 ng/ml	80% 100,000 IU/3 mo	Modality: DXA Site: LS, TH Fractures: +	13.8 after Rx, +12
Saki 2018	50	36	Mean: 10.3	IR	C	Solid tumor	<20 ng/ml	100% 200 IU/d	Modality: DXA Site: LS, FN, TH Fractures: NR	During or after completion of Rx
Any childhood cancer diagnosis										
Choi 2017	30	21	Median: 11.2	KR	L	Any type	<20 ng/ml	NR	Modality: DXA Site: TB Fractures: NR	Dx, 1, 6, 12
Esbenshade 2014	171	96	Median: 12.1	US	C	Any type	<20 ng/ml	1,2% Dose NR	Modality: DXA Site: LS, TB Fractures: NR	2.7 after Rx
Henderson 1998	37	NA	Mean: 7.3	US	L	Any type	<15 ng/ml	NR	Modality: DXA Site: LS, TH Fractures: NR	Dx, 5—8 interval

ALL=acute lymphoblastic leukemia; BMD=bone mineral density; C=cross-sectional; Dx=diagnosis; DXA=dual-energy X-ray absorptiometry; FN=femoral neck; IU=international units; L=longitudinal; LS=lumbar spine; NR=not reported; QCT=quantitative computed tomography; Rx=treatment; TB=total body; TBLH=total body less head; TH=total hip.
[1]International Organization of Standardization (ISO) country codes; [2]1 ng/ml = 2.5 nmol/L

Table 1. Study characteristics of the observational studies in children during or shortly after cancer treatment.

Study quality

There were significant concerns about the risk of bias in the included studies (Supplemental Table 2 and 3). The main limitations of the observational studies concerned low study participation rates, inadequate prognostic factor measurement (25OHD not measured by liquid chromatography-tandem mass spectrometry [gold standard] and/or analyzed at different timepoints), lack of adjustment for important confounders (no multivariable analysis), and suboptimal statistical analysis or reporting (correlations instead of risk estimates using a 25OHD and BMD Z-score threshold). The main limitations of the interventional studies concerned a lack of adequate randomization procedures, allocation concealment, or blinding of participants and personnel, as well as incomplete outcome data.

Vitamin D status, BMD status and fractures

Mean or median 25OHD levels and BMD Z-scores per timepoint are shown in Table 3. Mean or median 25OHD levels were below 20 ng/ml at one or more timepoints in seven studies[21,34,36,39,45,46,49] (44%), and below 12 ng/ml in four studies[21,39,45,46] (25%). Mean or median aBMD Z-scores at any skeletal site and at one or more timepoints were <0 in 12[21,34–36,38,41–43,45,46,48,49] of the 14 studies[21,34–38,41–43,45–49] (86%) that reported aBMD Z-scores during or (just) after treatment. Two studies which reported apparent and true vBMD Z-scores, respectively, found mean values below zero as well.[36,40] Because the timepoints as well as the 25OHD level threshold for VDD and BMD Z-score threshold for low BMD varied across the studies and did not allow comparisons, no comprehensive overview of the percentage of children with VDD or low BMD in the included studies was calculated. In addition, none of the included studies compared the incidence of symptomatic fractures with a healthy reference population, so we could not determine the incidence rate ratio of fractures in children with cancer.

Association between 25OHD levels and BMD Z-scores

None of the included studies assessed the association between VDD (using the threshold defined in the study) and low BMD (using a Z-score threshold) or fractures. Therefore, it was not possible to provide risk estimates for low BMD and fractures in children with VDD. In a study of 65 childhood ALL survivors, Jain *et al.*[36] reported a significant association (p=0.046) between low 25OHD levels (≤10 ng/ml, n=36) and lower height-adjusted TB BMD Z-scores (continuous) at a median of 52 months after cessation of treatment. However, there was no significant association between low 25OHD levels and height-adjusted LS, non-height adjusted LS, or TB BMD Z-scores. All 14 studies[21,34,35,37,38,40–43,45–49] that assessed the association between 25OHD levels as a continuous variable and BMD Z-scores found no significant association (Table 3).

Author (year)	No. of participants	Sex (M)	Age at baseline (years)	Country[1]	Design	Childhood cancer diagnosis	Intervention group	Control group	Outcome	Follow-up
Hematologic malignancies										
Demirsoy 2017	Intervention: 34 Controls: 59	16 34	Median: 3.7 Median: 8.9	TR	Quasi experimental study	ALL	Oral vitamin D3 (400-600 IU/day) + Ca carbonate (500-1000 mg/day) supplementation	Historical controls without vitamin D/Ca supplementation	BMD (g/cm^2, Z-score) Modality: DXA Site: LS, TB, TBLH Fractures: +	From diagnosis until completion of reinduction therapy (~ 8 months)
Díaz 2008	Intervention: 8 Controls: 8	5 4	Mean total: 5.5	CL	RCT	ALL	Oral 1,25(OH)2D (10-20 IU/day) + Ca carbonate (500 mg/day) supplementation	Ca carbonate (500 mg/d) supplementation	BMD (g/cm^2) Modality: DXA Site: LS, TB, TH Fractures: +	From diagnosis until 1 year into treatment
Orgel 2017	Intervention: 19 Controls: 10	13 6	Median: 15.2 Median: 14.6	US	RCT	ALL	Directly observed therapy: oral vitamin D3 (100,000 IU/2 months) + Ca carbonate (800 mg/day) in addition to standard of care	Standard of care: routine encouragement regarding activity and ad hoc nutritional monitoring	BMD (mg/cm^3) Modality: QCT Site: LS, femur Fractures: +	From end of induction until delayed intensification (median 6.7 months)

ALL=acute lymphoblastic leukemia; BMD=bone mineral density; Ca=calcium; DXA=dual-energy X-ray absorptiometry; IU=international units; LS=lumbar spine; M=male; QCT=quantitative computed tomography; RCT=randomized controlled trial; TB=total body; TBLH=total body less head; TH=total hip
[1]International Organization of Standardization (ISO) country codes

Table 2. Study characteristics of the interventional studies in children during or shortly after cancer treatment.

	25OHD (mean [SD]) ng/ml nmol/L*	LS aBMD Z-scores (mean [SD])	TB(LH*) aBMD Z-scores (mean [SD])	TH aBMD Z-scores (mean [SD])	FN aBMD Z-scores (mean [SD])	Fractures (%)	Association
Hematologic mailgnancies							
Boot 1999						16%	No significant correlation[1]
T1	115 (67)*	-0.67 (1.3)	0.02 (1.3)	NR	NR		
T2	60 (26)*	NR	NR	NR	NR		
T3	79 (36)*	NR	NR	NR	NR		
T4	63 (30)*	NR	NR	NR	NR		
T5	56 (32)*	NR	NR	NR	NR		
Bordbar 2016						NR	No significant association[2]
T1	20.4 (15.2)	-1.3 (1.2)	NR	NR	-1.9 (1.3)		
El-Ziny 2005						NR	No significant correlation[3]
T1	11.0 (5.3–29.0)[4]	-1.8 (-3.0– -0.1)[4]	NR	NR	NR		
T2	14.2 (5.5–26.8)[4]	-1.1 (-2.0– -0.4)[4]	NR	NR	NR		
T3	17.5 (10.3–38.5)[4]	-1.1 (-1.9– -0.4)[4]	NR	NR	NR		
El-Ziny 2007						NR	No significant correlation[3]
T1	8.5 (6.7–21.0)[4]	-0.3 (-1.6–0.6)[4]	NR	NR	NR		
T2	29.0 (16.0–49.0)[4]	-0.7 (-2.3–0.6)[4]	NR	NR	NR		
T3	12.0 (10.0–29.0)[4]	-0.9 (-2.2–0.6)[4]	NR	NR	NR		
Gunes 2010						NR	No significant correlation (P=0.06)[3] and association (p-value NR)
T1	21.0 (7.9)	-1.72 (0.83)	NR	NR	NR		
Halton 1995						10%	No significant correlation[3]
Total	17.0 (15.2)	NR	NR	NR	NR		
Boys	NR	-0.16	NR	NR	NR		
Girls	NR	-0.76	NR	NR	NR		
Jain 2017						18%	No significant association: P=0.196 (LS) P=0.068 (LS HA) P=0.089 (TB)[5] Significant association: P=0.046 (TB HA)[5]
T1	29.5 (35.9)*	-1.24 (1.21) -0.67 (1.11)[6]	-0.91 (1.00) -0.84 (0.92)[6]	NR	NR		

	25OHD (mean [SD]) ng/ml nmol/L*	LS aBMD Z-scores (mean [SD])	TB(LH*) aBMD Z-scores (mean [SD])	TH aBMD Z-scores (mean [SD])	FN aBMD Z-scores (mean [SD])	Fractures (%)	Association
Kadan-Lottick 2001						28%	No significant association: P=0.2[7]
Total	NR	NR	0.22 (0.96)	NR	NR		
BMD Z-score ≤ -1	43 (17)	NA	NA	NA	NA		
BMD Z-score > -1	37 (11)	NA	NA	NA	NA		
Kelly 2009						NR	No significant correlation[3]
Total	23.1 (6.0–36.9)	NR	NR	NR	NR		
On Rx < 12 mo (17%)	NR	NR	-0.46 (0.48)	NR	NR		
On Rx > 12 mo (41%)	NR	NR	-1.72 (0.33)	NR	NR		
Off Rx > 12 mo (41%)	NR	NR	-0.41 (0.31)	NR	NR		
Marinovic 2005						22%	No significant association[8]
Total	NR	NR	NR		NR		
Fracture + (22%)	10 (8.5–16.5)[4]	NA	NA	NR	NA		
Fracture – (78%)	10.5 (8–16)[4]	NA	NA	NA	NA		
				NA			
Mostoufi-Moab 2012						18%	No significant association[3]
T1	30.9 (4.1–93.6)		-0.84 (1.05)[9]	NR	NR		
T2	NR	NR	-0.51 (0.91)[9]	NR	NR		
		NR					
Solid tumors							
Bilariki 2010						21%	No significant correlation[10]
T1	19.7 (8.5)	-0.86 (1.11)	NR	-0.87 (0.98)	NR		Significant association P=0.002[11]
T2	20.5 (7.1)	NR	NR	NR	NR		
Fracture + (21%)	23.7 (7.4)	NA	NA	NA	NA		
Fracture – (79%)	18.7 (8.4)	NA	NA	NA	NA		
Saki 2018						NR	No significant association: P=0.991
T1	23.3 (18.3)	-1.4 (1.4)	NR	-1.6 (0.9)	-1.8 (1.3)		P=0.717[12]

	25OHD (mean [SD]) ng/ml nmol/L*	LS aBMD Z-scores (mean [SD])	TB(LH*) aBMD Z-scores (mean [SD])	TH aBMD Z-scores (mean [SD])	FN aBMD Z-scores (mean [SD])	Fractures (%)	Association
Any childhood cancer diagnosis							
Choi 2017						NR	No significant association[13]
Hematologic T1	12.6 (4.4–22.2)[4]	NR	0.70 (-1.40–2.50)[4]	NR	NR		
Hematologic T2	NR	NR	0.65 (-1.5–2.5)[4]	NR	NR		
Hematologic T3	NR	NR	0.10 (-1.6–1.3)[4]	NR	NR		
Hematologic T4	NR	NR	-0.80 (-1.7–1.3)[4]	NR	NR		
Solid T1	11.9 (9.3–47.9)[4]	NR	0.00 (-1.4–1.8)[4]	NR	NR		
Solid T2	NR	NR	-0.20 (-1.1–1.9)[4]	NR	NR		
Solid T3	NR	NR	-0.60 (-1.9–1.8)[4]	NR	NR		
Solid T4	NR	NR	-0.70 (-2.1–1.8)[4]	NR	NR		
Esbenshade 2014						NR	No significant correlation: ρ=0.10, P=0.374 (TB), ρ=0.09, P= 0.39 (LS)[3]
T1	29 (6–82)[4]	0.0 (-4.2–3.3)[4]	0.1(-4.2–3.6)[4]	NR	NR		No significant association: P=0.32[7]; P=0.41[14] (TB) P=0.81[7]; P=0.16[14] (LS)
Henderson 1998						NR	No significant correlation[15]
T1	NR	-0.46 (0.22)[16]	NR	-0.60 (0.21)[16]	NR		
T2	NR	NR	NR	NR	NR		
T3	NR	NR	NR	NR	NR		
T4	NR	-0.37 (0.27)[16]	NR	-0.48 (0.24)[16]	NR		

aBMD=areal bone mineral density; HA=height-adjusted; LS=lumbar spine; NA=not applicable; NR=not reported; Rx=treatment; SD=standard deviation; SE=standard error; T=time-point; TB=total body; TBLH=total body less head

[1]Between 25OHD and BMD Z-scores at diagnosis, during, and after treatment; [2]Between 25OHD levels and LS and FN BMD Z-scores; [3]Between 25OHD levels and BMD Z-scores; [4]Median (range); [5]Between low vitamin D levels (≤25 nmol/L) and BMD Z-scores; [6]Height-adjusted BMD Z-score; [7]Between 25OHD levels and BMD Z-score ≤-1; [8]Between 25OHD levels in patients with and without fractures; [9]Tibia cortical vBMD Z-score; [10]Between Δ25OHD and ΔBMD Z-scores; [11]Significantly higher 25OHD levels in patients with fractures; [12]Between 25OHD levels and LS and FN BMD Z-score ≤-2; [13]Between 25OHD and BMD Z-score at diagnosis; [14]Between 25OHD levels and BMD Z-score ≤-2; [15]Between 25OHD levels and ΔBMD Z-scores; [16]Mean (SE)

Table 3. Results of the observational studies.

		Intervention group (mean ± SD)			Control group (mean ± SD)			
		Baseline	End of study	Δ	Baseline	End of study	Δ	P-value
Hematologic malignancies								
Demirsoy 2017	LS BMD[1]	-0.6	-1.6	-1.0	NR	NR	NR	NR
	TB BMD[1]	0.1	-0.7	-0.8	NR	NR	NR	NR
	TBLH BMD[1]	0.2	-0.5	-0.7	NR	NR	NR	NR
	Fractures[2]	NA	NA	6%	NA	NA	2%	NR
Díaz 2008	LS BMD[3]	NR	NR	83	NR	NR	101	0.637
	TB BMD[3]	NR	NR	-73	NR	NR	26	0.834
	TH BMD[3]	NR	NR	16	NR	NR	31	0.834
	Fractures[2]	NA	NA	0%	NA	NA	0%	NR
Orgel 2017	LS vBMD[4]	249.3 ± 71.0	203.8 ± 77.1	-45.5	234.6 ± 52.0	201.4 ± 66.4	-33.2	0.432
	Femoral vBMD[4]	2091.4 ± 43.5	2093.1 ± 62.5	1.7	2081.7 ± 66.2	2090.9 ± 26.7	9.2	0.915
	Fractures[2]	NA	NA	0%	NA	NA	0%	NR

BMD=bone mineral density; LS=lumbar spine; NA=not applicable; NR=not reported; TB=total body; TBLH=total body less head; SD=standard deviation; vBMD=volumetric bone mineral density
[1]BMD Z-score; [2]Symptomatic fractures (pain); [3]BMD in g/cm^2; [4]vBMD in mg/cm^3

Table 4. Results from the interventional studies.

According to the GRADE assessment, there is very low quality evidence with conflicting results for the association between lower 25OHD levels and lower BMD Z-scores in children with cancer up to five years after cancer treatment (Supplemental Table 4).

Association between 25OHD levels and fractures

Two studies[34,39] assessed the association between vitamin D levels and symptomatic fractures (Table 3). Marinovic *et al.*[39] did not find a significant association between mean 25OHD levels in 37 children with ALL with (22%) and without (78%) a history of symptomatic fractures in the previous five years (10.0 vs. 10.5 ng/ml 25OHD) from diagnosis until a median follow-up of 38 months after cessation of treatment. Bilariki *et al.*[34] reported significantly higher mean levels of 25OHD at 13.8 months after treatment in 10 out of 52 children with a solid tumor who experienced symptomatic fractures from diagnosis until follow-up compared to those without fractures (23.7 vs. 18.7 ng/ml, p=0.002).

According to the GRADE assessment, very low quality evidence suggests that there is no increased risk of fractures for children with lower 25OHD levels up to five years after cancer treatment (Supplemental Table 4).

Effect of vitamin D supplementation on BMD and fractures

Table 4 summarizes the results of the three interventional studies in children with ALL. Demirsoy *et al.*[51] reported a significant increase in median (interquartile range, IQR) 25OHD levels in the intervention group from ALL diagnosis until completion of reinduction therapy (17.9 [IQR 10.9 to 23.7] vs. 23.5 [IQR 19.9 to 28.6] ng/ml, p=0.01). However, median BMD Z-score decreased significantly during this interval (LS BMD Z-score -0.6 [IQR -1.1to 0.2] vs. -1.6 [IQR -2.1 to -0.1], p=0.025; TB BMD Z-score 0.1 [IQR -0.5 to 0.9] vs. -0.7 [IQR -1.4 to 0.1], p=0.005; TB less head BMD Z-score 0.2 [IQR -0.2 to 1.5] vs. -0.5 [IQR -1.7 to 0.0], p=0.005). The study design did not allow a comparison of the difference of 25OHD levels and BMD during supplementation with the control group. Diaz et al.[50] and Orgel et al.[52] both found a greater increase or smaller decrease in BMD during the study period in the control group compared to the intervention group, indicating that the intervention was not effective. In all three studies, the percentage of children with symptomatic fractures was equal or higher in the interventional group compared to the control group.[50–52]

According to the GRADE assessment, very low quality evidence suggests that there is no significant effect of vitamin D supplementation on BMD and fracture

frequency in children with ALL up to five years after cancer treatment compared to controls (Supplemental Table 4).

Consensus recommendations

Table 5 shows our consensus recommendations to ensure an adequate vitamin D status in the context of bone health in children with cancer, which are mainly based on expert opinion (supported by international guidelines for the general population) as a result of the very low quality evidence identified by this systematic review. In summary, we recommend to encourage a diet adequate in calcium and vitamin D according to standard national guidelines (expert opinion), and to monitor 25OHD levels at diagnosis with subsequent measurements every 6 months at least throughout therapy (expert opinion). Vitamin D ± calcium supplementation is recommended in children with 25OHD levels <20 ng/ml (very low quality evidence and expert opinion).

DISCUSSION

In adult childhood cancer survivors, there is a greater than expected proportion with BMD Z-scores ≤-1, and 10-20% have BMD Z-scores ≤-2.[5] The BMD trajectory in individual patients from cancer diagnosis until adulthood is still largely unknown. However, prevention of low BMD during therapy could conceivably reduce fracture risk in children with cancer and survivors. Patient-specific risk factors (age, race, and sex, for example),[5,11] are non-modifiable, and treatment-specific risk factors are challenging to modify without adversely affecting remission and cure rates. However, vitamin D supplementation, if effective, would be a simple and inexpensive intervention. Based upon very low quality evidence overall, we identified inconsistent findings regarding the association between lower 25OHD levels and lower BMD Z-scores, no significant association between lower 25OHD levels and fractures, and no significant effect of vitamin D supplementation on BMD and fractures in children with cancer (mainly hematologic malignancies) up to five years after cancer therapy. The very low quality of evidence calls into question whether the identified lack of effect is due to lack of evidence, or whether other factors explain the BMD decline and fractures in children with cancer, which effects are not modifiable by vitamin D supplementation.

The observational studies included in this review used different thresholds to define VDD. Fourteen studies assessed the association between 25OHD levels as a *continuous* variable and BMD Z-scores and reported no significant association.

We recommend adequate dietary vitamin D and calcium, i.e. 400 IU vitamin D and 200-1000 mg calcium (depending on age) per day, as recommended by the IOM. In addition, if national guidelines on vitamin D supplementation for certain groups (e.g. infants) in the general population are present, these also apply to children with cancer (expert opinion, supported by the IOM 2011 guideline[18]).
We recommend to monitor 25OHD at cancer diagnosis with subsequent measurements every six months, at least until cessation of treatment, in all children with cancer (expert opinion).
We recommend (additional) vitamin D (D_2 or D_3) supplementation in children with 25OHD levels below 20 ng/ml (initial dose: 2000 IU/day) throughout treatment, or higher doses if serum levels >20 ng/ml are not reached after three months (very low quality evidence and expert opinion). In addition, if the recommended daily amount of dietary calcium is not met, we recommend 500 mg calcium supplementation per day (expert opinion).

IOM=institute of medicine; IU=international units; 25OHD=25-hydroxyvitamin D

Table 5. Consensus recommendations to ensure an adequate vitamin D status in the context of bone health in children with cancer.

Notably, the only study that assessed the association between VDD according to a *threshold*, in this case 25OHD levels ≤10 ng/ml, and BMD Z-scores reported a significant association.[36] It is important to note that using vitamin D as a continuum makes a meaningful evaluation of a potential association with BMD difficult. Although this methodology eliminates the problem of having to choose an arbitrary threshold for VDD, it is associated with another methodological issue: in the general population, a relationship between 25OHD and BMD has been observed in patients with vitamin D insufficiency or deficiency, but not in patients with a vitamin D replete state.[53] Because most of the observational studies in this systematic review analyzed a correlation between 25OHD levels (including replete 25OHD values) and BMD Z-scores, this might have led to false negative results.

Only two studies assessed the association between 25OHD levels and fractures. One study[34] reported significantly higher mean levels of 25OHD in children with fractures compared to those without fractures. However, both studies measured 25OHD levels in the patients after the fractures (if present) had already occurred. This significant finding may thus reflect the fact that after the fracture had been diagnosed, vitamin D supplementation may have been more frequently recommended (and taken) in children with fractures compared to those without.

There was very low quality evidence to suggest that vitamin D supplementation has no significant effect upon BMD and fracture risk in children with ALL. These results are similar to those of an RCT in 275 long-term childhood ALL survivors by Kaste *et al*., who found no significant effect of nutritional counseling with supplementation (1,000 mg/day calcium and 800 IU/day cholecalciferol) or placebo for two years on LS BMD Z-scores.[54] However, the doses of vitamin D

supplementation utilized in the three included interventional studies varied significantly. Furthermore, most included studies were hampered by (very) small sample sizes, had a short follow-up, were performed in children with leukemia and not with other types of cancer, and failed to adjust for important confounders such as body mass index (BMI) and skin tone. These limitations also apply to the observational studies.

In children and adults without cancer, large studies have established the relationship between VDD and bone mineralization defects (rickets and osteomalacia in children, osteomalacia in adults), generalized decrease in BMD, as well as muscle weakness, at a critical cut-off of 12 ng/ml.[18,19] Recent meta-analyses of vitamin D trials demonstrated that the effect of vitamin D supplementation on BMD and fracture risk is only significant in adults with baseline 25OHD levels lower than 16 ng/ml,[55,56] and a meta-analysis in children identified a similar threshold.[23] This indicates that there seems to be a minimum requirement of 25OHD, and that supplementation only benefits estimates of bone strength when this requirement is not met (i.e in vitamin D deficient children). More recent studies also failed to show an effect of (high dose) vitamin D supplementation when applied to children generally (i.e. regardless their 25OHD status).[57,58]

It is likely that low BMD and increased fracture risk in pediatric cancer patients and recent childhood cancer survivors is even more multifactorial in etiology than in the general population. The cancer itself, its treatment, or their consequences such as weakness of bone due to previous bone marrow infiltration by the oncologic disease, glucocorticoid use, osteotoxic effects of chemotherapy and radiotherapy, immobility, malnutrition, or endocrine deficiencies could be such additional (potentially confounding) etiologies.[5,9,26,59–61] These factors may impact BMD more severely and in a larger proportion of children with cancer than low vitamin D levels, and their effects on BMD and fracture risk may not be prevented or overcome by vitamin D supplementation alone.

This systematic review with consensus recommendations may be a first step towards the development of an evidence-based clinical practice guideline for bone health in children with cancer. The knowledge gap that this systematic review has identified, could be overcome by prospective, adequately powered studies addressing the risk of low BMD (Z-score ≤-1 or -2) and fractures for children with cancer at different 25OHD cut-offs, and the effect of vitamin D (and calcium) supplementation on estimates of bone strength. To provide guidance to clinicians until this new evidence has emerged, we have provided strong

recommendations on the basis of the current very low quality evidence and expert opinion (supported by international guidelines for the general population).

We propose that ensuring adequate vitamin D status and mitigating modifiable bone problems in children with cancer are important. According to the Institute of Medicine (IOM), the minimal daily requirement of vitamin D and calcium in children is 400 IU and 200-1100 mg (depending on age), respectively.[18] A diet adequate in vitamin D and calcium should be encouraged.[18,62] Another natural way to acquire vitamin D is through sunlight exposure; however, we abstain from recommendations in this regard given the potential adverse effects on skin health.[63] If national guidelines on vitamin D supplementation for certain groups (e.g. infants) in the general population are present, these also apply to children with cancer. For several reasons, it is conceivable that not all children with cancer will be able to meet the minimal daily requirement of vitamin D and calcium, at least not during all treatment phases. We suggest that in these children, it is reasonable to monitor the 25OHD status regularly instead of supplementing all children (although the harms and costs of standard supplementation appear minimal[23]), since the added benefit of vitamin D supplementation in children and adults with normal vitamin D levels has not been demonstrated,[23,56] and children with cancer undergo frequent phlebotomy. We therefore recommend measurement of 25OHD levels at cancer diagnosis with subsequent measurements every six months, at least until cessation of treatment. In addition, we think it is reasonable to continue 25OHD surveillance throughout the first years of follow-up, however, the frequency may be lower as it may depend upon the frequency of follow-up visits. Although elevated PTH (and alkaline phosphatase) levels provide definitive evidence of clinically significant VDD, we do not recommend universal PTH surveillance, amongst others due to financial constraints in some regions. However, measurement of PTH may be of additional value in children in whom VDD is clinically suspected or in situations when vitamin D concentrations may be unreliable, such as in children with obesity. In these cases, an elevated PTH level is helpful to diagnose VDD, and may diagnose VDD earlier, preventing more severe consequences.

In children with 25OHD levels below 20 ng/ml, we recommend supplementation with vitamin D (D_2 or D_3) throughout treatment at an initial dose of 2000 IU vitamin D per day, as well as 500 mg calcium per day if the recommended daily amount of dietary calcium is not met. This is consistent with the widely-used, global consensus statement in children without cancer by Munns *et al*.[14] Measurement of 25OHD levels after three months could verify adequate dosing

and compliance in patients receiving supplementation. Higher doses may be needed if serum 25OHD levels >20 ng/ml are not reached at this point. Each 1000 IU/day of vitamin D_3 in addition to what a child is currently ingesting will raise the level of 25OHD by 10 ng/ml after a few weeks.[64] The BMI of the patient and the assay that was used need to be taken into consideration in this regard.[65,66] The risk of vitamin D toxicity is considered negligible using our recommended doses.[14] A more extensive report on vitamin D monitoring, titration and its caveats, possible other beneficial effects of vitamin D than bone strength, as well as long-term follow-up recommendations,[67] were not within the scope of this systematic review nor our consensus recommendations.

In conclusion, this systematic review identified that the risk of low BMD during and shortly after cancer treatment for children with VDD has not yet been adequately studied. Very low quality evidence showed inconsistent results for the association between low vitamin D status and reductions in BMD parameters. Similarly, the relationship between 25OHD status and fractures as well as the effect of vitamin D supplementation has not been sufficiently studied to draw meaningful conclusions. Adequately powered prospective studies assessing the risk of low BMD and fractures for children with all types of cancer at different 25OHD cut-offs, as well as the effect of vitamin D (and calcium) supplementation to improve the BMD-fracture pathway in this population are needed. On the other hand, it is well-established that a small, critical amount of vitamin D is needed to prevent overt disturbances in mineral ion metabolism (i.e. hyperparathyroidism and hypocalcemia) in both the healthy and cancer setting. To prevent severe VDD causing overt skeletal effects, children should receive adequate intakes of calcium and vitamin D through diet to meet targets recommended by the IOM 2011 guidelines.[18] Because of the frequency of VDD and low BMD in children on, or who have received, cancer therapy, children undergoing cancer therapy and recent childhood cancer survivors should have routine 25OHD surveillance in order to detect critical VDD that would require supplementation beyond routine preventative measures.

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SUPPLEMENTAL MATERIAL

TITLE: Vitamin D supplementation for children with cancer: a systematic review and consensus recommendations.

Table of contents

1) Pubmed

Search 1: Childhood cancer	Leukemia[MeSH] OR leukemi* [tiab] OR leukaemi* [tiab] OR "childhood ALL" [tiab] OR AML [tiab] OR lymphoma [MeSH] OR lymphom* [tiab] OR hodgkin* [tiab] OR non-hodgkin* [tiab] OR sarcoma [MeSH] OR sarcom* [tiab] OR "sarcoma, Ewing" [MeSH] OR Ewing* [tiab] OR osteosarcom* [tiab] OR "Wilms Tumor" [MeSH] OR wilms* [tiab] OR nephroblastom* [tiab] OR neuroblastoma [MeSH] OR neuroblastom* [tiab] OR rhabdomyosarcoma [MeSH] OR rhabdomyosarcom* [tiab] OR teratoma [MeSH] OR teratom* [tiab] OR "carcinoma, hepatocellular" [MeSH] OR hepatom* [tiab] OR hepatocellular* [tiab] OR hepatoblastoma [MeSH] OR hepatoblastom* [tiab] OR "neuroectodermal tumors, primitive" [MeSH] OR PNET* [tiab] OR medulloblastoma [MeSH] OR medulloblastom* [tiab] OR retinoblastoma [MeSH] OR retinoblastom* [tiab] OR meningioma [MeSH] OR "brain neoplasms" [MeSH] OR "central nervous system neoplasms" [MeSH] OR glioma [MeSH] OR brain tumor* [tiab] OR brain tumour* [tiab] OR "brain cancer" [tiab] OR brain neoplasm* [tiab] OR central nervous system neoplasm* [tiab] OR "central nervous system cancer" [tiab] OR central nervous system tumor* [tiab] OR central nervous system tumour* [tiab] OR intracranial tumor* [tiab] OR intracranial tumour* [tiab] OR "intracranial cancer" [tiab] OR intracranial neoplasm* [tiab] OR meningiom* [tiab] OR gliom*[tiab] OR "childhood oncology" [tiab] OR "pediatric oncology" [tiab] OR "paediatric oncology" [tiab] OR "childhood cancer" [tiab] OR "pediatric cancer" [tiab] OR "paediatric cancer" [tiab] OR childhood tumor* [tiab] OR pediatric tumor* [tiab] OR paediatric tumor* [tiab] OR childhood tumour* [tiab] OR pediatric tumour* [tiab] OR paediatric tumour* [tiab] OR childhood neoplasm* [tiab] OR pediatric neoplasm * [tiab] OR paediatric neoplasm* [tiab] OR childhood malignanc* [tiab] OR pediatric malignanc* [tiab] OR paediatric malignanc* [tiab]
Search 2: Children	Pediatrics [MeSH] OR "young adult" [MeSH] OR child [MeSH] OR adolescent [MeSH] OR infan* [tiab] OR toddler* [tiab] OR minors [MeSH] OR minor* [tiab] OR boy* [tiab] OR girl* [tiab] OR kid* [tiab] OR child* [tiab] OR schoolchild* [tiab] OR adolescen* [tiab] OR juvenil* [tiab] OR youth* [tiab] OR teen* [tiab] OR pubescen* [tiab] OR pediatric* [tiab] OR paediatric* [tiab]
Search 3: Survivors	"Cancer survivors" [MeSH] OR survival [MeSH] OR surviv* [tiab]

Search 4: Late effects	"Long term adverse effects" [MeSH] OR "late effect" [tiab] OR "late effects" [tiab] OR "late side effect" [tiab] OR "late side effects" [tiab] OR "late adverse effect" [tiab] OR "late adverse effects" [tiab] OR "long term" [tiab] OR aftercare [tiab] OR "follow up" [tiab]
Search 5: Vitamin D	"Vitamin d"[MeSH] OR "vitamin d"[tiab] OR "calciferol"[tiab] OR "vitamin d3"[tiab] OR "vitamin d2"[tiab] OR cholecalciferol [tiab] OR calcitriol [tiab] OR calcefediol [tiab] OR 25 ohd* [tiab] OR 1,25(OH)2D [tiab] OR 24,25(OH)2D [tiab] OR marker* [tiab] OR biomarker* [tiab] OR laborator* [tiab] OR blood* [tiab] OR serum level* [tiab]
Search 6: Outcome	Osteoporosis [MeSH] OR osteoporos* [tiab] OR osteopeni* [tiab] OR "bone mineral density" [tiab] OR "bone density" [tiab] OR "bone loss" [tiab] OR "bone health" [tiab] OR "bone turnover" [tiab] OR "bone morbidity" [tiab] OR "bone morbidities" [tiab] OR "bone fragility" [tiab] OR "bone mass" [tiab] OR fracture* [tiab] OR "broken bone" [tiab]
Combined:	1 AND (2 OR 3 OR 4) AND 5 AND 6 = 320 hits

2) Embase

Search 1: Childhood cancer	'Leukemia'/exp OR 'lymphoma'/exp OR 'nephroblastoma'/exp OR 'sarcoma'/exp OR 'Ewing sarcoma'/exp OR 'meningioma'/exp OR 'retinoblastoma'/exp OR 'neuroblastoma'/exp OR 'teratoma'/exp OR 'liver cell carcinoma'/exp OR 'hepatoblastoma'/exp OR 'medullablastoma'/exp OR 'glioma'/exp OR 'central nervous system tumor'/exp OR 'childhood cancer'/exp OR 'childhood leukemia'/exp OR 'acute lymphoblastic leukemia'/exp OR 'acute myeloid leukemia'/exp OR 'Hodgkin disease'/exp OR 'neuroectoderm tumor'/exp OR leukemi*:ab,ti OR leukaemi*:ab,ti OR 'childhood ALL':ab,ti OR AML:ab,ti OR lymphom*:ab,ti OR hodgkin*:ab,ti OR non-hodgkin*:ab,ti OR sarcom*:ab,ti OR Ewing*:ab,ti OR osteosarcom*:ab,ti OR wilms*:ab,ti OR nephroblastom*:ab,ti OR neuroblastom*:ab,ti OR rhabdomyosarcom*:ab,ti OR teratom*:ab,ti OR hepatom*:ab,ti OR hepatocellular*:ab,ti OR hepatoblastom*:ab,ti OR PNET*:ab,ti OR medulloblastom*:ab,ti OR retinoblastom*:ab,ti OR 'brain tumor*':ab,ti OR 'brain tumour*':ab,ti OR 'brain cancer':ab,ti OR 'brain neoplasm*':ab,ti OR 'central nervous system neoplasm*':ab,ti OR 'central nervous system cancer':ab,ti OR 'central nervous system tumor*':ab,ti OR 'central nervous system tumour*':ab,ti OR 'intracranial tumor*':ab,ti OR 'intracranial tumour*':ab,ti OR 'intracranial cancer':ab,ti OR 'intracranial neoplasm*':ab,ti OR

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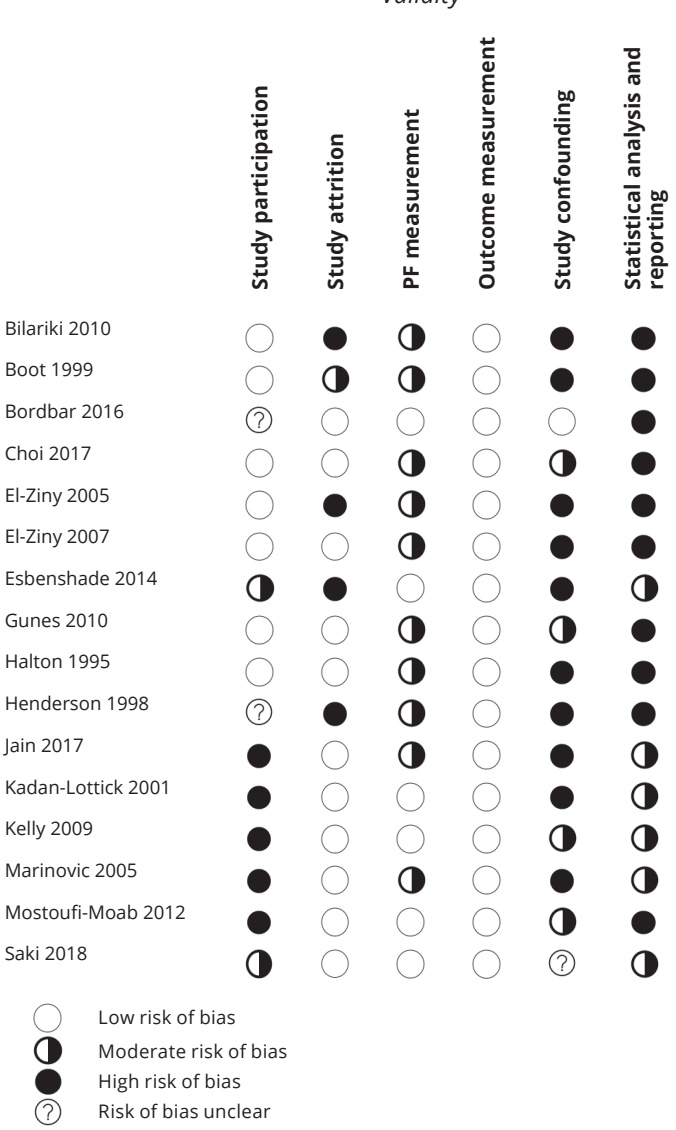
Search 2: Children	'Child'/exp OR 'infant'/exp OR 'adolescent'/exp OR 'pediatrics'/exp OR infan*:ab,ti OR toddler*:ab,ti OR minor*:ab,ti OR boy*:ab,ti OR girl*:ab,ti OR kid*:ab,ti OR child*:ab,ti OR schoolchild*:ab,ti OR adolescen*:ab,ti OR juvenil*:ab,ti OR youth*:ab,ti OR teen*:ab,ti OR pubescen*:ab,ti OR pediatric*:ab,ti OR paediatric*:ab,ti
Search 3: Survivors	'Childhood cancer survivor'/exp OR 'cancer survival'/exp OR 'survival'/exp OR surviv*:ab,ti
Search 4: Late effects	'long term survival'/exp OR 'late effect':ab,ti OR 'late effects':ab,ti OR 'late side effect':ab,ti OR 'late side effects':ab,ti OR 'late adverse effect':ab,ti OR 'late adverse effects':ab,ti OR 'long term':ab,ti OR aftercare:ab,ti OR 'follow up':ab,ti
Search 5: Vitamin D	'Vitamin D'/exp OR 'vitamin d':ab,ti OR 'calciferol':ab,ti OR 'vitamin d3':ab,ti OR 'vitamin d2':ab,ti OR 'cholecalciferol':ab,ti OR 'calcitriol':ab,ti OR 'calcefediol':ab,ti OR '25 ohd*':ab,ti OR 1,25OH2D:ab,ti OR 24,25OH2D:ab,ti OR marker*:ab,ti OR biomarker*:ab,ti OR laborator*:ab,ti OR blood*:ab,ti OR 'serum level*':ab,ti
Search 6: Outcome	'Osteoporosis'/exp OR osteoporos*:ab,ti OR osteopeni*:ab,ti OR 'bone mineral density':ab,ti OR 'bone density':ab,ti OR 'bone loss':ab,ti OR 'bone health':ab,ti OR 'bone turnover':ab,ti OR 'bone morbidity':ab,ti OR 'bone morbidities':ab,ti OR 'bone fragility':ab,ti OR 'bone mass':ab,ti OR fracture*:ab,ti OR 'broken bone':ab,ti
Combined:	1 AND (2 OR 3 OR 4) AND 5 AND 6 = 1219 hits

3) Cochrane

Search 1: Childhood cancer	Leukemi*:ab,ti OR leukaemi*:ab,ti OR 'childhood ALL':ab,ti OR AML:ab,tiORlymphom*:ab,tiORhodgkin*:ab,tiORnon-hodgkin*:ab,ti OR sarcom*:ab,ti OR Ewing*:ab,ti OR osteosarcom*:ab,ti OR wilms*:ab,ti OR nephroblastom*:ab,ti OR neuroblastom*:ab,ti OR rhabdomyosarcom*:ab,ti OR teratom*:ab,ti OR hepatom*:ab,ti OR hepatocellular*:ab,ti OR hepatoblastom*:ab,ti OR PNET*:ab,ti OR medulloblastom*:ab,ti OR retinoblastom*:ab,ti OR 'brain tumor*':ab,ti OR 'brain tumour*':ab,ti OR 'brain cancer':ab,ti OR 'brain neoplasm*':ab,ti OR 'central nervous system neoplasm*':ab,ti OR 'central nervous system cancer':ab,ti OR 'central nervous system tumor*':ab,ti OR 'central nervous system tumour*':ab,ti OR 'intracranial tumor*':ab,ti OR 'intracranial tumour*':ab,ti OR 'intracranial cancer':ab,ti OR 'intracranial neoplasm*':ab,ti OR meningiom*:ab,ti OR gliom*:ab,ti OR 'childhood oncology':ab,ti OR 'pediatric oncology':ab,ti OR 'paediatric oncology':ab,ti OR 'childhood cancer':ab,ti OR 'pediatric cancer':ab,ti OR 'paediatric cancer':ab,ti OR 'childhood tumor*':ab,ti OR 'pediatric tumor*':ab,ti OR 'paediatric tumor*':ab,ti OR 'childhood tumour*':ab,ti OR 'pediatric tumour*':ab,ti OR 'paediatric tumour*':ab,ti OR 'childhood neoplasm*':ab,ti OR 'pediatric neoplasm*':ab,ti OR 'paediatric neoplasm*':ab,ti OR 'childhood malignanc*':ab,ti OR 'pediatric malignanc*':ab,ti OR 'paediatric malignanc*':ab,ti
Search 2: Children	Infan*:ab,ti OR toddler*:ab,ti OR minor*:ab,ti OR boy*:ab,ti OR girl*:ab,ti OR kid*:ab,ti OR child*:ab,ti OR schoolchild*:ab,ti OR adolescen*:ab,ti OR juvenil*:ab,ti OR youth*:ab,ti OR teen*:ab,ti OR pubescen*:ab,ti OR pediatric*:ab,ti OR paediatric*:ab,ti
Search 3: Survivors	Surviv*:ab,ti
Search 4: Late effects	'Late effect':ab,ti OR 'late effects':ab,ti OR 'late side effect':ab,ti OR 'late side effects':ab,ti OR 'late adverse effect':ab,ti OR 'late adverse effects':ab,ti OR 'long term':ab,ti OR aftercare:ab,ti OR 'follow up':ab,ti
Search 5: Vitamin D	'Vitamin d':ab,ti OR 'calciferol':ab,ti OR 'vitamin d3':ab,ti OR 'vitamin d2':ab,ti OR 'cholecalciferol':ab,ti OR 'calcitriol':ab,ti OR 'calcefediol':ab,ti OR '25 ohd*':ab,ti OR 1,25OH2D:ab,ti OR 24,25OH2D:ab,ti OR marker*:ab,ti OR biomarker*:ab,ti OR laborator*:ab,ti OR blood*:ab,ti OR 'serum level*':ab,ti

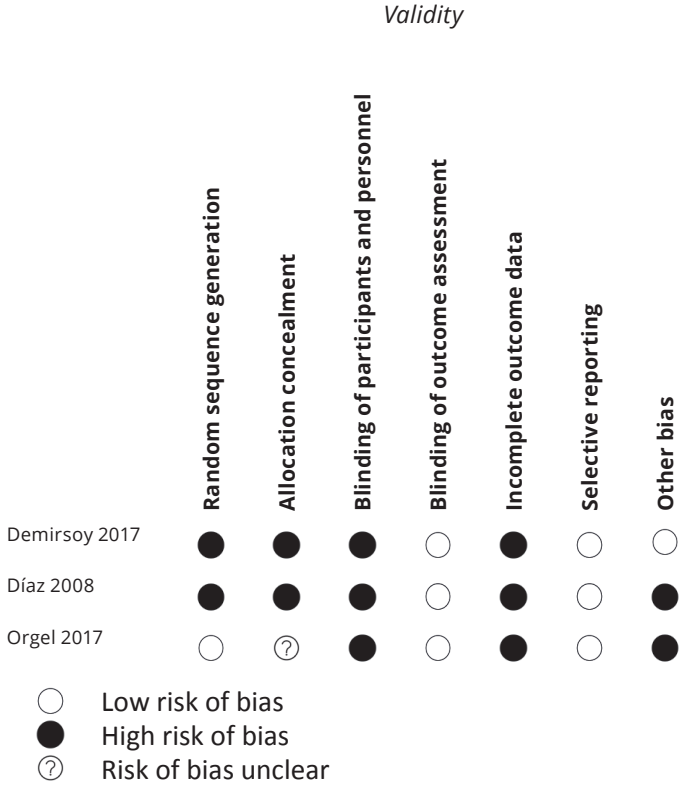
Search 6: Outcome	Osteoporos*:ab,ti OR osteopeni*:ab,ti OR 'bone mineral density':ab,ti OR 'bone density':ab,ti OR 'bone loss':ab,ti OR 'bone health':ab,ti OR 'bone turnover':ab,ti OR 'bone morbidity':ab,ti OR 'bone fragility':ab,ti OR 'bone mass':ab,ti OR 'bone morbidities':ab,ti OR fracture*:ab,ti OR 'broken bone':ab,ti
Combined**:**	1 AND (2 OR 3 OR 4) AND 5 AND 6 **= 109** hits

Supplemental Table 1. Search strategies for the Pubmed, Embase and Cochrane databases.



PF=prognostic factor

Supplemental Table 2. Critical appraisal of the observational studies using the QUIPS risk of bias tool.



Supplemental Table 3. Critical appraisal of the interventional studies using the Cochrane risk of bias tool.

Quality assessment								
Study design	**Study limitations**	**Inconsistency**	**Indirectness**	**Imprecision**		**Effect size**	**Dose-response**	**Plausible confounding**
Observational studies *What is the risk of lower BMD Z-scores for lower serum 25OHD levels in children with cancer up to five years after cancer therapy?*								
Longitudinal and cross-sectional cohort studies[1] (+4)	Very serious[2] (-2)	Serious[3] (-1)	Not serious	Serious[4] (-1)	Unlikely	No large effect size	Dose-response effect not assessed	No plausible confounding
Overall quality of the evidence: ⊕⊖⊖⊖ VERY LOW **Conclusion:** There is conflicting evidence for the association between lower serum 25OHD levels and lower BMD Z-scores in children with cancer up to five years after cancer therapy. **Number of studies and participants:** 16 studies, 873 participants								
Observational studies *What is the risk of fractures for lower serum 25OHD levels in children with cancer up to five years after cancer therapy?*								
Cross-sectional cohort studies[5] (+2)	Very serious[2] (-2)	Not serious	Not serious	Serious[4] (-1)	Unlikely	No large effect size	Dose-response effect not assessed	No plausible confounding
Overall quality of the evidence: ⊕⊖⊖⊖ VERY LOW **Conclusion:** No increased risk of fractures for lower serum 25OHD levels in children with cancer up to five years after cancer therapy. **Number of studies and participants:** 2 studies, 89 participants								
Interventional studies *What is the effect of vitamin D supplementation on BMD and fractures in children with cancer up to five years after cancer therapy?*								
Two RCTs, one quasi-experimental study[6] (+2)	Very Serious[7] (-2)	Not serious	Serious[8] (-1)	Serious[4] (-1)	Unlikely	No large effect size	Dose-response effect not assessed	No plausible confounding
Overall quality of the evidence: ⊕⊖⊖⊖ VERY LOW **Conclusion:** No significant effect of vitamin D supplementation on BMD and fractures in children with ALL up to five years after cancer therapy compared to controls. **Number of studies and participants:** 3 studies, 61 participants, 77 controls								

BMD=bone mineral density; RCT=randomized controlled trial

[1]Initial score +4 assigned, as evidence from these observational study designs is the best available for prognostic questions

[2]Downgraded for risk of bias, as the QUIPS tool showed significant risk of bias for study participation, prognostic factor measurement, study confounding and statistical analysis and reporting

[3]Downgraded for inconsistency, as one study reported a significant association between lower 25OHD levels and lower BMD

[4]Downgraded for imprecision, as the sample size of most studies was very small

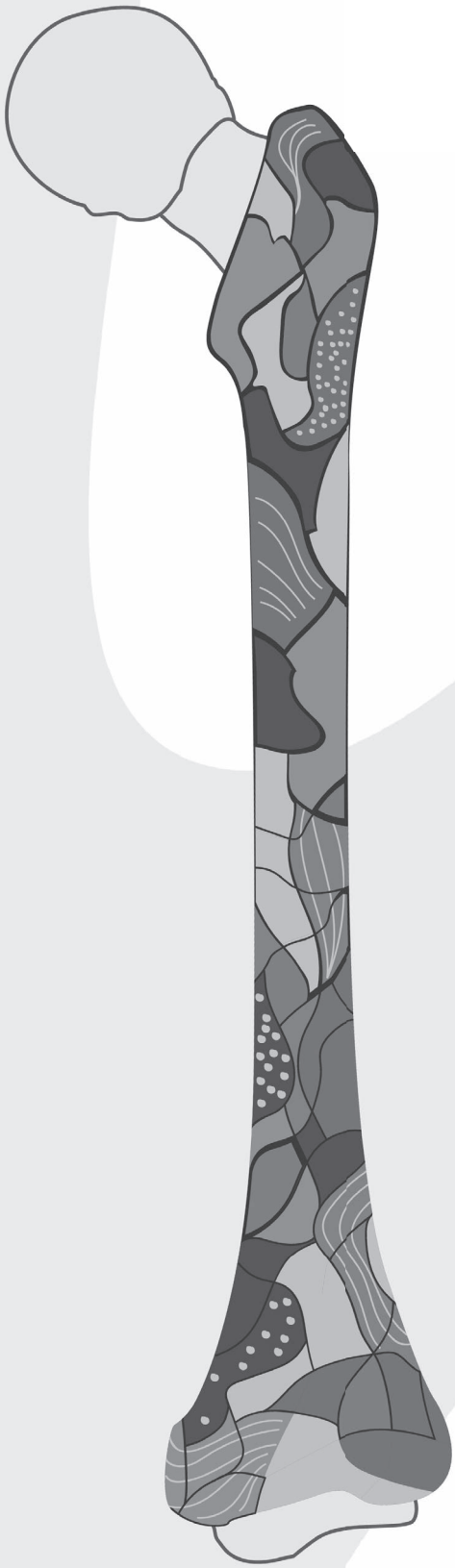
[5]Initial score +2 assigned, as the studies were longitudinal for BMD but cross-sectional for fractures: the association between 25OHD levels and a *history* of fractures was assessed

[6]Initial score +2 assigned, as the majority of the studies was no RCT

[7]Downgraded for risk of bias, as the Cochrane Risk of Bias tool showed significant risk of bias for random sequence generation, allocation concealment, incomplete outcome data, and other bias

[8]Downgraded for indirectness, as all interventional studies were performed in children with acute lymphoblastic leukemia, and therefore the results might not be generalizable to children with other cancer diagnoses

Supplemental Table 4. GRADE assessment of the total body of observational and interventional studies.



CHAPTER 8

Frailty and sarcopenia within the earliest national Dutch childhood cancer survivor cohort (n=2,003): a DCCSS-LATER Study

Jenneke E. van Atteveld, Demi T.C. de Winter, Vincent G. Pluimakers, Marta Fiocco, Rutger A.J. Nievelstein, Monique G.G. Hobbelink, Leontien C.M. Kremer, Martha A. Grootenhuis, Heleen Maurice-Stam, Wim J.E. Tissing, Andrica C.H. de Vries, Jacqueline J. Loonen, Eline van Dulmen-den Broeder, Helena J. van der Pal, Saskia M.F. Pluijm, Margriet van der Heiden-van der Loo, A. Birgitta Versluijs, Marloes Louwerens, Dorine Bresters, Hanneke M. van Santen, Imo Hoefer, Sjoerd A.A. van den Berg, Jaap den Hartogh, Jan H.J. Hoeijmakers, Sebastian J.C.M.M. Neggers*, and Marry M. van den Heuvel-Eibrink*, on behalf of the Dutch LATER study group

*These authors contributed equally to this study

Under review The Lancet Healthy Longevity.

ABSTRACT

Background: Childhood cancer survivors seem to be at increased risk of frailty and sarcopenia, two partly overlapping aging phenotypes that have been associated with adverse health outcomes. However, evidence on prevalence and risk factors of frailty and sarcopenia is limited. We investigated this in a well-defined national cohort of Dutch childhood cancer survivors diagnosed from 1963-2001.

Methods: 2,003 childhood cancer survivors aged 18-45 years at invitation were included (mean age at participation 33.1±7.2 years). We defined prefrailty and frailty according to modified Fried criteria, and sarcopenia based on the EWGSOP2 definition. Associations between demographic, treatment-related, endocrine, as well as lifestyle-related factors and both conditions were estimated with multivariable logistic regression models.

Findings: In survivors with complete frailty measurements (n=1,114, 55.6% of participants) or complete sarcopenia measurements (n=1,472, 73.5%), the percentage of prefrailty, frailty, and sarcopenia was 20.3%, 7.4%, and 4.4%, respectively. In the model for prefrailty in the full cohort (n=2,003), underweight and obesity, cranial irradiation (CRT), total body irradiation (TBI), cisplatin dose ≥600 mg/m^2, growth hormone deficiency (GHD), hyperthyroidism, bone mineral density (BMD, Z-score ≤-1 and >-2, and Z-score ≤-2), and folic acid deficiency were statistically significant. For frailty, associated factors included age at diagnosis between 10-18 years, underweight, CRT, TBI, cisplatin dose ≥600 mg/m^2, higher carboplatin doses, cyclophosphamide equivalent dose ≥20 g/m^2, hyperthyroidism, BMD Z-score ≤-2, and folic acid deficiency. Male sex, lower body mass index (continuous), CRT, TBI, hypogonadism, GHD, and vitamin B12 deficiency were significantly associated with sarcopenia.

Interpretation: Our findings show that frailty and sarcopenia occur already at a mean age of 33 years in childhood cancer survivors, i.e., conceivably more than three decades earlier than in the general population. Early recognition and interventions for endocrine disorders and dietary deficiencies may be crucial in minimizing the risk of (pre)frailty and sarcopenia in this population.

INTRODUCTION

The improving survival of children with cancer leads to a continuously growing population of childhood cancer survivors.[1] These survivors are at increased risk of developing several conditions that appear to be related to aging in the general population, such as metabolic syndrome (including type 2 diabetes), cardiovascular disease, neurological decline, and low bone mineral density (BMD).[2] As these conditions seem to occur already during young adulthood in survivors, this suggests that a process of premature aging occurs, which includes frailty as an important component. Frailty is a state of vulnerability, i.e., a reduced physiologic reserve defined by a cluster of five physical ability measurements. Frailty has been associated with a marked susceptibility to adverse health outcomes such as chronic diseases and death in the general population and in survivors.[3,4]

The term sarcopenia, or muscle failure, has long been used to describe low lean mass. In addition to low lean mass, new insights recognized the importance of muscle strength when characterizing muscle failure, which led to its inclusion in the definition of sarcopenia.[5] Despite some overlap between frailty and sarcopenia, they are often described as separate conditions.[6] Similar to frailty, sarcopenia is associated with an increase in adverse outcomes such as excess morbidity and mortality in the general population.[7]

Studies in two American childhood cancer survivor cohorts have shown that 6-8% of survivors (mean age 30-37 years, mean time since cancer diagnosis 25-30 years) were frail,[4,8] and that 3.5% of survivors were sarcopenic.[9] In addition, several demographic, disease- and treatment-related, endocrine, and lifestyle-related factors were shown to be associated with (pre)frailty in these cohorts.[4,8,10,11] However, the prevalence and risk factors of frailty in a representative cohort of European survivors have not been investigated so far, and studies that assessed clinical risk factors for sarcopenia (defined as both low lean mass and low muscle strength) are lacking. If frailty and sarcopenia are indeed universally relevant conditions in survivors, a better understanding of their risk factors could enhance insights in underlying biological mechanisms, as well as facilitate timely identification and targeted interventions. The aim of this cross-sectional study was to assess the prevalence and explore risk factors of (pre)frailty and sarcopenia in a national cohort of Dutch childhood cancer survivors diagnosed from 1963-2001.

METHODS

Patients

The current study is part of the Dutch Childhood Cancer Survivor Study (DCCSS) LATER cohort.[12] This cohort consists of 6,165 survivors who were: 1) at least five years after childhood cancer diagnosis; 2) diagnosed in a Dutch pediatric oncology center from 1963-2001; 3) between 0-19 years of age at cancer diagnosis; and 4) alive at cohort formation in 2008. We deemed 2,169 survivors ineligible due to reasons such as death, living abroad, attained age younger than 18 years or older than 45 years, or previous refusal for any late-effects studies. Hence, 3,996 adult survivors were eligible and invited for this cross-sectional study. Written informed consent was obtained from all participants.

Definition of (pre)frailty and sarcopenia

According to modified Fried criteria,[3] prefrailty and frailty were defined as the presence of ≥2 or ≥3 of the following criteria: low lean mass, low muscle strength, exhaustion, slowness, or low physical activity. We defined sarcopenia as the presence of both low lean mass and low muscle strength as proposed by the EWGSOP2.[13] We aligned the definitions and thresholds of each component to a large extent with those in previous childhood cancer survivor studies[4,14] to be able to compare our data with previously published results. However, we chose to use age- and sex-specific normative values for each component.

Low lean mass

DXA was performed to assess appendicular lean mass (ALM) divided by height squared (kg/m^2). Normative values from the DXA manufacturer were used to calculate ALM Z-scores. Appendicular lean mass was classified as low in case of an age- and sex-specific Z-score ≤-1.5.

Low muscle strength

Muscle strength was measured with a hand-held dynamometer (Jamar, Sammons Preston Rolyan, Bolingbrook, IL, USA) using a standardized procedure. We calculated the mean of two measurements to determine muscle strength for each side. A Z-score ≤-1.5 at one or both sides was considered low. Age- and sex-specific normative values from the dynamometer manufacturer were used.[15,16]

Exhaustion

The subscale vitality of the Dutch version of the MOS-SF-36 Health Survey[17] was used as proxy measure for exhaustion. Age- and sex-specific normative values from

the general Dutch population were available.[17] We classified scores 1.5 standard deviation (SD) below the Dutch mean as low vitality, which indicates exhaustion.

Slowness

The subscale physical function of the Dutch version of the MOS-SF-36 Health Survey[17] was used as proxy measure for slowness. Age- and sex-specific normative values from the general Dutch population were available.[17] We classified scores 1.5 SD below the Dutch mean as low physical functioning, which indicates slowness. In addition, we performed a six-minute walking test (6MWT) in a subcohort of survivors (n=309) to assess the correlation between survey results and distance covered during the 6MWT.

Low physical activity

The validated SQUASH (Short QUestionnaire to ASsess Health enhancing physical activity) questionnaire was used to assess regular physical activity (including commuting, household, work or school, and leisure-time activities).[18] We converted each activity to a metabolic equivalent of task (MET) value to determine activity intensity using the 2011 compendium by Ainsworth and colleagues.[19] The number of minutes spent on moderate-to-vigorous physical activity per week was compared with that in age- and sex-matched young adults from the general Dutch population (Lifelines cohort).[20] Values below the 20th percentile were considered low.

Potential risk factors

For all eligible survivors, we retrieved sex, age at cancer diagnosis, attained age, disease- and all treatment-related data from medical records. The latter included cancer diagnosis, chemotherapy regimens and total cumulative doses, radiotherapy fields and (fractionated) dose, hematopoietic stem cell transplantation (HSCT), and amputation surgery for primary diagnoses as well as recurrences. Intention-to-treat cumulative corticosteroid doses were determined based on treatment protocols and converted to prednisone equivalent doses.[21] If the treatment protocol was missing, it was estimated based on disease type and treatment decade. All additional data were collected during a one-day outpatient clinic visit at one of the six late-effect clinics in the Netherlands between May 2016 and February 2020. Height and weight were obtained to calculate body mass index (BMI, weight/height^2). We measured BMD and fat mass using Dual-Energy X-ray absorptiometry (DXA; Hologic, Marlborough, MA). A medical history was taken to assess fractures that occurred from five

years after cancer diagnosis onwards. In addition, we registered if survivors had ever been diagnosed with endocrine disorders. Survivors completed various questionnaires, including questionnaires regarding individual health behaviors. Furthermore, blood samples were obtained after an overnight fast. We assessed free thyroxine (FT4), thyroid stimulating hormone (TSH), insulin-like growth factor 1 (IGF-1), 25-hydroxyvitamin D (25OHD), vitamin B12, homocysteine, and folic acid levels. Definitions of the potential risk factors that we assessed are shown in Supplementary Table 1.

Statistical analysis

Characteristics of study participants were compared to those of non-participants and underlying cohort using a Chi-Square test. A Fisher's exact p-value was employed when the number of observations was lower than five. Risk factors for (pre)frailty and sarcopenia were first assessed using univariable logistic regression analyses. Chemotherapy and radiotherapy dose thresholds were chosen based on clinical relevance or previous reports in the literature. Potential risk factors identified in univariable analysis (with a p-value <0.2) together with demographic factors known to be associated with (pre)frailty or sarcopenia from previous literature (i.e. sex, attained age, and BMI) were incorporated in multivariable models. When two collinear risk factors were identified in univariable analysis (i.e. HSCT and total body irradiation [TBI]), the risk factor with the largest effect size was included. We made separate multivariable models for potential demographic and treatment-related as well as endocrine and lifestyle-related risk factors for (pre)frailty and sarcopenia. In addition, when interaction was suspected based on literature from the elderly, this was assessed by adding an interaction term. As survivors with an amputation have per definition lower ALM detected by DXA, models were adjusted for amputation surgery when the number of observations was at least five. All analyses were performed in R version 4.0.3 (Vienna, Austria).[22]

RESULTS

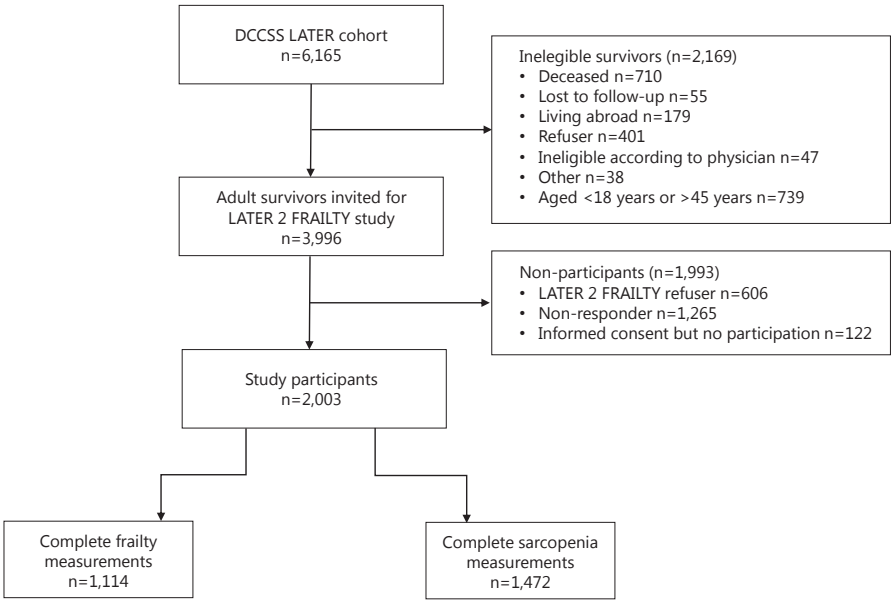
Participants

Of 3,996 eligible survivors, 2,003 (50.1%) participated in this study (Figure 1). Baseline characteristics of the study cohort are shown in Table 1. The mean attained age of participants was 33.1±7.2 years, and the median follow-up time since cancer diagnosis was 25.3 (interquartile range 20.3-31.3) years. The participating cohort was representative with regard to age at cancer diagnosis and study invitation, follow-up time, and surgery frequency compared to non-participants. However,

participants were more often female, had a different distribution of cancer diagnoses, and received more often all evaluated types of cancer treatment compared to non-participants. We obtained complete frailty measurements from n=1,114 survivors (55.6% of participants) and complete sarcopenia measurements from n=1,472 survivors (73.5%). Participants with complete frailty measurements were representative regarding the same aspects as all participants, and additionally regarding all evaluated types of radiotherapy, HSCT, platinum, vinca-alkaloid, methotrexate, and steroid frequency (data not shown).

Prevalence of (pre)frailty and sarcopenia

Table 2 shows the prevalence of the separate frailty components and of each frailty score in the full cohort (n=2,003) and in complete cases (n=1,114). Low muscle strength was the most prevalent frailty component (20.6%), and low lean mass the least prevalent (12.0%). Slowness was present in 13.7% of survivors, and self-reported physical function (used to indicate slowness) was significantly correlated with distance covered during the 6MWT ($p<0.001$); correlation was low to moderate ($r=0.39$, 96%CI=0.28-0.49). In the representative group of 1,114 survivors with complete measurements, the prevalence of prefrailty, frailty,



Abbreviations: DCCSS LATER=Dutch Childhood Cancer Survivor LATER Study.

Figure 1. Flowchart of study participants.

Characteristics	Participants (n=2,003) N (%)	Non-participants (n=1,993) N (%)	Underlying cohort (n=6,165) N (%)	P-value participants vs. non-participants	P-value participants vs. underlying cohort
Sex				<0.001	0.004
Male	1,037 (51.8)	1,217 (61.1)	3,433 (55.7)		
Female	966 (48.2)	776 (38.9)	2,731 (44.3)		
Transgender	0 (0.0)	0 (0.0)	1 (0.02)		
Primary childhood cancer (ICCC)				<0.001	<0.001
Leukemias, myeloprofiferative diseases and myelodysplastic diseases	748 (37.3)	696 (34.9)	2,094 (34.0)		
Lymphomas and reticulo endothelial neoplasms	373 (18.6)	349 (17.5)	1,062 (17.2)		
CNS and miscellaneous intracranial and intraspinal neoplasms	192 (9.6)	298 (15.0)	844 (13.7)		
Neuroblastoma and other peripheral nervous cell tumors	119 (5.9)	94 (4.7)	324 (5.3)		
Retinoblastoma	10 (0.5)	13 (0.7)	33 (0.5)		
Renal tumors	237 (11.8)	200 (10.0)	596 (9.7)		
Hepatic tumors	18 (0.9)	28 (1.4)	52 (0.8)		
Bone tumors	90 (4.5)	84 (4.2)	370 (6.0)		
Soft tissue and other extraosseous sarcomas	134 (6.7)	129 (6.5)	450 (7.3)		
Germ cell tumors, trophoblastic tumors, and neoplasms of gonads	60 (3.0)	78 (3.9)	232 (3.8)		
Other malignant epithelial neoplasms and malignant melanomas	20 (1.0)	23 (1.2)	102 (1.7)		
Other and unspecified malignant neoplasms	2 (0.1)	1 (0.1)	6 (0.1)		
Age at diagnosis (yr)*				0.99	<0.001
0-4	998 (49.8)	994 (50.0)	2,727 (45.3)		
5-9	553 (27.6)	551 (27.7)	1,628 (27.1)		
10-14	366 (18.3)	359 (18.0)	1,285 (21.4)		
15-17	86 (4.3)	85 (4.3)	376 (6.3)		

Characteristics	Participants (n=2,003) N (%)	Non-participants (n=1,993) N (%)	Underlying cohort (n=6,165) N (%)	P-value participants vs. non-participants	P-value participants vs. underlying cohort
Age at invitation[#]				0.53	<0.001
<18	NA	NA	49 (1.2)		
18-29	771 (38.5)	522 (37.6)	1,313 (32.9)		
30-39	871 (43.5)	629 (45.3)	1,511 (37.9)		
≥40	361 (18.0)	236 (17.0)	1,118 (28.0)		
Follow-up time since childhood cancer diagnosis[¶]				0.21	<0.001
10-19	466 (23.3)	432 (21.7)	981 (20.4)		
20-29	916 (45.7)	956 (48.0)	1,931 (40.1)		
30-39	544 (27.2)	546 (27.4)	1,393 (29.0)		
40-49	77 (3.8)	59 (3.0)	460 (9.6)		
50-59	0 (0.0)	0 (0.0)	46 (1.0)		
Radiotherapy[a‡]					
Any radiotherapy	676 (33.7)	566 (28.4)	2,527 (41.2)	<0.001	<0.001
Cranial[¥]	320 (16.0)	180 (13.0)		0.015	
Abdomen/pelvis	148 (7.4)	63 (4.6)		<0.001	
Total body	83 (4.2)	28 (2.0)		<0.001	
Chemotherapy[a‡]					
Any chemotherapy	1,784 (89.1)	1,603 (80.4)	5,005 (81.7)	<0.001	<0.001
Alkylating agents	1,015 (53.3)	581 (43.9)		<0.001	
Anthracyclines	1,067 (53.8)	628 (45.7)		<0.001	
Platinum	298 (14.9)	168 (12.1)		0.021	

Characteristics	Participants (n=2,003) N (%)	Non-participants (n=1,993) N (%)	Underlying cohort (n=6,165) N (%)	P-value participants vs. non-participants	P-value participants vs. underlying cohort
Vinca alkaloids	1,589 (79.4)	1,015 (73.2)		<0.001	
Methotrexate	939 (46.9)	588 (42.4)		0.010	
Glucocorticoïds	1,165 (58.2)	738 (53.2)		0.004	
Hematopoietic stem cell transplantation[a]				<0.001	0.23
Autologous	54 (2.7)	33 (1.7)	155 (2.6)		
Allogeneic	95 (4.7)	54 (2.7)	231 (3.9)		
Surgery[a¡]					
Any surgery	965 (48.2)	1,003 (50.3)	3,185 (52.2)	0.14	0.002
Amputation	42 (2.1)	29 (2.1)		0.99	

Abbreviations: CNS=central nervous system; ICCC=International Classification for childhood cancer; NA=not applicable (survivors <18 yrs or >45 yrs excluded); yr=year
[a]For primary cancer and recurrences
*Not reported for survivors refusing registration
[#]Not reported for survivors refusing participation
[¶]Not reported for survivors refusing registration and those who are ineligible due to reasons such as death, lost to follow-up, or living abroad
[¡]Subgroup data not reported for survivors refusing participation
[¥]Including cranial irradiation for brain tumors and craniospinal irradiation

Table 1. Baseline characteristics of the study cohort.

	No. of measurements (% of participants)	No. of events (%)
Separate frailty components		
Low lean mass	1,536 (76.7)	185 (12.0)
Low muscle strength	1,830 (91.4)	377 (20.6)
Exhaustion	1,473 (73.5)	275 (18.7)
Slowness	1,485 (74.1)	204 (13.7)
Low physical activity	1,698 (84.8)	283 (16.7)
	Full cohort (n=2,003) N (%)	**Complete cases (n=1,114) N (%)**
Frailty score		
0	1,110 (56.6)	597 (53.6)
1	529 (27.0)	291 (26.1)
2	207 (10.6)	144 (12.9)
3	86 (4.4)	59 (5.3)
4	27 (1.4)	20 (1.8)
5	3 (0.2)	3 (0.3)
All values missing	41 (2.0)	NA

Abbreviations: NA=not applicable; No.=number.

Table 2. Prevalence of frailty components and frailty scores in childhood cancer survivors.

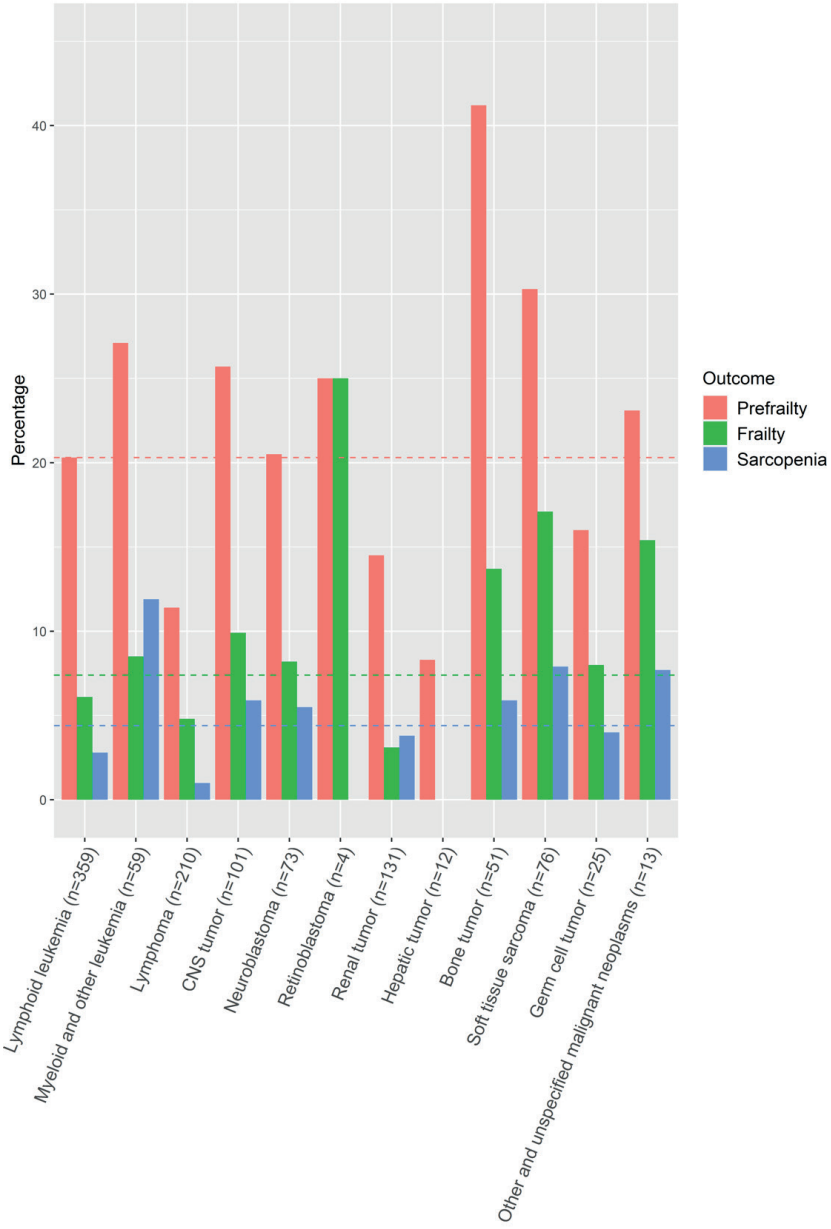
and sarcopenia was 20.3%, 7.4%, and 4.4%, respectively. Overall, the contribution of each frailty component to frailty scores was remarkably equal (Supplementary Figure 1). The frequency of prefrailty, frailty, and sarcopenia per cancer diagnosis is depicted in Figure 2. Prefrailty and frailty frequencies were higher than the average across all diagnoses among survivors of bone tumor, soft tissue sarcoma, central nervous system tumor, myeloid leukemias, and other and unspecified malignant neoplasms, whereas sarcopenia was most frequent among survivors of myeloid leukemias.

Factors associated with (pre)frailty

Risk factors for prefrailty and frailty estimated from univariable models in the full cohort (n=2,003) are presented in Supplementary Table 2.

Demographic and treatment-related factors

In the multivariable models for prefrailty and frailty including demographic and treatment-related factors, BMI category was significantly associated with prefrailty (Table 3). Underweight (odds ratio [OR]=3.38) as well as obesity (OR=1.67) were



Abbreviations: CNS=central nervous system.
Dashed lines represent outcome frequencies across all diagnoses.

Figure 2. Frequency of prefrailty, frailty, and sarcopenia per cancer diagnosis in survivors with complete frailty measurements (n=1,114).

	Prefrailty		Frailty		Sarcopenia	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Sex (male)	1.19 (0.92-1.56)	0.19	0.84 (0.55-1.28)	0.42	4.56 (2.26-9.17)	**<0.001**
Attained age (per year)	0.98 (0.95-1.02)	0.32	0.97 (0.94-1.01)	0.10	1.00 (0.96-1.05)	0.83
Age at diagnosis						
0 to 10 years	-	-	Ref	Ref	-	-
10 to 18 years	-	-	1.94 (1.19-3.16)	**0.008**	-	-
Follow-up time (per year)	0.99 (0.96-1.02)	0.48	-	**-**	-	-
Body mass index*					0.52 (0.45-0.60)	**<0.001**
Underweight	3.38 (1.92-5.95)	**<0.001**	3.09 (1.42-6.69)	**0.004**	-	-
Normal	Ref	Ref	Ref	Ref	-	-
Overweight	0.71 (0.52-0.99)	**0.043**	0.66 (0.39-1.15)	0.14	-	-
Obese	1.67 (1.14-2.43)	**0.008**	1.31 (0.71-2.42)	0.39	-	-
Cranial irradiation#	2.07 (1.47-2.93)	**<0.001**	2.65 (1.59-4.34)	**<0.001**	3.87 (1.80-8.31)	**0.001**
Total body irradiation	3.17 (1.77-5.70)	**<0.001**	3.28 (1.48-7.28)	**0.003**	4.52 (1.67-12.2)	**0.003**
Corticosteroid dose (PED)						
0 g/m^2	-	-	-	-	Ref	Ref
<10 g/m^2	-	-	-	-	0.38 (0.19-0.76)	**0.006**
≥10 g/m^2	-	-	-	-	0.58 (0.16-2.18)	0.42
Cisplatin dose						
0 mg/m^2	Ref	Ref	Ref	Ref	-	-
<600 mg/m^2	1.02 (0.59-1.77)	0.94	1.31 (0.62-2.78)	0.47	-	-
≥600 mg/m^2	3.75 (1.82-7.74)	**<0.001**	3.93 (1.45-10.67)	**0.007**	-	-
Carboplatin dose (per g/m^2)	1.08 (0.97-1.20)	0.16	1.15 (1.02-1.31)	**0.026**	-	-
Alkylating dose (CED)						
0 g/m^2	Ref	Ref	Ref	Ref	-	-
<20 g/m^2	0.85 (0.65-1.12)	0.25	1.26 (0.80-1.99)	0.32	-	-
≥20 g/m^2	1.64 (0.82-3.28)	0.16	3.90 (1.65-9.24)	**0.002**	-	-

Abbreviations: CED=cyclophosphamide equivalent dose; CI=confidence interval; No.=number; OR=odds ratio; PED=prednisone equivalent dose; Ref=reference
The model for prefrailty was adjusted for amputation (significantly associated with prefrailty in a univariable model)
*Adjusted for amputation. Analyzed as a continuous variable for sarcopenia
#Including cranial irradiation for brain tumors and craniospinal irradiation

Table 3. Demographic and treatment-related risk factors for prefrailty, frailty, and sarcopenia using multivariable logistic regression analysis.

significantly associated with increased odds of prefrailty, whereas overweight was significantly associated with reduced odds of prefrailty (OR=0.71) compared to survivors with a normal BMI. A similar pattern was observed for frailty, but only the effect of underweight reached statistical significance (OR=3.09). To investigate the effect of BMI category on prefrailty for males and females, an interaction term was added to the multivariable model. In Supplementary Figure 2, the odds ratio's along with 95% confidence intervals are shown. This interaction term could not be added to the model for frailty due to small sample size. Age at diagnosis between 10 to 18 years (OR=1.94) was significantly associated with frailty compared with survivors diagnosed below 10 years of age (Table 3).

Previous treatment with cranial irradiation (CRT, OR=2.07), TBI (OR=3.17), and a total cumulative cisplatin dose ≥600 mg/m^2 (OR=3.75) significantly increased the risk of prefrailty in a multivariable model that was adjusted for amputation surgery (Table 3). CRT (OR=2.65), TBI (OR=3.28), cisplatin dose ≥600 mg/m^2 (OR=3.93), a higher cumulative dose of carboplatin (per g/m^2, OR=1.15), and a cyclophosphamide equivalent dose (CED) ≥20 g/m^2 (OR=3.90) were significantly associated with frailty. Especially survivors treated with a CRT dose ≥25 Gy were at increased risk of prefrailty (0 Gy=15.2%, 1-24 Gy=18.1%, 25+ Gy=24.3%, p=0.001) and frailty (0 Gy, 5.2%; 1-24 Gy, 4.8%; 25+ Gy, 11.3%, p=0.003). A sensitivity analysis only including survivors with complete frailty measurements showed similar results (Supplementary Table 3). However, in this model for prefrailty, cisplatin dose ≥600 mg/m^2 was not significant (OR=1.87, 95%CI=0.71-4.95).

Endocrine and lifestyle-related factors

In the multivariable model for prefrailty that included endocrine and lifestyle-related factors adjusted for patient characteristics and amputation surgery, GHD (OR=2.25), hyperthyroidism (OR=3.72), BMD Z-score ≤-1 and >-2 (OR=1.80), as well as BMD Z-score ≤-2 (OR=3.37) were all statistically significant (Table 4). Hyperthyroidism (OR=2.87) and BMD Z-score ≤-2 (OR=2.85) were significantly associated with frailty. Survivors with hypogonadism also had increased odds of prefrailty (OR=1.48, 95%CI=0.77-2.83) and frailty (OR=2.27, 95%CI=0.98-5.27), and survivors with GHD of frailty (OR=1.85, 95%CI=0.80-4.28), but this was not statistically significant. Of the survivors with GHD, 39.4% was being treated with GH replacement therapy at the time of the study.

	Prefrailty		Frailty		Sarcopenia	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	**P-value**
Hypogonadism	1.48 (0.77-2.83)	0.24	2.27 (0.98-5.27)	0.057	3.96 (1.40-11.18)	**0.009**
Growth hormone deficiency	2.25 (1.23-4.09)	**0.008**	1.85 (0.80-4.28)	0.15	4.66 (1.44-15.15)	**0.010**
Hyperthyroidism	3.72 (1.63-8.47)	**0.002**	2.87 (1.06-7.76)	**0.038**	-	**-**
Bone mineral density*						
Z-score >-1	Ref	Ref	Ref	Ref	Ref	Ref
-2< Z-score ≤-1	1.80 (1.31-2.47)	**<0.001**	1.42 (0.84-2.39)	0.19	1.48 (0.73-3.02)	0.28
Z-score ≤-2	3.37 (2.20-5.15)	**<0.001**	2.85 (1.54-5.29)	**0.001**	0.98 (0.40-2.42)	0.97
Severe vitamin D deficiency#	1.31 (0.88-1.95)	0.18	-	**-**	0.94 (0.40-2.24)	0.89
Vitamin B12 deficiency¶	-	**-**	-	**-**	6.26 (2.17-1.81)	**0.001**
Folic acid deficiency¥	1.87 (1.31-2.68)	**0.001**	2.04 (1.20-3.46)	**0.008**	-	-

Abbreviations: CI=confidence interval; No.=number; OR=odds ratio
Models adjusted for sex, attained age, BMI, and each other variable in the model. The model for prefrailty was also adjusted for amputation (significantly associated with prefrailty in a univariable model)
*At one or more skeletal sites (lumbar spine, total body, or total hip)
#25OHD levels <30 nmol/L
¶Vitamin B12 levels <150 pmol/L or vitamin B12 levels ≥150 and <220 pmol/L and homocysteine levels >19 µmol/L
¥Folic acid levels <6.8 nmol/L

Table 4. Endocrine and lifestyle-related risk factors for prefrailty, frailty, and sarcopenia using multivariable logistic regression analysis.

Folic acid deficiency (present in 14.9% of survivors) was significantly associated with prefrailty (OR=1.83) and frailty (OR=2.04) in multivariable analysis (Table 4). Severe vitamin D deficiency (present in 12.6%) had an odds ratio of 1.31 for prefrailty (95%CI 0.88-1.95). Vitamin B12 deficiency (present in 3.6%) was not significantly associated with prefrailty or frailty. Only 13.0% of survivors with at least one of these biochemical vitamin deficiencies had multiple deficiencies (Supplementary Figure 3).

Factors associated with sarcopenia

Risk factors for sarcopenia estimated from univariable models are presented in Supplementary Table 4.

Demographic and treatment-related factors

In multivariable analysis for sarcopenia, male sex (OR=4.56), lower BMI (continuous, OR=0.52), CRT (OR=3.87), and TBI (OR=4.52) were statistically significant (Table 3). Survivors treated with a CRT dose ≥25 Gy had a higher frequency of sarcopenia compared to those treated with <25 Gy (0 Gy=4.0%, 1-24 Gy=3.2%, 25+ Gy=8.1%), but this did not reach statistical significance (p=0.078).

Endocrine and lifestyle-related factors

Hypogonadism (OR=3.96), GHD (OR=4.66), and vitamin B12 deficiency (OR=6.26) were significantly associated with sarcopenia, whereas BMD Z-score ≤-1 and >-2, BMD Z-score ≤-2, folic acid deficiency, and severe vitamin D deficiency were not (Table 4). We added the interaction terms sex*GHD and sex*hypogonadism to this model to test for sex differences in the effect of endocrine disorders on sarcopenia, but low numbers hampered this analysis; 82% of survivors with sarcopenia and GHD, and 92% of survivors with sarcopenia and hypogonadism were male, respectively.

Interaction between treatment-related and endocrine factors

In addition to the models described above, we intended to build an additional model for prefrailty, frailty, and sarcopenia including all factors and interaction terms such as CRT*GHD, to assess whether associations between for example CRT and our outcomes were different for survivors with and without endocrine disorders. However, this was not possible due to small sample size.

DISCUSSION

In this national Dutch childhood cancer survivor cohort at a mean age of 33 years (median follow-up 25 years), the prevalence of prefrailty, frailty, and sarcopenia was 20.3%, 7.4%, and 4.4%, respectively. These frequencies are comparable with previous American studies in survivors with similar follow-up time,[4,8,9] indicating that this phenomenon is inherent to cancer treatment. In addition, we identified novel associations between demographic, treatment-related, endocrine, as well as lifestyle-related factors and (pre)frailty and sarcopenia in survivors.

The frailty prevalence that we confirmed in our study is conceivably high for this young adult population, illustrated by the fact that it exceeds the prevalence of community-dwelling elderly aged 65-74 years.[23] Since frailty covers broad aspects of the multimorbidity associated with aging, this suggests that childhood cancer survivors age faster than healthy peers. Our finding that reduced BMD, another condition that is typically observed in elderly, was independently associated with (pre)frailty, supports this interpretation. The associations that we found between genotoxic anti-cancer treatments and (pre)frailty as a proxy of aging is consistent with the growing notion that DNA damage is central to multimorbidity and the process of systemic aging in the general population.[24]

The observed association between underweight and (pre)frailty is conceivably linked with the presence of low lean mass in these individuals, as lower fat mass Z-score was not associated with (pre)frailty in univariable analysis. The relationship between obesity and prefrailty (mainly observed in female survivors) is thought to be twofold. First, obese individuals have a greater risk of disability and impaired physical function.[25] Second, obesity is linked with a proinflammatory state, which may be part of the physiological basis of frailty.[26] Alternatively, as in the general population, altered body composition (i.e. reduced lean mass and increased fat mass) may be the result of systemic aging as reflected by (pre)frailty, although this often happens without concomitant changes in BMI.[27] The bimodal pattern in the relationship between BMI and (pre) frailty has also been observed in elderly.[28]

We postulate that many of the identified treatment-related risk factors for (pre)frailty and sarcopenia affect these adverse outcomes not only through direct DNA damage but also through endocrine disorders. Our findings are in line with previously reported univariable associations between GHD and primary hypogonadism and frailty in survivors.[10,11] Moreover, in our study, GHD and hypogonadism were *independently* associated with one or more

aging phenotypes, highlighting the importance of both disorders. In addition, we found that hyperthyroidism was significantly associated with (pre)frailty, which is in accordance with a large prospective study in elderly which showed that higher FT4 levels were associated with incident frailty.[29] CRT (especially doses ≥25 Gy) and TBI were consistently associated with prefrailty, frailty, and sarcopenia, which may be through GHD, primary or secondary hypogonadism, or hyperthyroidism.[10,30,31] Results in this study are in line with previous literature showing an association between cisplatin doses ≥600 mg/m^2 and frailty.[8] We additionally found that higher doses of carboplatin and high doses of alkylating agents (≥20 g/m^2) were significantly associated with frailty. This may in part be through (primary) hypogonadism,[11,32] but alkylating agents and platinum-based drugs may impact the development of frailty by causing DNA damage as well.[33]

It is important to identify survivors at risk for (pre)frailty and sarcopenia early, as these phenotypes are characterized by an increased susceptibility to multiple morbidities and excess mortality. Our study identified several novel risk factors for (pre)frailty and sarcopenia in survivors, which could aid in the identification of at-risk individuals and targeted intervention. Primary prevention through dose reduction or changes in administration of associated treatment modalities without jeopardizing anti-tumor efficacy would be optimal, but this is not always possible.[34] However, for some agents such as platinum, achieving lower cumulative doses or alternative compounds are for some disease types being explored.[35] In the meantime, interventions such as nutritional support and physical activity (especially resistance exercise) have been shown to attenuate hallmarks of ageing in the general population.[36,37] In addition, our data suggest that treating hyperthyroidism as well as adequate supplementation in case of hypogonadism, GHD, or folic acid, and vitamin B12 deficiencies may have the potential to prevent or remediate frailty or sarcopenia in survivors. However, causality cannot be proven in a cross-sectional study. Although counterintuitive, there is for example also evidence that attenuation of the GH/IGF-1 somatotropic axis, which also occurs with natural aging and after DNA damage, is actually part of a beneficial response that shifts priorities from growth to maintenance and resilience mechanisms which aim to retard (accelerated) aging.[38] This emphasizes not only the importance of surveillance of endocrine deficiencies,[39] but also of adequate endocrine counseling and close monitoring of survivors receiving hormone replacement.

An alternative approach would be the use of senolytics. Genotoxic anti-cancer treatments (including radio- and most chemotherapies) induce cellular senescence, a state of irreversible growth arrest, which also encompasses a

senescence-associated secretory phenotype (SASP).[40] Senescent cells secrete numerous pro-inflammatory mediators that promote sterile inflammation, disrupt tissue structure and function, and thereby contribute to local and systemic aging-associated pathologies. Senolytics, agents that selectively eliminate senescent cells and diminish SASP,[41] have been reported to reduce inflammation and alleviate frailty in irradiated mice and elderly humans.[42,43] A clinical trial in American childhood cancer survivors is already underway (ClinicalTrials.gov Identifier: NCT04733534).

Our results need to be interpreted in the context of some limitations. First, there was no control group of 'healthy' young adults available to compare our (pre) frailty and sarcopenia prevalence with. Second, several characteristics of the participants differed significantly from the non-participants or underlying cohort, which could indicate selection bias. However, from all non-participants, detailed treatment data were only available from non-responders and not from refusers, and absolute differences were small. Third, we used low physical functioning as a proxy of slowness, although this correlated significantly with distance covered during the 6MWT in a subgroup. Lastly, we defined hypogonadism as survivors that had ever been diagnosed with this disorder, which induced a conceivable underestimation of its true prevalence.

In conclusion, in this national Dutch cohort of childhood cancer survivors with a mean age of 33 years, we showed that (pre)frailty and sarcopenia conceivably occur more than three decades earlier than in the general population and identified novel risk factors. These findings help to target individuals at high-risk of these debilitating aging phenotypes, and provide insights into new opportunities to potentially prevent them in upfront treatment but also during adult survivorship, which could increase survival and quality of life. In particular, our findings suggest that early identification and adequate counseling for endocrine disorders, as well as supplementation of dietary deficiencies may be crucial in minimizing the risk of (pre)frailty and sarcopenia for childhood cancer survivors. Future interventional studies are needed to assess the effect of these strategies.

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Covariate	Measurement and definition	Source
Body mass index	BMI was derived from height and weight measures (weight/height^2) and adjusted for amputation using estimated total body weight percentages of the amputated limb.	Amputee coalition
Body mass index category	Underweight: BMI <18.5 kg/m^2 Normal: BMI ≥18.5 kg/m^2 and <25 kg/m^2 Overweight: BMI ≥25 kg/m^2 and <30 kg/m^2 Obese: BMI ≥30 kg/m^2	WHO
Bone mineral density	Low BMD: Z-score ≤-1 Very low BMD: Z-score ≤-2 BMD was measured at the lumbar spine (L1-L4), total body, or total hip by dual-energy X-ray absorptiometry (Hologic, Marlborough, MA). Lumbar spine and total body BMD were measured in all centers, and total hip BMD in three centers.	ISCD
Fracture	Any fracture that occurred from five years after cancer diagnosis onwards, assessed by medical history.	NA
Heavy drinking	Males: >14 alcoholic consumptions per week (self-report) Females: >7 alcoholic consumptions per week (self-report)	NIAAA
Hypogonadism	Ever diagnosed with hypogonadism, assessed using medical charts.	NA
Growth hormone deficiency	Ever diagnosed with GHD, assessed using medical charts, or low IGF-1 levels according to age with a plausible reason to have GHD (e.g. treated with CRT). IGF-1 levels were assessed using the IDS-iSYS assay.	Manufacturer and expert opinion
Hyper-thyroidism	FT4 levels >24.3 pmol/L and TSH levels <0.56 mU/L. FT4 and TSH levels were assessed using the Fujirebio Lumipulse assay. Of note: FT4 was calibrated to the RMP. The threshold for hyperthyroidism for FT4 was derived from *de Grande et al.* (Clin Chem. 2017 Oct;63(10):1642-1652). TSH was calibrated according to the IFCC harmonization recommendation. The threshold for hyperthyroidism for TSH was derived from *Thienpont et al.* (Clin Chem. 2017 Jul;63(7):1248-1260).	De Grande et al. 2017 Thienpont et al. 2017
Hypo-thyroidism	FT4 levels <11 pmol/L or TSH levels >10 mU/L. FT4 and TSH levels were assessed using the Fujirebio Lumipulse G assay.	Manufacturer and expert opinion
Vitamin D deficiency	25OHD levels <50 nmol/L. 25OHD levels were assessed using the Fujirebio Lumipulse G assay.	Manufacturer
Severe vitamin D deficiency	25OHD levels <30 nmol/L. 25OHD levels were assessed using the Fujirebio Lumipulse G assay.	Manufacturer
Elevated homocysteine	Homocysteine levels >19 µmol/L. Homocysteine levels were assessed using the Cobas 6000 c501 assay.	Manufacturer
Vitamin B12 deficiency	Vitamin B12 levels <150 pmol/L or vitamin B12 levels ≥150 and <220 pmol/L with elevated homocysteine levels. Homocysteine and vitamin B12 levels were assessed using the Cobas 6000 c501 and c601 assay, respectively.	UpToDate
Folic acid deficiency	Folic acid levels <6.8 nmol/L. Folic acid levels were assessed using the Cobas 6000 c601 assay.	WHO

Abbreviations: 25OHD=25-hydroxyvitamin D; BMI=body mass index; BMD=bone mineral density; CRT=cranial irradiation; GHD=growth hormone deficiency; FT4=free thyroxine; IFCC=International Federation of Clinical Chemistry; IGF-1=insulin growth factor 1; ISCD=international society of clinical densitometry; NA=not applicable; NIAAA=national institute on Alcohol Abuse and Alcoholism; RMP=reference measurement procedure; TSH=thyroid stimulating hormone; WHO=world health organization

Supplementary Table 1. Definition of covariates.

	Prefrailty			Frailty		
	No. (%)	**OR (95% CI)**	**P-value**	**No. (%)**	**OR (95% CI)**	**P-value**
Demographics						
Sex						
Male	174 (17.1)	1.11 (0.87-1.41)	0.40	58 (5.7)	0.93 (0.64- 1.35)	0.70
Female	149 (15.7)	Ref	Ref	58 (6.1)	Ref	Ref
Attained age (per year)		0.99 (0.97-1.00)	0.10		0.99 (0.96-1.02)	0.42
First tertile	123 (18.8)			44 (6.7)		
Second tertile	103 (15.8)			40 (6.1)		
Third tertile	97 (15.0)			32 (4.9)		
Body mass index*						
Underweight	29 (42.0)	3.81 (2.30-6.32)	**<0.001**	12 (17.4)	3.43 (1.75-6.74)	**<0.001**
Normal	166 (16.0)	Ref	Ref	60 (5.8)	Ref	Ref
Overweight	68 (12.0)	0.71 (0.53-0.97)	**0.029**	23 (4.0)	0.69 (0.42-1.12)	0.13
Obese	53 (22.7)	1.55 (1.09-2.19)	**0.014**	17 (7.3)	1.28 (0.73-2.24)	0.38
Fat mass (per Z-score)		1.49 (1.30-1.71)	**<0.001**		1.54 (1.24-1.90)	**<0.001**
First tertile	63 (11.6)			20 (3.7)		
Second tertile	87 (16.0)			24 (4.4)		
Third tertile	137 (25.2)			54 (9.9)		
Age at diagnosis						
0 to 10 years	247 (16.2)	Ref	Ref	81 (5.3)	Ref	Ref
10 to 18 years	76 (17.3)	1.08 (0.82-1.43)	0.59	35 (8.0)	1.54 (1.02-2.33)	**0.039**
Follow-up time (per year)		0.98 (0.97-1.00)	0.062		0.98 (0.95-1.01)	0.12
First tertile	127 (19.4)			50 (7.6)		
Second tertile	104 (15.9)			35 (5.3)		
Third tertile	92 (14.1)			31 (4.8)		
Treatment-related factors						
HSCT‡		2.33 (1.56-3.48)	**<0.001**		2.94 (1.71-5.04)	**<0.001**
Yes	38 (29.9)			18 (14.2)		
No	282 (15.5)			97 (5.3)		
Total body irradiation		2.69 (1.67-4.34)	**<0.001**		3.35 (1.79-6.26)	**<0.001**
Yes	27 (33.3)			13 (16.0)		
No	293 (15.7)			101 (5.4)		
Cranial irradiation		1.66 (1.23-2.23)	**0.001**		1.97 (1.27-3.04)	**0.002**
Yes	71 (22.9)			30 (9.7)		
No	250 (15.2)			85 (5.2)		

	Prefrailty			Frailty		
	No. (%)	**OR (95% CI)**	**P-value**	**No. (%)**	**OR (95% CI)**	**P-value**
Cranial irradiation dose						**0.003**
0 Gy	250 (15.2)	Ref	Ref	85 (5.2)	NA	
<25 Gy	15 (18.1)	1.23 (0.69-2.19)	0.48	4 (4.8)		
≥25 Gy	54 (24.3)	1.79 (1.28-2.51)	**0.001**	25 (11.3)		
Abdominal irradiation		0.65 (0.39-1.10)	0.11		0.55 (0.22-1.37)	0.20
Yes	17 (11.6)			5 (3.4)		
No	303 (16.8)			109 (6.0)		
Platinum		1.42 (1.04-1.94)	**0.026**		1.62 (1.02-2.57)	**0.040**
Yes	61 (20.9)			25 (8.6)		
No	261 (15.6)			91 (5.5)		
Cisplatin		1.61 (1.09-2.36)	**0.016**		1.69 (0.96-2.98)	0.070
Yes	38 (23.2)			15 (9.1)		
No	284 (15.8)			101 (5.6)		
Cisplatin dose						
0 mg/m^2	284 (15.8)	Ref	Ref	101 (5.6)	Ref	Ref
<600 mg/m^2	23 (19.2)	1.26 (0.79-2.02)	0.33	10 (8.3)	1.53 (0.77-3.00)	0.22
≥600 mg/m^2	14 (35.9)	2.98 (1.53-5.81)	**0.001**	5 (12.8)	2.47 (0.94-6.45)	0.065
Carboplatin		1.23 (0.81-1.88)	0.34		1.71 (0.95-3.07)	0.072
Yes	29 (19.2)			14 (9.3)		
No	293 (16.2)			102 (5.6)		
Carboplatin dose (per g/m^2)		1.11 (1.00-1.23)	**0.040**		1.15 (1.03-1.30)	**0.015**
First tertile	8 (16.7)			4 (8.3)		
Second tertile	10 (20.4)			6 (12.2)		
Third tertile	9 (18.8)			3 (6.3)		
Alkylating agents		1.00 (0.78-1.28)	0.99		1.47 (0.98-2.19)	0.061
Yes	162 (16.3)			67 (6.7)		
No	142 (16.3)			41 (4.7)		
Alkylating dose (CED)						
0 g/m^2	142 (16.3)	Ref	Ref	41 (4.7)	Ref	Ref
<4 g/m^2	61 (15.3)	0.93 (0.67-1.29)	0.67	20 (5.0)	1.07 (0.62-1.86)	0.80
≥4 - <8 g/m^2	46 (16.5)	1.02 (0.71-1.46)	0.93	20 (7.2)	1.57 (0.90-2.72)	0.11
≥8 - <20 g/m^2	40 (15.0)	0.91 (0.62-1.33)	0.63	17 (6.4)	1.39 (0.77-2.48)	0.27
≥20 g/m^2	15 (28.8)	2.09 (1.12-3.90)	**0.021**	10 (19.2)	4.83 (2.27-10.31)	**<0.001**
Corticosteroids		0.78 (0.61-0.99)	**0.038**		0.61 (0.42-0.89)	**0.011**
Yes	151 (14.8)			47 (4.6)		
No	172 (18.3)			69 (7.3)		

	Prefrailty			Frailty		
	No. (%)	**OR (95% CI)**	**P-value**	**No. (%)**	**OR (95% CI)**	**P-value**
Corticosteroid dose						
0 g/m^2	172 (18.3)	Ref	Ref	69 (7.3)	Ref	Ref
<10 g/m^2	133 (14.2)	0.74 (0.58-0.95)	**0.018**	39 (4.2)	0.55 (0.37-0.82)	**0.004**
≥10 g/m^2	18 (20.7)	1.17 (0.68-2.01)	0.58	8 (9.2)	1.28 (0.59-2.76)	0.53
Methotrexate		0.93 (0.73-1.19)	0.57		0.82 (0.56-1.20)	0.31
Yes	146 (15.9)			49 (5.3)		
No	176 (16.9)			67 (6.4)		
Vinca alkaloids		0.62 (0.47-0.81)	**<0.001**		0.61 (0.40-0.92)	**0.020**
Yes	232 (14.9)			82 (5.3)		
No	90 (22.2)			34 (8.4)		
Anthracyclines		1.02 (0.80-1.30)	0.87		1.00 (0.68-1.46)	0.98
Yes	172 (16.5)			60 (5.8)		
No	146 (16.2)			52 (5.8)		
Amputation		2.60 (1.32-5.12)	**0.007**		NA	0.29[a]
Yes	13 (33.3)			4 (10.3)		
No	310 (16.1)			112 (5.8)		
Endocrine factors						
Hypogonadism		2.34 (1.51-3.64)	**<0.001**		3.00 (1.68-5.38)	**<0.001**
Yes	31 (30.4)			15 (14.7)		
No	292 (15.7)			101 (5.4)		
Growth hormone deficiency		2.91 (1.93-4.40)	**<0.001**		3.47 (2.01-5.99)	**<0.001**
Yes	38 (34.5)			18 (16.4)		
No	282 (15.4)			98 (5.3)		
Hyperthyroidism		4.04 (2.12-7.71)	**<0.001**		4.31 (1.93-9.60)	**<0.001**
Yes	17 (43.6)			8 (20.5)		
No	295 (16.0)			104 (5.7)		
Hypothyroidism		NA	0.70[a]		NA	1.00[a]
Yes	2 (18.2)			0 (0.0)		
No	310 (16.6)			112 (6.0)		
Bone mineral density[#]						
Z-score >-1	121 (12.2)	Ref	Ref	42 (4.3)	Ref	Ref
2< Z-score ≤-1	94 (22.9)	2.13 (1.58-2.87)	**<0.001**	28 (6.8)	1.65 (1.01-2.70)	**0.046**
Z-score ≤-2	58 (38.9)	4.57 (3.12-6.68)	**<0.001**	23 (15.4)	4.11 (2.39-7.06)	**<0.001**
Fracture		0.91 (0.70-1.18)	0.49		0.91 (0.60-1.39)	0.67
Yes	96 (15.8)			34 (5.6)		
No	216 (17.1)			77 (6.1)		

	Prefrailty			Frailty		
	No. (%)	**OR (95% CI)**	**P-value**	**No. (%)**	**OR (95% CI)**	**P-value**
Lifestyle-related factors						
Smoking						
Never	200 (17.1)	Ref	Ref	68 (5.8)	Ref	Ref
Former	32 (11.9)	0.66 (0.44-0.98)	**0.039**	13 (4.9)	0.82 (0.45-1.52)	0.54
Current	55 (19.6)	1.18 (0.84-1.65)	0.32	20 (7.1)	1.24 (0.74-2.09)	0.41
Heavy drinking[¶]		0.89 (0.41-1.90)	0.76		NA	0.77[a]
Yes	8 (15.1)			2 (3.8)		
No	282 (16.7)			100 (5.9)		
Vitamin D deficiency[¤]		1.14 (0.89-1.46)	0.30		1.08 (0.73-1.60)	0.69
Yes	125 (17.8)			44 (6.3)		
No	187 (15.9)			68 (5.8)		
Severe vitamin D deficiency[§]		1.61 (1.15-2.24)	**0.005**		1.21 (0.70-2.09)	0.50
Yes	53 (23.0)			16 (7.0)		
No	259 (15.7)			96 (5.8)		
Vitamin B12 deficiency[€]		1.40 (0.76-2.56)	0.28		1.34 (0.53-3.41)	0.54
Yes	14 (21.5)			5 (7.7)		
No	298 (16.4)			106 (5.8)		
Folic acid deficiency[¥]		1.91 (1.41-2.59)	**<0.001**		2.29 (1.48-3.56)	**<0.001**
Yes	70 (25.4)			30 (10.9)		
No	242 (15.1)			81 (5.1)		
Elevated homocysteine[ǁ]		1.90 (1.22-2.97)	**0.004**		1.47 (0.72-2.99)	0.29
Yes	29 (26.6)			9 (8.3)		
No	283 (16.0)			102 (5.8)		

Abbreviations: CED=cyclophosphamide equivalent dose; CI=confidence interval; HSCT=hematopoietic stem cell transplantation; NA=not available (n<5 in one of the cells); No.=number; OR=odds ratio; Ref=reference

*Adjusted for amputation

‡Only HSCTs with myeloablative conditioning regimens were included

[a]Fisher exact p-value

#At one or more skeletal sites (lumbar spine, total body, or total hip)

¶>14 alcoholic consumptions per week for males, and >7 for females

¤25OHD levels <50 nmol/L

§25OHD levels <30 nmol/L

€Vitamin B12 levels <150 pmol/L or vitamin B12 levels ≥150 and <220 pmol/L and homocysteine levels >19 µmol/L

¥Folic acid levels <6.8 nmol/L

ǁHomocysteine levels >19 µmol/L

Supplementary Table 2. Risk factors for prefrailty and frailty using univariable logistic regression analysis in the full cohort (n=2,003).

	Prefrailty		Frailty	
	OR (95% CI)	**P-value**	**OR (95% CI)**	**P-value**
Sex (male)	1.13 (0.82-1.55)	0.46	0.82 (0.50-1.32)	0.41
Attained age (per year)	0.98 (0.94-1.02)	0.24	0.97 (0.94-1.01)	0.14
Age at diagnosis				
0 to 10 years	-	-	Ref	Ref
10 to 18 years	-	-	1.77 (1.00-3.12)	0.050
Follow-up time (per year)	0.99 (0.95-1.03)	0.55		
Body mass index*			0.99 (0.93-1.05)	0.70
Underweight	6.32 (2.84-14.07)	**<0.001**		
Normal	Ref	Ref	NA	
Overweight	0.71 (0.48-1.05)	0.088		
Obese	1.57 (0.97-2.53)	0.064		
Cranial irradiation	1.64 (1.05-2.55)	**0.030**	1.88 (1.00-3.55)	**0.050**
Total body irradiation	2.56 (1.17-5.59)	**0.019**	3.41 (1.27-9.13)	**0.015**
Cisplatin dose				
0 mg/m^2	Ref	Ref	Ref	Ref
<600 mg/m^2	0.98 (0.51-1.89)	0.95	1.22 (0.50-3.00)	0.66
≥600 mg/m^2	1.87 (0.71-4.95)	0.21	3.36 (1.09-10.40)	**0.035**
Carboplatin dose (per g/m^2)	1.11 (0.98-1.26)	0.10	1.17 (1.00-1.35)	**0.045**
Alkylating dose (CED)				
0 g/m^2	Ref	Ref	Ref	Ref
<20 g/m^2	0.81 (0.58-1.13)	0.21	0.99 (0.60-1.65)	0.97
≥20 g/m^2	2.24 (0.96-5.24)	0.062	3.03 (1.03-8.89)	**0.043**

Abbreviations: CED=cyclophosphamide equivalent dose; CI=confidence interval; NA=not applicable; No.=number; OR=odds ratio; Ref=reference
The model for prefrailty was adjusted for amputation (significantly associated with prefrailty in a univariable model)
*Adjusted for amputation. Analyzed as a continuous variable in the model for frailty to retain sufficient power in this subset.

Supplementary Table 3. Demographic and treatment-related risk factors for prefrailty and frailty using multivariable logistic regression analysis in complete cases (n=1,114).

		Sarcopenia	
	No. (%)	**OR (95% CI)**	**P-value**
Demographics			
Sex			
Male	50 (76.9)	3.09 (1.72-5.56)	**<0.001**
Female	15 (23.1)	Ref	Ref
Attained age (per year)		0.97 (0.94-1.01)	0.096
First tertile	26 (5.2)		
Second tertile	23 (4.7)		
Third tertile	16 (3.3)		
Body mass index* (per kg/m²)		0.55 (0.48-0.62)	**<0.001**
First tertile	59 (12.2)		
Second tertile	5 (1.0)		
Third tertile	0 (0.0)		
Fat mass (per Z-score)		1.00 (0.75-1.33)	0.99
First tertile	22 (4.2)		
Second tertile	24 (4.7)		
Third tertile	19 (4.3)		
Age at diagnosis			
0 to 10 years	54 (4.7)	Ref	Ref
10 to 18 years	11 (3.5)	0.75 (0.38-1.44)	0.38
Follow-up time (per year)		0.99 (0.95-1.02)	0.51
First tertile	23 (4.7)		
Second tertile	24 (4.8)		
Third tertile	18 (3.7)		
Treatment-related factors			
HSCT‡		9.13 (5.10-16.3)	**<0.001**
Yes	20 (23.5)		
No	45 (3.3)		
Total body irradiation		10.39 (5.38-20.07)	**<0.001**
Yes	15 (27.3)		
No	49 (3.5)		
Cranial irradiation		1.66 (0.90-3.06)	0.10
Yes	14 (6.5)		
No	50 (4.0)		
Cranial irradiation dose			0.078[a]
0 Gy	50 (4.0)	NA	
<25 Gy	2 (3.2)		
≥25 Gy	12 (8.1)		

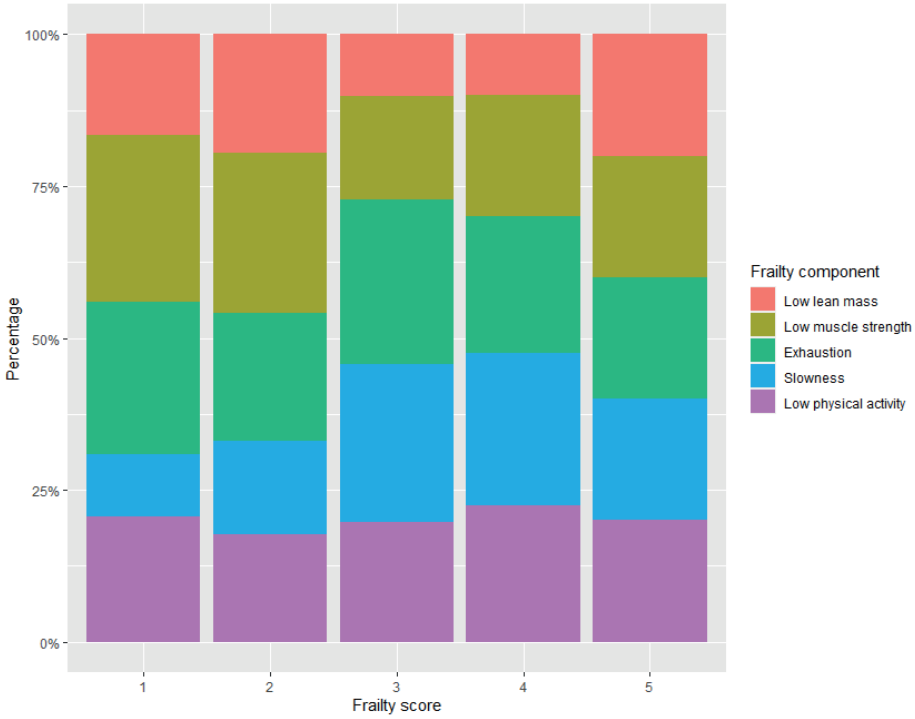
	Sarcopenia		
	No. (%)	**OR (95% CI)**	**P-value**
Abdominal irradiation		NA	0.31[a]
Yes	2 (2.0)		
No	62 (4.5)		
Platinum		1.11 (0.56-2.22)	0.76
Yes	10 (4.8)		
No	55 (4.4)		
Cisplatin		1.30 (0.55-3.08)	0.55
Yes	6 (5.6)		
No	59 (4.3)		
Cisplatin dose (cat)			0.44[a]
0 mg/m^2	59 (4.3)	NA	
<600 mg/m^2	4 (4.8)		
≥600 mg/m^2	2 (8.0)		
Carboplatin		1.25 (0.53-2.95)	0.62
Yes	6 (5.4)		
No	59 (4.3)		
Alkylating agents		1.21 (0.71-2.06)	0.49
Yes	33 (4.4)		
No	24 (3.7)		
Alkylating dose (CED, per g/m^2)		1.01 (0.98-1.05)	0.53
First tertile	7 (2.6)		
Second tertile	15 (6.3)		
Third tertile	11 (4.5)		
Corticosteroids		0.63 (0.38-1.03)	0.068
Yes	28 (3.5)		
No	37 (5.5)		
Corticosteroid dose			
0 g/m^2	37 (5.5)	Ref	Ref
<10 g/m^2	21 (2.8)	0.50 (0.29-0.78)	**0.014**
≥10 g/m^2	7 (11.5)	2.23 (0.95-5.24)	0.066
Methotrexate		0.80 (0.49-1.33)	0.40
Yes	28 (3.9)		
No	37 (4.9)		
Vinca alkaloids		0.50 (0.29-0.86)	**0.012**
Yes	44 (3.7)		
No	21 (7.2)		

	Sarcopenia		
	No. (%)	**OR (95% CI)**	**P-value**
Anthracyclines		0.99 (0.59-1.65)	0.96
Yes	33 (4.2)		
No	29 (4.3)		
Amputation		NA	1.00
Yes	1 (4.0)		
No	64 (4.4)		
Endocrine factors			
Hypogonadism		6.02 (3.03-11.96)	**<0.001**
Yes	12 (19.0)		
No	53 (3.8)		
Growth hormone deficiency		4.06 (2.03-8.13)	**<0.001**
Yes	11 (14.1)		
No	54 (3.9)		
Hyperthyroidism		NA	0.34[a]
Yes	2 (7.4)		
No	62 (4.4)		
Hypothyroidism		NA	0.27[a]
Yes	1 (14.3)		
No	63 (4.4)		
Bone mineral density[#]			
Z-score >-1	18 (1.9)	Ref	Ref
2< Z-score ≤-1	27 (7.1)	3.92 (2.13-7.21)	**0.046**
Z-score ≤-2	19 (13.7)	8.16 (4.17-15.99)	**<0.001**
Fracture		0.86 (0.50-1.49)	0.60
Yes	19 (4.0)		
No	45 (4.6)		
Lifestyle-related factors			
Smoking		0.67 (0.35-1.26)	0.22
Never	39 (4.5)		
Former or current	13 (3.0)		
Heavy drinking[¶]		NA	0.40[a]
Yes	0 (0.0)		
No	52 (4.1)		
Vitamin D deficiency[¤]		1.21 (0.72-2.01)	0.47
Yes	26 (4.9)		
No	38 (4.1)		

	Sarcopenia		
	No. (%)	**OR (95% CI)**	**P-value**
Severe vitamin D deficiency[§]		1.92 (1.00-3.67)	**0.050**
Yes	12 (7.5)		
No	52 (4.0)		
Vitamin B12 deficiency[€]		5.08 (2.26-11.38)	**<0.001**
Yes	8 (17.4)		
No	56 (4.0)		
Folic acid deficiency[¥]		0.94 (0.46-1.93)	0.86
Yes	9 (4.2)		
No	55 (4.4)		
Elevated homocysteine[ǁ]		3.68 (1.85-7.34)	**<0.001**
Yes	11 (12.9)		
No	53 (3.9)		

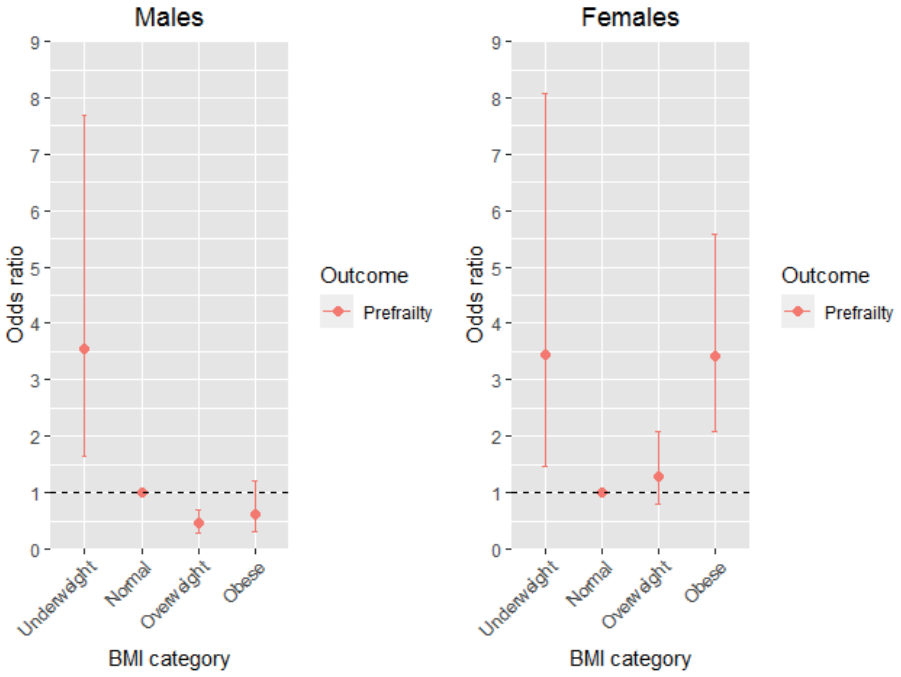
Abbreviations: CED=cyclophosphamide equivalent dose; CI=confidence interval; HSCT=hematopoietic stem cell transplantation; NA=not available (n<5 in one of the cells); No.=number; OR=odds ratio; Ref=reference
*Adjusted for amputation
[‡]Only HSCTs with myeloablative conditioning regimens were included
[a]Fisher exact p-value
[#]At one or more skeletal sites (lumbar spine, total body, or total hip)
[¶]>14 alcoholic consumptions per week for males, and >7 for females
[¤]25OHD levels <50 nmol/L
[§]25OHD levels <30 nmol/L
[€]Vitamin B12 levels <150 pmol/L or vitamin B12 levels ≥150 and <220 pmol/L and homocysteine levels >19 μmol/L
[¥]Folic acid levels <6.8 nmol/L
[ǁ]Homocysteine levels >19 μmol/L

Supplementary Table 4. Risk factors for sarcopenia using univariable logistic regression analysis (n=1,472).



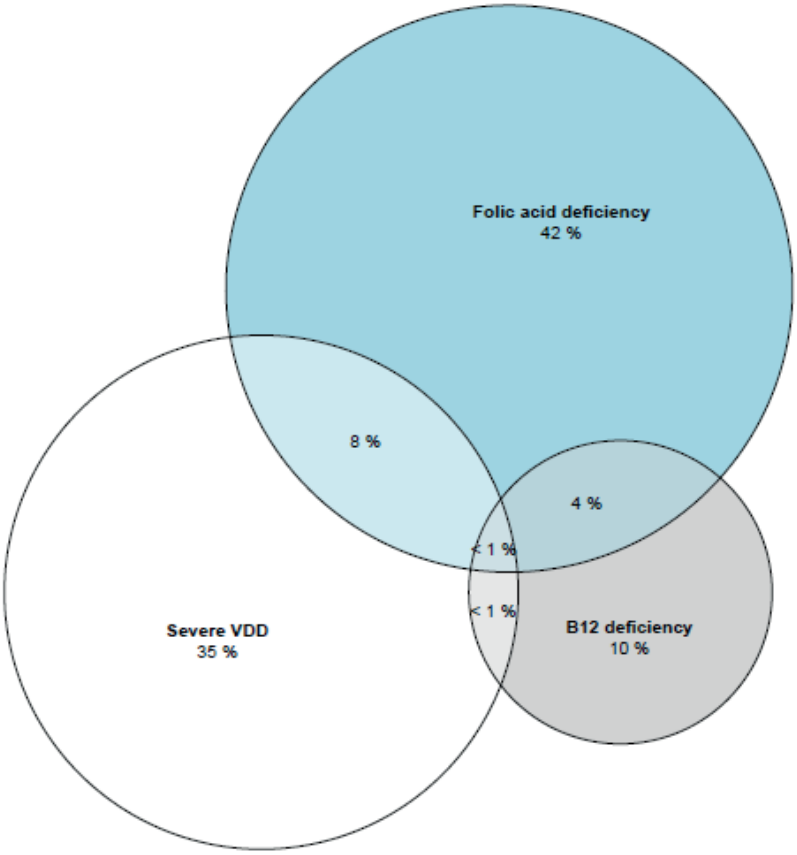
For each frailty score, the absolute percentage of the frailty components that were present in survivors with this frailty score is depicted.

Supplementary Figure 1. Contribution of frailty components to the frailty score in survivors with complete frailty measurements (n=1,114).



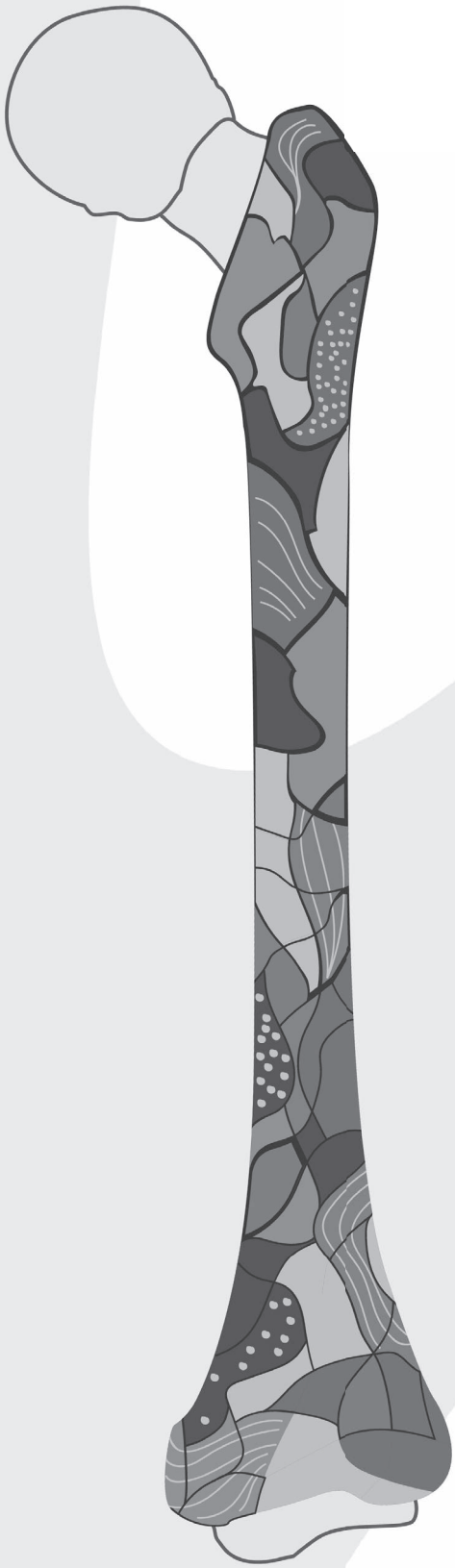
Abbreviations: BMI=body mass index.

Supplementary Figure 2. Visualization of the interaction effect between BMI category and sex in the multivariable model for prefrailty shown in Table 2.



Abbreviations: B12=vitamin B12; VDD=vitamin D deficiency
All survivors with at least one biochemical vitamin deficiency (n=684) represent 100%.

Supplementary Figure 3. Euler diagram of biochemical vitamin deficiencies among childhood cancer survivors.



CHAPTER 9

General discussion and future perspectives

The aim of this thesis was to increase knowledge on the prevalence of, and risk factors for bone toxicity and accelerated aging in children with cancer and childhood cancer survivors, to identify those childhood cancer survivors that may benefit from bone mineral density surveillance, and to identify potential interventions for these sequelae through identification of novel modifiable risk factors. The findings in this thesis may have considerable implications with regard to (primary) prevention, timely detection, and interventions for individuals at high-risk of these conditions, as well as for future research, as discussed below.

OSTEONECROSIS DUE TO ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) TREATMENT

Improving early detection and treatment

It had been well-established that children older than 10 years were at increased risk of osteonecrosis.[1] This thesis showed that symptomatic, *severe* osteonecrosis during or shortly after ALL treatment especially occurred in children aged 15 to 18 years (Chapter 2). Awareness about the specific increased risk in this age group may lead to earlier recognition and detection of severe osteonecrosis. As the absolute risk of osteonecrosis was shown to be very high for these patients (about one third developed symptomatic osteonecrosis, of which more than half of the cases concerned severe osteonecrosis), we would urge clinicians to perform an MRI of affected joints in case of the slightest persistent bone pain during or shortly after treatment in these patients if the pain cannot be explained by vincristine neuropathy. Such an aggressive case-finding approach may enhance early identification of children with severe osteonecrosis, which facilitates intervention before the affected joint has collapsed. This could potentially reduce the risk of long-term morbidity and increase consequent quality of life. We would not advise routine osteonecrosis screening by MRI due to the current lack of evidence that asymptomatic lesions are clinically significant, as well as the fact that there are limited effective treatment options.

It has been previously shown by our group that osteonecrosis symptoms resolved in only 40% of patients who were treated conservatively within five years.[2] Conservative treatment of osteonecrosis may include weight-bearing restrictions and pharmacologic treatment with for example bisphosphonates, prostacyclin analogs, or statins.[3,4] Although some of these interventions seem to alleviate pain, it is unclear whether they actually prohibit osteonecrosis progression and enhance functional performance. Moreover, during the period

that we performed our studies, preclinical research from our colleagues from St. Jude Children's Research Hospital (Memphis, TN, USA) suggested that zoledronic acid may reduce the antileukemic efficacy of chemotherapy based on research in xenograft models.[5] As antileukemic efficacy naturally remains the key priority, we are currently investigating the oncological safety of the use of bisphosphonates and recombinant human parathyroid hormone by assessing their influence on in vitro chemotherapy sensitivity of acute lymphoblastic leukemia cells.

In addition, the extent of, and risk factors for long-term (i.e. more than five years after ALL diagnosis) osteonecrosis-related morbidity, the impact on quality of life and societal participation, as well as their relationship with radiological findings remain currently unknown. This will be investigated by our group in future studies. Insights into clinical and radiological risk factors for long-term morbidity could aid clinical decision making *during* ALL treatment. This is challenging, as an undeniably effective intervention is currently not available for patients with osteonecrosis. Reducing or discontinuing corticosteroids may be considered, but this needs to be carefully weighed against the need for ongoing corticosteroid therapy from an oncological standpoint, especially in teenagers with ALL.[4]

Surgical interventions such as core decompression are being used in patients with osteonecrosis in an attempt to prevent joint collapse, but results are not convincing.[6,7] It is certainly not advised in patient with lower grade osteonecrosis, as 40% of osteonecrosis is self-limiting. A joint replacement may be indicated for patients with grade IV (Ponte di Legno grading) or grade V (Niinimäki classification) osteonecrosis with persistent pain and functional limitations, resulting in significant functional improvements.[3,4,7] However, this is undoubtedly an unfavorable outcome in these young individuals given the short life span of prostheses relative to the life expectancy of children. Therefore, more research focused on possibilities to prevent, timely diagnose (e.g. by assessing the predictive value of biomarkers), and better treat osteonecrosis is needed. As osteonecrosis has been associated with BMD decline[8,9] (Chapter 3),[10] results of these studies may have beneficial effects beyond reducing osteonecrosis-related morbidity.

Prevention

Efforts to prevent osteonecrosis are of key importance, as effective treatment options are limited and associated morbidity may be severe.[3] The only evidence-based strategy to prevent osteonecrosis in the context of acute lymphoblastic leukemia (ALL) that had been published prior to this thesis consisted of administering shorter pulses dexamethasone.[11] In this randomized controlled

trial in American children aged 10 to 21 years with high-risk ALL, a novel alternate-week schedule of dexamethasone (10 mg/m2 per day on days 0–6 and 14–20) was compared with standard continuous dexamethasone (10 mg/m2 per day on days 0–20) in permuted blocks within double or single delayed intensification phases, respectively. As this finding had not been replicated in Europe, we compared historical data of our national Dutch cohort of children with ALL treated according to three consecutive Dutch protocols over approximately 20 years (1997-2015). We did not find a significant difference in the cumulative incidence of symptomatic osteonecrosis for children treated according to the most recent Dutch ALL protocols that administered short pulses dexamethasone versus earlier protocols that administered long pulses dexamethasone during post-consolidation.[12] We postulate that there may indeed be a protective effect of shorter pulses dexamethasone on osteonecrosis occurrence, but that this may be attenuated by recent intensification of other treatment components such as (PEG-)asparaginase. This finding is subsequently supported by studies from other groups, in which the role of asparaginase on the development of osteonecrosis is being more and more recognized as well.[13] For example, increased rates of orthopedic toxicity (including osteonecrosis) have also been observed in late-generation compared to early-generation Dana-Farber Cancer Institute ALL Consortium protocols, which included PEG-asparaginase instead of *E. coli* asparaginase.[14] These authors suggested that the observed increased osteonecrosis risk in late-generation protocols may be driven by the pharmacokinetic drug interaction between PEG-asparaginase and dexamethasone, leading to higher dexamethasone exposure. This is in line with our previously reported finding that administration of the combination of dexamethasone and asparaginase may lead to reduced levels of antithrombin and protein S, resulting in a hypercoagulable state.[15] The insight into the detrimental effect of the combination of dexamethasone and asparaginase on the skeleton may provide opportunities for primary prevention of osteonecrosis. We suggest that future studies (in mice and humans) assess the effect of certain regimen modifications on the development of osteonecrosis in a randomized fashion, such as alternating the administration of dexamethasone and asparaginase. However, as the synergistic effect of dexamethasone and (PEG-)asparaginase may also have contributed to the increased survival of children on recent ALL protocols that intensified (PEG-)asparaginase, clinical trials that closely monitor leukemic responses are needed to obtain results in a safe oncological setting. In addition, the implementation of therapeutic drug monitoring of asparaginase in recent pediatric ALL protocols (e.g. ALLTogether1) may prove beneficial with regard to osteonecrosis occurrence.

In the meantime, other efforts are being made to reduce osteonecrosis (resulting from dexamethasone and asparaginase exposure). Recently, hypertension was shown to be a risk factor for osteonecrosis in children with ALL, and administration of antihypertensive drugs led to a decrease in the frequency of osteonecrosis resulting from chemotherapy in mice.[16] The effect of intensive compared to conventional antihypertensive therapy is currently being investigated in children with ALL in the USA (ClinicalTrials.gov Identifier: NCT04401267). Likewise, the fact that hyperlipidemia was shown to increase the risk of osteonecrosis[17] led to the initiation of a trial investigating the effect of lipid lowering agents (i.e. fish oil) in children with ALL in Denmark (ClinicalTrials.gov Identifier: NCT04209244).

The results of our findings were integrated in the standard of care protocol for osteogenic toxicities in the Princess Máxima Center for Pediatric Oncology (Appendix).

REDUCED BONE MINERAL DENSITY AND FRACTURES AMONG CHILDHOOD CANCER SURVIVORS

Prediction of survivors at high risk of reduced BMD

Prior to this thesis, it had been shown that low BMD (Z-score ≤-1) and very low BMD (Z-score ≤-2) are common in long-term childhood cancer survivors.[18] In addition, many studies had assessed demographic, treatment-, and lifestyle-related risk factors for reduced BMD.[18–20] However, it remained unknown which individual survivors were at highest absolute risk of reduced BMD. Within the framework of this thesis project, we developed prediction models for low and very low BMD in a large cohort of childhood cancer survivors from St. Jude Children's Research Hospital, and externally validated these models in a single-center Dutch cohort (Chapter 4).[21] We found that low and very low BMD could be quite adequately predicted for an individual survivor based on the clinical factors sex, height, weight, attained age, previous treatment with cranial or abdominal irradiation, and smoking status. The fact that we were able to identify high-risk survivors is important, as it guides clinicians in which survivors may benefit from BMD assessment by dual-energy X-ray absorptiometry (DXA). It may also inform cost-benefit assessments and aid financially, as insurance companies may now be convinced that they should cover costs of DXA scans for survivors at high risk of (very) low BMD. Moreover, it supports health care professionals to take the combined effect of multiple risk factors into account when assessing a survivor's risk of reduced BMD, and not just one treatment-related risk factor, which makes

predictions more specific. The discriminative capacity of the prediction models may be further improved when the effect of additional determinants such as endocrine disorders (e.g. hypogonadism or growth hormone deficiency [GHD]) will be evaluated.

In addition, equally treated survivors show differences in BMD, which suggests a role for genetic susceptibility in developing reduced BMD. In the general population, many single nucleotide polymorphisms (SNPs) were shown to be associated with BMD.[22,23] Currently available studies in survivors used a candidate gene or whole exome sequencing approach, were hampered by small sample sizes, lacked replication or functional validation, or mainly included ALL survivors.[24–26] We will assess the genetic susceptibility to reduced BMD in survivors by a candidate SNP as well as by a genome-wide association study (GWAS) approach in our national childhood cancer survivors included in the DCCSS-LATER study. This may potentially lead to the development of polygenic risk scores by adding replicated SNPs to the prediction models, thereby possibly increasing the diagnostic performance of the models. Moreover, these genetic susceptibility studies may also identify novel (therapy-specific) SNPs and associated genes, which could provide further insights into the mechanisms of therapy-related bone loss and underlying pharmacokinetic processes.

Internationally harmonized recommendations for BMD surveillance

Clinical practice guidelines are important to provide consistent, evidence-based care. Several national guidelines for BMD surveillance in childhood cancer survivors had existed for some years, but these guidelines all lacked a systematic review of the literature.[27–30] As a result, high-risk groups and timing of surveillance varied considerably across these guidelines, hampering effective implementation and adherence. In this thesis project, we developed internationally harmonized BMD surveillance recommendations for childhood, adolescent, and young adult cancer survivors under the umbrella of the International Late Effects of Childhood Cancer Guideline Harmonization Group (IGHG, Chapter 5).[31] BMD surveillance is now recommended for survivors treated with cranial/craniospinal or total body irradiation using DXA at entry into long-term follow-up (between two to five years after completion of therapy), and if normal (Z-score >–1), again at 25 years of age. These recommendations are on the conservative side, as we decided (after careful consideration of the benefits and harms of BMD surveillance) to only recommend BMD surveillance for treatment-related risk factors with at

least moderate quality evidence for very low BMD (Z-score ≤-2). For example corticosteroids did not meet these strict criteria. We therefore think that it is likely that this surveillance strategy will lead to early identification of very low BMD in survivors without conducting many unnecessary evaluations. However, the timing of the recommended surveillance strategy was largely based on consensus. Especially since only low quality evidence for an association between low BMD and fractures was identified in survivors, the yield of our surveillance recommendations needs to be investigated in future studies.

Despite its widespread use and being considered the gold standard for BMD measurement, the recommended surveillance modality DXA has several limitations. Most importantly in children, DXA measures the bone mineral density of a three-dimensional bone two-dimensionally.[32] As a result, DXA provides areal bone mineral density (in g/cm^2) and not volumetric bone mineral density (in g/cm^3), which leads to a systematic underestimation of bone mineral density in small individuals. However, volumetric BMD of the vertebrae can be approached by calculating the bone mineral apparent density using estimates of vertebral body depth.[33] Second, DXA only provides information about bone mineral density and not about other factors that are important for bone strength such as bone geometry and microarchitecture. Other diagnostic modalities such as quantitative computed tomography and quantitative ultrasound provide this information in addition to bone mass,[34] but these modalities are currently mainly considered research techniques as they have other disadvantages and clear associations with fracture risk still need to be established.[35] An advantage of DXA scans is that besides BMD, it can reliably measure body composition.

It is important to recognize that developing guidelines does not automatically result in their use.[36] To facilitate clinical implementation, involvement of all relevant people in guideline development is essential, and the developed proposal for change should ideally be evidence-based, globally and clinically relevant, feasible, and attractive.[37] Several strategies for successful guideline implementation have been developed, which can be classified as provider- or workflow-focused.[36] For example guideline dissemination, education and training of professionals, social interaction (e.g. educational outreach visits and marketing), and providing automated decision support systems and standing orders may aid in optimal clinical implementation.[36,38] Our particular surveillance guideline was drafted by 36 experts from 10 different countries, reviewed by several survivor representatives, and presented at international conferences. We believe that embedment within long-term follow-up services can be further

improved by developing (local) surveillance protocols per disease or treatment modality, ideally with automated reminders in the electronic medical records. After successful implementation, our guideline will conceivably lead to early identification of reduced BMD, improved quality of care, patient outcomes, and cost-effectiveness.[36] Nevertheless, surveillance guideline implementation is an ongoing process, which also requires repeated updates based on novel solid evidence.

We think that our prediction models for low and very low BMD (Chapter 4) and our BMD surveillance guideline (Chapter 5) are a helpful addition to each other and can be used concurrently. In the surveillance guideline, we extensively evaluated the literature to provide insight in which factors put survivors at a higher *relative* risk for low or very low BMD. In addition, we drafted recommendations for the surveillance modality to be used, timing of surveillance, and interventions when reduced BMD is identified. These recommendations were based on literature in childhood cancer survivors as well as guidelines from the general population or expert opinion when evidence in survivors was insufficient. After carefully weighing the benefits and harms of BMD surveillance, we were able to provide clear guidance on which survivors might benefit when from BMD surveillance using this approach. However, for example for survivors treated with corticosteroids, no recommendations could be formulated for or against BMD surveillance due to conflicting evidence. We recommended that this surveillance decision should be made by the childhood, adolescent, and young adult cancer survivor and health-care provider together, after careful consideration of the potential harms and benefits and additional risk factors. Our externally validated prediction model is the only available tool that enables physicians to calculate a survivor's *absolute* risk of (very) low BMD based on *multiple* risk factors. For example for survivors treated with steroids, its use provides an evidence-based way to further support the surveillance decision for an individual survivor. However, several known risk factors for low BMD such as TBI, hypogonadism, and growth hormone deficiency could not be incorporated in this prediction model. Currently, we are making efforts to validate and further improve our risk prediction models by adding these and other factors in the national Dutch survivor cohort (DCCSS-LATER cohort). In addition, it will be of value to validate the models in survivors aged younger than 18 and over 40 years, as well as in non-white survivors. This may lead to their incorporation in clinical practice surveillance guidelines and general use for surveillance in survivors.

Identification of novel determinants for reduced BMD in childhood cancer survivors

Prior to this thesis, our group and others had gained much knowledge on the prevalence, risk factors, and consequences of reduced BMD during and after childhood cancer treatment. For example, our group had shown that BMD determines fracture rate in children with ALL[39], and identified many novel clinical and (pharmaco)genetic risk factors for reduced BMD in children with cancer and survivors.[40–46] We were recently also able to create and validate a prediction model for bone fragility during ALL therapy.[47] However, the prevalence and determinants of reduced BMD had not been assessed in any national cohort of childhood cancer survivors. Through intensive collaboration, we were able to establish a national cohort of Dutch childhood cancer survivors (the DCCSS-LATER cohort) that had been treated for childhood cancer between 1963 and 2001 to perform such a study (Chapter 7). In this study, we identified several novel risk factors for reduced BMD and further supported the evidence for previously suggested risk factors in childhood cancer survivors, including male sex, underweight, shorter follow-up time, total body irradiation, cranial irradiation, carboplatin, alkylating agents, hypogonadism, growth hormone deficiency, hyperthyroidism, low physical activity, severe vitamin D deficiency, vitamin B12 deficiency, and folic acid deficiency. Vitamin B12 and folic acid deficiency are potential novel modifiable risk factors for reduced BMD. Whether supplementation of these vitamins will indeed lead to prevention or improvement of reduced BMD needs to be evaluated in interventional studies.

At this point, the most important unanswered question was probably whether reduced BMD is associated with fractures, and whether interventions for low BMD will lead to less fractures in survivors. This had been investigated and confirmed in elderly adults for several interventions.[48] However, as low BMD in younger adults often originates from a different cause than in older adults,[49] these findings are not automatically generalizable to children, adolescents, and young adults.

Towards early identification and preventing fractures in adult childhood cancer survivors

Risk and risk factors of fractures

In the framework of this thesis project, we were able to address part of this unanswered question in our national cohort of childhood cancer survivors (DCCSS-LATER cohort, Chapter 7). First, by taking into account sex- and age-

adjusted person years at risk, we showed that childhood cancer survivors are at increased risk of experiencing any first fracture. The risk of any first fracture was 3.5 times increased for male survivors and 5.4 times increased for female survivors compared with the general population. Second, we showed in a multivariable model that any fracture, long bone fracture, and fragility fracture that occurred more than 5 years after discontinuation of therapy were significantly associated with reduced BMD in adult childhood cancer survivors with a median follow-up of 25 years. Moreover, very low lumbar spine BMD (Z-score ≤-2) was identified as the most important risk factor for long bone and fragility fractures. These findings underscore the relevance of our prediction model for reduced BMD and our recommendations for BMD surveillance, as it is now more likely that treatment of reduced BMD may indeed prevent fractures in survivors. However, the influence of reduced BMD on incident future fractures needs to be validated in a longitudinal study.

In addition to reduced BMD and clinical fractures, this thesis has expanded knowledge on the risk of and risk factors for vertebral fractures in childhood cancer survivors, as these are indicative of osteoporosis. We were the first to investigate this in a representative single center cohort of very long-term survivors of all types of childhood cancer. We found that 13.3% of childhood cancer survivors had a prevalent vertebral fracture, and that the vast majority of these fractures were asymptomatic. The latter finding is in line with two studies that investigated vertebral fractures in short- and long-term survivors of childhood ALL.[50,51] This illustrates that current estimates of bone fragility among survivors may be underestimated. Therefore, vertebral imaging may be considered in survivors of childhood cancer, especially in those with very low BMD. Based on our findings, routine vertebral imaging may even be considered in survivors treated with spinal radiotherapy or platinum drugs, who have GHD, or those who have limited physically activity. However, it is unclear whether these factors increase the risk independently of for example older age and BMD. Therefore, these results need to be validated in larger cohorts to allow replication using multivariable models.

Prevention of and interventions for reduced BMD during and after cancer treatment

Ideally, BMD decline during childhood cancer therapy (e.g. glucocorticoid-induced BMD decline) would be prevented. In adults who are on long-term corticosteroid treatment, bisphosphonates have been shown to prevent bone loss[52] and are prophylactically administered in patients at high risk of fracture.[53] However, in

young individuals, this therapy is typically considered a last option and reserved for those with overt bone fragility.[54] This is mainly due to concerns about conditions related to chronic bone turnover suppression in children, including atypical femur fractures and osteonecrosis of the jaw, the lower absolute fracture risk in young individuals, as well as the lower efficacy of bisphosphonates in low bone turnover states. In addition, bisphosphonate administration has been suspected to interfere with leukemia treatment efficacy.[5]

Adequate dietary vitamin D and calcium intake are also important to optimize bone mineralization, and some clinicians routinely supplement vitamin D during childhood cancer treatment in an attempt to prevent BMD decline. However, the effect of universal vitamin D supplementation on bone strength remained unknown prior to this thesis. We therefore systematically reviewed the literature on this topic, and found very low quality evidence for a beneficial effect of vitamin D supplementation during childhood cancer therapy on BMD and fracture frequency (Chapter 6).[55] We subsequently generated consensus recommendations based on literature in other pediatric populations and expert opinions. We recommend dietary vitamin D/calcium intake in keeping with standard national guidelines, and periodic 25OHD monitoring to detect levels <20 ng/ml. Vitamin D/calcium supplementation is only recommended in children with low levels. This strategy has now been implemented in the standard of care protocol for osteogenic toxicities in the Princess Máxima Center (Appendix).

In addition to acute bone loss, we and others have shown that childhood cancer treatment may lead to several endocrine disorders that have been associated with reduced BMD after treatment, such as GHD and hypogonadism (Chapter 7).[56–58] Growth and sex hormone replacement therapy have been shown to arrest bone loss in deficient individuals.[59–62] Despite this and other health benefits (e.g. improved body composition), there are several considerations to take into account when deciding to initiate hormone replacement therapy. Growth hormone replacement requires a daily subcutaneous injection,[63] which may be burdensome for survivors. In addition, there have been concerns about administering growth hormone in cancer survivors due to presumed risk of secondary tumors.[64] However, a recent meta-analysis showed that the risk of secondary cancers is not increased in adults with hypopituitarism treated with growth hormone versus non-replacement,[65] and a large cohort with long-term follow-up of patients treated with recombinant human growth hormone during childhood recently showed that all-cause mortality was associated with underlying diagnosis and not with growth hormone therapy.[66] Also in childhood

cancer survivors, the risk of a second neoplasm does not seem to be increased after growth hormone therapy.[67] Sex hormone replacement therapy has also several risks, such as a slight increase in breast or prostate cancer rates and strokes.[68,69] We advise shared decision making when counseling about hormone replacement therapy, after carefully weighing the benefits and risks, which is in line with the IGHG recommendations for cancer treatment-related endocrine disorders.[70] If replacement therapy is initiated by an endocrinologist, surveillance of patients is warranted.

When no underlying condition is identified, measures to optimize bone health remain to consist primarily of lifestyle interventions with regard to nutrition and physical activity.[31] This is important for all children with cancer and survivors, but especially when (very) low BMD is expected or detected. If these interventions are insufficient, pharmacological treatment with bisphosphonates may decrease fracture risk, but treatment indications for young individuals are limited (as previously discussed). Therefore, novel bone-modifying agents for children, adolescents, and young adults are needed. For example human parathyroid hormone,[71] Denosumab (a RANKL inhibitor),[72] Sclerostin (a Wnt signaling pathway inhibitor),[73] and odanacatib (a cathepsin K inhibitor)[74] have shown promising results on BMD in preclinical models and in adults with osteoporosis, but merit further investigation in younger individuals.

ACCELERATED AGING IN CHILDHOOD CANCER SURVIVORS

In the general population, low BMD is typically observed in elderly,[48] which led to our hypothesis that childhood cancer survivors may age faster in general. Indeed, two American studies have indicated that frailty, an important component of physiologic decline, occurs earlier in childhood cancer survivors compared with the general population.[75,76] Radiotherapy and chemotherapy conceivably induce permanent alternations in DNA structure and function in nonmalignant cells.[77] Together with impaired cellular repair mechanisms and certain lifestyle choices, this may lead to reduced molecular integrity and functional decline.[77,78] This explanation is in line with accumulating evidence that DNA damage plays a central role in the physiologic aging process as well.[79] In this thesis**,** we showed that frailty and sarcopenia seem to occur more than three decades earlier in childhood cancer survivors compared to the general population based on data from our national cohort of the earliest Dutch childhood cancer survivors treated

from 1963 to 2001 in the Netherlands (DCCSS-LATER cohort, Chapter 8). The fact that the frequencies that we found for these phenotypes in survivors with a mean age of 33 years are in line with those reported in the American studies with a similar follow-up time, indicates that accelerated aging may be inherent to cancer treatment. We identified novel associations between demographic, treatment-related, endocrine, and lifestyle-related factors and (pre)frailty and sarcopenia, which helps to identify survivors at high risk of accelerated aging, as well as reveals new possibilities to prevent these conditions in upfront treatment and during adult survivorship. In particular, we found that several endocrine disorders (i.e. GHD, hypogonadism, hyperthyroidism, and very low BMD) and vitamin deficiencies (i.e. folic acid deficiency and vitamin B12 deficiency) were significantly associated with (pre)frailty or sarcopenia in adult childhood cancer survivors.

The diagnosis of frailty and sarcopenia in survivors is clinically meaningful. One previous study in childhood cancer survivors (mean follow-up 25 years) showed that frailty was significantly associated with several grade 3 to 4 chronic conditions, including adverse respiratory, gastrointestinal, liver, genitourinary, neurologic, psychiatric, and second malignancy conditions, but not with adverse musculoskeletal/integument or endocrine/metabolic and breast conditions.[76] Associations between other chronic conditions observed in the Dutch LATER cohort and prefrailty, frailty, and sarcopenia need to be further evaluated in future studies. In the general population, it has already been shown that frailty is a distinct syndrome with some overlap with disability and comorbidities.[80] Moreover, we learned from longitudinal childhood cancer survivor studies that baseline frailty (i.e. frailty at start of the study) was associated with the onset of chronic conditions and excess mortality.[76,81] The development of (polygenic risk) prediction models for (pre)frailty and sarcopenia could aid in identifying survivors at high risk of being affected, providing opportunities for targeted surveillance and intervention.

Whether the with (pre)frailty or sarcopenia associated endocrine disorders and vitamin deficiencies precede (pre)frailty or sarcopenia and are therefore modifiable risk factors, or are a result of systemic aging, remains unknown (Chapter 8). For anabolic hormones, two conceivable scenarios for this association have been described in the general population.[82] First, in response to DNA damage and physical stress, an appropriate compensatory decline in anabolic hormone levels occurs in order to prioritize maintenance and healing over growth.[83,84] If such a decrease persists due to the severity of the damage for example, low anabolic hormone levels would merely represent a state of

physiological stress. Alternatively, as anabolic hormones play an important role in muscle building, low levels could conceivably contribute to frailty and sarcopenia.[85] Hyperthyroidism and higher FT4 serum concentrations have been associated with prevalent but not incident frailty in males from the general population.[86,87]

Testosterone supplementation has been shown to increase muscle mass and strength in men.[88] In adults with GHD, some studies have shown that treatment with recombinant human growth hormone increased muscle mass and strength.[89,90] For patients with hyperthyroidism, no intervention studies with thyroid surgery, radioactive iodine, or thyroid suppressive drugs are available.

Also for vitamin deficiencies, insights can be gleaned from the general population. Vitamin D deficiency has been associated with incident frailty in multiple studies in elderly,[91–93] and vitamin D supplementation has been shown to improve muscle strength and balance, but not muscle mass or the risk of falls.[94–96] Although severe vitamin D deficiency (25OHD levels <30 nmol/L) showed an odds ratio for prefrailty of 1.41 (Chapter 8), this association was not significant (p=0.053). We found that folic acid and vitamin B12 deficiency were significantly associated with (pre)frailty or sarcopenia. Earlier studies in elderly revealed that high homocysteine levels were associated with frailty,[97] and a contribution of low vitamin B12 and folic acid levels to this association has been suggested.[98,99] Longitudinal studies are needed to establish the possible causal effects of endocrine disorders and vitamin deficiencies on (pre)frailty and sarcopenia in the general population and childhood cancer survivors. In addition, randomized controlled trials could adequately investigate the effect of hormone replacement therapy and vitamin supplementation on (pre)frailty and sarcopenia.

As for low bone mineral density, the current hallmark of frailty and sarcopenia management is to improve lifestyle habits. Exercise interventions are key in this respect, because they have shown to increase muscle mass, strength, and functional ability in the general population.[100–102] Furthermore, nutritional interventions may have (additional) benefits, but evidence for this is less convincing.[103,104] Future studies, as described above, are needed to identify novel effective preventive or interventional measures. Given the phenotype, tailoring these interventions for survivors to the needs of geriatric patients may be beneficial.

IMPLEMENTATION AND IMPACT

In this chapter, we discussed the place of our prediction model (Chapter 4), BMD surveillance guideline (Chapter 5), and consensus recommendations for vitamin D supplementation (Chapter 6) in the care for children with cancer and childhood cancer survivors. In Chapter 7 and 8, we identified associations between folic acid deficiency, vitamin B12 deficiency, and severe vitamin D deficiency and (pre) frailty, sarcopenia, and reduced BMD in childhood cancer survivors. Whether supplementation of these vitamins will indeed lead to prevention or improvement of these conditions still needs to be determined. In the meantime, we argue that routine assessments of these vitamin deficiencies during late-effect clinic visits and supplementation in case of deficits is reasonable, as this could significantly improve a survivors condition while potential harms seem little.

Our findings are especially important in the context of multiple toxicity and late effects due to childhood cancer treatment, because—as frailty and fractures have been associated with the onset of other chronic conditions—it is possible that the whole condition of survivors will improve as a result of our research and suggested future interventional studies. Moreover, our results highlight the importance of a life course approach to (bone) health in childhood cancer survivors regarding care and research, as our and other studies have shown that it is important to keep learning from adverse effects of treatment administered during childhood. Based on our findings, future preclinical and clinical studies may be initiated in order to better understand the mechanisms behind toxicity, as well as to assess the effect of primary cancer treatment adaptations or interventions during survivorship. Such an approach is becoming more and more important now that new treatment strategies such as immunotherapy and other targeted treatment regimens are being implemented of which the full spectrum of early and late side-effects in children still has to be determined.

ALL is the most common type of childhood cancer, and corticosteroids are an important part of this treatment. We therefore believe that some of our results apply not only to pediatric oncology, but also to children on long-term corticosteroid treatment for other diseases. This applies, for example, to our recommendations for monitoring vitamin D. Also, our *Survivor Information Brochure* on the benefits and harms of BMD surveillance after treatment with corticosteroids (that is included in our international BMD surveillance guideline) could be used in a different clinical setting.

REMAINING GAPS IN KNOWLEDGE AND FUTURE PERSPECTIVES

We believe that the research described in this thesis is an important step towards improving bone strength and preventing fractures and accelerated aging in children with cancer and childhood cancer survivors while using 'conventional' pediatric cancer treatment. In addition, we identified several gaps in knowledge that need to be addressed in future research (Table 1). These include directions for future research with regard to risk, detection, prevention, and treatment of (incident) osteonecrosis, reduced BMD, vertebral and non-vertebral low-trauma fractures, (pre)frailty, and sarcopenia in childhood cancer survivors.

Domain	Directions for future research
Symptomatic osteonecrosis	Effect of certain regimen modifications on the development of symptomatic osteonecrosis, such as alternating the administration of dexamethasone and asparaginase
	Predictive effect of biomarkers
	Prevention of symptomatic osteonecrosis, such as the effect and safety of bone modifying, anti-hypertensive, or lipid lowering agents on the development of symptomatic osteonecrosis
	Extent of, and risk factors (including radiological abnormalities) for osteonecrosis-related morbidity in very long-term survivors of childhood ALL, as well as its impact on quality of life (including physical performance and participation)
	Adverse effect of immunotherapy and other novel targeted treatment approaches on the prevalence of symptomatic osteonecrosis
Bone mineral density and fractures	Effect of corticosteroids on the risk of low and very low BMD with increasing follow-up time
	Independent effect of TBI and HSCT on the risk of low and very low BMD
	Risk and risk factors of low and very low BMD in childhood survivors older than 40 years
	Risk and risk factors of incident low-trauma vertebral and non-vertebral fractures in multivariable models, including treatment-related risk factors and other risk factors such as reduced BMD, a history of fractures and maternal hip fracture etc. (included in the FRAX® fracture risk profile for older adults), the most frequent sites of fractures, and the disability and impact on quality of life resulting from low-trauma fractures
	Further improvement and validation of prediction models (including genetic, demographic, lifestyle, and treatment factors) for low and very low BMD, and development of a prediction model for low-trauma fractures
	Genetic susceptibility markers to cancer therapy-related reduced BMD and fractures
	Risk and risk factors of impaired bone structure and its association (± BMD) with low-trauma fractures
	BMD trajectory and latency time of low-trauma fractures from cancer diagnosis into very long-term follow-up

Domain	Directions for future research
	Association between QCT, pQCT and QUS measurements and fracture risk
	Effect and safety of novel bony modifying agents (e.g. rPTH, Denosumab etc.) on BMD and fracture incidence
	Yield of the IGHG BMD surveillance recommendations
	Effect of physical activity, hormone replacement therapy, vitamin supplementation, and other nutritional interventions on low BMD, very low BMD, and fractures
Frailty and sarcopenia	Development of prediction models for the occurrence of (pre)frailty and sarcopenia
	Genetic susceptibility to cancer therapy-related (pre)frailty and sarcopenia
	Possible causal effects of endocrine disorders and vitamin deficiencies on (pre) frailty and sarcopenia using longitudinal studies
	Effect of physical activity, hormone replacement therapy, vitamin supplementation, and other nutritional interventions on (pre)frailty and sarcopenia

Abbreviations: ALL=acute lymphoblastic leukemia; BMD=bone mineral density; HSCT=hematopoietic stem cell transplantation; IGHG=International Late Effects of Childhood Cancer Guideline Harmonization Group; (p)QCT=(peripheral) quantitative computed tomography; QUS=quantitative ultrasound; rPTH=recombinant parathyroid hormone; TBI=total body irradiation

Table 1. Bone toxicity and accelerated aging due to childhood cancer treatment: domains for future research.

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APPENDIX

Standard of care protocol for diagnosis, management, and prevention of osteogenic toxicities in children with cancer (especially ALL) treated at the Princess Máxima Center for Pediatric Oncology

1. Bone mineral density and fractures

Definition

Osteoporosis in children: the presence of vertebral fractures is indicative of osteoporosis. In the absence of vertebral fractures, the diagnosis of osteoporosis is indicated by the presence of both a clinically significant fracture history and BMD Z-score ≤ -2.0. A clinically significant fracture history is one or more of the following: 1) two or more long bone fractures by age 10 years; 2) three or more long bone fractures at any age up to age 19 years.[1]

Diagnostics

- There is no indication for routine BMD surveillance by dual-energy X-ray absorptiometry (DXA) during treatment for childhood cancer.
- In children with ALL, younger age and relatively low body weight at ALL diagnosis can reasonably and easily predict bone mineral density at diagnosis and therefore bone mineral density course during therapy (http://lsbmd-risk-calculator.azurewebsites.net/).[2] This can be used to identify individual children at increased risk of fracture and bone fragility.
- A DXA scan (lumbar spine and whole body less head) at diagnosis is only recommended in the case of a history of clinically significant fractures. A DXA scan can be performed from the age of 4 years.
- Conventional X-rays are indicated for clinically suspected fractures.
- In case of acute back pain at ALL diagnosis or during therapy, a spinal X-ray is recommended (diagnosis of vertebral fractures using the Genant method).[3]

Prevention and intervention

All patients:

- A good nutritional status with sufficient calcium and vitamin D intake must be guaranteed.[4]
- This includes 400 IU/day of vitamin D and 200-1000 mg/day of dietary calcium, depending on age.[5] However, dietary calcium intake was shown to be often insufficient in Dutch children during ALL treatment.[6] If this is the case, 1 daily CalcichewD3 supplement (500-600mg Calcium + 10 microgram Cholecalciferol/day in 1 dose) is recommended.[4,8]

- Measure 25OHD levels at cancer diagnosis and every 6 months thereafter.[4]
- Start vitamin D supplementation in children with a deficiency (levels < 20 ng/ml). Initial dose: 2000 IU/day, higher dose if levels >20 ng/ml are not reached at 3 month follow-up.[4]
- It is important to assess which possibilities for movement and muscle strengthening are possible within the patient's capacity to exercise. If specific advice is needed: consult a physiotherapist.

In case of osteoporosis:

- In principle, do not adjust oncological therapy (only in consultation with the chairman of the protocol committee)
- Treatment options are limited. Bisphosphonates can be considered in the small group of children with severe bone fragility with a low potential for BMD restitution and vertebral body reshaping (= especially older children).[7]

Treatment with bisphosphonates

In the very rare case that bisphosphonates are considered (only after communication with the protocol chair), the adequate choice of drug and dosage for a particular patient can be determined in consultation with the local pediatric endocrinologist. Treatment can occur according to the scheme of Ward et al.[7]

- Pamidronic acid i.v.
  - Age <2 years: 0.5 mg/kg/day for 3 days, every 2 months; maximum dose 9 mg/kg/year
  - 2 years: 0.75 mg/kg/day for 3 days, every 3 months; maximum dose 9 mg/kg/year
  - ≥ 3 years: 1.0 mg/kg/day for 3 days, every 4 months; maximum dose 9 mg/kg/year
  - First cycle: half dose due to acute phase response. Infusion in NaCl 0.9% in 4 hours.
  - Alternative (lower) dose: 1 mg/kg/day in NaCl 0.9% in 4 hours, max 90mg/day, 1x/3 months

- Zoledronic acid iv is increasingly used in the clinic because it needs to be given less often and the infusion time is shorter than that of pamidronic acid.
  - Age <2 years: 0.025 mg/kg every 3 months (maximum 0.1 mg/kg/year)
  - ≥2 years: 0.05 mg/kg every 6 months (maximum 0.1 mg/kg/year)

- Oral bisphosphonates (risodronate, alendronate) are available, but their use is not encouraged in children. A child must sit upright for 30-60 minutes

after ingestion due to a fairly high risk of erosive gastritis/ulcer. This appears to be difficult to achieve in children and this has an influence on treatment adherence. This potential side effect is also highly undesirable with steroid use. However, this option can be considered in consultation with the pediatric endocrinologist and chair of ALL CIE.

- Risedronate (Actonel) (off label)
  - o <40 kg: 15 mg/dose, 1x per week
  - o ≥40 kg: 30-35 mg/dose, 1x per week
    Take the tablet sitting/standing upright with plenty of water at least 30 minutes before breakfast, do not lie down for 30 minutes after taking it. Only to be prescribed by a specialist with experience with this product.

- Alendronic Acid (Fosamax, Bonasol) (off label)
  - o 3 to 18 years: 10 mg/day in 1 dose
  - o < 30 kg: 35-40 mg/week
  - o 30kg 70mg/week
  - o Alternative: 1-2 mg/kg/week, max. 70 mg/week
    Take the tablet sitting/standing upright with plenty of water at least 30 minutes before breakfast, do not lie down for 30 minutes after taking it. Only to be prescribed by a specialist with experience with this product.

2. Symptomatic Osteonecrosis

Definition

Osteonecrosis in children with cancer: persisting pain in the extremities, not related to vincristine-induced neuropathy, in combination with characteristic findings on MRI.

- The clinical severity of osteonecrosis can be graded using the Ponte di Legno Toxicity Working Group criteria (PTWG criteria, Table 1).[1]
- Occurs mainly in patients with acute lymphoblastic leukemia or Non-Hodgkin lymphoma who are treated with corticosteroids and asparaginase.
- Risk groups: age >10 years (especially 15-18 years)[3,4]. Other risk factors that are mentioned in the literature, but that are less firmly established are: female sex, higher BMI, higher cumulative dose of corticosteroids, and hyperlipidemia.[5]

Diagnostics

- Diagnostic standard: MRI (Flair image) of the joint with the most complaints.
- A radiological classification of the severity of osteonecrosis is the Niinimäki classification (Table 2).[6]

- List the classification (MRI) and grade (PTWG) in the summary section of the patient chart.
- Asymptomatic patient: currently no indication for screening.

Prevention and intervention

- In case of PTWG or CTCAE grade 3/4, adjustments to the oncological treatment protocol can be considered:
  1. Reduce blocks of corticosteroids
  2. Halt the corticosteroids.[5,7,10]
- Adjustments only after consultation with the chairman of the protocol committee.
- Reduce weight-bearing activities as much as possible (use of crutches or wheelchair if necessary) and start non-joint-loading training aimed at preserving joint mobility and muscle strength under the guidance of an expert pediatric (oncology) physiotherapist.
- Adequate pain relief.
- Effect of bisphosphonates, lipid-lowering drugs (statins), blood pressure lowering drugs and anticoagulants have not been proven, only a favorable effect on pain has been described with bisphononates.[7-9]
- Be careful with surgical interventions, but consider a consultation of orthopedics to assess baseline severity and prognosis.
- Only in patients with larger lesions in the subchondral region (Niinimäki radiological grade 4), joint preservation surgery (core decompression, osteotomy, bone grafting) may be considered at an early stage to prevent joint collapse (with associated poor long-term outcomes).[10] Caution is advised, however, as at least 40% of all symptomatic ON is reversible.[3]

Grade	Grading osteonecrosis
1	Asymptomatic with findings only by MRI.
2	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions only outside joint lines in non-weight-bearing bones.
3	Symptomatic, not limiting or only slightly limiting self-care activity of daily living. Lesions in weight-bearing bones or affecting joint lines in non-weight-bearing bones.
4	Symptomatic with deformation by imaging of one or more joints and/or substantially limiting self-care activity of daily living.

Table 1. Ponte di Legno Toxicity Working Group criteria (PTWG criteria).[1]

Grade	Location of osteonecrosis: Weight-bearing bone*, Long bone	Weight-bearing bone*, Short bone	Non-weight-bearing bone, Long bone	Non-weight-bearing bone, Short bone
0	No osteonecrosis	No osteonecrosis	No osteonecrosis	No osteonecrosis
I	—	—	Diaphysis or metaphysis (0%)	Body (0%)
II	Diaphysis or metaphysis (0%)	Body (0%)	Epiphysis (<30%)	Surface (<30%)
III	Epiphysis (<30%)	Surface (<30%)	Epiphysis (≥30%)	Surface (≥30%)
IV	Epiphysis (≥30%)	Surface (≥30%)	—	—
V	Deformation of Joint**	Deformation of Joint**	Deformation of Joint**	Deformation of Joint**

The area of articular surface involvement (in percentage) is presented in parentheses.

*Weight-bearing bones comprise the lower extremities, the pelvis and lumbar vertebrae.

** Includes any deformation of the bone from sub-chondral local collapse to total destruction of the joint.

If there is multiple separate osteonecrotic lesions around a single joint, the overall grading is based on the grading assigned to the most severe lesion.

Definition of the location of osteonecrosis:

In *diaphysis or metaphysis of long bone*, the location of osteonecrosis is named by the affected bone.

In *epiphysis of long bone*, the location of osteonecrosis is named by the closest joint.

In *short bone*, the location of osteonecrosis is named by the affected extremity, except the location of osteonecrosis in *vertebra*, which is classified as "spine".

Table 2. Niinimäki radiological classification system osteonecrosis.[6]

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Osteonecrosis

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ENGLISH SUMMARY

The survival of children with cancer has greatly improved over recent decades, with current 5-year overall survival rates approximating 80% in high-income countries. As a result, the population of survivors is growing, and research on acute and long-term side effects of childhood cancer treatment to improve quality of life for survivors has become increasingly important. The aim of this thesis was to increase knowledge on the prevalence of, and risk factors for bone toxicity and accelerated aging among children with cancer and childhood cancer survivors, and to identify those childhood cancer survivors that may benefit from bone mineral density (BMD) surveillance. The identification of modifiable risk factors may potentially lead to novel interventions to prevent and treat these sequelae.

Prior to this thesis, the only effective measure to prevent symptomatic osteonecrosis (shown in an American study) was to administer shorter pulses dexamethasone during delayed intensification of childhood acute lymphoblastic leukemia (ALL) treatment. To assess this effect in Dutch treatment protocols, we included a national cohort of 1470 children treated for ALL between 1997 and 2015 according to the DCOG ALL-9, ALL-10 or ALL-11 protocol. In this study, we found no significant effect of shorter pulses dexamethasone (administered in the asparaginase-intensified ALL-10/11 protocols) compared to longer pulses dexamethasone (ALL-9) on the development of symptomatic osteonecrosis. We suggest that the protective effect of shorter pulses dexamethasone on osteonecrosis may be attenuated by recent intensification of asparaginase treatment, highlighting the relevance of therapeutic context when interpreting results of treatment-related toxicity. In addition, we observed that especially children aged 15-18 years developed symptomatic osteonecrosis (age 15-18 years 31.4% vs. 10-14 years 14.3% vs. 1-9 years 1.2%), and that the osteonecrosis was most often severe in this age group.

Our group had previously shown that in children with ALL, BMD decline during treatment is more pronounced in children with symptomatic osteonecrosis compared to children without symptomatic osteonecrosis. The decline in bone density starts right after ALL diagnosis but becomes more substantial following osteonecrosis diagnosis. This indicates that restriction of weight-bearing activities, that is generally advised to patients with osteonecrosis, could aggravate BMD decline. Subsequently, the association between osteonecrosis and BMD decline has been confirmed by a large American study in children with

ALL. In a narrative review about this association, we discussed common risk factors and possible mechanisms for osteonecrosis and BMD decline, including immobilization (i.e. weight-bearing restrictions) and its clinical implications.

Although many studies had identified risk factors for low and very low BMD in childhood cancer survivors, it remained unknown which survivors were at highest absolute risk of reduced BMD and might benefit from BMD assessment by dual-energy X-ray absorptiometry (DXA). In collaboration with the American St. Jude Lifetime Cohort (n=2,032), we successfully developed models for the prediction of low and very low BMD in adult survivors of childhood cancer, and externally validated these models in a single-center Dutch cohort (n=403). The models included male sex, lower weight, shorter height, younger attained age, smoking, and cranial and abdominal irradiation, and showed an area under the curve of 0.72 (validation: 0.69) for low BMD and 0.76 (validation: 0.72) for very low BMD. This means that our validated models, using easily measured predictors, correctly identified BMD status in most white adult survivors. To facilitate its clinical use, we designed an online calculator that can be used by clinicians to calculate the absolute risk of low and very low BMD for an individual survivor.

Clinical practice surveillance guidelines are important for timely diagnosis and treatment of childhood cancer survivors with reduced BMD as well. Discordances across current late effects guidelines necessitated international harmonisation of recommendations for bone mineral density surveillance to enhance implementation and adherence. We developed an international guideline for BMD surveillance with a panel of 36 experts from 10 countries. We carefully evaluated the evidence, and BMD surveillance is now recommended for survivors treated with cranial/craniospinal or total body irradiation using DXA at entry into long-term follow-up (between two to five years after completion of therapy), and if normal (Z-score >–1), again at 25 years of age. In addition, recommendations for the management of reduced BMD in survivors of childhood cancer were drafted. These recommendations facilitate evidence-based care for survivors internationally, which could improve bone mineral density parameters and prevent fragility fractures.

Vitamin D deficiency and low BMD are common in children with cancer, which has internationally led to universal vitamin D supplementation in these children. However, it remained unsettled whether this enhances bone strength. We addressed this issue by carrying out a systematic review with consensus recommendations from several international experts. Nineteen studies were

included. One study which analyzed 25-hydroxyvitamin D (25OHD) as a threshold variable (≤10 ng/ml) found a significant association between 25OHD levels and BMD Z-scores, while continuous 25OHD levels were not significantly associated with BMD Z-scores in 14 observational studies. We found neither a significant association between lower 25OHD levels and fractures (2 studies), nor between vitamin D/Ca supplementation and BMD or fracture frequency (3 studies) (very low quality evidence). Further research that assesses the rationale and effect of vitamin D and calcium supplementation in children with cancer is needed; until then, we recommend dietary vitamin D and calcium intake in keeping with national guidelines, and periodic 25OHD monitoring to detect levels <20 ng/ml. Vitamin D and calcium supplementation is recommended in children with low levels, to maintain levels ≥20 ng/ml.

Prior to this thesis, we and others had identified multiple risk factors for low and very low BMD in childhood cancer survivors. However, the prevalence and risk factors for reduced BMD in a national cohort of childhood cancer survivors, as well as risk factors for other important indicators of bone fragility (e.g. vertebral and non-vertebral fractures) were lacking. In the general population, reduced BMD is mainly of concern in elderly. Therefore, we hypothesized that childhood cancer survivors may also age faster in general. Frailty and sarcopenia are two important components of physiologic decline. Except for the results from one American cohort study, the risk and risk factors for (pre)frailty and sarcopenia remained largely unknown. We assessed the prevalence of and risk factors for low and very low BMD, vertebral and non-vertebral fractures, (pre)frailty, and sarcopenia in a national cohort of the earliest treated Dutch childhood cancer survivors (n=2,003). In this study, we showed that childhood cancer survivors have a 3.5 times (males) or 5.4 times (females) increased risk of experiencing any fracture, and also the frequency of vertebral fractures seems to be increased in this group (13.3%). For the first time, we were able to show in a multivariable model that reduced BMD (especially very low lumbar spine BMD) is indeed significantly associated with a history of fractures in childhood cancer survivors, which highlights the importance of timely identification and treatment of survivors with reduced BMD. Reduced BMD was also significantly associated with prefrailty and frailty, which supports our finding that childhood cancer survivors age earlier: prefrailty, frailty, and sarcopenia seem to occur 30 years earlier in survivors compared with the general population. We confirmed and identified several novel associations between previous cancer treatment (i.e. high-dose cisplatin, carboplatin, and alkylating drugs), endocrine disorders (i.e. growth hormone deficiency, hypogonadism, and hyperthyroidism) and vitamin

deficiencies (i.e. folic acid deficiency, vitamin B12 deficiency, and severe vitamin D deficiency) and reduced BMD, (pre)frailty, and sarcopenia in adult childhood cancer survivors. As many of these factors may be modifiable risk factors, these findings may be the first step in novel ways to prevent and treat these adverse conditions.

In conclusion, the studies described in this thesis have led to improved identification of individuals at high risk of bone toxicity, as well as to newly discovered determinants of bone toxicity and accelerated aging as a result of childhood cancer treatment. Our research is an important step towards improving bone strength and preventing fractures and accelerated aging in this vulnerable group. In addition, we identified gaps in knowledge and domains for future research on bone toxicity and accelerated aging during and after treatment for childhood cancer.

NEDERLANDSE SAMENVATTING

De overleving van kinderen met kanker is de afgelopen decennia sterk verbeterd. Tegenwoordig overleeft ongeveer 80% van de kinderen met kanker ten minste vijf jaar in landen met een hoog inkomen. Als gevolg hiervan groeit de populatie van overlevenden en wordt onderzoek naar acute bijwerkingen en bijwerking op lange termijn van de behandeling van kinderkanker om de kwaliteit van leven te verbeteren steeds belangrijker. Het doel van dit proefschrift was om de kennis over de prevalentie van en risicofactoren voor bottoxiciteit en versnelde veroudering bij kinderen met kanker en overlevenden van kinderkanker te vergroten, en om die overlevenden van kinderkanker te identificeren die baat kunnen hebben bij het screenen van de botmineraaldichtheid (BMD). De identificatie van aanpasbare risicofactoren kan mogelijk leiden tot nieuwe interventies om deze gevolgen te voorkomen en te behandelen.

Voorafgaand aan dit proefschrift was de enige effectieve maatregel om symptomatische osteonecrose te voorkomen (aangetoond in een Amerikaanse studie) het toedienen van kortere pulsen dexamethason tijdens de verlate intensiveringsfase van de behandeling van acute lymfatische leukemie bij kinderen. Om dit effect in Nederlandse behandelprotocollen te beoordelen, hebben we een nationaal cohort van 1470 kinderen geïncludeerd die tussen 1997 en 2015 voor ALL werden behandeld met het DCOG ALL-9-, ALL-10- of ALL-11-protocol. In deze studie vonden we geen significant effect van kortere pulsen dexamethason (toegediend in de asparaginase-geïntensiveerde ALL-10/11-protocollen) in vergelijking met langere pulsen dexamethason (ALL-9) op de ontwikkeling van symptomatische osteonecrose. We suggereren dat het beschermende effect van kortere pulsen dexamethason op osteonecrose kan worden afgezwakt door recente intensivering van behandeling met asparaginase, wat de relevantie van de therapeutische context benadrukt bij het interpreteren van studies naar behandelingsgerelateerde toxiciteit. Daarnaast lieten we zien dat vooral kinderen van 15-18 jaar symptomatische osteonecrose ontwikkelden (leeftijd 15-18 jaar 31,4% vs. 10-14 jaar 14,3% vs. 1-9 jaar 1,2%), en dat de osteonecrose het vaakst ernstig was in deze leeftijdsgroep.

Onze groep had eerder aangetoond dat bij kinderen met ALL de BMD afname in de loop van de behandeling meer uitgesproken is bij kinderen met symptomatische osteonecrose dan bij kinderen zonder symptomatische osteonecrose. De afname van de botdichtheid begint direct na ALL diagnose, maar wordt aanzienlijk na diagnose van osteonecrose. Dit geeft aan dat beperking van gewichtdragende

activiteiten, die over het algemeen wordt geadviseerd aan patiënten met osteonecrose, de BMD afname kan verergeren. Vervolgens is de associatie tussen osteonecrose en BMD afname bevestigd door een groot Amerikaans onderzoek bij kinderen met ALL. In een verhalende review over deze associatie bespraken we veelvoorkomende risicofactoren en mogelijke mechanismen voor osteonecrose en BMD afname, inclusief immobilisatie (d.w.z. gewichtsdragende beperkingen) en de klinische implicaties ervan.

Hoewel veel onderzoeken risicofactoren voor een lage en zeer lage BMD bij overlevenden van kinderkanker hadden geïdentificeerd, bleef het onbekend welke overlevenden het hoogste absolute risico hadden op verminderde BMD en mogelijk baat zouden hebben bij botdichtheid meting middels dual-energy X-ray absorptiometry (DXA). In samenwerking met het Amerikaanse St. Jude Lifetime Cohort (n=2.032) hebben we met succes voorspelmodellen ontwikkeld voor lage en zeer lage BMD bij volwassen overlevenden van kinderkanker, en deze modellen extern gevalideerd op een Nederlands cohort uit één ziekhuis (n=403). De modellen omvatten mannelijk geslacht, lager gewicht, kortere lengte, jongere leeftijd, roken en bestraling van de schedel en buik, en vertoonden een oppervlakte onder de curve van 0,72 (validatie: 0,69) voor lage BMD en 0,76 (validatie: 0,72) voor zeer lage BMD. Dit betekent dat onze gevalideerde modellen, met behulp van gemakkelijk te meten voorspellers, de BMD status correct identificeerden bij de meeste blanke volwassen overlevenden. Om het klinische gebruik ervan te vergemakkelijken, hebben we een online rekenmachine gemaakt die door clinici kan worden gebruikt om het absolute risico van een lage en zeer lage BMD voor een individuele overlevende te berekenen.

Richtlijnen voor screening zijn belangrijk voor een tijdige diagnose en behandeling van overlevenden van kinderkanker met een verminderde BMD. Inconsistenties in de huidige richtlijnen voor late effecten maakten internationale harmonisatie van aanbevelingen voor het screenen van botmineraaldichtheid noodzakelijk om de implementatie en het gebruik te verbeteren. We hebben een internationale richtlijn voor het screenen van BMD ontwikkeld met een panel van 36 experts uit 10 landen. We hebben bestaand bewijs zorgvuldig geëvalueerd, en BMD screening wordt nu aanbevolen voor overlevenden die worden behandeld met craniale/craniospinale of totale lichaamsbestraling met DXA bij aanvang van de langetermijn follow-up (tussen twee en vijf jaar na voltooiing van de therapie), en indien normaal (Z-score >–1), opnieuw op 25-jarige leeftijd. Daarnaast werden aanbevelingen opgesteld voor de behandeling van verminderde BMD bij overlevenden van kinderkanker. Deze aanbevelingen vergemakkelijken

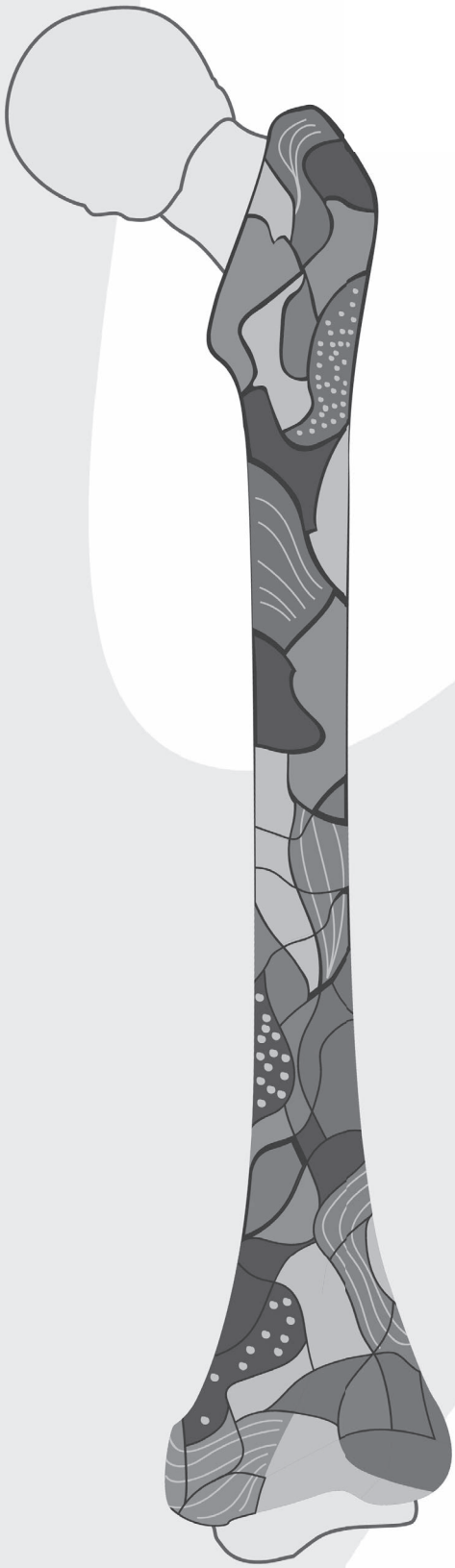
internationaal evidence-based zorg voor overlevenden, wat BMD parameters zou kunnen verbeteren en fracturen zou kunnen voorkomen.

Het veel voorkomen van vitamine D deficiëntie en een lage BMD heeft geleid tot universele vitamine D suppletie voor kinderen met kanker. Het blijft echter onzeker of dit de botsterkte verbetert. We hebben dit probleem uitgezocht door een systematische review uit te voeren en consensusaanbevelingen gemaakt met verschillende internationale experts. Negentien studies werden geïncludeerd. Een studie waarin 25-hydroxyvitamine D (25OHD) met als drempelvariabele ≤10 ng/ml werd geanalyseerd, vond een significant verband tussen 25OHD-spiegels en BMD Z-scores, terwijl continue 25OHD-spiegels niet significant geassocieerd waren met BMD Z-scores in 14 observationele studies. We vonden geen significant verband tussen lagere 25OHD-spiegels en fracturen (2 studies), noch tussen vitamine D/Ca-suppletie en BMD of fractuurfrequentie (3 studies) (bewijs van zeer lage kwaliteit). Verder onderzoek dat de grondgedachte en het effect van vitamine D en calciumsuppletie bij kinderen met kanker beoordeelt, is nodig; tot die tijd raden we inname van vitamine D/Ca via de voeding aan in overeenstemming met de nationale richtlijnen, en periodieke 25OHD monitoring om niveaus <20 ng/ml te detecteren. Vitamine D/Ca suppletie wordt aanbevolen bij kinderen met een laag gehalte, om gehaltes ≥20 ng/ml te handhaven.

Voorafgaand aan dit proefschrift hadden wij en anderen meerdere risicofactoren geïdentificeerd voor lage en zeer lage BMD bij overlevenden van kinderkanker. Echter ontbraken de prevalentie en risicofactoren voor verminderde BMD in een nationaal cohort van overlevenden van kinderkanker, evenals risicofactoren voor andere belangrijke indicatoren van botfragiliteit (bijv. vertebrale en niet-vertebrale fracturen). In de algemene bevolking is een verminderde BMD vooral een punt van zorg bij ouderen. Daarom veronderstelden we dat overlevenden van kinderkanker in het algemeen ook sneller ouder zouden kunnen worden. Kwetsbaarheid (frailty) en sarcopenie zijn twee belangrijke componenten van fysiologische achteruitgang. Afgezien van de resultaten van één Amerikaans cohortonderzoek, bleven het risico en de risicofactoren voor (pre)frailty en sarcopenie grotendeels onbekend. We onderzochten de prevalentie van en risicofactoren voor lage en zeer lage BMD, vertebrale en niet-vertebrale fracturen, (pre)frailty en sarcopenie in een nationaal cohort van de vroegst behandelde Nederlandse overlevenden van kinderkanker (n=2.003). In deze studie toonden we aan dat overlevenden van kinderkanker een 3,5 keer (mannen) of 5,4 keer (vrouwen) verhoogd risico hebben om een fractuur te krijgen, en ook de frequentie van wervelfracturen lijkt verhoogd te zijn in deze groep (13.3%). Voor het eerst

konden we in een multivariabel model aantonen dat een verminderde BMD (vooral een zeer lage BMD van de lumbale wervelkolom) inderdaad significant geassocieerd is met een voorgeschiedenis van fracturen bij overlevenden van kinderkanker, wat het belang van tijdige identificatie en behandeling van overlevenden met een verminderde BMD benadrukt. Een verminderde BMD was ook significant geassocieerd met prefrailty en frailty, wat onze bevinding ondersteunt dat overlevenden van kinderkanker sneller verouderen: prefrailty, frailty en sarcopenie lijken 30 jaar eerder op te treden bij overlevenden vergeleken met de algemene bevolking. We bevestigden en identificeerden verschillende nieuwe associaties tussen eerdere kankerbehandeling (d.w.z. hoge doses cisplatin, carboplatin en alkylerende geneesmiddelen), endocriene aandoeningen (d.w.z. groeihormoondeficiëntie, hypogonadisme en hyperthyreoïdie) en vitaminetekorten (d.w.z. foliumzuurdeficiëntie, vitamine B12 deficiëntie, en ernstige vitamine D deficiëntie) en verminderde BMD, (pre) frailty en sarcopenie bij volwassen overlevenden van kinderkanker. Omdat veel van deze factoren risicofactoren kunnen zijn waar op aangegrepen kan worden, zijn deze bevindingen waarschijnlijk de eerste stap om deze ongunstige aandoeningen te voorkomen en te behandelen.

Concluderend, de studies beschreven in dit proefschrift hebben geleid tot een verbeterde identificatie van individuen met een hoog risico op bottoxiciteit, evenals tot nieuw ontdekte determinanten van bottoxiciteit en versnelde veroudering als gevolg van de behandeling van kinderkanker. Ons onderzoek is een belangrijke stap in het verbeteren van de botsterkte en het voorkomen van fracturen en versnelde veroudering bij deze kwetsbare groep. Daarnaast identificeerden we hiaten in kennis en domeinen voor toekomstig onderzoek naar bottoxiciteit en versnelde veroudering tijdens en na de behandeling van kinderkanker.



ADDENDUM

A

List of abbreviations

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Dankwoord

LIST OF ABBREVIATIONS

ALL	Acute lymphoblastic leukemia
ALM	Appendicular lean mass
ASP	Asparaginase
AUC	Area under the curve
BMD	Bone mineral density
BMI	Body mass index
CCG	Children's Oncology Group
CED	Cyclophosphamide equivalent dose
CI	Confidence interval
CIsON	Cumulative incidence of symptomatic osteonecrosis
CNS	Central nervous system
CRT	Cranial irradiation
DCCSS	Dutch Childhood Cancer Survivor Study
DCOG	Dutch Childhood Oncology Group
DEXA	Dexamethasone
DXA	Dual-Energy X-ray absorptiometry
FN	Femoral neck
FT4	Free thyroxine
GHD	Growth hormone deficiency
HR	Hazard ratio
HSCT	Hematopoietic stem cell transplantation
IGF-1	Insulin-like growth factor 1
IGHG	International Late Effects of Childhood Cancer Guideline Harmonization Group
IQR	Interquartile range
LS	Lumbar spine
MRG	Medium risk group
MRI	Magnetic Resonance Imaging
OR	Odds ratio
PTH	Parathyroid hormone
PTWG	Ponte di Legno Toxicity Working Group
QCT	Quantitative computed tomography
QUS	Quantitative ultrasound
ROC	Receiver operating characteristic
SDS	Standard deviation score
SIR	Standardized incidence ratio
sON	Symptomatic osteonecrosis
TB	Total body
TBI	Total body irradiation
TBLH	Total body less head

TH	Total hip
TSH	Thyroid stimulating hormone
VDD	Vitamin D deficiency
VFA	Vertebral fracture assessment
$1,25(OH)_2D$)	1,25-dihydroxyvitamin D
25OHD	25-hydroxyvitamin D
6MWT	Six-minute walking test

CURRICULUM VITAE

Jenneke Elizabeth van Atteveld was born in Amsterdam (the Netherlands) on January 28, 1993. She graduated cum laude from secondary school in 2011 (Mendelcollege, Haarlem). That same year, she started her medical training at Utrecht University and obtained her medical degree in 2017. She dedicated her final year to pediatrics, and performed her last clinical and research internships at the Princess Máxima Center for Pediatric Oncology in Utrecht, the Netherlands. She also performed an internship at the Dana-Farber Cancer Institute/Boston Children's Hospital in Boston, MA, USA. In December 2017, she started her PhD trajectory at the Princess Máxima Center for Pediatric Oncology under the supervision of prof. dr. Marry M van den Heuvel-Eibrink and dr. Sebastian J.C.M.M. Neggers, which resulted in this thesis. During this trajectory, she took part in the Training of Upcoming Leaders In Pediatric Science (TULIPS) PhD curriculum 2019-2021, and was part of several committees for TULIPS. She combined her research activities with the supervision of master student and later fellow PhD student Demi T.C. de Winter. She is also an Editor for 4Pediatrics, an email service that provides a monthly overview of the last published abstracts of original articles, trials and reviews in the four leading pediatric journals. As of March 2022, she works as a pediatric resident at the Meander Medical Center in Amersfoort, the Netherlands.

LIST OF PUBLICATIONS

This thesis

van Atteveld JE, Pluijm SMF, Ness KK, Hudson MM, Chemaitilly W, Kaste SC, Robison LL, Neggers SJCMM, Yasui Y, van den Heuvel-Eibrink MM, Wilson CL. Prediction of low and very low bone mineral density among adult survivors of childhood cancer. *J Clin Oncol. 2019 Sep 1;37(25):2217-2225.*

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van Atteveld JE[#], Verhagen IE[#], van den Heuvel-Eibrink MM, van Santen HM, van der Sluis IM, Di Iorgi N, Simmons JH, Ward LM, Neggers SJCMM. [#]Shared first authorship. Vitamin D supplementation for children with cancer: A systematic review and consensus recommendations. *Cancer Med. 2021 Jul;10(13):4177-4194.*

van Atteveld JE, de Winter DTC, Pieters R, Neggers SJCMM, van den Heuvel-Eibrink MM. Recent perspectives on the association between osteonecrosis and bone mineral density decline in childhood acute lymphoblastic leukemia. *Faculty Reviews. 2021 10:(57).*

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on behalf of the Dutch LATER study group. [§]Shared first authorship. [¥]Shared last authorship. Risk and determinants of reduced bone mineral density and fractures in a national cohort of Dutch childhood cancer survivors (n=2,003): a DCCSS-LATER Study. *Lancet Diabetes Endocrinol. 2022 Dec 9:S2213-8587(22)00286-8.*

van Atteveld JE, de Winter DTC, Pluimakers VG, Fiocco M, Nievelstein RAJ, Hobbelink MGG, Kremer LCM, Ronckers CM, Grootenhuis MA, Maurice-Stam H, Tissing WJE, de Vries ACH, Loonen JJ, van Dulmen-den Broeder E, van der Pal HJ, Pluijm SMF, van der Heiden-van der Loo M, Versluijs AB, Louwerens M, Bresters D, van Santen HM, Hoefer I, van den Berg SAA, den Hartogh J, Hoeijmakers JHJ, Neggers SJCMM[‖], van den Heuvel-Eibrink MM[‖], on behalf of the Dutch LATER study group. [‖]Shared last authorship. Frailty and sarcopenia within the earliest national Dutch childhood cancer survivor cohort (n=2,003): a DCCSS-LATER Study. *Under review The Lancet Healthy Longevity.*

Other publications

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Pluimakers VG, **van Atteveld JE**, de Winter DTC, Fiocco M, Bolier M, Nievelstein RAJ, Janssens GOR, Bresters D, van der Heiden-van der Loo M, de Vries ACH, Louwerens M, van der Pal HJ, Pluijm SMF, Ronckers CM, Versluijs AB, Kremer LCM, Loonen JJ, van Dulmen-den Broeder E, Tissing WJE, van Santen HM, 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.*

PHD PORTFOLIO

Name: Jenneke van Atteveld
PhD period: December 2017 – February 2022
Research School: Clinical and Translational Oncology (Utrecht University)
Department: Pediatric Oncology
(Princess Máxima Center for Pediatric Oncology)
Promotor: Prof. dr. Marry M. van den Heuvel-Eibrink
Co-promotor: Dr. Sebastian. J.C.M.M. Neggers

1. PhD training	**Year**
Courses	
Regression Analysis – Boerhaave, LUMC (online)	2022
Academic Writing in English – GSLS, UU (online)	2020
Adobe Illustrator – GSLS, UU (online)	2020
Basic Methods and Reasoning in Biostatistics – Boerhaave, LUMC	2020
Advances in Genome-Wide Association Studies – NIHES, Erasmus MC	2020
SNP Course XVI: SNPs and Human Diseases – MolMed, Erasmus MC	2019
Basic Human Genetics Course: Genetics for Dummies – MolMed, Erasmus MC	2019
Basic Course on Regulation and Organization for Clinical Investigators (BROK) – NFU	2018
Seminars and Workshops	
Research Retreat Princess Máxima Center	2019, 2021
Joint Retreat Kitz - Princess Máxima Center (online)	2020
CTO PhD retreat	2019, 2020
PhD Retreat Van den Heuvel-Eibrink Group	2019, 2020
SKION LATER Research Day	2018
Weekly Research Seminars Princess Máxima Center	2017 - 2022
Weekly PhD Meetings Van den Heuvel-Eibrink Group	2017 - 2022

Conferences

Society for Endocrinology British Endocrine Society annual conference (SfE BES), Harrogate, England. *Invited speaker.*	2022
54th Congress of the International Society for Paediatric Oncology (SIOP), Barcelona, Spain. *Oral presentation*.	2022
10th International Conference on Children's Bone Health (ICCBH), Dublin, Ireland. *Oral presentation*.	2022
53rd Congress of the International Society for Paediatric Oncology (SIOP), virtual. *Oral presentation*.	2021
MASCC/ISOO Supportive Care in Cancer Annual Meeting, virtual. *Invited speaker.*	2021
2nd Annual Meeting of the of the European Society for Paediatric Oncology (SIOP-Europe), virtual. *Invited speaker.*	2021
12th Biennial Childhood Leukemia and Lymphoma Symposium, virtual. *Poster presentations.*	2021
51st Congress of the International Society for Paediatric Oncology (SIOP), Lyon, France. *Oral presentation.*	2019
60th American Society of Hematology (ASH) Annual Meeting and Exposition, San Diego, CA, USA. *Poster presentation*.	2018
50th Congress of the International Society for Paediatric Oncology (SIOP), Kyoto, Japan. *Poster presentation*.	2018
22nd Pan-European Network for Care of Survivors after Childhood and Adolescent Cancer (PanCare) meeting, Paris, France. *Oral presentation*.	2018
59th American Society of Hematology (ASH) Annual Meeting and Exposition, Atlanta, GA, USA.	2017
49th Congress of the International Society for Paediatric Oncology (SIOP), Washington D.C., USA.	2017

2. Teaching activities

Supervising a fellow PhD student	2021 - 2022
Supervising a master student	2019 - 2020
Role-play actor for the Teach the Teachers course – UMCU	2018 - 2022

3. Other activities

TULIPS alumni network committee	2021 - 2022
Organization of the TULIPS Grant Writing & Presenting Weekend	2021
Editor at 4Pediatrics	2020 - 2022
Training of Upcoming Leaders In Pediatric Science (TULIPS) PhD curriculum	2019 - 2021
Research internship at the Dana-Farber Cancer Institute / Boston Children's Hospital	2017

4. Awards

ICCBH New Investigator Award	2022
SIOP Young Investigator Award	2021
Dutch Society of Pediatrics/TULIPS Young Investigator Audience Award	2021
SIOP Young Investigator Award	2019

DANKWOORD

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Voor het onderzoek in dit proefschrift heb ik mogen samenwerken met vele co-auteurs, zowel nationaal als internationaal. Ik heb veel van jullie geleerd en genoten van het samen nadenken over onderzoeksopzet, methodologie, en de interpretatie en verwoording van de resultaten. Mijn speciale dank gaat uit naar *dr. R.L. Mulder, prof. dr. M. Fiocco, dr. S.M.F. Pluijm, prof. dr. L.C.M. Kremer, prof. dr. R. Pieters, drs. J. den Hartogh* en *dr. L.M. Vrooman*. Beste *Renée*, dank voor het delen van al je kennis over richtlijnontwikkeling, maar vooral ook bedankt voor

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Beste collega's van het CB LATER, beste *Margriet, Nynke, Lennart, Anke* en *Lucienne*. Het werk dat jullie hebben verzet voor de LATER studie is niet te beschrijven. Jullie nauwkeurigheid en onuitputtelijke aandacht hebben de data zo sterk gemaakt als ze zijn.

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