Osteoporos Int (2012) 23:665–674
DOI 10.1007/s00198-011-1605-2

ORIGINAL ARTICLE

Predictors of bone loss in childhood hematologic malignancies: a prospective study

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Received: 15 September 2010 / Accepted: 1 March 2011 / Published online: 8 April 2011

Abstract
Summary Twenty-nine children with malignancies and age, gender-matched controls were prospectively studied over 14 months. Patients had higher parathyroid hormone (PTH) levels and fat mass, lower bone mass, and bone mass increments at follow-up than controls. Lean mass, age at diagnosis, systemic and intrathecal therapy were predictors of bone mass changes on adjusted analyses.
Introduction Children with hematologic malignances have low bone mass. We prospectively investigated anthropometric, clinical, and hormonal predictors of changes in bone mass in children receiving cancer therapy.
Methods Twenty-nine children, mean age of 9±2.9 years and 32 age and gender-matched controls, were studied. Seven had completed their course 40±22 weeks prior, while 22 were still receiving therapy for 80±28 weeks. Age at diagnosis, calcium intake, exercise activity, systemic corticosteroids in dexamethasone (Dex) dose, and methotrexate (MTX), and intrathecal MTX therapy received within follow-up period were assessed. Routine chemistries, PTH, 25-hydroxy vitamin D (25-OHD), bone remodeling markers, bone mass, and body composition were measured at baseline and 14 months.
Results Patients had lower exercise activity, sun exposure, and bone markers levels than controls. They had higher PTH levels and fat mass, lower bone mass at the spine, hip, and total body, and lower increments at these sites on follow-up. Predictors of bone mass changes on univariate analyses were: age at diagnosis (R=−0.50 to −0.44, p< 0.05), Dex–MTX doses (R=−0.58 to −0.41, p<0.05), intrathecal therapy (p<0.03),% changes in lean mass (R= 0.37 to 0.54, p<0.04), 25-OHD levels (R=0.39, p<0.03), and PTH levels (R=−0.47 to −0.41, p<0.05). Lean mass, age at diagnosis, systemic and intrathecal therapy were predictors of bone mass changes on adjusted analyses.
Conclusion This study provides insight into the pathophysiology of bone loss in children receiving cancer therapy and possible interventions to optimize their skeletal health.

Keywords Age at diagnosis · Chemotherapy · Childhood malignancies · Intrathecal therapy · Prospective bone loss

Abbreviations

MTX	Methotrexate
CS	Corticosteroids
Dex	Dexamethasone
S-Ca	Serum calcium
S-P	Serum phosphorus
S-Mg	Serum magnesium
S-Cr	Serum creatinine
TSP	Total serum protein
PTH	Parathyroid hormone
25-OHD	25-hydroxy vitamin D
ALKP	Alkaline phosphatase
S-Osteocalcin	Serum osteocalcin
S-Crosslaps	Serum crosslaps
BMD	Bone mineral density
BMC	Bone mineral content
BMAD	Bone mineral adjusted density

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Introduction

Children with cancer have reduced bone mineral density (BMD) [1–8], decrements that may not be totally reversible [9]. This is particularly relevant in view of the improved survival of these children, thus increasing their risk for osteoporotic fractures in elderly life. Furthermore, a recent study reported vertebral compression fractures as an under-recognized complication of newly diagnosed acute lymphoblastic leukemia (ALL), the most common childhood malignancy and the one with the highest survival rate [10, 11]. Predictors of BMD deficits in survivors of childhood cancer were extensively reviewed by the Children Oncology Group [9]. The primary disease itself in acute lymphoblastic leukemia, chemotherapy including methotrexate (MTX) and glucocorticoids, and radiation therapy, were consistent predictors [9, 12–14]. Treatment may also lead to hypogonadism, a risk factor for low bone mass in children and adolescents [15, 16]. Indeed, we have recently demonstrated in a cohort of 41 children with cancer that bone maturation, a biological readout of sexual maturation, MTX and intrathecal therapy, were all significant predictors of bone mass Z-score at the spine and total body; bone maturation accounted for the greatest proportion in Z-score variance [7].

Studies evaluating bone loss and its predictors prospectively in children receiving therapy for malignancies are scarce and were either conducted in the induction phase or after completion of chemotherapy. None to our knowledge were conducted past the early induction phase [17–19]. Moreover, despite the fact that inadequate calcium and vitamin D nutritional status are risk factors for BMD deficits [9], and that low vitamin D and/or high PTH levels have been noted in few studies in children with cancer, the impact and contribution of abnormalities in calciotropic hormones to bone loss in children with cancer remains unknown.

In this study, we systematically investigated the impact of known anthropometric, clinical, and hormonal predictors of bone loss, prospectively measuring all such variables in a cohort of children followed past the initial high-dose chemotherapy induction period.

Methods

Study design

This is a case–control follow-up study of a cohort of children with hematologic malignancies receiving therapy at the Children's Cancer Center of Lebanon, (affiliated with St. Jude Children's Research Hospital, Memphis, TN, USA), at the American University of Beirut Medical Center and their 32 age and gender-matched controls [7]. Although the originally planned follow-up was supposed to be at 1 year, the mean follow-up period was 14±1 month, range 12–17 months. This was due to chemotherapy complications that delayed the follow-up at 1 year for some of the patients. Anthropometric, hormonal, and densitometric characteristics of the study cohort at study entry were previously reported in detail [Table 1; 7].

Inclusion criteria were a diagnosis of childhood blood malignancy, age >3 years, and Tanner stage 1. Only prepubertal children were studied at study entry to avoid the anticipated confounding effects of puberty on bone metabolism; furthermore, most children with ALL are prepubertal. Exclusion criteria included onset of puberty, a history of metabolic bone disease, or history of using drugs known to affect bone mass before the diagnosis of cancer. Age and gender-matched prepubertal, healthy control children were recruited from the pediatric outpatient clinic and from patients' siblings. The number of control subjects was 32. Twenty-six patients had acute lymphoblastic leukemia, and 3 had lymphoma, 2 with leukemia/lymphoma, and 1 with Hodgkin's lymphoma. This reflects the usual patient mix in general and of the patient populations at the Children Cancer Center in particular. The children with ALL were treated on the Children's Cancer Center of Lebanon ALL protocol, which is adopted from the St. Jude TOTAL XV protocol. Patients received induction including prednisone for 4 weeks, and consolidation with four courses of high-dose MTX. They also received Dex as part of reinduction and as pulses during maintenance [20]. The majority of children, 21/29 of patients and 21/32 of the controls subjects remained at Tanner stage one at follow-up. The proportion of subjects who matured beyond Tanner stage 1 was not significantly different between the two groups (p=0.51). The study was approved by the Institutional Review Board at the American University of Beirut; written assents were obtained from children, and written informed consents from their parents or guardians.

Data collection

Height and weight were measured and plotted on standardized updated growth curves of the Center for Disease Control. Body mass index (BMI) was calculated as the weight in kilograms divided by height in square meters. Pubertal status was assessed by two physicians according to the established criteria (SM, AA) [21]. Sun exposure and exercise activity were assessed at entry and follow-up. Calcium intake assessed through a Food Frequency Questionnaire targeting diary items (milk, yogurt, pizza, cheese, and ice cream). It was based on a previously validated instrument tested in Lebanese youth that showed dairy products provided almost 80% of total daily calcium intake [22], as shown in the NHANES II survey [23].

Table 1 Demographic, anthropometric, clinical and lifestyle characteristics (variables expressed as mean (SD), or median [range] for non-normally distributed variables) of study subjects at follow-up

Number of follow-ups	Patients 29	Controls 32	*P* values
Demographics			
Male/female	16/13	17/15	
Age (years)	9.1(2.8)	8.8(2.6)	0.70
Age at diagnosis (years)	6.0(2.9)	–	–
Lifestyle			
Calcium intake (mg/day)	663(388)	883(436)	0.05
Exposure to sun (h/week)	6(4.9)	13(6.2)	<0.001
Exercise (h/week)	3(4.1)	12(7.7)	<0.001
Anthropometric			
Height (cm)	131(16)	130(13.8)	0.88
Weight (kg)	28[17–65]	26[15–46]	0.15
BMI (kg/m²)	17[14–27]	15.7[13–23]	0.009
Body composition parameters			
Lean mass (kg)	20[12–44]	20.5[10–31]	0.38
Fat mass (kg)	8.5[5–26]	6.2[3–17]	0.04
% Fat mass	29(6.37)	24(6.03)	0.004
Fat mass index (kg/m^2)	4.9[3–12]	3.7[2–8]	0.005
Clinical data			
Total cumulative Dex mg	1,250(478)	–	–
Total cumulative MTX dose in mg	14,261(6803)	–	–
Total cumulative Dex dose in mg/m^2 within 1 year	405(355)	–	–
Total cumulative MTX dose in mg/m^2 within 1 year	1,822(1390)	–	–
Total cumulative intrathecal steroids in Dex in mg within 1 year (N=14)	3.9(3.2)	–	–

Information on cumulative dose of corticosteroids (CS) and MTX, intrathecal therapy received within the follow-up period, radiation dose, and site was obtained from the medical records by the pediatric oncologist (SM). Systemic doses of MTX and CS received within the follow-up periods were both converted into milligrams and arithmetically added to derive a cumulative Dex-MTX dose. Dex-MTX were used as a combination in the multivariate analyses because correlations with BMD outcomes were more consistent across analyses than using each drug separately.

Biochemical and hormonal status

Fasting blood was drawn and routine serum biochemistries were measured at follow-up using standard methods. Laboratory specific age and sex-matched reference data was used when available. Adjusted serum calcium was calculated using the following formula: adjusted [Ca](mmol/L)=total [Ca] (mmol/L)+0.02+(40−)[albumin] (g/L).

Hormonal values were measured in the Institutional Endocrine Core Laboratory. These included the measurement of intact PTH, serum 25-OHD, serum Osteocalcin (OC), and serum CrossLaps (CTX). Values for calciotropic hormones were expressed as value at follow-up, mean of baseline and follow-up, and difference between the two. Samples were stored at −20°C until analyzed. Full details on assays performed are provided in Appendix I.

Bone maturation and bone mineral density measurements

All patients had their bone age measured at study entry only, and the parameter bone age minus chronologic age, an index of skeletal maturation was derived [7]. Bone age was not repeated at follow-up due to budgetary limitations. Bone mineral content (BMC) and BMD of the lumbar spine, total hip (as a stand-alone study) and subtotal BMD and BMC, and composition were measured using dual-energy X-ray absorptiometry (DXA) with a Hologic 4500A densitometer (pediatric software, software version 12.4, Hologic, Bedford, MA, USA). Spine and subtotal body are the accepted skeletal sites of interest in pediatric studies [24]. Hip BMD and BMC were analyzed when the scan

image reflected good edge detection in a subset of subjects (N=14). Because of the limitations of DXA measurements in children, including the impact of bone size on DXA-derived BMD [24], bone area, BMC were also evaluated. The densitometer pediatric database was used to derive site-specific Z-scores [7]. In our center, the mean±SD precision for BMD measurements, expressed as CV%, for 280 same-day duplicate scans performed during the study duration was less than 1.2% at spine and hip. In the pediatric population, the CV for same day duplicates at our center for BMD for total body was 0.6±0.4%, subtotal body was 0.8±0.6%, and total hip was 0.9±0.6 for 16 same day duplicate measurements. Because inclusion of the head BMD in the calculation of total BMD may lower the predictive value of some parameters for this variable, subtotal body measurements (head excluded) were used to evaluate serial changes over time [24]. There are no norms for body less head BMD/BMC; therefore, subtotal Z-scores for that variable were not presented in Table 2. The software determines BMC and BMD, fat mass and non-fat soft tissue mass identified as lean mass (lean mass=total mass)−(fat mass+bone mass). Lumbar spine BMAD was also calculated using the formula: BMC/area$^{3/2}$ [7].

Statistical analyses

The primary outcomes of interest were bone mass changes of the lumbar spine and sub-total body. The secondary outcomes were bone mass changes of the hip and bone remodeling markers (OC and CTX). The predictors of interest for both primary and secondary outcomes included age at diagnosis, lean mass, the calciotropic hormones, 25-OHD and PTH, markers of bone remodeling, cumulative dose of Dex, cumulative dose of MTX, cumulative dose of chemotherapy (Dex and MTX merged), and intrathecal therapy received during follow-up. These were expressed as the level at follow-up or mean of baseline and follow-up values.

Statistical analyses were performed using SPSS software for Windows statistical program, version 16 (SPSS, Chicago, IL, USA). Patients and controls were compared using two-tailed t tests for normally distributed variables. Normally distributed variables data were expressed as means±SD; a p value<0.05 was considered as statistically

Table 2 Serum biochemistries of mineral metabolism, calciotropic hormones and changes in densitometric measures and subtotal lean mass (variables expressed as mean (SD)) at follow-up

	Patients (N=29)	Controls (N=32)	P values
Biochemical studies			
S–Ca (mg/dl)	9.0(0.3)	9.5(0.2)	<0.001[a]
S−P (mg/dl)	5.0(0.6)	5.1(0.6)	0.68
S−Mg (mg/dl)	2.0(0.2)	2.2(0.2)	<0.001
S−Cr (mg/dl)	0.36(0.09)	0.41(0.13)	0.08
TSP; (ALB/GLO) (g/l)	68 (5.6) 45/23(2.6/5.0)	75 (4.2) 47/28(2.1/4.0)	<0.001
Calciotropic hormones			
25-OHD (ng/ml)	15(8)	19(9)	0.08
PTH (pg/ml)	52(27)	21(12)	<0.001
Serum markers of bone remodeling			
ALKP (IU/l)	208(73)	230(68)	0.23
S-Osteocalcin (ng/ml)	81(42)	169(77)	<0.001
S-Crosslaps (pg/ml)	170(229)	692(490)	<0.001
Z-scores and bone mass changes			
Lumbar spine Z-score	−1.2(1.2)	−0.5(1.0)	0.02
Total hip Z-score (N=14)	−1.5(1.2)	−0.4(0.9)	<0.001
Total body Z-score	−1.2(1.1)	−0.6(0.9)	0.028
% Change BMD Lumbar spine(g/cm^2)	6.0(8.4)	8.1(6.5)	0.28
% Change BMC lumbar spine(g)	9.6(13.6)	15.9(14.9)	0.09
% Change BMD hip[b] (g/cm^2)	8.1(9.1)	8.1(5.1)	0.98
% Change BMC hip (g)	14.0(14.6)	16.7(12.2)	0.45
% Change BMD subtotal body(g/cm^2)	8.2(5.4)	8.5(5.9)	0.88
% Change BMC subtotal body(g)	12.0(8.7)	15.5(5.0)	0.07
% Change BMD total body (g/cm^2)	4.8(4.9)	6.5(2.8)	0.11
% Change BMC total body(g)	7.3(7.2)	11.4(4.3)	0.01
% Change subtotal lean mass	20.7(9.0)	18.8(6.0)	0.34

[a] Adjusted calcium was not significantly different between the two groups (see text)

[b] Hip BMD measured in 14 subjects (in whom ROI could be well-defined)

significant. Non-normally distributed data were expressed as median and range. Correlation coefficients were calculated to determine the relationships between different predictors and outcomes. Pearson's correlation coefficients were derived for normally distributed variables, and Spearman's correlation coefficients for non-normally distributed variables. Partial correlations were implemented to select predictors to be included in multivariate analyses. Multivariate linear regression models were built using an all enter method; the outcome was the percent changes in bone mass (BMC, BMD, or BMAD) and the independent variables were significant predictors on bivariate analyses that remained significant in partial correlations. The independent variables were entered by sequential blocks, and the R^2 due to the additional entry of each block was calculated, allowing the determination of the amount of variance in the outcome measure (percent changes in BMD, BMC, or BMAD) explained by each independent block. The models were built with and without adjustment for age at diagnosis. In all models, Dex-MTX and intrathecal therapy were entered as last block, as it was only received by 14 study subjects, in order to investigate the strength of their association with bone mass changes, after correcting for all other significant predictors.

Results

Characteristics of patients and controls at follow-up

Demographic, anthropometric, lifestyle, and clinical characteristics of the study subjects

The patient population consisted of (16 boys, 13 girls), 26 with ALL (7 high risk, 5 intermediate risk, 14 low risk), and 2 with leukemia/lymphoma and 1 with Hodgkin's lymphoma. Mean age at diagnosis was 6.0±2.8 year, mean age at study entry was 7.7±2.8 years, mean age at follow-up was 9.1±2.8 years. Twenty-two patients were still receiving chemotherapy, with a mean duration of 80± 28 weeks at follow-up, and seven had finished 40±22 weeks prior to study entry. The mean cumulative dose of steroid dose received during the follow-up period was 405± 355 mg/m^2 of Dexamethasone (Dex) dose, mean cumulative dose of MTX received was 1,822±1390 mg/m^2. Intrathecal therapy was administered to 14 subjects with a mean cumulative dose of intrathecal steroids expressed in Dex dose of 3.9±3.2 mg. Radiotherapy was administered to one patient as cranial radiation treatment at the dose of 12 Gy. At follow-up, there were no differences in age, height, weight, and lean mass between patients and controls, but patients had significantly lower calcium intake, sun exposure, and exercise activity (Table 1). However, patients had significantly higher BMI, and fat mass parameters including percent of fat mass, and fat mass index compared to controls (Table 1). The higher fat mass indices had been noted at study entry [7].

Serum biochemistries and calciotropic hormones

Patients had significantly lower serum calcium, magnesium, and total protein than the control subjects (Table 2). However, serum calcium adjusted for albumin did not differ between the two groups. Both patients and controls had low 25-OHD levels, with a mean level that was lower in the patient group: 24.1% of patients and 12.5% of controls had a serum 25-OHD level below 10 ng/ml, 79.3% of patients and 62.5% of controls had a serum 25-OHD level below 20 ng/ml ($p<$ 0.01), and only six patients and five control subjects had 25-OHD levels above ≥25 ng/ml. The mean PTH level was twice as high in patients compared to control subjects (Table 2). Patients had substantially lower serum levels of bone turnover markers including OC and CTX that were on the average reduced by 51–75% compared to controls (Table 2). Serum CTX measured at follow-up were positively correlated with the variable cumulative Dex-MTX merged within 1 year in milligrams (R=0.42, p= 0.03). There were positive associations between serum PTH levels at follow-up and total cumulative dose of CS, (R= 0.47, p=0.01), and total cumulative dose of MTX, (R=0.45, p=0.02) received within the follow-up period.

Skeletal parameters: Z-scores and changes in bone mass

Patients had lower Z-scores at the lumbar spine, hip and total body, compared to controls, at follow-up (Table 2). They also experienced a consistent trend for smaller increments in bone mass, expressed as % change in BMC, at all three skeletal sites at follow-up, differences that achieved significance at the total BMC (Table 2). There were no differences between the two groups in percent change BMAD.

Subgroup analyses within the patient group revealed no significant differences in bone mass changes between the two genders, between those who completed their treatment (N=7) and the 22 still receiving therapy, and no differences could be detected between those whose Tanner stage had changed (total number was 11) and those in whom it did not (data not shown).

Correlates of bone mass and bone remodeling in patients

Bone mass changes

Significant predictors of bone mass changes are detailed in Table 3. Age at diagnosis was negatively correlated with bone mass changes, both in BMD and BMC at the hip

(R=−0.50 and −0.44, $p\leq0.05$) and BMD at the spine (R=−0.45, $p<0.05$). Changes in lean mass were positively correlated with changes in bone mass at subtotal body and total hip sites (R=0.37–0.54, $p\leq0.05$). There was no effect of fat mass on changes in bone mass. Serum 25-OHD level at follow-up, and difference, positively correlated with bone mass change at subtotal BMD and BMC (R=0.39–0.42, $p<$ 0.03)(Table 3), but mean 25-0HD did not show significant correlations. There were significant negative correlations between both mean PTH and follow-up PTH level and bone mass changes at the subtotal body (R=−0.37 to −0.47; $p<$ 0.05), but PTH difference did not show any significant correlations (Table 3). Cumulative MTX dose negatively correlated with percent change subtotal BMC, whereas cumulative dose of Dex correlated negatively with percent changes bone mass BMD and BMC at the hip and subtotal body (Table 3). Cumulative dose of Dex (milligrams), MTX (milligrams), or the merged variable received within the follow-up period were negatively correlated with bone mass changes at the subtotal body and hip, both BMD and BMC (R=−0.54 to −0.41, $p\leq0.05$).

Similarly, intrathecal therapy received within the follow-up period was negatively correlated with bone mass changes at the subtotal body and hip, both BMD and BMC (R=−0.58 to −0.41, $p\leq0.05$). Bone maturation delay at study entry did not correlate with any changes in bone mass at follow-up (data not shown). Similarly, there were no significant correlations between % change lumbar spine BMAD and any of the anthropometric, hormonal, and chemotherapy variables.

Bone remodeling

There was no correlation between mean or follow-up serum OC, or serum CTX and bone mass changes at any skeletal site (data not shown).

Adjusted regression analyses to evaluate predictors of low bone mass

Partial adjustments

The predictive effect of 25-OHD on bone mass changes disappeared after adjustment for PTH levels, and the predictive effect of PTH levels on bone mass changes disappeared after adjustment for cumulative Dex-MTX dose. After controlling for mean OC, the predictive effect of cumulative Dex-MTX received within follow-up remained significant at the subtotal body and hip, both BMD and BMC (R=−0.54 to R=−0.41, $p\leq0.05$). The following predictors that were significant on bivariate analyses and persisted in partial correlations were retained in multivariate analyses: % changes in lean mass, cumulative Dex-MTX therapy within follow-up, and intrathecal therapy.

Table 3 Coefficient between anthropometric parameters, hormonal, and chemotherapy variable with changes in bone mass at lumb

	% change L1-L4 BMC	% change L1-L4 BMD	% change subtotal BMC	% change subtotal BMD	% change total hip BMC	% change total hip BMD
Anthropometric parameters						
Age at diagnosis	–	−0.45*	–	–	−0.44*	−0.50*
Height	–	–	–	–	–	–
% change subtotal lean mass	–	–	–	0.37*	0.54**	0.51**
Hormonal variables						
25-OHD at follow up	–	–	–	0.39*	–	–
25-OHD difference	–	–	–	0.42*	–	–
PTH at follow up	–	–	–	−0.47*	–	−0.41*
PTH mean	–	–	–	−0.37*	–	–
Chemotherapy treatment						
Total cumulative intrathecal steroids in Dex in mg within 1 year	–	–	−0.43*	−0.58**	−0.41*	−0.41*
Total cumulative Dex in mg within 1 year	–	–	−0.43*	−0.58**	−0.41*	−0.41*
Total cumulative MTX in mg within 1 year	–	–	−0.47**	–	–	–
Total cumulative merged Dex-MTX in mg within 1 year	–	–	−0.54**	−0.48*	−0.41*	−0.41*

*$P\leq0.05$

**$P\leq0.001$

–No statistical significance detected

Multivariate model

Changes in lean mass were significantly associated with changes in bone mass at the total hip BMC and BMD ($p<0.005$ and $p=0.03$ respectively, Tables 4 and 5). A similar positive association was present at subtotal body BMD ($p=0.015$) Cumulative Dex-MTX dose within follow-up remained negatively associated with changes in subtotal body BMC ($p=0.025$, Table 4), with similar trends for changes in subtotal BMD and total hip BMD (Table 5).

Intrathecal therapy remained a significant negative predictor for changes in BMC at the lumbar spine and total hip BMC (Table 4). Intrathecal therapy accounted for 20% of the variance in changes in lumbar spine BMC, and for 26% of variance in changes in total hip BMC; whereas cumulative Dex-MTX dose explained 35% of variance in subtotal body BMC (Table 4). Integrating cumulative Dex alone instead of cumulative Dex-MTX showed a negative correlation β(SE)=−0.007(0.004), $p=0.088$ at the subtotal BMC.

When age at diagnosis was entered in the models, the predictive impact of changes in lean mass, but not of intrathecal therapy or Dex-MTX, on changes in total hip BMC, $R^2=19\%$, $p=0.01$, and subtotal BMD, $R^2=14\%$, $p=0.05$, remained significant.

Discussion

This prospective study demonstrates substantially lower markers of bone formation and bone resorption, and lower increments in bone mass in children receiving consolidation cancer therapy, compared to control subjects. They also had high prevalence of hypovitaminosis D and elevated PTH levels; the latter were correlated with the cumulative doses of steroids and MTX received within the follow-up period, and inversely associated with bone mass increments. Changes in lean mass, systemic MTX or CS therapy, intrathecal therapy, and age at diagnosis were significant predictors of bone mass increments; only lean mass remained a significant predictor when age at diagnosis was also entered in the model.

We are aware of only two prospective studies investigating bone mass changes in children with malignancies, both conducted by the same group within the first year of cancer therapy initiation. Decrements in bone mass of 8% in volumetric and area BMD at the femoral neck, and of 2.1% in volumetric lumbar spine BMD were noted within the first 6 months in 46 young children, median age of 8 years [18]. These persisted in a sub-group followed for 1 year, resulting in substantial deficits in bone mass of 11–16% compared with age and gender-matched controls [17]. Bone recovery may be possible, as demonstrated by the significant increase in total body bone mass in 37 children with ALL, median age 8 year, 2 years after therapy completion. The increments were larger in the subgroup evaluated more than 1.5 years since therapy completion, but the deficit in total body BMD remained [19]. Patients in our study were, for the most, evaluated in the consolidation phase, thus explaining the observed bone mass increments, albeit smaller than in controls. Indeed, they were studied

Table 4 Linear regression models assessing the relationship between changes in lean mass and treatment and changes in BMC at the lumbar spine, total hip and total body (blocks entered sequentially)

		% change lumbar spine BMC	% change total hip BMC	% change subtotal body BMC
Block 1	% changes subtotal lean mass			
	β (SE)		1.02(0.26)	0.29(0.19)
	95% CI		0.320–1.587	−0.064–0.614
	p value		0.005*	0.106
	ΔR^2		0.29	0.08
Block 2	Dex-MTX merged (mg)			
	β (SE)			−0.002(0.001)
	95% CI			−0.004–0.000
	p value			0.025*
	Intrathecal therapy (yes/no)			
	β (SE)	13.59(5.85)	12.20(4.88)	
	95% CI	1.480–25.691	2.090–22.326	
	p value	0.03*	0.020*	
	ΔR^2	0.20	0.26	0.35
	R^2	0.21	0.54	0.43

*$P\leq0.05$

Table 5 Linear regression models assessing the relationship between changes in lean mass and treatment and changes in BMD at the lumbar spine, total hip, and total body (blocks entered sequentially)

		% change lumbar spine BMD	% change total hip BMD	% change subtotal body BMD
Block 1	% changes subtotal lean mass			
	β (SE)	0.18(0.20)	0.47(0.20)	0.31(0.12)
	95% CI	−0.227–0.584	0.061–0.871	0.07–0.56
	p value	0.37	0.03*	0.015*
	Δ R^2	0.032	0.19	0.22
Block 2	Dex-MTX merged (mg)			
	β (SE)	−0.001(0.001)	−0.002(0.001)	−0.001(0.001)
	95% CI	−0.004–0.001	−0.004–0.000	−0.002–0.000
	p value	0.18	0.08	0.07
	Intrathecal therapy (yes/no)			
	β (SE)	2.13(3.52)	2.16(3.46)	1.82(1.99)
	95% CI	−5.149–9.413	−5.015–9.330	−2.29–5.94
	p value	0.55	0.54	0.37
	Δ R^2	0.14	0.18	0.20
	R^2	0.18	0.37	0.41

*$P \leq 0.05$

after completion of the initial high-dose therapy phase during which changes in bone mass may be most negatively affected [17]. However, most patients were still on therapy and thus in a period during which anticipated catch up bone mass increments were not yet possible [19].

Although the impact of nutritional factors on bone health in general and pediatric bone health in particular is well-described [9], their impact in children on cancer therapy was not adequately investigated. Such children are particularly vulnerable to nutritional deficiencies in calcium and 25-OHD due to anorexia and avoidance of sun exposure while on chemotherapy. Calcium intake when evaluated was suboptimal [19] as we have demonstrated; and in the few studies that measured vitamin D, levels were indeed low [12, 17–19]. Furthermore, the biological readout for low calcium absorption, be it from low nutritional intake and/or hypovitaminosis D, namely PTH, when measured was also found to be elevated in another study conducted in 20 patients with ALL [25]. In our study, we specifically investigated the associations between calciotropic hormones and bone loss prospectively. The positive associations between PTH levels and cumulative doses of steroids and MTX within the follow-up period are consistent with a deleterious effect of such therapy on intestinal calcium absorption, as is known to occur with these agents [9], thus explaining the rise in PTH levels. Furthermore, the negative association between PTH levels and prospective changes in bone mass, their positive association with serum 25-OHD levels, and the disappearance of such association when adjusting for PTH levels, support the possibility for a biological relevance for a negative impact of these abnormalities in calciotropic hormones on the children's skeletal heath. This possibility is, however, merely based on observed associations and biological plausibility, and can only be asserted in a calcium/vitamin D trial. We are unaware of any such trials, but one group of investigators is initiating such intervention in survivors of childhood leukemia who are at least 5 years into remission [26].

High PTH levels and low 25-OHD have both been associated with increased adiposity [27, 28], that can in these patients be explained by corticosteroid therapy and decreased activity. The relationship between fat mass and bone mass is a complex one [29], but fat mass, in contrast to lean mass, did not correlate with changes in bone mass in this prospective study.

The deleterious effect of chemotherapy, namely CS and MTX, on skeletal health has been extensively reviewed by the Children Oncology Group, Skeletal Toxicities Task Force [9]. Steroid exert their deleterious effects through multiple pathways including decreased osteoblastic activity, reflected in the low serum OC level in our study, increased bone resorption, interference with growth hormone IGF1, reduced muscle strength, and disturbances in calcium balance at the gut and kidney [9]. MTX has a direct cytotoxic effect on the osteoblasts reducing formation of new bone and bone volume [9]. It is possible that combination therapy with both, such as used in patients with ALL who constitute the majority of our study group, may result in synergy. Most studies available to date suggest that the noted BMD deficits are not totally

reversible [9, 13]. The low markers of bone remodeling despite high PTH levels confirm a suppressive effect of chemotherapy treatment on bone remodeling. Failure to recover BMD deficits has been linked to cumulative doses of steroids exceeding 9,000 mg/m^2 (of prednisone equivalent) or 40,000 mg/m^2 of MTX [9]. Our study is somewhat unique in view of its evaluation of multiple risk factors concomitantly, its prospective nature, and the inclusion of controls, and in that, it studied patients past the first few months of receiving high-dose chemotherapy. It shows unequivocally the deleterious impact of systemic and intrathecal therapy on bone mass changes in the adjusted analyses and as importantly that such therapy accounts for a substantial proportion of bone mass variance. Age at diagnosis has been shown to affect bone morbidity including fractures in previous studies [30], but the exact pathophysiologic basis for such observation in a prepubertal population is unclear. In our study, known predictors of bone mass changes such as chemotherapy and intrathecal therapy disappeared from the model with the introduction of age at diagnosis, underscoring the pivotal role of early prevention measures to decrease chemotherapy-induced bone loss in children/adolescents. The limitations of our study include its modest sample size, the fact that most, but not all, patients had ALL, included subjects with different risk stratification, studied at various stages of chemotherapy administration, and had few who entered puberty during the follow-up period.

This prospective study demonstrates impaired bone mass increments in children receiving chemotherapy during the consolidation phase, or after therapy completion, compared to healthy controls. It also underscores the powerful relationship between cumulative chemotherapy received and calciotropic hormones, and the significant elevations in PTH levels that were inversely correlated with bone mass increments. This raises interesting possibilities for nutritional therapeutic interventions during or after chemotherapy completion in an attempt to reverse such PTH increments, and possibly optimize bone mass recovery.

Acknowledgments We would like to thank the children and their parents for study participation and acknowledge Ms Randa Shahine for assistance in study progress, Ms Samia Mroueh for BMD analyses, and Ms Carmen Hajj-Chahine for the hormonal assays. This study was supported by an investigator-initiated grant funded by Novartis Pharmaceuticals.

Conflicts of interest None.

Appendix I

Hormonal assays Serum 25-OHD was measured by a competitive protein-binding assay, RIA (radioimmunoassay) with inter- and intra-assay CV (coefficient of variation) between 5% and 8.2% for values between 15.6 and 60.5 ng/ml (IDS, Immuno-Diagnostic Systems, Boldon, UK), 1,25-$(OH)_2$D by RIA with inter- and intra-assay CV between 9.3% and 13.6% for values between 8.6 and 56.8 pg/ml, serum PTH with ELSA-PTH, IRMA (immunoradiometric assay) with inter-and intra-assay CV <7% for values between 6 and 887 pg/ml (CIS Bio International, Gif-sur-Yvette, France), serum *C-TX* by ECLIA (electrochemiluminescence immunoassay) *(Elecsys*) with inter- and intra-assay CV between 1% and 5.5% for values between 140 and 359 pg/ml (Roche Diagnostics), serum OC by IRMA (immunoradiometric assay) (CIS Bio International, Gif-Sur-Yvette, France), with inter- and intra-assay CV between 1.2% and 5.2% for values between 20.4 and 220 ng/ml, TSH by ECLIA *(Elecsys)* with inter- and intra-assay CV between 4.3% and 5.3% for values between 0.2 and 7.9 μU/ml, FT4 by ECLIA (*Elecsys*) with inter- and intra-assay CV between 2.77% and 4.08% for values between 1 and 3.2 ng/dl, testosterone by RIA (CIS Bio International, Gif-Sur-Yvette, France), with inter- and intra-assay CV between 3.8% and 7.5% for values between 46 and 763 ng/dl, estradiol by IRMA with inter- and intra-assay CV between 3.5% and 4.9% for values between 347 and 2,609 pg/ml (CIS Bio International, Gif-Sur-Yvette, France), IGF1 by IRMA with inter-and intra-assay CV between 3.2% and 5.9% at values between 39.1 and 509 ng/ml (CIS Bio International, Gif-Sur-Yvette, France).

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