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Original Article

Modeling Pathways for Low Bone Mass in Children With Malignancies

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Abstract

Children with malignancies have low bone mass. Pathways for metabolic bone disease were investigated in children with cancer by concomitantly assessing lifestyle, clinical, and biochemical predictors of bone mass. Forty-one children who were receiving cancer therapy for 61 weeks and 39 controls were studied. Data on lifestyle factors, biochemical and hormonal parameters, dual-energy X-ray absorptiometry bone mass measurements, body composition, and bone age were obtained. Compared with controls, patients had higher weight percentile and fat mass, a 6-month delay in bone age, and lower estradiol levels. They also had higher parathyroid hormone levels and lower bone remodeling markers and bone mass. Age, height, lean mass, fat mass, and bone maturation correlated positively with several bone mass variables, so did serum estradiol, testosterone, and markers of bone remodeling. Conversely, corticosteroids, methotrexate (MTX), and intrathecal therapy negatively correlated with bone mass at the spine and hip ($R = -0.33$ to 0.40, $p < 0.04$). In the adjusted analyses, bone maturation, serum osteocalcin level, MTX, and intrathecal therapy were significant predictors of lumbar spine and total body Z-scores, bone maturation accounting for the largest proportion in Z-score variance. Chemotherapy-induced delay in bone maturation and suppression of bone modeling are major pathophysiologic pathways predicting bone mass in children with malignancies.

Key Words: Bone disease; malignancies; model; pathophysiology; pediatric.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, and current cure rates reach approx 80% *(1)*. In view of the improved survival of children with cancer after treatment, concerns about the long-term consequences of childhood cancer and its treatment on bone health have been raised *(1−5)*.

Children with cancer have a reduced bone mineral density (BMD) *(5−8)*, and these decrements persist up to several years after cure and into adulthood *(2,9)*. Contributing factors for decreased BMD are many *(10)*. These include the disease itself, systemic or intrathecal treatment with corticosteroids (CS) and methotrexate (MTX), radiotherapy, treatment-induced growth hormone deficiency, hypogonadism, limited physical activity, and nutritional deficiencies such as low calcium intake and hypovitaminosis D *(10)*. The latter is a worldwide public health problem affecting all age groups including children *(11−15)*. Children with malignancies may have low calcium intake, low vitamin D, and increased calcium excretion from high-dose steroid therapy and therefore are at risk for secondary hyperparathyroidism *(1)*, all additional risk factors for low bone density. The specific effect of low vitamin D on bone mass in children with cancer has not been investigated, and the relative impact of the above listed risk factors on bone mass is unclear. The aims of our study were to

1. Investigate the association between calciotropic hormones, serum 25-hydroxyvitamin D (25-OHD), and

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parathyroid hormone (PTH) and skeletal health in children with malignancies.
2. Propose a paradigm for the pathophysiology of bone loss, taking into account known predictors of low bone mass within the same study group. The predictors include anthropometric, lifestyle, clinical, and biochemical variables.

Such an approach would help establish a hierarchy for interventions to prevent or treat metabolic bone diseases in such children, a pressing issue in light of the increased survival and improved cure rates of children with cancer.

Methods

Study Design

This is a cross-sectional, observational, case-control study of 41 consecutive Lebanese children, 35 with ALL, 1 with acute monocytic leukemia, 3 with lymphomas (2 non-Hodgkin and 1 Hodgkin), and 2 with solid tumors (1 medulloblastoma and 1 Ewing sarcoma), who received therapy at the Children's Cancer Center of Lebanon (affiliated with St Jude Children's Research Hospital, Memphis, TN) at the American University of Beirut Medical Center within 1 year. Inclusion criteria were a diagnosis of childhood malignancy, age >3 years, and Tanner stage 1. Only prepubertal children were studied 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. Children were treated with standard oncology protocols, the Children's Cancer Center ALL1 protocol that is adopted from the St Jude TOTAL XV ALL experimental protocol *(16)*.

Age- and gender-matched prepubertal healthy control children were recruited from the pediatric outpatient clinic and from patients' siblings. There were 2 prepubertal patients who were relatively older, aged 15.4 and 12.5 years, and for whom no controls could be matched (because normal controls with a comparable age would have reached puberty). Therefore, the number of control subjects was 39. The study was approved by the Institutional Review Board at the American University of Beirut; written assents were obtained from children and/or written informed consents from their parents or guardians.

Data Collection

Information on age at diagnosis, type of malignancy, time on cancer therapy (weeks of therapy), cumulative dose of CS and MTX, intrathecal therapy, radiation dose, and site was obtained from the medical records. Height and weight were measured and plotted on standardized updated growth curves of the Center for Disease Control to derive height and weight percentiles. Body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared. Pubertal status was assessed by 2 physicians according to the established criteria for Tanner *(17)*. Exercise activity and sun exposure were evaluated, and calcium intake was assessed through a Food Frequency Questionnaire targeting dairy items (milk, yogurt, pizza, and ice cream). The questionnaire was based on a previously validated dietary calcium questionnaire in Lebanese youth demonstrating that dairy products provided almost 80% of total daily calcium intake *(18)*, findings that are consistent with the NHANES II survey results *(19)*. Information regarding calcium and vitamin D supplementation was also obtained.

Biochemical and Hormonal Status

Fasting blood was drawn and routine serum biochemistries and urinary calcium and creatinine (second-void urine) levels were measured using standard methods. Creatinine clearance was derived using the Schwartz formula (CrCl = [0.55 $\times$ Ht in cm]/serum creatinine [mg/dL]). Laboratory-specific age- and sex-matched reference data were used. Hormonal values were measured in the Institutional Endocrine Core Laboratory. These included the measurement of free thyroxine (FT4), thyroid-stimulating hormone (TSH), testosterone (T), estradiol, intact PTH, insulin growth factor 1 (IGF1), serum 25-OHD, serum 1,25-dihydroxyvitamin D (1,25-OHD or calcitriol), serum osteocalcin (OC), and serum C-terminal cross-links of collagen b-CrossLaps (CTX). Samples were stored at -70°C until analyzed. Full details on assays are provided in Appendix.

BMD Measurement and Bone Age

Bone mineral content (BMC) and BMD were measured within 1 week of the blood and urine samples. BMC and BMD of the lumbar spine (LS), total hip, and subtotal body 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). Spine and total body are the recommended skeletal sites for bone mass measurements in pediatric studies *(20)*. Because of the limitations of DXA measurements in children, including the impact of bone size on DXA-derived BMD *(20)*, bone area, BMC, BMC adjusted for height (BMC/Ht), and adjusted BMD (BMAD *(21)*) were also evaluated. Hip BMD and BMC were analyzed when the scan image reflected good edge detection in a subset of subjects, 7 patients and 7 controls, in whom the region of interest (ROI) was well delineated. Because the inclusion of the head BMD in the calculation of total body BMD may lower the predictive value of some parameters for this variable, subtotal body measurements (head excluded) were used in the analyses *(20)*. The software determines BMC and BMD, fat mass index, and nonfat soft-tissue mass identified in the software as lean mass (lean mass = total mass $-$ [fat mass + bone mass]). Bone age was determined by 2 bone radiologists with an X-ray of left hand and wrist using the Greulich and Pyle method. The variable bone age minus chronologic age (BA $-$ CA) was derived, as an index of delay in skeletal growth, and a mean value below zero would indicate significant delay in bone maturation *(22)*.

Statistical Analyses

The primary outcomes of interest were BMD and BMC of the LS and subtotal body. The secondary outcomes were BMD and BMC of the hip, BA − CA, and bone remodeling markers (OC and CTX). The predictors of interest for both primary and secondary outcomes included age; height; body composition; exercise; calcium intake; bone growth delay (BA − CA); the calciotropic hormones 25-OHD and PTH; IGF1; sex steroids; thyroid hormone; bone remodeling markers; and cumulative doses of chemotherapy, including CS, MTX, and intrathecal therapy.

Statistical analysis was performed using SPSS software for Windows statistical program, version 14 (SPSS, Chicago, IL). Patients and controls were compared using 2-tailed *t*-tests for normally distributed variables and Mann-Whitney nonparametric test for variables that did not have a normal distribution. For normally distributed variables, data were expressed as means ± SD, whereas nonnormally distributed variables were expressed as median (range). Normality was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests. A p value <0.05 was considered statistically significant. Correlation coefficients were calculated to determine the relationships between different variables (predictors and outcomes) using the Pearson correlation coefficient for normally distributed variables and Spearman correlation for nonnormally distributed data. Predictors that were significant in the bivariate analyses were entered into a multivariate regression model. Because Z-score adjusts for age and gender, Z-score was the main outcome used in the multivariate regression models. The p values were not adjusted for multiple testing.

Results

Characteristics of Patients and Controls With Comparisons

Demographic, Anthropometric, Lifestyle, and Clinical Characteristics of the Study Subjects

There were 41 prepubertal patients (22 boys and 19 girls), 35 with ALL (7 high-risk, 8 intermediate-risk, and 20 low-risk ALL), 2 with solid tumors (1 Ewing sarcoma and 1 medulloblastoma), and 4 with lymphoma. Mean age at diagnosis was 6.0 ± 3.0 years, mean age at study entry was 7.7 ± 2.8 years, mean duration of cancer treatment was 61 ± 47 weeks (range: 2−146 weeks), mean cumulative dose of steroids received was 757 ± 507 mg/m^2 of dexamethasone (DEX) equivalent, and median cumulative dose of MTX received was 11,840 (range: 0−23,140) mg/m^2. Intrathecal therapy was administered to 32 subjects with a mean cumulative dose of intrathecal steroids expressed in DEX equivalent 6.9 ± 5.2 mg. Radiotherapy was used in 6 patients with a median of 15 Gy (range: 8−39 Gy), 4 of them had cranial radiation (3 patients with ALL and 1 with medulloblastoma), 1 patient with ALL had mediastinal radiation, and 1 patient with Ewing sarcoma had right fibula radiation. There were no differences in age, height, weight, BMI, calcium intake, and exercise activity between patients and controls (Table 1). Patients had less sun exposure and tended to exercise less than controls (Table 1). Four patients took calcium supplement and 3 took vitamin D supplements, whereas only 1 control subject took calcium tablets. Although there was no difference in lean mass between the 2 groups, patients had significantly higher fat mass parameters, including percentage of fat mass, and fat mass index compared with controls (Table 1). Few more patients had a personal history of fractures validated by X-ray than controls: the numbers were 6 patients (16%) and 2 controls (5%) (p = not significant). Patients sustained their fractures before the diagnosis of their malignancy, 3 had arm fractures, 2 had leg fractures, and 1 had a shoulder fracture. Both controls had arm fractures.

Serum and Urinary Biochemistries and Calciotropic Hormones

All subjects had a normal serum creatinine, protein, phosphorus, and magnesium, and there were no differences in S-Ca between the 2 groups (Table 2), but patients had a higher estimated CrCl. Both patients and controls had low 25-OHD levels, but there was no difference between the 2 groups (Table 2). Only 3 patients and 2 control subjects had 25-OHD levels above the minimally desirable range (>20 ng/mL) *[14]*, and 2 of these patients were already receiving calcium and vitamin D supplementation (Table 2). Calcitriol levels were comparable between the 2 groups. Conversely, the mean PTH level in patients was twice that in controls, and 39% of the patients had levels above the upper limit of normal. Patients had significantly lower serum levels of bone turnover markers, including alkaline phosphatase, OC, and CTX, which were on the average reduced by 30−40% compared with controls (Table 2).

Other Hormonal Parameters

The 2 groups had comparable serum IGF1 and FT4 levels, but patients had slightly yet significantly higher TSH levels (Table 3, $p < 0.03$). Serum estradiol levels were lower, and serum testosterone and luteinizing hormone levels were higher in patients than in controls (Table 3).

Skeletal Parameters: Bone Age and Densitometry

Mean bone age in patients was 6.1 ± 3.4 years for a mean chronologic age of 7.7 ± 2.8 years, whereas the mean bone age in controls was 7.8 ± 2.8 years for a mean chronologic age of 7.7 ± 2.6 years ($p < 0.009$ for bone age between the 2 groups). In patients, the mean bone age minus chronologic age (BA − CA) was −0.5 ± 0.8 years (−6.2 ± 10 months), and this parameter was significantly different from zero ($p = 0.001$), confirming significant bone delay in this group. BA − CA was not different between the patients who received intrathecal therapy (n = 32) and those who did not (N = 9, p = not significant). Patients had significantly lower BMD Z-scores at the LS and total body, with a similar trend at the total hip, compared with controls (Table 2). There were no gender differences in Z-score at any skeletal site (data not shown). The difference in the Z-scores between patients and controls varied between 0.5 and 1.3 SD, depending on the skeletal site (Table 2). There was no difference in BMC, bone area, and

Table 1
Demographic, Anthropometric, Clinical, and Lifestyle Characteristics of Study Subjects[a]

Variable	Patients	Controls	*p* Values
N baseline	41	39	
Demographics			
Male/female	22/19	20/19	—
Age (yr)	7.7 (2.8)	7.7 (2.6)	0.97
Age at diagnosis (yr)	6.0 (3.0)	—	—
Anthropometric			
Height (cm)	123.7 (15.7)	123.3 (13.8)	0.91
Weight (kg)	23 (15−52)	23 (13−43)	0.48
BMI (kg/m^2)	16 (12.7−30)	16 (12.4−26.7)	0.10
Body composition parameters			
Lean mass (kg)	18.5 (6.6)	18.4 (5.4)	0.95
Fat mass (kg)	7.1 (4−22)	5.4 (3−18)	0.001
% fat mass	30 (7)	25 (6)	<0.001
Fat mass index (kg/m^2)	5.5 (2)	4.2 (2)	0.006
Clinical			
Weeks on cancer treatment	61 (47)	—	—
Cumulative steroid dose (DEX, mg/m^2)	757 (507)	—	—
Cumulative MTX dose (mg/m^2)	11,840 (0−23,140)	—	—
Cumulative IT steroids in DEXA (mg) (N = 32)	6.9 (5.2)	—	—
Radiation (Gy) (N = 6)	15 (8−39)	—	—
Lifestyle			
Calcium intake (mg/d)	750 (347)	779 (359)	0.71
Exposure to sun (h/wk)	7.0 (0−35)	16.7 (3−30)	<0.001
Exercise (h/wk)	20 (11)	24 (11)	0.09

Abbr: DEX, dexamethasone; IT, intrathecal.
[a]Variables are expressed as mean (SD) or median (range), depending on normality of their distribution.

BMC/height at any skeletal site, except for hip BMC/Ht measuring 0.08 ± 0.02 g/cm in patients and 0.10 ± 0.02 g/cm in controls ($p = 0.012$). LS BMAD measured 0.822 ± 0.100 g/cm^3 in patients and 0.871 ± 0.135 in controls ($p = 0.07$).

Correlates of Bone Mass, Bone Remodeling, and Bone Maturation in Patients

Bone Mass

Significant predictors of bone mass are detailed in Table 4. Age was a significant predictor of BMD and BMC at the spine, hip, and subtotal body ($R = 0.39-0.84$, $p < 0.05$) but negatively correlated with bone mass Z-score at all sites ($R = -0.34$ and -0.42, $p < 0.05$). Height was a significant predictor of BMD and BMC at the spine, hip, and subtotal body ($R = 0.48-0.91$, $p < 0.002$). Lean mass and fat mass also correlated with spine, hip, and total body BMC and BMD ($R = 0.57-0.92$, $p < 0.001$ for lean mass, and $R = 0.47-0.50$, $p < 0.001$ for fat mass). Exercise correlated with total hip Z-score ($R = 0.39$, $p < 0.03$). BA − CA, an index of bone maturation, correlated with spine and hip Z-scores ($R = 0.52-0.56$, $p < 0.002$). CA and BA − CA were negatively correlated ($R = -0.46$, $p < 0.005$). 25-OHD levels negatively correlated with spine, hip, and subtotal body BMD but not with BMD Z-scores (Table 4). These correlations persisted after adjusting for age, BMI, and gender (data not shown). No correlations were found between urinary Ca/Cr, PTH, or IGF1 levels and bone mass at any skeletal site. Serum estradiol and serum testosterone levels positively correlated with bone mass at all sites ($R = 0.35-0.79$, $p < 0.05$). Corticosteroid cumulative dose negatively correlated with spine Z-score ($R = -0.33$, $p = 0.04$), and MTX cumulative dose negatively correlated with spine BMD and with spine Z-score ($R = -0.33$ and -0.40). Merging cumulative doses of CS and MTX did not further strengthen the correlation coefficient with bone mass measures compared with MTX alone (data not shown). Intrathecal therapy negatively correlated with both spine and hip Z-scores ($R = -0.35$, $p < 0.05$).

Bone Remodeling and Bone Maturation

OC positively correlated with bone maturation, expressed as BA − CA ($R = 0.31$, $p = 0.047$), and negatively correlated with MTX dose ($R = -0.39$, $p = 0.01$). Corticosteroid dose and intrathecal therapy negatively correlated with bone maturation ($R = -0.31$ to 0.33, $p < 0.04$). None of the predictors studied correlated with serum CTX levels.

Table 2
Serum and Urinary Biochemistries of Mineral Metabolism, Calciotropic Hormones, and Densitometric Measures[a]

Variable	Patients (n = 41)	Controls (n = 39)	*p* Values
Biochemical			
S-Ca (mg/dL)	9.5 (0.5)	9.4 (0.3)	0.21
S-P (mg/dL)	5.1 (0.6)	5.2 (0.5)	0.41
S-Mg (mg/dL)	2.1 (0.4)	2.2 (0.2)	0.75
S-Cr (mg/dL)	0.4 (0.3−0.8)	0.5 (0.3−0.6)	<0.001
Ur-Ca/Cr	0.05 (0.0−0.41)	0.05 (0.01−0.31)	0.49
Creatinine clearance	179 (30)	141 (18)	<0.001
TSP (ALB:GLO) (g/L)	66(6);44/22(3/5)	75(4);46/28(2/3)	<0.001
Calciotropic			
25-OHD (ng/mL)	13 (5)	13 (5)	0.95
1,25-OHD (pg/mL)[b]	53 (30)	52 (12)	0.81
PTH (pg/mL)	49 (30)	23 (10)	<0.001
Serum markers of bone remodeling			
ALKP (IU/L)	172 (63)	237 (59)	<0.001
S-OC (ng/mL)	63 (30)	100 (37)	<0.001
S-CrossLaps (pg/mL)	1035 (447)	1439 (634)	0.001
BMD			
LS BMD (g/cm^2)	0.500 (0.075)	0.522 (0.076)	0.212
LS BMC (g)	19.08 (5.62)	19.53 (5.63)	0.724
LS Z-score	−1.0 (1.0)	−0.5 (1.0)	0.035
Subtotal BMD (g/cm^2)	0.562 (0.088)	0.592 (0.112)	0.20
Subtotal BMC (g)	555.04 (167.58)	590.38 (176.39)	0.36
Total body Z-score	−1.0 (1.0)	−0.6 (0.97)	0.05
Total hip BMD (g/cm^2)[c]	0.569 (0.138)	0.661 (0.132)	0.23
Total hip BMC (g)[c]	15.96 (4.95)	17.35 (4.42)	0.60
Total hip Z-score[c]	−2.3 (1.4)	−1.0 (1.5)	0.14

Abbr: TSP, total serum protein; ALB, albumin; GLO, globulin; OC, osteocalcin; ALKP, alkaline phosphatase; BMD, bone mineral density; BMC, bone mineral content; PTH, parathyroid hormone; 25-OHD, 25-hydroxyvitamin D; LS, lumbar spine; ROI, region of interest; S-Ca, serum calcium; S-P, serum phosphorus; S-Mg, serum magnesium; S-Cr, serum creatinine; Ur-Ca, urinary calcium.

[a]Variables are expressed as mean (SD) or median (range), depending on normality of their distribution.

[b]1,25-$(OH)_2$ D was measured in 23 patients and 32 controls.

[c]Hip BMD, BMC, and Z-scores were measured in 14 subjects (in whom ROI could be well defined).

Adjusted Regression Analyses to Evaluate Predictors of Low Bone Mass

Partial Adjustments

Because chemotherapy can negatively affect bone mass directly or indirectly through its impact on bone modeling, sex steroids, and bone maturation, as shown above, partial adjustments were implemented to elucidate the biological pathways involved in the negative effect of chemotherapy on bone (*see* Fig. 1) before performing multivariate linear regression models. After adjustment for MTX and CS, the relationship between bone markers and bone mass disappeared, whereas after adjustment for OC, the relationship between MTX and CS and bone mass persisted at the LS Z-score ($r = -0.33$, $p = 0.04$).

Similarly, after adjustment for CTX, the relationship between MTX and CS and bone mass persisted at the LS BMD and LS Z-score ($r = -0.31$ and $r = -0.39$, $p = <0.05$). After adjustment for estradiol, the relationship between MTX and CS and bone mass persisted at the LS BMD and LS Z-score ($r = -0.34$ and $r = -0.43$, $p < 0.05$). After adjustment for testosterone, the relationship between MTX and CS and bone mass persisted at the LS BMD and LS Z-score ($r = -0.37$ and $r = -0.41$, $p < 0.01$). After adjustment for BA − CA, the relationship between MTX and CS and bone mass persisted at the LS BMD and LS Z-score ($r = -0.34$, $p = 0.03$).

Multivariate Model

Significant predictors of bone mass Z-score in bivariate analyses, which persisted in partial correlations, were entered in the multivariate model in blocks by categories: anthropometric, hormonal, and chemotherapy related (Table 5).

Table 3
Hormonal Studies in Patients and Control Subjects[a]

Variable	Patients (n = 41)	Controls (n = 39)	*p* Values	Normal range
TSH (μU/mL)	2.6 (1.4)	2.1 (0.9)	0.03	0.27−4.2
FT4 (ng/dL)	1.3 (0.3)	1.3 (0.1)	0.36	0.93−1.7
IGF1 (ng/mL)	143 (72)	145 (90)	0.93	1−4 yr: 700−4700 ng/mL; 5−9 yr: 1100−7100 ng/mL; 10−12 yr: 2100−8900 ng/mL
E2 (pg/mL)	0.7 (0−11)	3.2 (0−14)	0.04	NA
T (ng/dL)	11.4 (1−80)	5.3 (1−38)	<0.001	1 yr: 12−21 ng/dL; 1−6 yr: 3−32 ng/dL; 7−12 yr: 3−68 ng/dL
LH (mIU/mL)	0.17 (0.41)	Undetectable	<0.001	>2 yr: female < 0.2 mIU/mL

Abbr: TSH, thyroid-stimulating hormone; FT4, free thyroxin; IGF1, insulin growth factor 1; E2, estradiol; T, testosterone; LH, luteinizing hormone; NA, not available.

[a]Variables expressed as mean (SD) or median (range), depending on normality of their distribution.

BA − CA, OC, and MTX therapy remained significant independent predictors of bone mass Z-score at the spine, and BA − CA and MTX therapy were independent predictors of total body Z-score and total body, whereas age and intrathecal therapy lost significance with these adjustments (Table 5). BA − CA accounted for the largest proportion in bone mass variance, explaining 32% of Z-score variance at the spine and 33% of Z-score variance at the total body, followed by chemotherapy that explained 10% variance of LS Z-score and 5.6% of variance of total body Z-score.

Discussion

Prepubertal patients treated for childhood malignancy, most of whom have ALL, have lower bone density compared with age- and gender-matched peers. The negative metabolic bone profile in these children is in large part because of chemotherapy-induced delay in sexual maturation and therefore bone maturation and suppressed bone modeling, as detailed in the model derived from the study.

Risk factors for low BMD in childhood cancer include male sex, age at diagnosis, low calcium and vitamin D intake, low physical activity, hypogonadism, growth hormone deficiency, reduced body size, cranial irradiation, and high dose of chemotherapy *(2,5,8,10,23−25)*, many were again demonstrated in this study. Patients had comparable height, weight, BMI, and lean mass compared with controls but significantly higher fat mass indices, as previously demonstrated *(2,26,27)*. The higher fat mass indices reflect a combination of decreased physical activity and the effect of glucocorticoid therapy. A high fat mass may result in an overestimation of DXA-derived BMD, and bone density in such patients may in reality be even lower than recorded *(2)*. The relationship between bone mass and fat mass is a complex one *(28)*. Fat is an endocrine organ, a source of estrogen with its protective effect on bone; and although some studies have demonstrated a positive relationship between fat mass and bone mass, this has not been a consistent finding *(29,30)*. Fat is also a source of adipokines and inflammatory cytokines, some with a negative effect on bone. Circulating cytokines were not measured in this study. Lean mass is one of the most important, if not the most important single known predictor of bone mass *(28)*, and lean mass was highly correlated with bone mass at multiple sites in patients in this study. Although steroids may negatively affect lean mass and thus bone mass, patients did not have a lower lean mass than controls.

Despite comparable calcium intake and vitamin D levels in patients and controls, both being below desirable targets, patients had substantially higher PTH levels, and in 40% of subjects, they exceeded the upper limit of normal. Arikoski et al. *(6,23)* previously showed high PTH after 6 months of starting chemotherapy *(6)* and at completion of the treatment *(23)* when hypocalcemia was present. Boot et al. *(24)* also showed a significant increase of PTH in children with ALL after 1 year of treatment. The high PTH levels probably reflect decreased intestinal calcium absorption, most likely because of steroid-induced decrements in the expression of calcium channels in the duodenum *(30)* and possibly because of MTX-induced mucositis. In addition, they may in part be explained by the relative hypercalciuria induced by steroids *(30)*, as shown in this study. The high PTH levels noted herein may ultimately have a negative impact on bone if chronically sustained. Indeed, although we did not find any significant correlation between baseline PTH levels and bone mass parameters at study entry in the present analyses, a negative impact was detected in longitudinal follow-up 1 year later *(31)*.

Table 4
Correlations (*R* Values) Between Anthropometric Parameters and Hormonal and Chemotherapy Variables With Bone Mass at LS, Subtotal Body, and Total Hip of Study Subjects

Variable	L1−L4 BMC	L1−L4 BMD	L1−L4 Z-score	Subtotal BMC	Subtotal BMD	Total hip BMC	Total hip BMD	Total hip Z-score
Anthropometric parameters								
Age	0.84**	0.62**	−0.34*	0.81**	0.78**	0.80**	0.39*	−0.42*
Height	0.91**	0.67**	—	0.89**	0.87**	0.86**	0.48**	—
Lean mass	0.91**	0.68**	—	0.89**	0.90**	0.92**	0.57**	—
Fat mass	0.48**	0.48**	—	0.47**	0.50**	0.44**	—	—
BA − CA	—	—	0.56**	—	—	—	—	0.52**
Hormonal variables								
25-OHD	—	−0.42*	—	—	−0.35*	−0.32*	−0.50**	—
Testosterone	0.68**	0.51**	—	0.79**	0.61**	0.78**	0.40*	—
Estradiol	0.45**	0.35*	—	0.43*	0.43*	0.43*	—	—
OC	—	—	0.31*	—	—	—	0.40*	0.41*
CrossLaps	0.34*	0.37*	—	0.31*	0.33*	—	—	—
Chemotherapy treatment								
DXA cumulative dose	—	—	−0.33*	—	—	—	—	—
MTX cumulative dose	—	−0.33*	−0.40*	—	—	—	—	—
Intrathecal therapy	—	—	−0.35*	—	—	—	—	−0.35*

Abbr: —, no statistical significance was detected; BMD, bone mineral density; OC, osteocalcin; BMC, bone mineral content; LS, lumbar spine.
*$p \leq 0.05$.
**$p \leq 0.001$.

Calcium intake and vitamin D levels were suboptimal not only in subjects but also in controls, reflecting observations worldwide in general and in our country in particular *(11,18,15)*. However, patients are particularly vulnerable to such deficiencies because of the added negative effect of steroids and possibly MTX on intestinal calcium absorption. Optimal calcium and vitamin D have been proposed in patients who have completed treatment of their malignancies by the long-term follow-up guidelines of Children's Oncology Group *(10)*. Indeed, BMD was normal in a group of children having completed treatment for ALL, a group that had adequate calcium and vitamin D intake *(32)*. Calcium and vitamin D reduced but did not completely prevent bone loss, in children with nephritic syndrome on steroids *(33)*. The inverse correlation between 25-OHD and BMD in this study remained unexplained, despite adjustments for potential confounders.

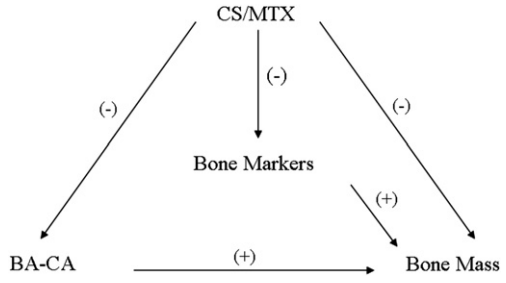

Fig. 1. Pathways for low bone mass in patients with cancer.

Growth hormone deficiency was not a salient feature in this study group because only a small proportion received radiation and in only 1 subject did it reach the dose of 18 Gy associated with growth hormone deficiency *(10)*. The high testosterone level in patients with the delay in bone maturation suggests a potential indirect effect of chemotherapy on the aromatase enzyme, suppressing its activity and thus contributing to low bone maturation and bone mass. Estradiol levels were indeed lower in patients than in controls and positively correlated with bone mass at multiple sites. Few studies assessed gonadal function in survivors of childhood cancer *(34−36)*, but to our knowledge, this is the first study to emphasize this effect at the time of chemotherapy. Skeletal maturation is a very important predictor of bone mass and bone mineral accrual and is in large part driven by sex steroids. Previous studies evaluating bone age in children with malignancies, all from the same group, did not show a difference between BA and CA *(6,7,23)*. However, we are unaware of any studies comparing BA in children with malignancies with age-matched controls, or using the calculated index BA − CA, and assessing its specific impact in the setting of a multivariate model exploring pathways for low bone mass in such a population. Older children had the most significant delay in bone maturation, and older age had indeed been shown to be a predictor of low bone mass in such patients. The additional information provided in our study is the concomitant evaluation of several anthropometric, lifestyle, clinical, and hormonal parameters to elucidate the major pathways explaining the observed low

Table 5
Linear Regression Models Showing the Impact of Treatment on Bone Density Z-Score at the LS, Total Hip, and Total Body Adjusted for Age, Delay in Bone Maturation, and OC

Variable	LS Z-score	Total hip Z-score	Total body Z-score
Block 1			
Age			
β (SE)	−0.06 (0.05)	−0.14 (0.07)	−0.06 (0.05)
p Value	0.2	0.05	0.2
BA − CA (mo)			
β (SE)	0.03 (0.01)	0.02 (0.01)	0.04 (0.01)
p Value	0.03	0.3	0.02
$\triangle R^2$	0.320	0.310	0.330
Block 2			
OC (ng/mL)			
β (SE)	1.6 (0.005)	0.009 (0.007)	−0.002 (0.005)
p Value	0.03	0.1	0.7
$\triangle R^2$	0.028	0.097	0.002
Block 3			
MTX (mg/m^2)			
β (SE)	-6×10^{-5} (0.00)	-5×10^{-5} (0.00)	-5×10^{-5} (0.00)
p Value	0.02	0.1	0.05
Intrathecal therapy (yes/no)			
β (SE)	−0.54 (0.3)	−0.58 (0.6)	−0.55 (0.4)
p Value	0.1	0.3	0.09
$\triangle R^2$	0.096	0.037	0.056

Note: Values were adjusted for all predictors entered in the models. $\triangle R^2$ refers to R^2 changes after adding blocks sequentially. BA − CA = bone age minus chronologic age.
Abbr: BMI, body mass index; OC, osteocalcin; LS, lumbar spine.

bone mass and to demonstrate the substantial impact of delayed bone maturation and impaired remodeling on these pathways.

This study has limitations, including its cross-sectional nature, the fact that patients were recruited into the study at varying intervals from the start of chemotherapy and that 2 radiologists assessed bone age. Other limitations include its relatively small sample size, the inclusion of solid tumors with ALL, and the patient mix regarding therapies received (intrathecal steroids and radiation therapy), thus limiting subgroup analyses. These also limit the generalizability of the findings to all patients with childhood malignancies. Nevertheless, the group consisted mostly of children with ALL, the commonest and most curable cancer in children today.

In this study, chemotherapy-induced delay in bone maturation and suppression of bone modeling were the main pathophysiologic pathways predicting low bone mass in children with malignancies, and older children were more affected. Improved calcium and vitamin D nutritional status aiming at normalizing PTH levels and novel targeted therapies that bypass the above pathways, which minimize the use of steroids, are needed to prevent bone loss that may be irreversible, in children receiving cancer therapies, at a critical period of bone mass accretion.

Appendix

Hormonal assays

Serum 25-OHD was measured by a competitive protein-binding assay, radioimmunoassay (RIA) with inter- and intra-assay coefficient of variation (CV) between 5% and 8.2% for values between 15.6 and 60.5 ng/mL (Immuno-Diagnostic Systems, Boldon, United Kingdom), 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, immunoradiometric assay (IRMA) with inter- and intra-assay CV $<7\%$ for values between 6 and 887 pg/mL (CIS Bio International, Gif-sur-Yvette, France), serum CTX by electrochemiluminescence immunoassay (ECLIA) (Elecsys) with inter- and intra-assay CV between 1% and 5.5% for values between 140 and 359 pg/mL (Roche Diagnostics, Manneheim, Germany), serum OC by IRMA (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 2609 pg/mL (CIS Bio International, Gif-Sur-Yuette, France), IGF1 by IRMA with inter- and intra-assay CV between 3.2% and 5.9% for values between 39.1 and 509 ng/mL (CIS Bio International, Gif-Sur-Yvette, France).

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