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Bone Loss and Vitamin D Deficiency in Children Undergoing Hematopoietic Cell Transplantation

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Abstract

Background—Hematopoietic cell transplantation (HCT) may be detrimental to bone health and vitamin D status in children.

Procedure—We conducted a prospective, multicenter cohort study to identify changes in bone health markers during the first 100 days after allogeneic HCT in 26 children. Bone mineral density (BMD), bone mineral content (BMC), and serum 25-hydroxyvitamin D (25OHD) concentrations were measured at baseline, 30 days, and 100 days after HCT.

Results—Mean (SD) BMD and BMC Z-scores (-0.48 ± 1.09 and -0.98 ± 1.26 , respectively) were normal at baseline. Repeated-measures analysis revealed significant declines in BMD and BMC Z-scores over the 100 day study period, when adjusted for age, sex, Tanner stage, lean mass, fat mass, resting energy expenditure, total energy intake, insulin sensitivity, serum phosphorus, and inpatient steroid intake. Adjusted mean (SE) 25OHD concentrations declined from 29.2 (3.1) ng/ml at baseline, to 17.7 (1.8) ng/ml at 100 days after HCT. Vitamin D deficiency (25OHD <20 ng/ml) was present in 50% of patients 100 days after HCT.

Conclusions—Significant bone loss and vitamin D deficiency occur in children in the first 100 days following allogeneic HCT. Strategies to diminish acute bone loss during HCT in children are needed.

Keywords

bone density; bone demineralization; hematopoietic cell transplantation; vitamin D

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INTRODUCTION

As survival rates after hematopoietic cell transplantation (HCT) have risen, attention has increasingly turned toward important aspects of health, including nutritional and metabolic outcomes. Low bone mineral density (BMD) has been reported among long-term survivors of HCT [1,2]. Children also have a high prevalence of asymptomatic fractures after HCT [3]. Children treated for leukemia who experience fractures have lower BMD [4] than those without fractures. In adult survivors of childhood acute leukemia, HCT increased the risk for low BMD compared to chemotherapy alone, when data were adjusted for diagnosis, age at diagnosis, follow-up duration, and corticosteroid use [4]. Since low BMD is likely linked to the development of fractures in children, the timing and duration of bone loss following HCT in children maybe an important consideration in the development of appropriate treatment or preventive interventions [5].

Vitamin D deficiency has been associated with low bone mass and osteoporosis in adult patients after HCT [6], and is also common in pediatric HCT recipients [7,8]. While the prevalence of vitamin D deficiency among long-term survivors of pediatric cancer appears to be similar to that of the general population [9,10], changes that occur in the immediate post HCT period may coincide with bone loss in this population.

In a prospective study of children undergoing HCT, mean loss of BMD was 5% six months after HCT, and in half of the subjects, bone density losses continued through one year after HCT [5]. Acute changes in BMD and biomarkers associated with bone health, such as vitamin D, in the first months after HCT are unknown. Prior studies have not reported bone morbidities at 30 and 100 days after pediatric HCT. We hypothesized that significant bone loss accrues in children during the first 100 days following HCT, and that this bone loss is accompanied by vitamin D deficiency.

METHODS

We performed an ancillary study as part of a multi-center, randomized, double-blind controlled clinical trial of two approaches to the provision of parenteral nutrition (PN) to pediatric HCT patients: (1) the standard of care ("standard PN") in which energy intake was provided in the amount of 140% of estimated basal metabolic rate calculated by standard reference equations [11], and (2) an alternative strategy ("experimental PN") in which energy intake was titrated to match resting energy expenditure (REE) measured by indirect calorimetry. Study methods [12] and main results [13,14] have been published. The Institutional Review Boards of Boston Children's Hospital and UCLA Mattel Children's Hospital approved the protocol. The study was registered in [ClinicalTrials.gov](https://clinicaltrials.gov) ID: NCT00115258. The primary outcome of the main study was body composition as measured by dual energy x-ray absorptiometry (DXA). The aim of this ancillary study was to evaluate changes in bone density and bone biomarkers among children undergoing HCT.

Children who were scheduled for their first myeloablative, matched related or unrelated donor allogeneic HCT at Boston Children's Hospital (n = 21) or UCLA Mattel Children's Hospital (n = 5) who were greater than or equal to 6 years of age were eligible. Underweight

(body mass index [BMI] z-score <-2) and overweight (BMI z-score >2) children were excluded. Subjects with unrelated donors received low dose steroids for graft-versus-host disease (GVHD) prophylaxis (1 mg/kg/dIV methylprednisolone from day +7 to day +21 after HCT, then tapered for grade of GVHD to an oral dose by hospital discharge).

Preparation for HCT included either total body irradiation (1400 cGy) or busulfan (0.8 mg/kg every 6 hr, intravenously, adjusted for levels) in addition to cyclophosphamide or other chemotherapeutic agents. GVHD prophylaxis included calcineurin-inhibitors, methotrexate and corticosteroids. Standard medications included oral non-absorbable antibiotics for gut decontamination, ursodeoxycholic acid, and vitamin E for venoocclusive disease prophylaxis [15], and leucovorin calcium for recovery from methotrexate, when used for GVHD prophylaxis.

Prior to the preparative regimen, baseline study measures were obtained, including anthropometric measures, REE measurement, whole body DXA, dietary intake assessment, and laboratory studies. A modified homeostasis model assessment was used to evaluate insulin sensitivity (HOMA index, defined as the product of fasting glucose in mM and fasting insulin in mU/L divided by 22.5). Bloods were drawn following an overnight fast of oral intake; intravenous fluids and PN were not discontinued. In addition to the study measures, standardized demographic and clinical assessments were performed by medical record review throughout the study period, including Tanner stage at admission for HCT (as assessed by the admitting clinician), medication use, and blood chemistries.

Nutritional Management

PN was initiated when and if oral energy intake declined to less than the baseline REE measured calories for more than 3–5 days, but not earlier than day 0. Total protein goals were constant among subjects at 1.5–2.0 g/kg/day for children 6–13 years, and 1.0–1.5 g/kg/day for children over 13 years [16]. Serum electrolytes were monitored daily and additives to the PN were adjusted as indicated. Intravenous pediatric trace elements, including zinc, copper, manganese and chromium, were provided daily to all subjects. PN was discontinued when oral energy intake reached 50% of the most recent measured REE, or at the time of preparation for hospital discharge, whichever came first.

As part of the PN solutions, pediatric intravenous multivitamin solutions containing 400 international units (IU) vitamin D daily were provided to subjects 6–11 years of age and 10 ml of adult intravenous multivitamin solutions containing 200 IU vitamin D daily were provided to subjects over 11 years of age. When biochemical vitamin D deficiency (serum 25OH vitamin D <20 ng/ml) was noted, oral supplementation of ergocalciferol (50,000 IU once weekly for 6 weeks) was recommended, followed by retesting. All subjects for whom supplementation was recommended were able to take oral medications by mouth. For patients with concentrations between 20 and 30 ng/ml, standard oral multivitamins containing 400–800 IU vitamin D were recommended with clinical monitoring of other risk factors (e.g., dietary intake, season, and sun exposure).

Anthropometrics and Dietary Intake

Body weight and standing height were measured at baseline, 30 days, and 100 days after transplantation. Oral dietary intake was recorded daily throughout the hospitalization using calorie counts and 24-hr recall methodology. Nutrient analysis software (Nutritionist Pro, ESHA Food Processor, and Nutrient Data Systems) and pharmacy specifications for parenteral solutions were used to calculate nutrient intake.

Body Composition and Bone Health Assessment

Whole body DXA was performed at baseline, day 30 and day 100 in the anterior–posterior position by a certified densitometry technologist using the Hologic Discovery A[®] (Hologic, Inc., Bedford, MA) scanner, which generates X-rays at two energy levels (100 and 70 kV). DXA scanners were cross-calibrated between the two sites using a whole body phantom, and weekly whole body phantom scans were performed locally as part of routine quality control. A series of transverse scans were made from the head to toe at 2 mm intervals. Area, body weight, fat mass, bone mineral content, and lean tissue mass were recorded in grams for each region. Areal BMD was calculated and reported in g/cm². Percent body fat was determined from the summation of the fat mass of each component divided by the total body mass, multiplied by 100. BMD and BMC Z-scores were determined using age-appropriate normative data [17]. BMC Z-scores were calculated for male subjects up to 17 years of age and female subjects up to 16 years of age due to the age limit of the normative data [17]. Data from both sites were analyzed and interpreted at Boston Children's Hospital using the Hologic Pediatric Upgrade for children and adolescents up to age 20 years [18]. Interpretations were performed at the primary coordinating site by a bone health specialist (CG).

Biochemical Assessments

Laboratory biomarkers of bone health were analyzed at baseline, day 30, and day 100 visits. Bone specific alkaline phosphatase (BSAP), an indicator of osteoblast activity; urinary N-telopeptide (NTX), an indicator of bone resorption which was corrected for urinary creatinine levels; parathyroid hormone (PTH); and 25OH vitamin D (25OHD) were included in this assessment. In addition, serum levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) were analyzed as markers of inflammation.

Data Analysis

The patients' clinical characteristics, baseline DXA bone measures, and biochemical markers were compared between the two experimental groups by *t*-test for continuous measures and Fisher exact test for binomial and multinomial proportions. Changes in dichotomous outcomes between 0, 30, and 100 days were assessed by the Cochrane–Armitage trend test.

The time course of each bone measure and marker was analyzed by repeated-measures analysis of variance (ANOVA) with an unstructured covariance model and main effects for time (baseline, day 30, day 100) and treatment group (experimental or standard PN). A time $\times$ treatment interaction term was included to test whether the time course differed between

groups. The following covariates were uniformly included in each repeated measures model, either a priori or because they showed influence on the various endpoints: sex, age, Tanner stage, lean mass and fat mass (by DXA), resting energy expenditure, total energy intake, serum phosphorus, inpatient steroid intake, and insulin sensitivity. Daily vitamin D intake was added to the model for 25OHD because of its strong influence on the serum level (approximately 1 ng/ml per added $\mu\text{g/day}$). The ANOVA provided an overall test for time trend as well as estimates and standard errors for the adjusted mean level at each time and the change between baseline and the later times.

When the hypothesis of null trend (equal levels at baseline, day 30, and day 100) was rejected at $P < 0.05$, the principle of closed testing permitted pairwise comparisons between baseline and each later time using the same critical P -value, without compromising the familywise Type I error rate [19]. SAS software (version 9.3, Cary, NC) was employed for all computations.

RESULTS

Thirty patients were consented to the study, three patients subsequently became ineligible and one patient withdrew yielding a total of 26 evaluable patients. One patient died after baseline measures; two patients died and two patients transferred care after the day 30 measurements. Baseline characteristics of the study cohort are reported in Table I. Mean weight, height, and BMI were normal for age among study participants. Seventeen of the 26 enrolled patients had a diagnosis of leukemia. Twelve patients had a matched sibling donor and 14 had a matched unrelated donor. Five (19%) of the 26 patients received TBI as part of their conditioning regimen. At baseline, mean BMD, and BMC Z-scores were normal. Five (19%) of the subjects were diagnosed with GVHD following HCT; three with gastrointestinal GVHD (grades 2 and 3) and two with skin GVHD (grade 1 and 4).

Measures of bone health as assessed by DXA are summarized in Table II. Significant declines in covariate-adjusted mean BMD, BMD Z-score, BMC, and BMC Z-score were observed over the course of the 100-day study period. The time course did not differ between the experimental and standard PN groups ($P > 0.30$ for interaction). At baseline, 2 of 26 subjects (8%) had BMD Z score below -2 . The prevalence of low scores increased to 5 of 25 (20%) at 30 days and 6 of 21 (29%) at 100 days after HCT ($P = 0.08$ for trend).

Figure 1 shows that the baseline adjusted mean BMD Z-score was within normal limits at -0.45 and declined by 0.5 Z-score units over the 100-day study period. Baseline adjusted mean BMC Z-score was -1.01 and significantly declined over time to -1.54 by day 100.

Biochemical measures of bone health are reported in Table III. The mean serum BSAP level was marginally higher at baseline in the experimental PN group (41.9 vs. 26.2 $\mu\text{g/L}$) and declined significantly after HCT ($P = 0.003$), whereas in the standard PN group the trend was not significant ($P = 0.57$; $P = 0.02$ for interaction). Mean serum NTX did not change significantly over the 100-day study period ($P = 0.59$). Mean serum PTH was normal at baseline and increased significantly over time ($P = 0.04$). As inflammatory markers, serum IL-6 and TNF-alpha were examined as potential covariates in the multivariate models;

however, they were not significant contributors to the observed bone changes over the time course of the study, nor did they correlate with serum PTH or NTX.

Serum 25OHD concentrations declined significantly after HCT (Figure 2). From a baseline level of 29.2 ng/ml, near the threshold of vitamin D sufficiency (30 ng/ml), the covariate-adjusted mean fell into the vitamin D-deficient range (under 20 ng/ml): 19.7 ng/ml at 30 days and 17.7 ng/ml at 100 days. At baseline, 6 of 26 patients (23%) were vitamin D-deficient; at day 30, 18 of 25 (72%); and at day 100, 13 of 21 (62%) were deficient ($P = 0.03$ for trend). A similar, statistically significant rise was seen in the number of patients showing vitamin D-insufficiency.

DISCUSSION

We observed significant changes in markers of bone health among a cohort of children undergoing HCT. Reductions in BMD, BMC, serum BSAP, and 25OHD suggest that bone loss and vitamin D deficiency occur during the first 30 days following pediatric HCT. Most of these reductions persisted to day 100. These results suggest that most of the deleterious impact on bone health may occur during the immediate post-transplant period.

Several studies have described bone loss in survivors of pediatric HCT [1,2,4,20–22]. Greater bone loss has been observed in children treated with HCT compared to chemotherapy alone [4]. Bone loss has been associated with female sex [1,4], pre-pubertal age at transplant [22], and growth hormone deficiency [1,21]. Our findings suggest that a significant loss of bone occurs in the first 30 days after HCT in children, and thus timing may be an important consideration in the bone loss of HCT survivors. These observations may be related to the immediate impact of the preparative myeloablative treatment or other contributing factors at the time of HCT or during the first 30 days after transplantation.

Petryk et al. observed a decline in mean lumbar spine BMD Z-score of 0.5 SD from baseline to six months following HCT in a group of 19 children who had DXA measurements completed at baseline, six months, and one year after HCT [5]. Our study also supports the premise that BMD declines by 0.5 SD after HCT, but adds further evidence to suggest that the first 30 days following HCT may be the time period in which these bone losses are most likely to occur.

We also observed a concomitant reduction in serum 25OHD levels in this cohort, despite the provision of standard doses of parenteral vitamin D. Since oral vitamin D supplementation was recommended to patients who were identified as deficient at day 30, supplemental vitamin D intake may have affected the trend in vitamin D levels from day 30 to day 100. However, half of the patients remained deficient at day 100. These results raise concern about the adequacy of standard parenteral vitamin D dosing for children undergoing HCT. Frisk et al. found a relationship between low 25OHD levels and low BMD in a small group of adult long-term survivors of HCT who received total body irradiation [20]. Low bone mass and osteoporosis have been associated with low 25OHD levels and insulin resistance in HCT patients, suggesting a multifactorial relationship among long-term complications after HCT [6].

In addition to declines in the well-known marker of bone health, BMD, we also observed declines in BMC, which may be less confounded by bone size than BMD. As noted in our previous results, percent body fat increased over time with losses in lean body mass, especially in the group receiving standard PN [13]. With a significant decline in BMC in the standard PN group, and the trend towards significance in the experimental PN group once fat mass was removed from the analysis model, the rise in fat mass may have statistically mediated the decline in BMC in this population. These results justify the need for further investigation about BMC with measures of body composition among children undergoing HCT.

The biochemical markers of bone health examined in this study were consistent with our radiological findings. In the study by Petryk et al., a 30% decline in mean BSAP was observed at 100 days after HCT [5]. We observed a 44% decline in mean BSAP at 30 days after HCT, suggesting that a significant decrease in bone formation may occur during this time period. Declines in bone formation may accompany malnutrition [23], and as previously reported, our subjects lost a significant amount of weight and lean mass between baseline and 30 days after transplantation [14]. Mean values for NTX declined (although not significant and widely variable), implying less bone resorption may have occurred alongside the decline in bone formation. These changes in NTX support the results of a study that found a decrease in other urinary markers of bone resorption, deoxypyridinoline and pyridinoline, in the first 30 days following HCT [24]. Our findings suggest a possible uncoupling of bone formation and resorption in the first month after HCT that coincides with bone loss.

Since inflammation has been implicated as a mediator of bone loss in HCT recipients [25], the contribution of serum inflammatory markers IL-6 and TNF-alpha were considered in our multivariate models. In this study, measures of serum IL-6 and TNF-alpha did not significantly contribute to the observed bone changes over the course of the 100 day study period. Furthermore, in this study, these proresorptive cytokines did not correlate with either of the resorptive biomarkers measured, PTH and NTX. This does not rule out the possibility, however, that some degree of inflammation may relate to bone loss.

An important limitation to our study may be that the BMC and BMD were measured inclusive of the head, which may overestimate total body skeletal BMC and density. In our cohort, baseline BMC and BMD Z-scores were at or below the median for age, suggesting that if they are indeed overestimates of bone mineral, then the results may be even more worrisome for the bone health of HCT patients. Height-for-age deficits may influence the interpretation of BMC and BMD Z-scores [26]. Linear growth deficits can result from chronic illness or medical treatments. In this cohort, mean height-for-age Z-score was normal, and there was no significant difference between actual BMD Z-score and BMD Z-score predicted by height-for-age Z-score [mean difference -0.2 (95% CI -0.61, 0.20) $P = 0.31$]. However, individual height expectations might be impacted by genetic variations not accounted for in this analysis. The sample size of our study was small, and likely underpowered for changes in biochemical markers with wide variability, such as NTX. This study was also limited to children receiving allogeneic HCT, and results may be different in autologous HCT or in children with nonmyeloablative preparative regimens. Physical

activity, particularly weight bearing exercise, may improve bone health in childhood cancer survivors [27,28]. Although most children are minimally active due to their symptoms and side effects during the first 30 to 100 days after HCT, physical activity was not specifically measured in this study and could have contributed to changes in bone mineral measurements. Furthermore, we did not assess doses of cancer or transplant specific medications that could affect bone health [4,29]. Other limitations include the short-term follow-up period of 100 days and the lack of regional bone mineral measures.

In conclusion, we observed rapid and significant declines in bone health markers during HCT in a cohort of children undergoing allogeneic HCT. Potential causes for these changes should be explored, including the relationships between preparative chemotherapy, other medical treatments and lack of physical activity. Strategies to potentially attenuate bone loss in this setting could include weight-bearing exercise and perhaps more aggressive vitamin D supplementation. Further study is needed to examine these interventions in children undergoing HCT.

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Fig. 1.

Changes in bone mineral indicators over time in a cohort of children following HCT. Error bars = ± 1 standard error; adjusted for age, sex, Tanner stage, lean mass, fat mass, REE, energy intake, insulin sensitivity, inpatient steroid intake, and serum phosphorus;

*significant decline from baseline; time trend significant at $P < 0.0001$ for BMD Z-score, $P = 0.007$ for BMC Z-score.

Fig. 2.

Changes in serum 25OHD over time in children after HCT. Error bars = ± 1 standard error; adjusted for age, sex, Tanner stage, lean mass, fat mass, REE, energy intake, insulin sensitivity, serum phosphorus, inpatient steroid intake, and vitamin D intake; *significant decline from baseline; time trend significant, $P = 0.001$. Dashed line indicates level of vitamin D deficiency, 20 ng/ml.

TABLE I

Baseline Characteristics of 26 Children Undergoing HCT

Characteristic	Mean (SD) or n (%) ^a
Age, years	14.9 (4.2)
Weight, kg	52.6 (17.9)
Height, cm	154.7 (19.3)
Height-for-age Z-score	-0.39 (1.23) ^b
BMI Z-score	0.31 (0.83)
BMD Z-score	-0.48 (1.09)
BMC Z-score	-0.98 (1.26) ^c
25-OH vitamin D, ng/mL	29.0 (13.5)
Vitamin D deficiency	6 (23)
Pubertal stage ^b	
Pre-pubertal, Tanner 1	6 (23)
Pubertal, Tanner 2–4	8 (31)
Post-pubertal, Tanner 5	11 (42)
Sex	
Male	12 (46)
Donor type	
Sibling related	12 (46)
Unrelated	14 (54)
Diagnosis for transplantation	
Acute lymphoblastic leukemia	7 (27)
Acute myelogenous leukemia	7 (27)
Myelodysplastic syndrome	3 (12)
Chronic myelogenous leukemia	3 (12)
Lymphoma	2 (8)
Beta-thalassemia	2 (8)
Aplastic anemia	1 (4)
Kostmann syndrome	1 (4)

^a Combined experimental and standard PN treatment groups, n = 26. No significant differences between treatment groups;

^b n = 25;

^c Normative values available only for boys 17 year and under, girls 16 year and under; n = 17.

TABLE II
Time Course of Bone Mineral After Allogeneic HCT in Children, Measured by Whole-Body DXA.*

	Baseline			Day 30			Day 100			
	n	Mean	n	Mean	Change from baseline	P	Mean	Change from baseline	P	
BMD, g/cm ²	26	0.96 (0.02)	23	0.95 (0.02)	-0.01 (0.004)	0.02	21	0.92 (0.02)	-0.03 (0.006)	<0.0001
BMD Z-score	26	-0.45 (0.21)	23	-0.62 (0.24)	-0.17 (0.06)	0.007	21	-0.94 (0.24)	-0.50 (0.09)	<0.0001
BMC, g	26	1737 (69)	23	1668 (66)	-69 (8)	<0.0001	21	1609 (63)	-128 (19)	<0.0001
BMC Z-score ^a	17	-1.01 (0.37)	16	-1.45 (0.36)	-0.45 (0.15)	0.005	14	-1.54 (0.44)	-0.54 (0.16)	<0.0001

* Mean and change from baseline estimated by repeated-measures analysis, adjusted for age, sex, Tanner stage, lean mass, fat mass, REE, energy intake, insulin sensitivity, serum phosphorus, and inpatient steroid intake. *P* tests for non-zero change from baseline;

^a Normative values available only for boys 17 year and under, girls 16 year and under.

TABLE III

Changes in Biochemical Markers of Bone Health After Allogeneic HCT in Children

Marker	Adjusted mean (SE) ^a			<i>P</i> _{time} ^c	<i>P</i> _{int} ^d
	Baseline	Day 30	Day 100		
BSAP, µg/L	34.1 (4.5)	19.1 (2.8)	20.8 (3.3)	0.03	0.02
Experimental PN	41.9 (6.1)	15.7 (4.1)	24.2 (4.3)	0.003	
Standard PN	26.2 (6.4)	22.5 (3.8)	17.5 (5.0)	0.57	
N-telopeptide, nmol BCE/mM Cr	2859 (513)	1809 (414)	2108 (683)	0.33	0.59
PTH, pg/ml	42.0 (5.8)	53.1 (9.6)	69.1 (11.0)	0.04	0.09
25-OH vitamin D, ng/ml ^b	29.2 (3.1)	19.7 (2.2)	17.7 (1.8)	0.001	0.67
IL-6, pg/ml	5.3 (1.2)	4.3 (0.7)	6.6 (3.3)	0.63	0.80
TNF-alpha, pg/ml	2.3 (0.2)	2.8 (0.6)	2.5 (0.2)	0.46	0.07

^aFrom repeated-measures analysis, adjusted for age, sex, Tanner stage, lean mass, fat mass, REE, energy intake, insulin sensitivity, serum phosphorus, and inpatient steroid intake;

^bAdditionally adjusted for vitamin D intake;

^cTesting for non-zero time trend;

^dTesting for time × treatment interaction, i.e., difference in time course between experimental and standard PN groups.