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Impaired bone mineral density in pediatric patients with chronic graft-versus-host disease

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Abstract

Pediatric allogeneic hematopoietic stem cell transplant (AHSCT) recipients with chronic graft-versus-host disease (cGVHD) are at high risk of endocrinopathies, particularly impaired bone mineral density (BMD). However, rates of BMD impairment in pediatric AHSCT recipients with cGVHD have not been well documented. We describe 33 patients with cGVHD that were referred to the NIH for the Natural History of Clinical and Biological Factors Determining Outcomes in Chronic Graft-Versus-Host Disease Study (NCT 0092235) and underwent formal BMD assessments via dual-energy X-ray absorptiometry (DEXA). Not surprisingly, we found much higher rates of BMD impairment than previously reported for pediatric AHSCT recipients who were not stratified by presence or absence of cGVHD. Most of these patients, 73%, had a z-score ≤-2 in at least one anatomical site. While we expected the rate to be higher than that observed for pediatric AHSCT recipients on studies that did not analyze cGVHD patients separately, the rate was nonetheless extremely high. Furthermore, the overall rate of occult vertebral compression fractures (VCF) in our cohort was 17%, and 23% in patients with at least one z-score of ≤ -2. The rates of BMD impairment and VCF in our pediatric cohort were significantly higher than those of the adult AHSCT recipients that were concurrently enrolled on the same study at the NIH and had similar cGVHD severity. We found that older age at cGVHD diagnosis and greater number of

systemic therapies were associated with occult VCF. Also, intensity of current immunosuppression negatively impacted lumbar spine and total hip BMD in this cohort. Our study, while limited by small patient numbers and lack of a control AHSCT recipient group without cGVHD, indicates that children with cGVHD are at a greater risk for BMD impairment than previously appreciated. Given the rising incidence of cGVHD in AHSCT recipients and our findings, we recommend that pre-AHSCT DEXA be incorporated into routine pediatric pre-transplant screening studies. Having a baseline DEXA study could facilitate longitudinal monitoring of BMD in children who may be more susceptible to negative effects of AHSCT on BMD than adults. Also, given the high risk of BMD impairment in pediatric AHSCT recipients with cGVHD, such patients should undergo BMD evaluations upon developing cGVHD and continue to be monitored thereafter to allow intervention prior to progression of the BMD impairment to its severe manifestation, VCF.

Introduction

Allogeneic hematopoietic stem cell transplantation (AHSCT) has the potential to cure hematologic malignancies, as well as inherited and acquired disorders of hematopoietic cells[1-4]. A significant proportion of AHSCTs are being performed in children[1, 5]. Due to reductions is early transplant-related mortality (TRM), there is an increasing number of long-term survivors of AHSCT, who are at risk for a multitude of late effects[1, 2, 4, 6]. For pediatric AHSCT recipients, in particular, late-effects have a significant impact on quality of life (QOL) because AHSCT may interrupt growth and development, which may not resume in the post-AHSCT period[1, 7-9]. Furthermore, children should have a long life-expectancy, hence the burden of morbidity from a chronic condition, such as cGVHD, could be greater for a pediatric AHSCT recipient. Chronic graft-versus disease (cGVHD) is a major cause of non-relapse mortality following AHSCT, for both adult and pediatric patients[2]. While the incidence of cGVHD in pediatric AHSCT recipients is lower compared to adult AHSCT recipients, up to 50% of children undergoing AHSCT are affected, but actual rates of cGVHD incidence in the pediatric setting are estimated since most studies of AHSCT do not report pediatric data separately[3]. Overall, combined adult and pediatric, cGVHD prevalence is high due to a multitude of factors, including wide use of unrelated donors and peripheral blood-derived stem cells[1, 2].

Chronic GVHD, a highly inflammatory state of immune dysregulation, and the immunosuppressive regiments used to treat this condition, can cause damage to many organ systems[1]. First-line treatment for cGVHD continues to be systemic corticosteroids, with duration of therapy frequently extending to several years, often combined with calcineurin inhibitors. While both therapies have been shown to have deleterious effects on bone health in children and adults[4, 10, 11], the negative impact on bone health can be further enhanced during certain developmental periods of childhood and adolescence, such as puberty[7]. Peak bone mass in adulthood is largely dependent on appropriate BMD gain during childhood; thus, disruptions to this process may have long-lasting effects[8, 9].

The negative effects of systemic corticosteroids on bone density are multifactorial, with the primary insult on bone formation[12-14]. This is caused by a reduction in the number of osteoblasts, which is mediated via increased apoptosis of existing osteoblasts coupled with

reduced osteoblast replication and pre-cursor differentiation. Moreover, corticosteroids interfere with osteoblast function by inhibiting osteoblast gene transcription. Glucocorticoids also promote bone resorption by enhancing osteoclast activity. Additionally, systemic corticosteroids promote renal excretion of calcium and lead to decreased calcium absorption in the gastrointestinal tract. In combination, these processes lead to decreased bone mass[12-14].

Although children with cGVHD are likely to have high rates of impaired BMD the prevalence of BMD impairment in this patient population is not known. Current guidelines for monitoring bone-related late effects following AHSCT for children do not include separate recommendations for patients with cGVHD. The recommendations for all AHSCT recipients include dual-energy x-ray absorptiometry or quantitative computed tomography one year following transplantation[4, 15]. In addition, all AHSCT survivors, adults and children, are encouraged to have adequate calcium and vitamin D intake[4, 15]. Endocrine consultations are advised for children with BMD z-scores more than 2 standard deviations below the mean for age and for those with fractures[15]. Pediatric cGVHD patients would be expected to have additional risk factors for impaired bone health, given associated chronic inflammation, long-term use of corticosteroids and calcineurin inhibitors, nutritional and gastrointestinal complications of cGVHD, pubertal delays, poor growth, and decreased physical activity[8]. Based on our clinical experience with moderate and severe cGVHD, we hypothesized that this subset of pediatric cGVHD patients, would have higher rates of BMD impairment than previously suspected, and would be at high risk for occult vertebral compression fractures (VCFs). In this descriptive study, we aimed to measure this cohort's rate of BMD impairment, as well as investigate clinical features associated with BMD impairment and/or VCF.

Methods

Patients

A total of 47 pediatric patients between the ages of 2 to 19 years were enrolled on the Natural History Study of Clinical and Biological Factors Determining Outcomes in Chronic Graft-Versus-Host Disease (NCT 00092235) between February 2005 and June 2016. This study was conducted at the National Cancer Institute (NCI), National Institutes of Health (NIH) and was approved by the NCI Institutional Review Board (IRB). Most the patients were referred to the NIH by their extramural primary transplant team for advice and guidance on management of challenging cGVHD manifestations[16]. Witnessed, signed informed parental or guardian consent, along with patient assent were obtained prior to study enrollment.

During the week-long participation in this cross-sectional study, patients underwent a multi-disciplinary cGVHD evaluation by a transplant physician, ophthalmologist, dermatologist, dentist, psychologist, physiatrist, occupational therapist, and pain and palliative care specialist, with some patients having a pediatric endocrinology consultation. Organ involvement with cGVHD was assessed and graded per the NIH cGVHD consensus criteria[16, 17].

Of 47 patients, 8 did not undergo bone mineral density (BMD) evaluation by dual-energy X-ray absorptiometry (DEXA) due to age (<8 years old), 1 was excluded due to recent contrast administration for a clinically indicated diagnostic computerized tomography (CT) scan, 1 patient missed the DEXA appointment, which could not be re-scheduled within the week-long study period, and 4 had a recent DEXA's performed at an outside institution or at the NIH, but more than 3 months from the time of the cGVHD Natural History Study evaluation. The latter were excluded from BMD assessments because scans were performed on different equipment, and for the one patient who had a scan performed at the NIH, the clinical cGVHD scoring was not performed in temporal proximity to the scan. BMD was evaluated via DEXA at four anatomical sites, including lumbar spine, femoral neck, total hip, and forearm. As previously stated, patients 8 years and older were scheduled for the scan; however, there were 6 patients younger than 8 years old that were able to undergo DEXA, as they were able to comply with non-sedated imaging. Thus, data on 33 pediatric patients (age range of 3 to 18 years, mean age 11.5± 5.0) with DEXA scan measurements were further analyzed with regard to BMD, Figure 1. cGVHD, chronic graft-versus-host disease; BMD, bone mineral density; DEXA, Dual-Energy X-ray Absorptiometry; VCR, vertebral compression fracture.

Clinical Data Collection

Patient data obtained during the weeklong enrollment in the Natural History Study were recorded in the NIH Clinical Center electronic medical record (EMR). Standard demographic data, age and ethnicity, were collected, Table 1.

Available patient records from referring institutions were reviewed and summarized, and included the following parameters: date of HSCT, indication for HSCT (primary disease), disease status at HSCT, conditioning regimen (non-myeloablative versus myeloablative), whether total body irradiation was used in the conditioning regimen, stem cell source, donor relationship and gender, and degree of HLA match between host and donor, Table 2. Outside records were also reviewed for history of acute GVHD, cGVHD classification (classic, overlap, or late acute), age at cGVHD diagnosis, duration of cGVHD, and number of prior systemic therapies, Table 3. At the time of evaluation at the NIH, the following information was assessed: intensity of current immunosuppression, current prednisone/prednisone equivalent dose, and past and current calcineurin inhibitor therapy, Table 3. Clinical cGVHD scoring[16, 18, 19] was performed during the patient's visit to the NIH and included, sum of NIH organ scores for skin, mouth, eyes, GI tract, liver, lungs, joints and fascia, and genital tract in female patients, average NIH organ score, NIH global severity score, chronic GVHD activity assessment, and performance status (Karnofsky for patients 16 years of age and older and Lansky for patients younger than 16 years old)[20, 21], Table 3. Biochemical data, specific to the hypothesis of this study, were collected: serum 25-OH Vitamin D, 1,25-$(OH)_2$ Vitamin D, and ionized calcium, the data on the former two are provided in Table 1. Information regarding pubertal staging was documented according to the methods of Marshall and Tanner[22] on patients with a pediatric endocrine clinic evaluation (n=33)[23, 24], Table 1. The presence or absence of back pain and history of fractures at the time of diagnosis were recorded.

Height for each patient was measured by a pediatric nurse using a regularly calibrated stadiometer. Measurements were obtained three times, and the average was recorded in the EMR. Weight was determined using either a digital or mechanical scale. Body mass index (BMI) was expressed as body weight in kilograms divided by the square of height in meters (kg/m^2) as a weight-for-height index and was converted to percentiles and corresponding z-scores by using age- and gender-specific normative values for US children[25], Table 1.

Bone Densitometry

DEXA scans were performed on a single densitometer. Areal (two-dimensional) bone density was quantified by DEXA using a Hologic Discovery A scanner [Hologic Inc., Bedford, MA]. Bone mineral density (g/cm2) measurements were obtained in the anterior-posterior direction at the lumbar spine (LS, L1-L4), total hip, femoral neck and one-third distal radius using Hologic machines QDR 4500, Delphi, and Discovery A. Pediatric normative data were used to calculate age-, gender-, and height-adjusted z-scores, using the Bone Mineral Density in Childhood Study (BMDCS) calculator[24]. We defined low bone mineral density for age as BMD z-score of ≤ -2 SD according to the guidelines established by the International Society for Clinical Densitometry[24]. As previously stated, clinical and anthropometric data were obtained at the time of the DEXA examination, Table 1.

Vertebral Morphometry

Multichannel body CT scans obtained to evaluate pulmonary and visceral manifestations of cGVHD were available for 29 patients. Sagittal reformatted images obtained from these scans were retrospectively reviewed for the presence and grade of vertebral compression fractures (VCFs) by a single radiologist blinded to the details of the patients' clinical and laboratory data and detailed history. VCF assessment was performed on the T4 through L4 vertebral bodies according to the Genant semi-quantitative method[26]. The Genant semi-quantitative method includes grading of vertebral bodies according to the extent of the difference in height ratios from 100% when the anterior vertebral height is compared to the posterior height (defined as a wedge fracture), the middle height to the posterior height (biconcave fracture), and the posterior height to the posterior height of the adjacent vertebral bodies (crush fracture) according to the following severity score: grade 0 (normal), grade 1 (mild), grade 2 (moderate), and grade 3 (severe)[26-28]. The scores corresponded to the following reduction in vertebral height ratios: Grade 0: 20% or less; Grade 1: >20 to 25%; Grade 2: > 25 to 40%: Grade 3: >40%. Grade 0 was considered to be normal while grades 1, 2, or 3 were consistent with presence of fracture, Figure 2.

Variables Assessed and Statistical Analysis

Data for BMD z-score at the four different sites (lumbar spine, femoral neck, total hip, and forearm) were obtained and described, Figure 3. Impaired BMD was defined as the presence of a z-score ≤ -2 at any site (n=22) versus BMD greater than -2 for all sites and none missing (n=8). Three patients with DEXA measurement in only one site with BMD >-2 could not be included in this classification. For additional secondary analyses, impaired BMD was defined a second way: the presence of a z-score ≤ -2 in at least two sites (n=12), versus BMD ≤ -2 in only one site or none of the four sites without any missing (n=16). A total of

five patients with a DEXA measurement for only one anatomical site (two with BMD ≤-2 and the others with BMD >-2) could not be classified per this latter definition.

Patients were grouped three ways for comparisons: a) impaired BMD versus not (both definitions), b) presence of vertebral compression fracture (n=5) vs not (n=24), and c) no impaired BMD (n=8) vs presence of VCF (n=5).

Data were reported as frequencies and percentages, or means ± standard deviations, unless otherwise noted. Comparisons on the following variables were carried out between these patient groups, as applicable: cGVHD global NIH severity score, intensity of immunosuppression, TBI conditioning, age at cGVHD diagnosis, duration of cGVHD, number of systemic therapies, prior or current calcineurin inhibitor therapy, serum 25-OH Vitamin D level, puberty state, and body mass index (BMI) z-score. Categorical data were compared using the Fisher's exact test, and if singly-ordered, using the Kruskal-Wallis test. Continuous data were assessed for their distributional assumptions, and compared by the two-sample t-test. Correlations between certain clinical characteristics and BMD z-scores were done using Pearson's correlation coefficients. A two-sided p-value < 0.05 was considered statistically significant. While corrections for multiple comparisons were not carried out in this descriptive and exploratory study, 95% Confidence Intervals (CIs) were reported where statistically significant results were observed. Data were analyzed using SAS v9.4 (SAS Institute, Inc., Cary, NC).

Results

Patient Characteristics

Demographic and clinical characteristics of the 33 pediatric patients with cGVHD evaluated for bone mineral density impairments are described in Tables 1-3.

Bone Mineral Density Impairments

In the pediatric cGVHD patients with DEXA scans (age range 3 to 18 years old), only 27% (n=8) of patients had no impairment in BMD at any site as measured by DEXA, with 73% (n=22) of patients having at least one site involved, and 39% having 3 to 4 sites affected. The most common sites of BMD impairment were the femoral neck and total hip, with 54% (n=15) and 48% (n=13), respectively, with of all the patients having a z-score≤ -2 at these sites. The forearm was affected in 37% (n=10) of patients, and lumbar spine was affected in 28% (n=9) of patients. Overall, the entire cohort of pediatric cGVHD patients evaluated on our study had low BMD z-scores at the four evaluated sites, with femoral neck and total hip having lower z-score means compared to the other two sites (albeit not statistically significant), Figure 3.

In our cohort, the overall VCF rate for patients that had an evaluable DEXA and scout X-ray was 17% (5 out of 29), and 23% of patients with at least one site with z-score ≤-2 by DEXA (5 out of 22) were found to have an occult VCF, Figure 2. These fractures were scored by severity according to corresponding reduction in vertebral height ratios: two patients with Grade 1 (20 to 25%), and three patients with Grade 2 (25 to 40%). The mean duration of cGVHD preceding detection of vertebral fractures for these patients was 2.7 ± 0.7 years,

ranging from 1.9 to 3.6 years. In all five of these patients, at least one site had a BMD z-score ≤ -2. Two of five patients with an occult VCF had impaired lumbar spine BMD and at least two other sites were affected. None of the patients in our cohort, with and without impaired BMD, had a known history of fracture at the time of enrollment on study. In addition, occult VCF's were not associated with back pain or scoliosis. Only two patients (out of 33) received bisphosphonates prior to study entry. One of these patients had a z-score ≤ -2 at all four sites, and the other patient had impaired BMD at three sites with available DEXA data; there were no occult vertebral compression fractures detected during the study evaluation. No occult vertebral fractures were found in the six patients without BMD impairment.

Twenty-one (64%) patients included in this analysis had an NIH global severity score of 3 (maximum), with slightly but consistently higher proportions among patients with either type of BMD impairment or vertebral fractures (range 73%-100%). Generally, a majority of the patients with BMD impairment or vertebral compression factures (range 59%-80%) were on high intensity systemic immune-suppression (more than two agents with or without systemic corticosteroids). These observations, however, were not statistically different between the cohorts.

Older age at cGVHD diagnosis (13.5 years, 95% CI: 11.9, 15.1) and more systemic therapies (7.4, 95% CI: 5.1, 9.7) were associated with occult vertebral fractures, compared to patients without fractures (9.2 years, 95% CI: 7.0, 11.5; p=0.0018 for age, and 4.3, 95% CI: 3.2, 5.4; p=0.0148 for systemic therapies) or those without BMD impairment (7.8 years, 95% CI: 4.3, 11.4; p=0.0066 for age, and 4.3, 95% CI: 2.1, 6.5; p=0.0335 for systemic therapies). An inverse correlation was observed between intensity of immunosuppression and lumbar spine (r_p=-0.48, 95% CI: -0.53, 0.13; p=0.005) and total hip (r_p=-0.41, 95% CI: -0.50, 0.17; p=0.030) BMD z-scores, suggesting that patients with higher intensity of immunosuppression tended to have worse BMD z-scores at these sites. We did not find statistically significant differences between patients with any or at least two sites of impaired BMD compared to patients with one or no sites of BMD impairment for other relevant clinical parameters such as TBI conditioning, age at cGVHD diagnosis, duration of cGVHD, number of systemic therapies, prior or current calcineurin inhibitor therapy, serum 25-hydroxyergocalciferol (25-OH Vitamin D) level (Figure 4A), pubertal state, and BMI z-score. Approximately one half of the patients enrolled on our study had mild to moderate Vitamin D deficiency (25-OH Vitamin D level 11-32 ug/ml) and 12% had severe deficiency (25-OH Vitamin D level 10 ug/ml and lower). 25-OH Vitamin D level did not differ between patients with or without VCF. Approximately half of the patients on our study were on Vitamin D supplementation at the time of the evaluation.

Discussion

As hypothesized, BMD impairments in the pediatric cGVHD patients on our study appear to be more severe and more prevalent than previously reported in pediatric HSCT recipients[8, 10, 15, 29-31], particularly those without cGVHD.[4, 9, 15] For instance, in a study that evaluated bisphosphonate therapy for pediatric AHSCT recipients with low BMD, the approximate rate of osteopenia (z-score -1 to -2.4) and osteoporosis (z-score <2.5)[10],

combined, was approximately 60%. In another pediatric study, rate of osteopenia post-AHSCT as defined by a z-score < -1 at the lumbar spine (one site) was approximately 40%[31]. In our cohort, 88% of patients had a z-score < -1 in at least one site. Also, of the four patients with z-scores greater than -1, only one had a z-score available at more than one site; thus, the rate of any patient on our study having a z-score better than -1 at more than one anatomical site could be as low as 3% (1/33). In the former study, two sites were evaluated by DEXA, in the latter - one site, while in our study four anatomical sites were imaged. This could in part explain the higher prevalence of BMD impairment observed in our group compared to others; but, also suggests that that rate of BMD impairment in children with cGVHD may have been previously underestimated.

Adult transplant recipients receiving high-dose systemic corticosteroids for immunosuppression can develop substantial bone loss within months of transplantation[32]. Similarly, the use of systemic corticosteroids has been strongly linked to BMD impairment in pediatric AHSCT recipients[4, 10, 15]. When comparing our pediatric versus adult cGVHD cohort (concurrently enrolled on the same study) we observed a higher rate of significant BMD impairment, with DEXA z-score ≤-2 at three sites in 39% of pediatric versus17% of adult patients, despite both groups having a similarly high prevalence of severe cGVHD[33]. Pediatric AHSCT recipients are particularly vulnerable to bone health impairments. Prior clinical studies showed that AHSCT recipients under the age of 20 at the time of transplant are at a greater risk for BMD impairment than older recipients[9, 34]. Major acquisition of BMD occurs in the first decade of life in healthy children. The greatest bone density accrual takes place during adolescence, and peak trabecular bone mass is typically attained before age 20 in both girls and boys[35]. Perhaps, this provides an explanation for our finding that “older age” (pubertal) at the time of cGVHD diagnosis was correlated with presence of VCF. Not surprisingly, in our patient cohort, the femoral neck, a site rich in trabecular bone, was the most frequently affected location, consistent with what has been reported in adult patients with cGVHD[4, 12, 33, 36]. Also, corticosteroids have been shown to exert greater effects on trabecular than cortical bone, which could provide another reason for the observed BMD impairment pattern observed in AHSCT recipients on our study and others[13, 37].

When comparing our pediatric cGVHD cohort to another pediatric cohort on chronic immunosuppression and persistent systemic inflammation, such as inflammatory bowel disease (IBD), patients on our study appear to have a greater BMD impairment. For instance, in a pediatric Crohn's disease cohort, ~15% of patients were found to have a z-score ≤-2 by DEXA in both lumbar spine and femoral neck[38]. In our cohort, 26% of patients had a score ≤-2 at both sites. In IBD, malnutrition, decreased calcium and vitamin D absorption, systemic inflammation, delayed puberty, and prolonged corticosteroids are the known insults to bone health. These risk factors are common to both, IBD and cGVHD. However, pediatric cGVHD patients often have additional detrimental exposures contributing to impaired BMD, including TBI conditioning and calcineurin inhibitor exposure[9, 15, 30]. Importantly, all the patients on our study had received systemic calcineurin inhibitor therapy in the post AHSCT period. Furthermore, patients on our study receiving more systemic therapies, indicative of difficult to manage cGVHD, were found to have greater BMD impairments, i.e. higher incidence of VCF. Additional bone health insults from cGVHD itself, an inflammatory process, would be expected for patients with an already significant risk for BMD impairment

associated with AHSCT alone[1, 4, 10]; however, to our knowledge, this study is the first to specifically examine the rate of BMD impairment in pediatric cGVHD.

It is possible that the rates of BMD impairment measured on our study are higher than those in pediatric cGVHD patients treated at other centers given referral bias to our center for severe cGVHD. However, our study does indicate that pediatric patients with moderate to severe cGVHD are at an extremely high risk for BMD impairment. We found an alarmingly high occult VCF rate in our patient cohort. Glucocorticoid administration plays a critical role in the development of vertebral fractures, and as described, patients on our study had uniformly been exposed to systemic corticosteroids for extended periods. In our cohort, 23% of patients with at least one site with z-score ≤-2 by DEXA we found to have an occult VCF. It has been recently recognized that children with chronic diseases sustain vertebral fractures more often that previously suspected. Although vertebral fractures in children have been found to correlate with back pain, the majority, up to 70% with vertebral fractures in the setting of glucocorticoid treatment are asymptomatic, without back pain complaints nor known traumatic incidence history[39]. Of note, none of the patients on our study reported back pain at the time of evaluation. Therefore, surveillance for BMD impairments and VCFs, which can be expected to be asymptomatic, in children with cGVHD is prudent.

There are several limitations to our study. For instance, lateral thoracolumbar spine radiographs[26], which are the preferred imaging modality for evaluating compression fractures of vertebral bodies, were not obtained. Also, in adults, bone densitometry is validated for predicting risk of fracture; however, there is no consensus for applying the same parameters to predict or manage fracture risk in children[40]. Reduced BMD in children, as measured by DEXA, has been previously associated with increased fracture risk in otherwise healthy children[41-43]; but pediatric patients with chronic health conditions, such as patients on our study, often have pubertal and growth delays, and other risk factors, which are known to impact BMD independently[24, 27, 40]. Prior studies of BMD impairment in chronically ill children have been limited by small patient size, heterogeneity of clinical diagnoses, and presence of multiple risk factors for poor bone health, which, again, could not be evaluated independently[40]. Hence, DEXA z-scores alone are not typically used to establish the diagnosis of osteoporosis in children, and there are no widely agreed upon guidelines for establishing such a diagnosis in the pediatric setting[24, 27, 43]. Nonetheless, in our study, occult VCFs were detected only in children with impaired BMD in at least one site by DEXA. Given the lack of longitudinal assessment of BMD we were unable to determine the length of time that elapsed between the occurrence of occult VCFs prior to their incidental detection. The latter is of significance for ALL survivors whose rates of VCF in adulthood are approximately 20%, a decade post ALL treatment[44]. Additionally, previous studies in ALL patients identified a high rate of VCF at diagnosis due to acute effects of the leukemic process on bone. It is, therefore, plausible that in our cohort the VCFs could pre-date AHSCT, as three of the patients with VCF (3 out of 5) had ALL or AML as a primary indication for BMT (bone marrow transplantation). Also, ALL survivors are known to be at high risk for BMD impairment likely due to long term corticosteroid exposure, as treatment for ALL, and over half of the patients on our study were transplanted for leukemia.

Additional limitations of our study were that our sample sizes were small for most comparisons and confirmatory studies will be of value. Also, our study lacked a control cohort of age-matched pediatric AHSCT recipients without cGVHD. This could explain why other important known risk factors for BMD impairment, such as TBI conditioning, age at AHSCT, exposure to high dose corticosteroids, calcineurin inhibitor use, etc.[15], were not statistically comparable within our patient cohort, which was almost uniformly composed of patients with moderate to severe cGVHD and could not be further substratified. We could not overcome this limitation due to the nature of the referral pattern for our study.

The prevalence of mild to moderate Vitamin D deficiency (25-OH Vitamin D level 11-32 ug/ml) and severe deficiency (25-OH Vitamin D level 10 ug/ml and lower) we observed in patients on our study were similar to other pediatric AHSCT cohorts[45, 46], albeit patients on our study were mostly evaluated outside of the immediate post-AHSCT window[45] (3.5 years post AHSCT on average) and approximately half of the patients on our study were on Vitamin D supplementation at the time of the evaluation. Preventative measures to maintain adequate BMD include ensuring adequate calcium and vitamin D stores, encouraging regular weight-bearing physical activity, and identifying and treating hypogonadism[47]. It is well known that untreated hypogonadism results in reduced BMD[48]. There were four patients in our study who had hypogonadism, but were not on hormonal replacement. Three of them were boys, age 15-16, for whom optimal linear growth had not been achieved, hence the delay in initiation of treatment for hypogonadism. Nonetheless, the effect of hypogonadism on bone density for these patients was likely limited, given the short duration of low testosterone levels, than that of osteoporosis resulting from aging and hypogonadism several years in duration. For one female patient with hypogonadism (age 18), hormone replacement was attempted and resulted in worsening clinical status, and was thus discontinued.

A number of randomized controlled trials (RCTs) of bisphosphonates have been shown to prevent bone loss in adults after alloHCT[49-54]; however, no RCTs have evaluated bisphosphonates in children to prevent or treat impaired bone density after HCT[55]. If a child has a fragility fracture in the setting of a z-score ≤ -2, especially with compromised quality of life and/or pain, bisphosphonate therapy may be considered; however, the optimal duration of therapy, frequency of administration, and type of bisphosphonate to be used are not established[56]. A Cochrane review evaluating bisphosphonate therapy for children with secondary osteoporosis concluded that data are not currently adequate to justify these drugs as a standard therapy[57]. Bisphosphonates have been linked to rare but serious adverse events, including sub-trochanteric femoral fractures and osteonecrosis of the jaw[58]. Thus, bisphosphonate use in children remains controversial due lack of long term safety and efficacy data; and, further clinical studies are needed. As an alternative to bisphosphonates, a number of pharmacologic therapies have been used in the magagement of osteoporis in adults, including terapatide (recombinant parathyroid homeone), raloxifen (selective estrogen receptor modulator), romosozumab (monoclonal anti-sclerostin antibody), and denosumab (monoclonal antibody agaist RANKL)[59, 60]. While these agents have been shown to improve BMD in adults, they have not beem systematically studied in children. Denosumab has been used in a small number of pediatric patients with impaired BMD, such as osteogeneisis imperfecta, with resultant increases in BMD measurements without serious

adverse events[61]. Furthermore, a clinical trial to test whether denosumab can prevent bone complications in children receiving AHSCT is forthcoming[62].

To summarize, our study is, to our knowledge, the first to specifically evaluate BMD impairment in pediatric patients with cGVHD. These patients were predominantly graded as having moderate to severe cGVHD. Our findings indicate that the rates of BMD impairment in pediatric cGVHD patients are high and that current recommendations for identifying and managing these morbidities may need to be altered. For future prospective studies it would be prudent to capture BMD impairment before progression to its most severe sequela - VCF. Monitoring of BMD is the first step in clarifying how BMD impairments develop, which may allow the development of effective treatments. On our study, we were unable to determine when VCF occurred, as our patients did not have a pre-BMT nor post-BMT DEXA scans that predated detection of VCF. The pediatric AHSCT community should consider including a pre-AHSCT DEXA or quantitative CT scan (for those who are unable to undergo DEXA without sedation) for pediatric patients undergoing AHSCT. Such imaging could be included in the standard pre-BMT work-up and would be concordant with the recommendations of the International Society of Clinical Densitometry for other conditions associated with impaired BMD[63]. In addition, the amount of radiation exposure from a DEXA scan in young adults is similar to those of adults resulting in exposures that are a fraction of that delivered by a standard chest X ray, in smaller children it is similar to the latter[64]; and, sedation for DEXA can be avoided in most pediatric patients, as was the case on our study. Pre-BMT imaging would be of particular importance for patients with ALL and AML as the primary indication for AHSCT due to rates of BMD impairment in this group even without AHSCT being very high and potential presence of VCFs that could predate AHSCT. Current bone health recommendations advise BMD evaluation with either DEXA or quantitative CT at one year after transplant for all pediatric AHSCT recipients, with endocrine consultation for children with BMD z-score greater than two standard deviations below the mean for age[15]. In our cohort, one in five such patients, i.e. BMD z-score ≤-2 by DEXA in one site, already had a VCF at the time of the first post-BMT DEXA. Thus, we recommend that patients with cGVHD, who are at a greater risk of BMD impairment compared to other pediatric AHSCT recipients, should undergo a DEXA scan and be seen by an endocrinologist upon the initial diagnosis of cGVHD.

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References

1. Baird K, Cooke K, Schultz KR. Chronic graft-versus-host disease (GVHD) in children. Pediatr Clin North Am. 2010; 57:297–322. [PubMed: 20307722]

2. Arai S, Arora M, Wang T, et al. Increasing incidence of chronic graft-versus-host disease in allogeneic transplantation: a report from the Center for International Blood and Marrow Transplant Research. Biol Blood Marrow Transplant. 2015; 21:266–274. [PubMed: 25445023]
3. Zecca M, Prete A, Rondelli R, et al. Chronic graft-versus-host disease in children: incidence, risk factors, and impact on outcome. Blood. 2002; 100:1192–1200. [PubMed: 12149197]
4. Carpenter PA. Late effects of chronic graft-versus-host disease. Best Pract Res Clin Haematol. 2008; 21:309–331. [PubMed: 18503995]
5. D'Souza A, , Zhu X. Current Uses and Outcomes of Hematopoietic Cell Transplantation (HCT): CIBMTR Summary Slides, 2016 Vol. 2016. CIBMTR; 2016
6. Pidala J, Kurland B, Chai X, et al. Patient-reported quality of life is associated with severity of chronic graft-versus-host disease as measured by NIH criteria: report on baseline data from the Chronic GVHD Consortium. Blood. 2011; 117:4651–4657. [PubMed: 21355084]
7. Dvorak CC, Gracia CR, Sanders JE, et al. NCI, NHLBI/PBMTC first international conference on late effects after pediatric hematopoietic cell transplantation: endocrine challenges-thyroid dysfunction, growth impairment, bone health, & reproductive risks. Biol Blood Marrow Transplant. 2011; 17:1725–1738. [PubMed: 22005649]
8. Petryk A, Bergemann TL, Polga KM, et al. Prospective study of changes in bone mineral density and turnover in children after hematopoietic cell transplantation. J Clin Endocrinol Metab. 2006; 91:899–905. [PubMed: 16352681]
9. Petryk A, Polgreen LE, Zhang L, et al. Bone mineral deficits in recipients of hematopoietic cell transplantation: the impact of young age at transplant. Bone Marrow Transplant. 2014; 49:258–263. [PubMed: 24121211]
10. Carpenter PA, Hoffmeister P, Chesnut CH 3rd, et al. Bisphosphonate therapy for reduced bone mineral density in children with chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2007; 13:683–690. [PubMed: 17531778]
11. McClune BL, Polgreen LE, Burmeister LA, et al. Screening, prevention and management of osteoporosis and bone loss in adult and pediatric hematopoietic cell transplant recipients. Bone Marrow Transplant. 2011; 46:1–9. [PubMed: 20729922]
12. Weilbaecher KN. Mechanisms of osteoporosis after hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2000; 6:165–174. [PubMed: 10816024]
13. Woolf AD. An update on glucocorticoid-induced osteoporosis. Curr Opin Rheumatol. 2007; 19:370–375. [PubMed: 17551369]
14. Canalis E. Clinical review 83: Mechanisms of glucocorticoid action in bone: implications to glucocorticoid-induced osteoporosis. J Clin Endocrinol Metab. 1996; 81:3441–3447. [PubMed: 8855781]
15. Chow EJ, Anderson L, Baker KS, et al. Late Effects Surveillance Recommendations among Survivors of Childhood Hematopoietic Cell Transplantation: A Children's Oncology Group Report. Biol Blood Marrow Transplant. 2016; 22:782–795. [PubMed: 26802323]
16. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005; 11:945–956. [PubMed: 16338616]
17. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant. 2015; 21:389–401 e381. [PubMed: 25529383]
18. Baird K, Steinberg SM, Grkovic L, et al. National Institutes of Health chronic graft-versus-host disease staging in severely affected patients: organ and global scoring correlate with established indicators of disease severity and prognosis. Biol Blood Marrow Transplant. 2013; 19:632–639. [PubMed: 23340040]
19. Curtis LM, Grkovic L, Mitchell SA, et al. NIH response criteria measures are associated with important parameters of disease severity in patients with chronic GVHD. Bone Marrow Transplant. 2014; 49:1513–1520. [PubMed: 25153693]
20. Schag C, Heinrich R, Ganz P. Karnofsky performance status revisited: reliability, validity, and guidelines. Journal of Clinical Oncology. 1984; 2:7.

21. Lansky S, List M, Lansky L, Ritter-Sterr C, Miller D. The measurement of performance in childhood cancer survivors. Cancer. 1987; 60:6.
22. Marshall WA, Tanner JM. Variations in the pattern of pubertal changes in boys. Arch Dis Child. 1970; 45:13–23. [PubMed: 5440182]
23. Kalkwarf HJ, Zemel BS, Gilsanz V, et al. The bone mineral density in childhood study: bone mineral content and density according to age, sex, and race. J Clin Endocrinol Metab. 2007; 92:2087–2099. [PubMed: 17311856]
24. Gordon CM, Bachrach LK, Carpenter TO, et al. Dual energy X-ray absorptiometry interpretation and reporting in children and adolescents: the 2007 ISCD Pediatric Official Positions. J Clin Densitom. 2008; 11:43–58. [PubMed: 18442752]
25. Ogden CL, Kuczmarski RJ, Flegal KM, et al. Centers for Disease Control and Prevention 2000 growth charts for the United States: improvements to the 1977 National Center for Health Statistics version. Pediatrics. 2002; 109:45–60. [PubMed: 11773541]
26. Genant HK, Wu CY, van Kuijk C, Nevitt MC. Vertebral fracture assessment using a semiquantitative technique. J Bone Miner Res. 1993; 8:1137–1148. [PubMed: 8237484]
27. Halton J, Gaboury I, Grant R, et al. Advanced vertebral fracture among newly diagnosed children with acute lymphoblastic leukemia: results of the Canadian Steroid-Associated Osteoporosis in the Pediatric Population (STOPP) research program. J Bone Miner Res. 2009; 24:1326–1334. [PubMed: 19210218]
28. Ma J, Siminoski K, Alos N, et al. The choice of normative pediatric reference database changes spine bone mineral density Z-scores but not the relationship between bone mineral density and prevalent vertebral fractures. J Clin Endocrinol Metab. 2015; 100:1018–1027. [PubMed: 25494661]
29. Ruble K, Hayat MJ, Stewart KJ, Chen AR. Bone mineral density after bone marrow transplantation in childhood: measurement and associations. Biol Blood Marrow Transplant. 2010; 16:1451–1457. [PubMed: 20417715]
30. Le Meignen M, Auquier P, Barlogis V, et al. Bone mineral density in adult survivors of childhood acute leukemia: impact of hematopoietic stem cell transplantation and other treatment modalities. Blood. 2011; 118:1481–1489. [PubMed: 21596857]
31. Leung W, Ahn H, Rose SR, et al. A prospective cohort study of late sequelae of pediatric allogeneic hematopoietic stem cell transplantation. Medicine (Baltimore). 2007; 86:215–224. [PubMed: 17632263]
32. Stein E, Ebeling P, Shane E. Post-transplantation osteoporosis. Endocrinol Metab Clin North Am. 2007; 36:937–963. viii. [PubMed: 17983930]
33. Pirsl F, Curtis LM, Steinberg SM, et al. Characterization and Risk Factor Analysis of Osteoporosis in a Large Cohort of Patients with Chronic Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2016
34. Bhatia S, Ramsay NK, Weisdorf D, Griffiths H, Robison LL. Bone mineral density in patients undergoing bone marrow transplantation for myeloid malignancies. Bone Marrow Transplant. 1998; 22:87–90. [PubMed: 9678801]
35. Soyka LA, Fairfield WP, Klibanski A. Clinical review 117: Hormonal determinants and disorders of peak bone mass in children. J Clin Endocrinol Metab. 2000; 85:3951–3963. [PubMed: 11095413]
36. Lee WY, Kang MI, Baek KH, et al. The skeletal site-differential changes in bone mineral density following bone marrow transplantation: 3-year prospective study. J Korean Med Sci. 2002; 17:749–754. [PubMed: 12482996]
37. Natsui K, Tanaka K, Suda M, et al. High-dose glucocorticoid treatment induces rapid loss of trabecular bone mineral density and lean body mass. Osteoporos Int. 2006; 17:105–108. [PubMed: 15886861]
38. Gokhale R, Favus MJ, Karrison T, Sutton MM, Rich B, Kirschner BS. Bone mineral density assessment in children with inflammatory bowel disease. Gastroenterology. 1998; 114:902–911. [PubMed: 9558278]
39. Lentle B, Ma J, Jaremko JL, et al. The Radiology of Vertebral Fractures in Childhood Osteoporosis Related to Glucocorticoid Administration. J Clin Densitom. 2016; 19:81–88. [PubMed: 26653615]

40. Bachrach LK, Sills IN, Section on E. Clinical report-bone densitometry in children and adolescents. Pediatrics. 2011; 127:189–194. [PubMed: 21187316]
41. Goulding A, Grant AM, Williams SM. Bone and body composition of children and adolescents with repeated forearm fractures. J Bone Miner Res. 2005; 20:2090–2096. [PubMed: 16294262]
42. Clark EM, Ness AR, Bishop NJ, Tobias JH. Association between bone mass and fractures in children: a prospective cohort study. J Bone Miner Res. 2006; 21:1489–1495. [PubMed: 16939408]
43. Rauch F, Plotkin H, DiMeglio L, et al. Fracture prediction and the definition of osteoporosis in children and adolescents: the ISCD 2007 Pediatric Official Positions. J Clin Densitom. 2008; 11:22–28. [PubMed: 18442750]
44. Makitie O, Heikkinen R, Toiviainen-Salo S, Henriksson M, Puukko-Viertomies LR, Jahnukainen K. Long-term skeletal consequences of childhood acute lymphoblastic leukemia in adult males: a cohort study. Eur J Endocrinol. 2013; 168:281–288. [PubMed: 23197573]
45. Wallace G, Jodele S, Myers KC, et al. Vitamin D Deficiency in Pediatric Hematopoietic Stem Cell Transplantation Patients Despite Both Standard and Aggressive Supplementation. Biol Blood Marrow Transplant. 2016; 22:1271–1274. [PubMed: 27044905]
46. Myers KC, Howell JC, Wallace G, et al. Poor growth, thyroid dysfunction and vitamin D deficiency remain prevalent despite reduced intensity chemotherapy for hematopoietic stem cell transplantation in children and young adults. Bone Marrow Transplant. 2016; 51:980–984. [PubMed: 26974276]
47. Hautmann AH, Elad S, Lawitschka A, et al. Metabolic bone diseases in patients after allogeneic hematopoietic stem cell transplantation: report from the Consensus Conference on Clinical Practice in chronic graft-versus-host disease. Transpl Int. 2011; 24:867–879. [PubMed: 21557780]
48. Golds G, Houdek D, Arnason T. Male Hypogonadism and Osteoporosis: The Effects, Clinical Consequences, and Treatment of Testosterone Deficiency in Bone Health. Int J Endocrinol. 2017; 2017:4602129. [PubMed: 28408926]
49. Hari P, DeFor TE, Vesole DH, Bredeson CN, Burns LJ. Intermittent zoledronic Acid prevents bone loss in adults after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2013; 19:1361–1367. [PubMed: 23806773]
50. Tauchmanova L, Ricci P, Serio B, et al. Short-term zoledronic acid treatment increases bone mineral density and marrow clonogenic fibroblast progenitors after allogeneic stem cell transplantation. J Clin Endocrinol Metab. 2005; 90:627–634. [PubMed: 15546907]
51. Chae YS, Kim JG, Moon JH, et al. Pilot study on the use of zoledronic acid to prevent bone loss in allo-SCT recipients. Bone Marrow Transplant. 2009; 44:35–41. [PubMed: 19139732]
52. Grigg AP, Shuttleworth P, Reynolds J, et al. Pamidronate reduces bone loss after allogeneic stem cell transplantation. J Clin Endocrinol Metab. 2006; 91:3835–3843. [PubMed: 16835281]
53. Kananen K, Volin L, Laitinen K, Alfthan H, Ruutu T, Valimaki MJ. Prevention of bone loss after allogeneic stem cell transplantation by calcium, vitamin D, and sex hormone replacement with or without pamidronate. J Clin Endocrinol Metab. 2005; 90:3877–3885. [PubMed: 15797959]
54. Tauchmanova L, Selleri C, Esposito M, et al. Beneficial treatment with risedronate in long-term survivors after allogeneic stem cell transplantation for hematological malignancies. Osteoporos Int. 2003; 14:1013–1019. [PubMed: 14517597]
55. Saraff V, Hogler W. ENDOCRINOLOGY AND ADOLESCENCE: Osteoporosis in children: diagnosis and management. Eur J Endocrinol. 2015; 173:R185–197. [PubMed: 26041077]
56. Boyce AM, Gafni RI. Approach to the child with fractures. J Clin Endocrinol Metab. 2011; 96:1943–1952. [PubMed: 21734001]
57. Ward L, Tricco AC, Phuong P, et al. Bisphosphonate therapy for children and adolescents with secondary osteoporosis. Cochrane Database Syst Rev. 2007:CD005324. [PubMed: 17943849]
58. Kennel KA, Drake MT. Adverse effects of bisphosphonates: implications for osteoporosis management. Mayo Clin Proc. 2009; 84:632–637. quiz 638. [PubMed: 19567717]
59. Saag KG, Petersen J, Brandi ML, et al. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. N Engl J Med. 2017; 377:1417–1427. [PubMed: 28892457]
60. Qaseem A, Forciea MA, McLean RM, Denberg TD. Clinical Guidelines Committee of the American College of P. Treatment of Low Bone Density or Osteoporosis to Prevent Fractures in

Men and Women: A Clinical Practice Guideline Update From the American College of Physicians. Ann Intern Med. 2017; 166:818–839. [PubMed: 28492856]

61. Hoyer-Kuhn H, Franklin J, Allo G, et al. Safety and efficacy of denosumab in children with osteogenesis imperfect--a first prospective trial. J Musculoskelet Neuronal Interact. 2016; 16:24–32. [PubMed: 26944820]
62. Denosumab for Prevention of Bone Complications After Bone Marrow Transplantation in Children. https://clinicaltrials.gov/show/NCT02677246
63. Bianchi ML, Baim S, Bishop NJ, et al. Official positions of the International Society for Clinical Densitometry (ISCD) on DXA evaluation in children and adolescents. Pediatr Nephrol. 2010; 25:37–47. [PubMed: 19603190]
64. Blake GM, Naeem M, Boutros M. Comparison of effective dose to children and adults from dual X-ray absorptiometry examinations. Bone. 2006; 38:935–942. [PubMed: 16376161]

Highlights

- Children with cGVHD are at a greater risk for BMD impairment than previously appreciated.
- Over 70% of patients in our cohort had a DEXA z-score ≤-2 in at least one anatomical site.
- The overall rate of occult vertebral compression fracture was ~20% in our study.
- We recommend that pediatric patients undergo a BMD evaluation at the onset of cGVHD.
- A pre-BMT DEXA for pediatric patients can allow understanding of the trajectory of BMD impairment.

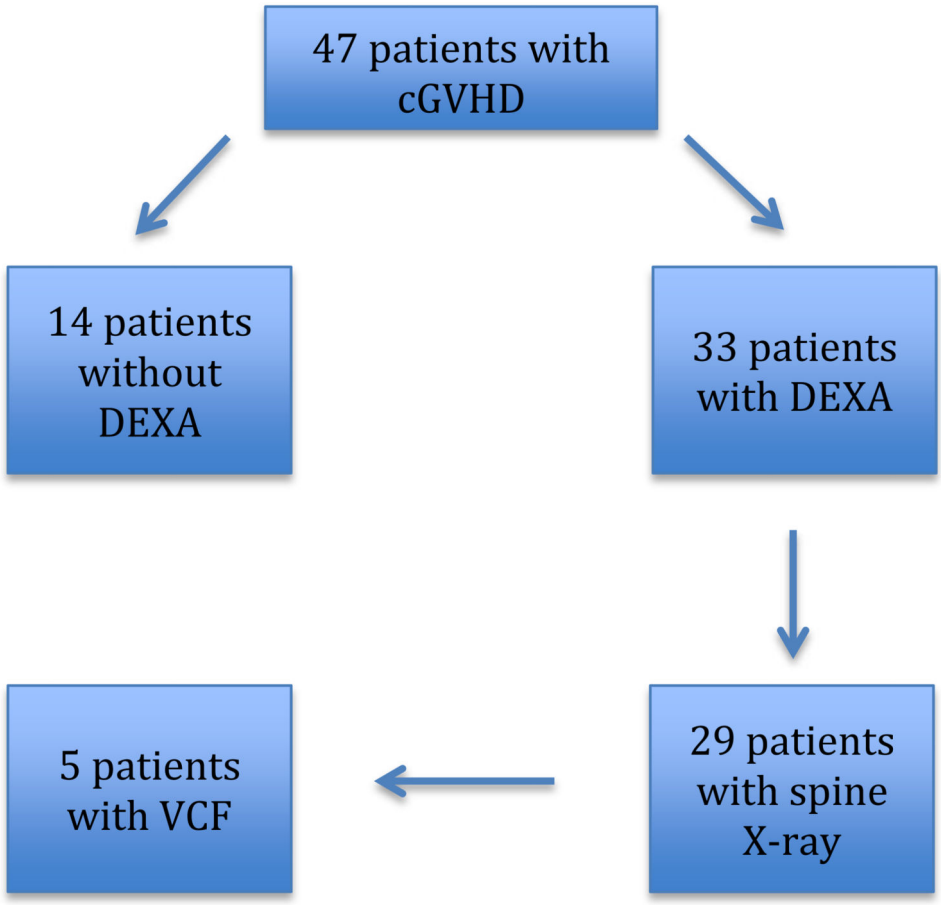

Figure 1.
Flow diagram of pediatric cGVHD patients enrolled on study, who underwent a BMD assessment and spine X-ray.

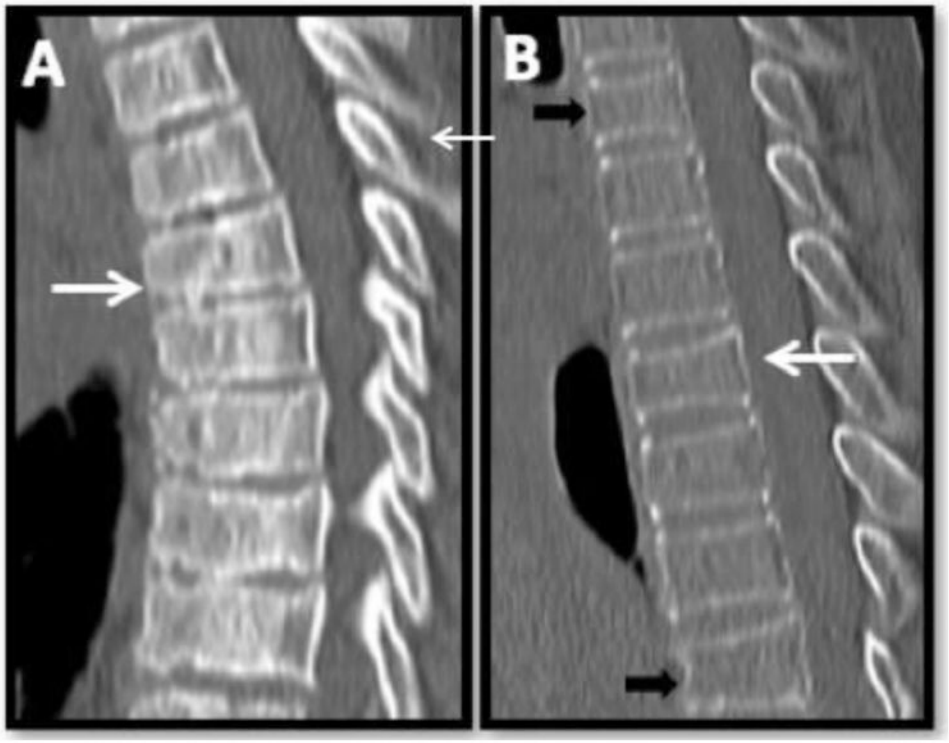

Figure 2.

Examples of VCFs (vertebral compression fractures) observed in our patient cohort. **(A)** CT of the lateral thorax of a 13-year-old female patient shows Genant grade 2 vertebral compression fracture in T7 vertebra (white arrow) with 33% reduction in anterior height. Note the irregularity of the spinal vertebral end plates. **(B)** CT of the lateral thorax of a 16-year-old male patient shows Genant grade 1 vertebral compression fracture in T8 vertebra (white arrow), with 23% reduction in anterior height. Anterior wedging in also noted in T5 and T11 vertebrae (black arrow heads). Note diffuse osteopenia of the spine.

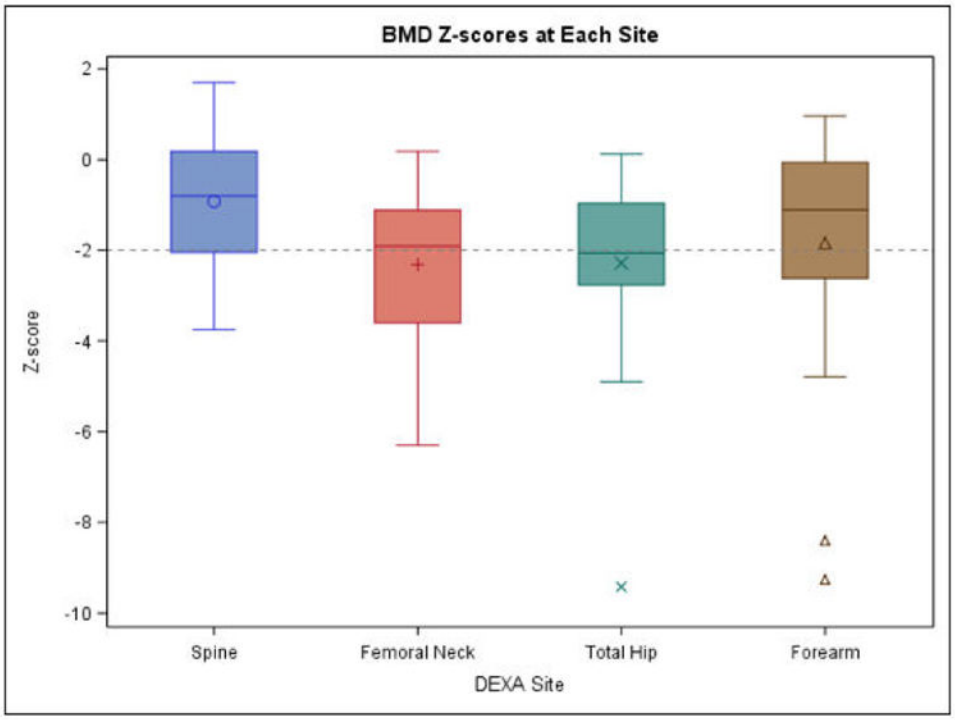

Figure 3.

A Box-and-Whiskers plot of the entire pediatric chronic graft-versus-host disease (cGVHD) cohort's bone mineral density (BMD) z-scores measured via dual-energy X-ray absorptiometry (DEXA) obtained at each anatomical site; spine, femoral neck, total hip, and forearm. The lengths of the boxes represent the interquartile ranges (25^{th} and 75^{th} percentiles] for each site, the symbols inside the boxes represent the group means, the horizontal line inside the boxes represents the group median z-score, and the vertical lines extending from the boxes are minimum and maximum values for each group.

Table 1

Demographic and clinical characteristics of pediatric cGVHD patients at the time of study entry.

Patient characteristics (n = 33, unless otherwise noted)	
Female / Male	10 (30.3%) / 23 (69.7%)
Age at DEXA, years	11.5 ± 5.0
Race (n=32)	
Asian	2 (6.3%)
Black/African	2 (6.3%)
White	27 (84.4%)
Other/Multiple race	1 (3.1%)
Ethnicity (n=30)	
Latino or Hispanic	4 (13.3%)
Non-Latino or Hispanic	26 (86.7%)
Pubertal	
Hypogonadism present and treated	2 (6.1%)
Hypogonadism present and untreated	4 (12.1%)
No hypogonadism	5 (15.1%)
Pre-Pubertal	22 (66.7%)
BMI	18.3 ± 4.6
BMI z-score (n=31)	
<-2	7 (22.6%)
between -1 and -2	4 (12.9%)
>-1	20 (64.5%)
Serum 25-OH Vitamin D Level	
Normal (33 ug/ml and above)	13 (39.4%)
Mild to moderate deficiency (11-32 ng/ml)	16 (48.5%)
Severe deficiency (10 ug/ml and lower)	4 (12.1%)
Serum 1,25-$(OH)_2$ Vitamin D Level (pg/ml)	59.4 ± 25.3
Vitamin D Supplementation	
Yes	16 (48.5%)
No	17 (51.5%)

Data are presented as mean ± standard deviation or number of patients (% of cohort). Percentages may not add up 100% due to rounding.
DEXA, Dual-Energy X-ray Absorptiometry; BMI, Body Mass Index.

Table 2
Transplant characteristics of pediatric patients evaluated on study

Characteristic, n=33 unless otherwise noted	
Primary Disease Characteristics	
ALL, AML and MDS	23 (69.7%)
HL	1 (3.0%)
Immunodeficiency	6 (18.2%)
Other non-malignant	3 (9.1%)
Disease status at HSCT for those with malignancies (n=32)	
Remission	30 (93.8%)
Persistent Disease	2 (6.3%)
Conditioning Regimen	
Non-myeloablative	5 (15.6%)
Myeloablative	27 (84.4%)
TBI used in conditioning	13 (40.6%)
Unknown	1 (3.0%)
Stem Cell Source	
Bone Marrow	18 (54.6%)
Peripheral Blood Stem Cells	8 (24.2%)
Cord Blood	7 (21.2%)
Donor Relationship	
Related	12 (36.4%)
Unrelated	21 (63.6%)
Donor Gender (n=25)	
Female, female recipient	4 (16.0%)
Female, male recipient	9 (36.0%)
HLA match	
Matched	17 (51.5%)
Mismatched	16 (48.5%)

Data are presented as number of patients (% of cohort). Percentages may not add up 100% due to rounding.
ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; MDS myleodysplastic syndrome; HL, Hodgkin lymphoma; HSCT, hematopoietic stem cell transplant; HLA, Human Leukocyte Antigen.

Table 3

Disease (cGVHD) and treatment characteristics among pediatric patients evaluated on study

Characteristic, n=33 unless otherwise noted	
History of acute GVHD	
Yes	29 (87.9%)
No	4 (12.1%)
cGVHD Classification (n=32)	
Classic	21 (65.6%)
Overlap	7 (21.9%)
Late acute	4 (12.5%)
cGVHD Onset (n=31)	
Progressive	21 (67.7%)
Quiescent	7 (22.6%)
De novo	3 (9.7%)
Age at cGVHD diagnosis, years	9.2 ± 5.4
Duration of cGVHD, days	1027.3 ± 1093.5
Number of prior systemic therapies for treatment of cGVHD	4.7 ± 2.5
Intensity of current immunosuppression, number of therapies	2.9 ± 1.2
Current prednisone/prednisone equivalent dose (mg/kg)	0.38± 0.63
Calcineurin Inhibitor Therapy	
Past Systemic	33 (100.0%)
Past Topical	10 (30.3%)
Current Systemic	17 (51.5%)
Current Topical	9 (27.3%)
Sum of NIH organ scores	6.4 ± 4.2
Average NIH organ scores	0.9 ± 0.6
NIH Global severity score	
Mild	1 (3.0%)
Moderate	9 (27.3%)
Severe	21 (63.6%)
cGVHD Activity Assessment (n=30)	
Active, irrespective of treatment	21 (70.0%)
Inactive, off treatment	2 (6.7%)
Inactive, on treatment	7 (23.3%)
Performance status	80.4 ± 14.0

Data are presented as mean ± standard deviation or number of patients (% of cohort). Percentages may not add up 100% due to rounding. Performance status (Karnofsky for patients 16 years of age and older and Lansky for patients younger than 16 years old).