Bone mineral density in children and young adults with neurofibromatosis type 1

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Abstract

Concern for impaired bone health in children with neurofibromatosis type 1 (NF-1) has led to increased interest in bone densitometry in this population. Our study assessed bone mineral apparent density (BMAD) and whole-body bone mineral content (BMC)/height in pediatric patients with NF-1 with a high plexiform neurofibroma burden. Sixty-nine patients with NF-1 (age range 5.2–24.8; mean 13.7±4.8 years) were studied. Hologic dual-energy X-ray absorptiometry scans (Hologic, Inc., Bedford, MA, USA) were performed on all patients. BMD was normalized to derive a reference volume by correcting for height through the use of the BMAD, as well as the BMC. BMAD of the lumbar spine (LS 2–4), femoral neck (FN), and total body BMC/height were measured and *Z*-scores were calculated. Impaired bone mineral density was defined as a *Z*-score −2. Forty-seven percent of patients exhibited impaired bone mineral density at any bone site, with 36% at the LS, 18% at the FN, and 20% total BMC/height. BMAD *Z*-scores of the LS (−1.60±1.26) were more impaired compared with both the FN (−0.54±1.58; *P*=0.0003) and the whole-body BMC/height *Z*-scores (−1.16±0.90; *P*=0.036). Plexiform neurofibroma burden was negatively correlated with LS BMAD (r_s=−0.36, *P*=0.01). In pediatric and young adult patients

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with NF-1, LS BMAD was more severely affected than the FN BMAD or whole-body BMC/height.

Introduction

The bone health of children and adolescents has become an increasingly recognized medical concern, and a number of genetic disorders of childhood are associated with impaired bone density. Neurofibroma-tosis type 1 (NF-1) is a variably penetrant autosomal dominant disorder with a birth incidence of ~1 in 2700 and prevalence of 1 in 4560 (Evans *et al.* 2010). NF-1 is caused by mutations in the neurofi-bromin gene on chromosome 17q11.2 (Marchuk *et al.* 1991). NF-1 is characterized by dermal and plexiform neurofibromas (PN) as well as café au lait macules, axillary and inguinal freckling, optic gliomas, and Lisch nodules (Gutmann *et al.* 1997, Lynch & Gutmann 2002, Ward & Gutmann 2005). Other common features within the diagnostic criteria are osseous abnormalities such as sphenoid wing dysplasia and tibial pseudarthrosis. Additional skeletal defects include bone cysts, spinal canal widening, vertebral body narrowing, rib-penciling, vertebral scalloping, and dural ectasia (Stevenson *et al.* 2007). Impaired BMD has been reported in several studies of children with NF-1; however, not all studies corrected for short stature (Dulai *et al.* 2007, Stevenson *et al.* 2007, Yilmaz *et al.* 2007, Brunetti-Pierri *et al.* 2008, Duman *et al.* 2008, Caffarelli *et al.* 2010).

In children and adolescents, the diagnosis of osteoporosis is made by a combination of densito-metric criteria plus the presence of both a clinically significant fracture history (Lewiecki *et al.* 2008). Impaired BMD in children is defined as a BMD *Z*-score that is −2.0, adjusted for age, gender, and body size. Reduced peak bone mass has been linked to an increased risk of osteoporosis earlier in adult life (Hui *et al.* 1990). NF-1 has been associated with compromised bone health in childhood that may leave patients vulnerable to fracture. Yilmaz *et al.* (2007) found that 31 pediatric NF-1 patients (mean age 9.9±4.1 years) with skeletal involvement were more likely to have a lumbar spine (LS) areal BMD (aBMD) lower than −2 compared with a control group. Using dual-energy X-ray absorptiometry (DXA) in 84 pediatric NF-1 patients (mean age 10.3±4.1 years), Stevenson *et al.* (2007) found that with or without skeletal abnormality NF-1 patients had a significant decrease in aBMD compared with control subjects. Dulai *et al.* (2007) studied 23 NF-1 patients (mean age 10.8 years) and showed that 30% had age-matched *Z*-scores below −1.5 for total body BMD. Duman *et al.* (2008) using DXA in 32 patients (mean age 8.38±3.75 years) showed significantly decreased BMD of the LS and femoral neck (FN) areas in NF-1 patients compared with controls. Similarly, Brunetti-Pierri *et al.* (2008) found significantly lower LS BMD in 73 NF-1 patients (mean age 16.0 years) compared with controls while Caffarelli *et al.* (2010) found significantly decreased FN aBMD and femur aBMD in 55 NF-1 patients (mean age 9.3±5.4 years) vs controls; both studies also corrected for height.

The impaired bone density in NF-1 may be associated with an increased risk of fracture. Fractures are a common occurrence in childhood, with an incidence rate in the general population ranging from 1.2 to 5% depending on the study (Jones 2004, Rennie *et al.* 2007, Donaldson *et al.* 2008). One study has evaluated the fracture rate in children with NF-1.

Fracture risk was evaluated in 103 Finnish children ages 3–16 years with NF-1 and were compared with age- and gender-matched controls. Children with NF-1 had a fracture incidence of 12/1000 person years vs an incidence of seven in control children, equal to a relative risk ratio of 3.4 (95% CI 1.5–7.8; Heerva *et al*. 2012).

One factor that may contribute to impaired BMD is 25-hydroxyvitamin D deficiency. Lammert *et al*. (2006) showed that 55 NF-1 patients had a mean serum 25-hydroxyvitamin D concentration of 14.0 ng/ml compared with 31.4 ng/ml in 58 healthy controls. Seitz *et al*. (2010) studied a subgroup of four adult NF-1 patients, who underwent DXA scans a year after oral administration of 25-hydroxyvitamin D supplements and showed significant improvement in spinal BMD *Z*-scores.

Chronic disease, poor nutrition, and tumor burden associated with NF-1 are additional factors that may affect BMD. In addition, calculations of BMD are usually underestimated in short individuals, particularly children, which necessitates the use of the bone mineral apparent density (BMAD) and whole-body bone mineral content (BMC) to correct for height. Many studies documenting a high prevalence of impaired BMD in pediatric NF-1 patients have failed to account for short stature in growing children, which leads to erroneous interpretation. The primary objective of this project was to characterize BMAD in pediatric NF-1 patients accounting for gender, age, ethnicity, and stature. Secondary aims sought to examine the prevalence of 25-hydroxyvitamin D deficiency in the patient cohort. Additionally, we analyzed the contribution of the PN tumor burden, scoliosis, scalloping, dural ectasia, and Eastern Cooperative Oncology Group Performance (ECOG) status to the BMAD. Our study is unique in that it is the first to examine the potential relationship between PN tumor burden and bone mineral density in children with NF-1.

Materials and methods

Subjects of protocol

Data were collected from a cohort of 69 children and young adults (ages 5–24 years) with a confirmed clinical diagnosis of NF-1 enrolled in a longitudinal study via retrospective chart review (National Institutes of Health Consensus Development Conference 1988). Collected baseline characteristics were gender, age, ethnicity, stature, ECOG performance score, 25-hydroxyvitamin D levels, and presence or absence of skeletal abnormalities (scoliosis, scalloping, dural ectasia, and history of fracture). All study tests were performed as part of the NF-1 Natural History study for children and adolescents (NCT00924196). Consent was obtained from each patient over 18 years, while parental consent and patient assent was obtained for those individuals under age 18 years after full explanation of the purpose and nature of all procedures used. The investigation was approved the Institutional Review Board of the Pediatric Oncology Branch, National Cancer Institute, at the NIH.

Assessment of bone density

Hologic DXA scans were analyzed to measure bone density. LS BMD of patients with spinal hardware was excluded because the devices cause artifacts on the DXA scan. Impaired BMAD for chronological age was defined as having a *Z*-score   −2.00 (Gordon *et*

al. 2008). Owing to the fact that BMD calculations are often underestimated in shorter pediatric patients, the BMD was normalized by correcting the values to adjust for differences in bone size through the use of the BMAD measurement, an estimation of volumetric bone density (g/cm^3). Values for BMC and area were used to calculate BMAD. BMAD Z-scores were calculated using published normative data for Hologic, as available on the Stanford applet: http://www-stat-class.stanford.edu/pediatric-bones (Bachrach *et al.* 1999). The normative data for the Stanford applet ages ranged from 9 to 25 years and is adjusted for age, gender, and race.

Hormone assays

25-Hydroxyvitamin D levels were defined as severely deficient (<10 ng/ml), mild-to-moderately deficient (10–24 ng/ml), and normal (25–80 ng/ml). Laboratory data assessing parathyroid hormone (PTH), calcium, 25-hydroxyvitamin D, and alkaline phosphatase were conducted within 6 months of the DXA. Elevated spot urinary calcium/creatinine ratio was defined as 0.3.

Tumor measurements

All patients had their PN tumor burden measured using the heuristics algorithm developed at NIH to analyze patient data through the MEDx (v3.42) visualization and analysis software (Sensor Systems, Inc., Sterling, VA, USA) using region-based and boundary-based segmentation (Solomon *et al.* 2004). Tumor burden was measured in cubic centimetre and percent tumor burden was calculated by dividing the tumor burden over the patient's body weight in kilogram.

Performance status

ECOG performance status is validated using scales and criteria are used to assess how a patient's disease affects their daily living abilities (Oken *et al.* 1982). In our cohort, scores ranged from 0 (fully active, able to carry on all pre-disease performance without restriction), 1 (restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature), to 2 (ambulatory and capable of all self-care but unable to carry out any work activities). ECOG status was assessed by interviews with study participants by a single nurse practitioner.

Statistical analysis

Baseline patient characteristics were analyzed as descriptive variables and summary statistics were generated. Paired *t*-tests were used to compare paired data (e.g. BMAD Z-scores at different sites), and two sample *t*-tests were used for comparing two independent groups. Tests for correlation included Pearson's correlation coefficient or Spearman's *rho*, as appropriate. The relationship between tumor burden and LS BMAD was explored with logistic and linear regression modeling. For logistic modeling, BMAD Z-scores were categorized as normal (−2) vs impaired (−2). The potential effects of patient characteristics hypothesized to be related to BMD such as age, gender, body mass index (BMI), Z-score, Tanner stage, prior PN therapy, and ECOG were considered in these models. Results are presented as mean±S.D., unless otherwise indicated. As appropriate,

multiple comparisons were adjusted by the step-down Bonferroni's method. A two-sided *P* 0.05 was considered statistically significant. Data were analyzed using SAS System Software version 9.2 (SAS Institute, Inc., Cary, NC, USA).

Results

Baseline characteristics are described in Table 1. One distinguishing characteristic of our patient cohort is the high prevalence PN, because these tumors have been known to develop in about 50% of individuals with NF-1; therefore, the 94% rate of PN in our study cohort is atypical (Tonsgard *et al.* 1998, Thakkar *et al.* 1999, Tucker *et al.* 2009). Seventy-one percent of the total patient cohort had been or were currently enrolled in one or multiple clinical trials with investigational drugs used to treat their PN. The most widely used therapies in our patient cohort included tipifarnib, peginterferon *α*-2b, pirfenidone, and sirolimus, with 41, 39, 26, and 13% of patients having received therapy with one of these agents respectively.

Patients had a low height SDS (-0.92 ± 1.17). While the majority of patient's laboratory data remained within the normal range (Table 1), it is notable that eight patients had elevated urine calcium levels, two patients had elevated PTH levels, and three patients had elevated alkaline phosphatase levels. Within the patient cohort, 30 (47.6%) had normal 25-hydroxyvitamin D status and 33 (52.4%) had moderate 25-hydroxyvitamin D deficiency. There were 14 patients (20.3%) with prior fractures and 42 patients (60.9%) had scoliosis.

Of the 69 patients, final data were available for FN BMAD in 53 patients, for LS BMAD in 50 patients, and for BMC/height in 51 patients; LS BMAD was excluded in three patients because of previously installed hardware, 12 because of age (i.e. under 9 years and applet did not allow for *Z*-score calculation), and one because of both, while three patients did not have a BMD measured at this site. Without correcting for short stature and using BMD values of -2 at any site to define impaired bone density, the prevalence of impaired BMD in this group would have been overestimated at 56%. In this study, 47% of patients had impaired BMAD at any bone site, while the expected frequency in the normal population is 2.3% at each individual bone site based on the normal distribution. The breakdown of impaired BMAD in our cohort consists of 36% at the LS, 18% at the FN, and 20% total BMC/height. BMAD *Z*-scores of the LS (-1.60 ± 1.26) were lower than those of the FN (-0.54 ± 1.58; *P*=0.0003; Fig. 1). The BMC/height *Z*-scores (-1.16 ± 0.90) were worse than those of the FN (-0.54 ± 1.58; *P*=0.007), while only slightly better than those of the LS (-1.60 ± 1.26; *P*=0.036) (Fig. 1). Tumor burden as a percent of body weight was inversely correlated with both the LS BMAD *Z*-scores ($r_s =-0.36$, *P*=0.01) and BMC/height ($r_s = -0.28$, *P*=0.048) (Fig. 2). The odds ratio of having a greater tumor burden was 1.26 (95% CI 1.006–1.575, *P*=0.04) in patients with LS BMAD *Z*-scores -2. Increased tumor burden, age, BMI *Z*-score, and Tanner stage were statistically significantly associated with impaired LS BMAD by univariable regression analysis, while gender, prior PN therapy, and ECOG score were not (Table 2). When the relation between tumor burden and LS BMAD was adjusted for these covariates, only BMI *Z*-score remained statistically significantly (*P*=0.002) associated with impaired BMAD. We asked the obvious question of whether patients with NF-1 and impaired BMAD were more likely to have a fracture than those NF-1

patients with normal BMAD. The likelihood of having a prior fracture was not statistically different in patients classified as having any BMAD Z site Z-score −2 vs those with normal BMAD.

As secondary analyses, we investigated the contribution of additional skeletal abnormalities and 25-hydroxyvitamin D deficiency to BMAD in NF-1 patients. Individuals with scoliosis had lower BMAD Z-scores of the FN than the patients without scoliosis ($P=0.007$; Table 3). Patients with scalloping or dural ectasia had worse LS BMAD Z-scores when compared with individuals without these defects (-1.61 ± 1.17 vs -0.31 ± 0.11, $P<0.0001$). Interestingly, the presence of a PN involving the LS was not associated with worse LS BMAD Z-scores in our study. Even though 52% of patients had moderate 25-hydroxy-vitamin D deficiency, there were no correlations with BMAD Z-scores for any of the three skeletal sites.

Discussion

Previous studies have analyzed bone density in patients with NF-1 (Dulai *et al.* 2007, Stevenson *et al.* 2007, Yilmaz *et al.* 2007, Brunetti-Pierri *et al.* 2008, Duman *et al.* 2008, Caffarelli *et al.* 2010). While more data exist for adults, a number of smaller studies have looked at impaired BMAD in children with NF-1, but not all of them corrected for height. Our study is unique in its examination of a large pediatric and young adult population with a significant PN burden, while looking at BMAD Z-scores to correct for discrepancies in height.

Despite all that is known about neurofibromin, it is still unclear how skeletal abnormalities evolve in these patients. Investigations in a mouse model of NF-1 by Yu *et al.* (2005) suggested that there was increased apoptosis of osteoprogenitors in the affected mice. Another study has shown increased osteoclast activity in NF-1 mice correlated with increased p21 function (Yang *et al.* 2006). A study of bone metabolism markers found that 26 children with NF-1 had lower levels of osteocalcin compared with normal controls (Duman *et al.* 2008). The Seitz *et al.* (2010) study looked at iliac crest biopsies in 14 NF-1 adult patients and found decreased trabecular thickness, as well as increased osteoblast and osteoclast activity, demonstrating increased bone turnover when compared with controls.

We found a 47% prevalence of impaired BMD among pediatric NF-1 patients. The results show that the LS was more affected with a 36% prevalence of impaired BMD and a mean BMAD Z-score of −1.7. The LS is predominantly composed of trabecular bone, compared with the FN that is primarily cortical bone. The pattern of more pronounced bone density impairment in the LS has been seen previously in other studies in pediatric patients with NF-1. Brunetti-Pierri *et al.* showed that 48% of patients had impaired LS BMD Z-scores using the criteria of a Z-score −1.5 (Brunetti-Pierri *et al.* 2008). Yilmaz *et al.* (2007) found that 12.5% of the pediatric patients had low LS BMD Z-scores based on a Z-scores criteria of −2. One potential explanation for low LS BMD is that trabecular bone of the LS may be more sensitive to mineralization problems in growing children (Brunetti-Pierri *et al.* 2008). These patients may potentially be at risk for asymptomatic vertebral compression fractures given the low LS BMD; however, this has yet to be investigated. Our study also showed that certain skeletal abnormalities including scoliosis, vertebral scalloping, and dural ectasia

were associated with lower BMAD *Z*-scores. Scoliosis affects up to 26% of individuals with NF-1, which could magnify the effects of impaired BMD (Friedman 2002). Interestingly, our NF-1 patients had a relatively low reported frequency of fracture (20.3%) compared with the percentage of fractures in the normal pediatric population in which the 42% of boys and 27% of girls report sustaining at least one fracture by the age of 16 years (Brinker & O'Connor 2004).

Our results indicated a correlation of larger tumor burden with a lower BMAD *Z*-score; however, the correlation was not strong. In addition to increased tumor burden, age, BMI *Z*-score, and Tanner stage were also statistically significantly associated with impaired LS BMAD by univariable analysis. A potential explanation for the correlation between tumor burden and decreased BMAD is that the PN may secrete a substance that has a negative impact on bone health, but this has not been investigated. Other mesenchymal tumors secrete fibroblast growth factor 23 as a paraneoplastic phenomenon, which may be part of the explanation of decreased bone density in patients with larger PN tumor burden (Ovesen *et al*. 2003, Ginde *et al*. 2009). However, arguing against this hypothesis is the normal serum calcium and PTH levels in these patients. Another hypothesis to explain the association of impaired BMAD with PN is linked to the pathogenesis of PN development in NF-1. As neurofibromin is a tumor suppressor, a second-hit mutation precedes the development of tumors including PN; mutations that predispose these individuals to develop PN may potentially affect bone if they occur early in development (Staser *et al*. 2012). When other factors were considered together, only BMI *Z*-score was statistically significantly associated with impaired BMAD, providing evidence that a number of variables work together to contribute to bone density. This could also indicate the effects of decreased physical activity as a contributor to poor bone health.

Despite the 52% of patients with moderate 25-hydroxyvitamin D deficiency, which we initially hypothesized would be associated with low bone density, no correlation was found between these two measures in the patient cohort. It is unclear how other factors might have affected 25-hydroxyvitamin D levels, as latitude, season, and skin tone have been shown to influence 25-hydroxyvitamin D levels (Ovesen *et al*. 2003, Ginde *et al*. 2009). Further investigation and longitudinal studies are warranted to better understand the relationship between 25-hydro-xyvitamin D and bone density in pediatric NF-1 patients.

One limitation of our study is the potential effects of tumor therapies on BMD. Within our patient cohort, 91% had PN, and the majority of these individuals were enrolled on clinical trials with investigational drugs for their PN. Studies have shown that several of these cancer treatments can negatively impact bone health, while a number of them are new agents without known bone-related effects. Methotrexate has been found to inhibit bone formation by blocking bone turnover and inhibiting the early differentiation of osteoblasts, which can potentially lead to methotrexate osteopathy characterized by osteoporosis, fractures, and bone pain (Holzer *et al*. 2003, Wilson *et al*. 2012). However, only two out of the 69 patients in our cohort received methotrexate treatment. Another limitation of our study is that a higher prevalence of PN in our cohort may make our results less generalizable to patients with NF-1 as a whole.

Understanding and identifying the elevated risks of low BMD in patients with NF-1 are critical in order to provide improved patient care. There are several ways that patients can minimize their risk such as participating in weight-bearing exercise, optimizing their calcium and 25-hydroxyvitamin D intake, and avoiding osteotoxic medications. The use of bisphosphonates in pediatric patients to improve BMD is controversial due to potential longitudinal affects on bone health and lack of randomized controlled studies (Marini 2003). Comprehensive management of the pediatric patient with NF-1 includes assessment of skeletal health, and our study provides important additional information about bone mineral density in this patient population.

Acknowledgments

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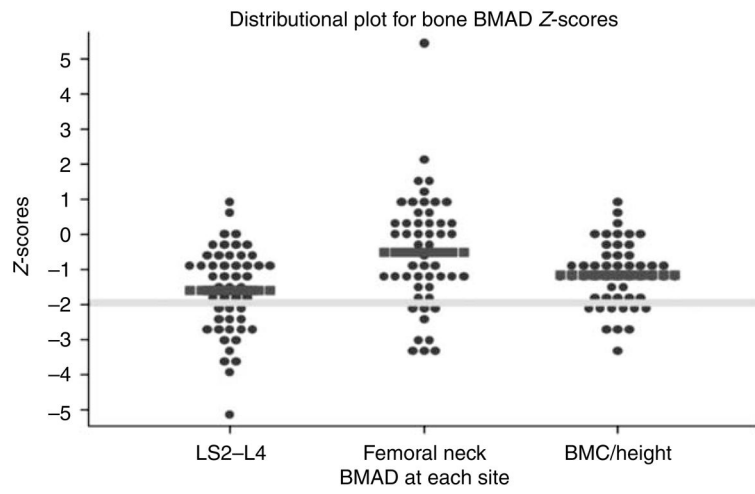Distributional plot for bone BMAD Z-scores

Figure 1.
Distributional plot for lumbar spine (LS2–L4) and femoral neck BMAD and BMC/height Z-scores. Thick yellow line is the −2.00 level Z-score. Thick red bars represent mean Z-scores.
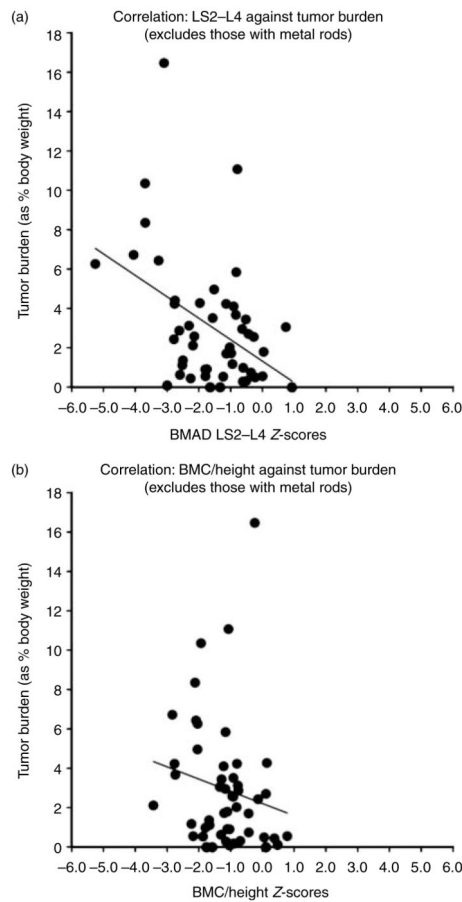
Figure 2.
(a) Correlation plot of tumor burden as a percent of body weight inversely correlated with the lumbar spine BMAD Z-scores ($r_s=-0.36$, $P=0.01$). Negative correlation marked by thin black line. (b) Correlation plot of tumor burden as a percent of body weight inversely correlated with the BMC/height ($r_s=-0.28$, $P=0.048$). Negative correlation marked by thin black line.

Table 1

Patient characteristics. All data are mean±S.D., unless otherwise indicated

		Male	Female
Females (%)	29 (42%)		
Males (%)	40 (58%)		
Age (years)	13.7±4.8		
Race (%)			
Asian	1 (1%)		
African-American	3 (4%)		
White	58 (84%)		
White/African	1 (1%)		
Hispanic	4 (6%)		
Other	2 (3%)		
Height SDS	−0.92±1.17		
BMI Z-score	0.03±1.2		
Tanner stages			
I		18 (45%)	9 (31%)
II		5 (13%)	2 (7%)
III		0 (0%)	1 (3%)
IV		1 (2%)	1 (3%)
V		15 (38%)	16 (55%)
No data		1 (2%)	
Tumor burden in cubic centimetre (median, 25th–75th percentiles)	781 (259–1734)		
Percent tumor burden (cc/kg; median, 25th–75th percentiles)	2 (0.53–4.23)		
Prior fracture history	14 (20%)		
Scoliosis present	42 (61%)		
Scalloping present	32 (46%)		
Dural ectasia present	38 (55%)		
Moderate 25-hydroxyvitamin D deficiency[a]	33 (52%)		
PTH (nl: 16–87 pg/ml)	39.4±20.9		
Serum calcium (nl: 2.05–2.50 mmol/l)	2.3±0.1		
Urine calcium/creatinine ratio	0.2±0.1		

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25 Vitamin D2 and D3 (nl: 25–80 ng/ml)   25.4±8.5

Alkaline phosphatase (U/l)

Age (years)	Male	Female	Age (years)	Male	Female
4–6	93–309	96–297	4–6	170±70	155±40
7–9	86–315	69–325	7–9	161±59	142±39
10–12	42–362	51–332	10–12	207±48	171±25
13–15	74–390	50–162	13–15	153±58	117±84
16–18	52–171	47–119	16–18	106±48	80±22
18	37–116	37–116	18	75±18	61±14

nl, standard normal values used for these measurements.

[a] Moderate 25-hydroxyvitamin D deficiency is defined as 10–24 ng/ml.

Table 2

Univariable regression modeling of potential contributing factors in the relationship between tumor burden and LS BMAD

Variable	R^2	P value
Gender	0.05	0.15
ECOG	0.05	0.13
Age	0.23	0.0009[*]
Tanner stage	0.30	0.0001[*]
BMI Z-score	0.30	0.0001[*]
Prior therapy	0.01	0.44

[*] Statistically significant.

Table 3

BMAD *Z*-scores of patients with and without scoliosis

Site	Patients with- out scoliosis (mean±S.D.)	Patients with scoliosis (mean±S.D.)	*P* value
Lumbar spine	−1.55±1.10	−1.64±1.38	0.8
Femoral neck	0.17±1.68	−1.01±1.34	0.0066
BMC/height	−1.15±0.79	−1.17±0.99	0.9