University of Cincinnati

Date: 2/3/2016

I, Halley M Wasserman M.D., hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
Low bone mineral density and fractures are highly prevalent in pediatric patients with Spinal Muscular Atrophy regardless of disease severity

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Committee member: Jane Khoury, M.S., Ph.D.
Low bone mineral density and fractures are highly prevalent in pediatric patients with Spinal Muscular Atrophy regardless of disease severity

A thesis submitted to the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of

Master of Science

in Clinical & Translational Research

In the Department of Environmental Health
Division of Epidemiology
of the College of Medicine

March, 2016

by

Halley M. Wasserman, MD

Doctor of Medicine, Northeast Ohio Medical University, June 2009
Bachelor of Science, Kent State University, August 2005

Committee Chair: Erin Haynes, Dr.P.H
Abstract:

Purpose: Fractures and poor bone health due to limited ambulation are significant concerns for patients with Spinal Muscular Atrophy (SMA). However, the prevalence of fractures, low areal bone mineral density (aBMD; Z-score ≤ -2.0) of the lateral distal femur (common fracture location in non-ambulatory children) and of osteoporosis by SMA subtype is not known.

Methods: We reviewed data from SMA patients ages 12 months to 25 years, seen at a single institution between January 2005 and January 2015. Fracture history was reported at annual clinic visits. aBMD was obtained from dual energy x-ray absorptiometry scans of the lumbar spine, total body, and lateral distal femur.

Results: Median age at initial SMA visit was 1.8 years, but differed by SMA subtype. DXA data were available on 69% of the sample: of these, 90% had a BMD Z-score ≤ -2.0 SD at time of first DXA. aBMD Z-scores at all sites was lower with worsening SMA severity, decreasing over time at the lateral distal femur. Fractures occurred in 36% of patients with the femur being the most common location (25 of 53 total fractures). Median age at first fracture was significantly younger with worsening SMA severity. 13% of patients had multiple fractures. Only 8.5% of patients fulfilled criteria for osteoporosis.

Conclusion: Low BMD is highly prevalent in SMA patients at the time of first DXA. Fracture frequency is also high with a predominance of femur fractures in all subtypes. However, few patients met ISCD diagnostic criteria for osteoporosis. Our data suggests poor bone health is a significant concern for SMA patients, but may be under-recognized using the 2013 International Society for Clinical Densitometry criteria for diagnosis of osteoporosis in children.
Acknowledgements

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Table of Contents:

Abstract...........................................................................................................ii
Acknowledgements........................................................................................iv
1. Introduction.................................................................................................1

2. Methods
   2.1 Bone Mineral Density...........................................................................3
   2.2 Fracture History.....................................................................................4
   2.3 Osteoporosis..........................................................................................4
   2.4 Statistical Analysis................................................................................4

3. Results
   3.1 Demographics.......................................................................................5
   3.2 Prevalence of low bone mineral density by SMA subtype.................5
   3.3 Fracture Frequency by SMA subtype......................................................7
   3.4 Bisphosphonate use by SMA patients....................................................7
   3.5 Osteoporosis Prevalence.......................................................................8

4. Discussion....................................................................................................8

5. Conclusion..................................................................................................12

Bibliography..................................................................................................14
Tables and Figures.........................................................................................18
List of Tables and Figures

Table 1: Demographics

Table 2: Bone Health Outcomes

Figure 1: Change in areal bone mineral density over time by skeletal site.
Introduction

Spinal Muscular Atrophy (SMA), an autosomal recessive neuromuscular disease due to mutations in the survival motor neuron gene 1 (SMN1), affects 1 in 6,000-10,000 live births and is the leading cause of death due to a genetic mutation in infants [1, 2]. This degenerative disease of the spinal cord and lower brainstem motor neurons causes progressive proximal muscle weakness resulting in varying degrees of hypotonic immobility and respiratory compromise. While there are no genotype-phenotype correlations, clinical severity is associated with the number of copies of a rescue gene, SMN2 [3]. Patients are typically characterized by their clinical phenotype: SMA Type 1 (SMA1) patients never sit independently; SMA Type 2 (SMA2) patients can sit but never stand or walk independently; and SMA Type 3 (SMA3) patients walk independently with a later loss of mobility [3, 4]. SMA Type 4 is an adult-onset disease with mild muscle weakness [4].

Without intervention, survival of the most severely affected children is poor with most patients dying before 24 months of life [5]. Recent advances in medical care have led to improved survival and quality of life [6]. However, these children now face complications due to chronic immobility that also impact those with milder phenotypes of SMA.

A major complication of chronic immobility is poor bone health. Weight-bearing activity during growth is an important stimulus for bone mass accrual [7-9]. Children who have limited weight-bearing activity are at risk for poor bone accrual and a marked decrease in peak bone mineral density (BMD) [10-13]. Children with SMA also have low muscle mass, which may lead to additional decreases in mechanical loading forces on the osteocyte. Low BMD increases risk for all types of fractures and development of osteoporosis [14, 15]. Despite these known complications of immobility, there is limited published literature on bone health in patients with
SMA. Retrospective studies report a high, but widely variable (9.3%-46%), fracture prevalence in SMA patients, with the distal femur being the most common fracture location [16-19]. Results from studies evaluating BMD in these children have been inconclusive; some studies reported that bone mineral parameters were not low in this population [20, 21], whereas others found that bone density was lower than expected for age[22, 23]. Furthermore, SMA patients were reported to have lower BMD Z-scores than did patients with other neuromuscular conditions [23]. No study has reported the prevalence of fractures or low BMD (Z-score ≤ -2.0) by SMA subtype, prevalence of low BMD of the lateral distal femur (an important fracture location in non-ambulatory children), nor prevalence of osteoporosis in this population. Thus, the degree and extent of poor bone health in SMA is not known.

In this study, we aimed to characterize bone health in pediatric patients with SMA by determining the prevalence of low BMD and fractures in this population. We also determined the prevalence of osteoporosis using the diagnostic criteria established in the 2013 International Society for Clinical Densitometry (ISCD) pediatric position statement.

2. Methods

We conducted a retrospective chart review of patients with a confirmed diagnosis of SMA seen at the Neuromuscular Comprehensive Care Center at Cincinnati Children’s Hospital Medical Center between January 2005 and January 2015. SMA subtypes were defined by the classic criteria [3, 4]. Patients were included in the analysis of bone health if they had a clinic visit between ages 1 year to 25 years. We selected this age range to exclude congenital fractures and fractures secondary to delivery in order to refine fracture analysis. This also excluded the most severely affected children who did not survive to one year of age. Additional exclusion criteria included use of systemic glucocorticoids or valproic acid, or diagnosis of another chronic
illness known to affect bone metabolism (e.g., malabsorption syndromes, inflammatory bowel disease, panhypopituitarism).

Data extracted from the medical records included sex, race, age at SMA diagnosis, SMA subtype, age at dual energy x-ray absorptiometry (DXA) scans, bisphosphonate use, and age at last encounter. This study was approved by the Institutional Review Board at Cincinnati Children’s Hospital Medical Center.

2.1 Bone mineral density:

Areal bone mineral density (aBMD) was measured by dual energy x-ray absorptiometry (DXA; Hologic 4500a). All DXA scans were obtained as part of routine clinical care where the standard protocol was to obtain annual DXA scans of the lumbar spine (LS), whole body (WB), and lateral distal femur (LDF) starting at three years of age. DXA scans were obtained prior to age 3 years in specific clinical scenarios, such as a fragility fracture. Limitations in positioning and/or spinal rod instrumentation prevented DXA scans at all sites from being obtained on each patient. LS and WB scans were acquired using standard positioning and analysis procedures. LDF scans were obtained and three regions of interest (R1, R2 and R3) were identified as described by Henderson et al [24] and Zemel et al [25]. All scans were reviewed (by H.W.) for image quality, positioning and artifact (spinal instrumentation, ports and movement). LS aBMD Z-scores were calculated using reference data from Kalkwarf et al [26] for ages 1 to 36 months, Kelly et al [27] for ages 37 to 60 months, Zemel et al [28] for ages 5 to 20 years. WB BMC and aBMD Z-scores were calculated using reference data from Kelly et al [27] for ages 37 to 60 months and from Zemel et al [28] for ages 5 – 20 years. LDF aBMD Z-scores were calculated
for children ages ≥3 years using reference data from Henderson et al [24]. aBMD Z-scores for each region of interest were the primary outcomes of interest.

2.2 Fracture History:

Patients were asked about fracture history at each clinic visit, in either the interdisciplinary neuromuscular clinic or the endocrine metabolic bone disease clinic, and recorded in the medical record. Data collected included age at first fracture, number of fractures, and location of fractures. Reported fractures were confirmed when possible by review of radiographic images (by H.W.), with documentation of radiographic fracture by radiologist or evidence of healing fracture on subsequent x-ray imaging. Fractures of the skull or of the digits were excluded as these do not usually constitute osteoporotic fractures.

2.3 Osteoporosis:

In 2013, the International Society for Clinical Densitometry (ISCD) released a pediatric position statement defining the criteria for osteoporosis. These guidelines, requiring evidence of atraumatic vertebral compression fractures, or a BMD Z-score ≤ -2.0 SD and two or more long bone fractures by 10 years of age or three or more long bone fractures by 19 years of age, were utilized to determine the prevalence of osteoporosis in our study sample [29].

2.4 Statistical Analyses:

Data were analyzed using SAS®, version 9.3 (SAS Institute, Cary, NC). Due to sample sizes and the distribution of variables, continuous data were summarized as medians with 25th and 75th percentiles and categorical data were summarized as frequency counts with percentages. Chi-square and Fisher’s exact tests were used, as appropriate, for group
comparisons of categorical variables. Nonparametric Kruskal-Wallis tests were used to compare continuous variables between groups at baseline. In order to account for multiple testing between groups, a False Discovery Rate adjusted P-value was calculated for each group comparison tested. Generalized linear mixed models with random effects (to account for the longitudinal nature of multiple visits by the same subject) were used to assess trends in BMD Z-scores over time, differences by SMA subtype and the group by time interaction. BMD Z-scores were analyzed with the three SMA types in the model as well as each SMA type separately modeled. Since the age at final measurement was greater in SMA3 compared to SMA1 and SMA2 patients due to the nature of the disease, we performed a sensitivity analysis by restricting the sample to individuals ages <16 years to determine if having similar age ranges for all three SMA types altered conclusions. Statistical significance was set a priori at α=0.05.

3. Results

3.1 Demographics:

A total of 102 SMA patients were evaluated at our institution, and 86 patients met inclusion criteria (SMA1: n=24; SMA2: n=44; SMA3: n=18). Table A shows the characteristics of the sample by SMA subtype. The majority of patients were Caucasian, and the sex distribution was similar across SMA subtypes. Age at initial clinic visit differed significantly by SMA subtype, youngest in SMA1 and oldest in SMA3, consistent with the onset of symptoms leading to the diagnosis. Patients with a mild SMA subtype were also more likely to be older at the time of last encounter than those with the more severe subtype.

3.2 Prevalence of low bone mineral density by SMA subtype:
DXA data were available on 59 patients (68.6% of the study sample). Of these, 90% had an aBMD Z-score of ≤-2.0 at any skeletal site at the first DXA scan. Median Z-scores by skeletal site for each SMA subtype are given in Table B. Patients with SMA1 were likely to have low aBMD Z-score at all skeletal sites; SMA2 patients were likely to have low BMD at the LS and the LDF, whereas patients with SMA3 were likely to have low aBMD at the LDF only. Patients with SMA1 had significantly lower BMD Z-scores at all skeletal sites compared those with SMA2 or SMA3, with a trend observed across the three subtypes.

Figures A.1-A.4 show longitudinal trends in aBMD at each skeletal site for individual patients. When including all SMA subtypes in mixed effect regression models, LS aBMD Z-scores significantly increased over time (p< 0.01). In contrast, WB aBMD Z-scores did not significantly differ over time. LDF aBMD Z-scores for all regions (R1, R2 [not shown] and R3) showed a significant group by time interaction indicating that the rate of change over time differed significantly by SMA subtype (all regions p< 0.002). For all LDF regions, patients with SMA3 had the fastest rate of decline in aBMD Z-scores compared to those with the other two subtypes. Patients with SMA2 had a faster rate of decline in LDF aBMD Z-scores at all regions compared to those with SMA1.

When the LDF aBMD was analyzed separately by SMA subtypes, a non-linear effect over time was found for all three SMA subtypes based on LDF region. Although patients with SMA1 showed an overall decrease in aBMD Z-scores at all LDF regions with time, there was an initial paradoxical increase followed by a decrease as patients aged. This overall decrease in LDF aBMD Z-score was also true for SMA2 and SMA3; however, the significance of the non-linear effect differed by region. For SMA2, there was an increase followed by a decrease in all regions, but this non-linear effect was only significant in R1 (p<0.001) and R2 (p=0.04). For SMA3, there
was a significant linear decrease over time in R1 (p=0.01), but a significant non-linear effect over time for R2 (p<0.001) and R3 (p<0.001). R3, an area associated with fracture risk in non-ambulatory children [30], showed the highest rates of overall decline for both SMA2 and SMA3. Among SMA3 patients, loss of ambulation was associated with lower aBMD Z-scores at R3 of the LDF compared to those children who maintained the ability to walk (p=0.02). There were no differences in aBMD Z-scores by ambulatory status at R1 and R2 of the LDF.

3.3 Fracture Frequency by SMA subtype:

Of the 86 patients providing fracture information, 31 reported at least one fracture (36%). We could radiographically verify 62% of these fractures. Occurrence of at least one long bone fracture did not differ significantly by SMA subtype. However, first reported fracture occurred at a younger age in those with more severe disease, with SMA1 patients reporting fractures at a significantly younger age than SMA3 patients. Although a trend was observed across the subtypes, the median age for first fracture in SMA 2 patients was not statistically different as compared with SMA1 or SMA3. Fracture of the distal femur accounted for 47% of all fractures and was the most common fracture location in all SMA subtypes. Eleven patients had multiple fractures (SMA1: n=4, SMA2: n=5, SMA3: n=2).

3.4 Bisphosphonate use by SMA patients:

Thirteen patients in the study sample were prescribed bisphosphonates (SMA1: n=8, SMA2: n=2, SMA3: n=3). Four patients received IV bisphosphonates (pamidronate or zolendronate) and nine received oral alendronate. Because of the small sample size, we were not able to assess for differences in BMD Z-scores or fracture risk between patients who received versus those who did not receive bisphosphonate therapy.
3.5 Osteoporosis Prevalence:

The overall prevalence of osteoporosis according to 2013 ISCD Pediatric Position Statement in this SMA sample was 8.5%. The percentage of patients with either SMA1 or SMA3 meeting criteria for osteoporosis was higher than patients with SMA2; however, this was not statistically significant.

4. Discussion

To our knowledge, this is the first study to assess both BMD and fracture prevalence in a sample of SMA patients inclusive of all subtypes of childhood disease. We found that children with SMA have a high prevalence of low BMD and fractures at a young age. Importantly, low BMD was common at the lateral distal femur, a site where fractures often occur in these patients. Despite the high prevalence of low BMD and fracture among children with SMA, only 8.5% met the 2013 ISCD criteria for osteoporosis.

Lack of ambulation and decreased mechanical loading forces are significant risk factors for poor bone mineral accrual. Multiple studies have documented low BMD in non-ambulatory children with a variety of neuromuscular disorders (e.g., cerebral palsy, Duchenne Muscular Dystrophy, myelomeningocele) [11, 13, 30, 31]. Few studies report BMD in SMA patients, and the extent of bone mineral deficits has been inconclusive. In a study of 8 children with SMA and 71 children with other neuromuscular disorders, Khatri et al found that LS aBMD Z-scores were lower in children with SMA (mean -2.25 ± 0.31) as compared to children with other neuromuscular disorders (Duchenne Muscular Dystrophy mean LS aBMD Z-score -1.71 ± 0.1) [23]. Neither steroid use nor vertebral fractures were controlled for in the Duchenne Muscular Dystrophy group, factors that could influence aBMD at LS. In a cross-sectional study, Kinali et
al reported that younger children with SMA had WB aBMD Z-scores in the normal range, whereas teenagers with SMA had low aBMD. They concluded that age, rather than disease severity and ambulatory status, had a more significant impact on BMD. However, this study only utilized WB aBMD, which may not accurately reflect aBMD of the peripheral skeleton, especially at weight-bearing sites, in young children as the head makes a large contribution to the whole body measure. Bone mineral apparent density, a measure that adjusts for vertebral size to account for smaller bone size in children, was recently reported for a sample of 30 patients with SMA identifying 50% of children with LS Z-scores <-1.5 SD[22]. In addition to small sample sizes, these previous reports are limited by the inclusion of primarily children with SMA2 or SMA3. Our study includes a larger sample of children and included those with SMA1. SMA1 children had the lowest aBMD at all sites as compared to milder phenotypes even at a younger age at presentation. Importantly, all patients, regardless of subtype, demonstrated a deficit in aBMD at the LDF, a site that has not been previously reported in the literature in children with SMA.

The LDF site is practical for measuring aBMD in children with contractures and spinal instrumentation, and it has also been shown to be associated with fracture risk in other non-ambulatory patients [30]. In our SMA cohort, aBMD was low at all regions of the LDF at the time of the first DXA scan in patients with SMA 1, SMA2, and SMA3, even before deficits were noted at the LS or WB. While our data suggest that SMA1 patients experience an initial increase in aBMD at the LDF site followed by a subsequent decline (albeit to a lesser degree than the milder phenotypes), this may be due to lack of aBMD data obtained during the adolescent years in this subgroup. While many SMA3 patients with had a LDF aBMD Z-score in the normal range at the time of the first DXA scan, there was a significant decline with age, especially at R3
where the risk of fracture in patients with low aBMD is greatest [30]. LDF R3 is comprised of primarily cortical bone, tissue that is responsive primarily to mechanical stimulation in early childhood [32]. Thus, lack of ambulation and low muscle forces may explain why SMA1 and SMA2 patients had lower aBMD Z-scores at this region than SMA3 patients, but SMA3 patients showed subsequent decreases in aBMD as weight-bearing declined. SMA3 patients were significantly older than SMA1 or SMA2 patients at the time of last encounter, thus it remains unknown if the more severely affected phenotypes show similar rates of decline in the adolescent years when peak bone mass accrual should occur.

Multiple studies report higher fracture prevalence in children with neuromuscular disorders as compared to healthy children. Fractures in children with neuromuscular disorders are of concern because they may lead to worsening contractures and loss of remaining mobility. Specifically, among patients with SMA, Granata et al reported 16 fractures in 10 children out of a cohort of 93 SMA patients (10.7%) [16]. This study did not categorize SMA patients by subtype, however given the data collection time period of 1974-1988 it is likely that most children were SMA3 or mildly affected SMA2. Fujak et al reported fracture history in a cohort of 131 patients with SMA; fractures occurred in 45.8% of the sample, with 53% occurring at the distal femur [19]. Most fractures in this sample occurred in children with SMA2 (46.9%) or SMA3 (51.5%) with only one SMA1 patient out of 11 sustaining a fracture. Multiple fractures occurred in 16% of the entire study sample. A limitation of their study is that only children referred for orthopedic evaluation were included, which may not accurately reflect the SMA population in general. Our findings of high fracture prevalence in SMA2 and SMA3 patients are consistent with these prior studies. In addition, we demonstrated that SMA1 patients also have
high prevalence of fractures, as nearly half of the patients sustained at least one fracture, the majority of which occurred at the distal femur.

Utilizing the current ISCD guidelines for diagnosis of osteoporosis in children, only 8.5% of SMA fulfilled criteria for osteoporosis. However, these criteria are quite stringent, and our results using these guidelines may underestimate the extent of bone disease in this population as many children were young and fractures will likely accrue as they transition from childhood and adolescence. Fracture patterns in our SMA cohort differed considerably from the general pediatric population in that fractures tended to occur at a younger age with preponderance for the lower extremities to be affected. Thus, while many children with SMA do not meet ISCD criteria for osteoporosis, these patients have significantly low BMD z-scores and a high prevalence of clinically pertinent fractures.

Although our study reports a comprehensive view of aBMD and fracture history across the pediatric SMA phenotypes, there are some limitations. First, because the study was conducted retrospectively, the frequency of bone health outcome monitoring was not consistent in all patients. DXA data were only available on 69% of our study sample, which may bias results toward patients who had a greater clinical suspicion of poor bone mineral density. However, it is important to note that of patients lacking DXA data, 63% were < 3 years of age at the time of last encounter. Thus, the main reason for missing BMD data was that clinicians had not ordered a screening DXA scan because the child had not reached an age where reference data for clinical DXA scans were readily available at the time. Height measurements in patients who are unable to stand are often inconsistent; segmental arm span, ulnar length, or supine length may be substituted for true standing height. This information was not consistently available for all study patients, thus we did not adjust our DXA BMD z-score for height. While this could
potentially affect the lumbar spine and whole body measures for children older than five years of age, we think the impact would have likely been small and not change our findings significantly as SMA children tend to be of normal stature. Further, there is no height-adjusted reference range for younger children or for the lateral distal femur site. Because some patients received medical care at local hospitals and only traveled to our institution for subspecialty care, not all fractures could be confirmed by review of radiographic imaging. In addition, we did not screen for vertebral compression fractures as spine x-rays were not part of routine clinical care. The overall slight increase of LS aBMD with age in this cohort may be due to asymptomatic vertebral compression fractures. While there were no symptomatic vertebral compression fractures reported in this cohort, recently published data from Vai et al suggest that asymptomatic fractures may be more prevalent than previously thought[22]. However, reported long bone fracture rates in this study are similar to findings in other studies. Finally, although it is tempting to speculate on the relationship between LDF DXA findings and the high prevalence of femur fractures, it is not possible to determine if LDF aBMD Z-score was predictive of fracture risk as there were too few patients in this study who had a DXA scan of this region prior to their first fracture.

5. Conclusions:

Fracture risk is high for children with SMA. Low aBMD may be the first indicator of this increased risk, and deficits in aBMD are apparent even at a very young age. Further work is necessary to determine the natural trajectory of aBMD changes at different skeletal sites especially in adolescent and young adult patients with SMA and to determine if low aBMD and propensity to fracture is related to immobility, muscle strength, or an inherent defect of SMA.
Importantly, more work is needed to identify effective interventions to delay the decline in BMD and prevent fractures in children with SMA.
Bibliography


[28] Zemel BS, Kalkwarf HJ, Gilsanz V, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black and non-black


Table 1: Demographics

<table>
<thead>
<tr>
<th></th>
<th>SMA 1 (most severe)</th>
<th>SMA 2 (intermediate)</th>
<th>SMA 3 (mild)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>24</td>
<td>44</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Female (%)</td>
<td>15 (63%)</td>
<td>23 (52%)</td>
<td>8 (44%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Caucasian (%)</td>
<td>20 (83%)</td>
<td>39 (89%)</td>
<td>15 (83%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Age (y) at initial</td>
<td>0.6 (0.3, 1.1)</td>
<td>2.0 (0.9, 4.4)</td>
<td>3.9 (2.1, 8.7)</td>
<td>0.0003&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>neuromuscular visit</td>
<td>[n=24]</td>
<td>[n=44]</td>
<td>[n=18]</td>
<td></td>
</tr>
<tr>
<td>Age (y) at loss of</td>
<td>N/A</td>
<td>N/A</td>
<td>8.4 (6.4, 9.7)</td>
<td>-</td>
</tr>
<tr>
<td>ambulation</td>
<td></td>
<td></td>
<td>[n=10]</td>
<td></td>
</tr>
<tr>
<td>Age (y) at last</td>
<td>7.6 (2.2, 12.4)</td>
<td>6.2 (3.5, 12.2)</td>
<td>12.9 (7.7, 17.9)</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;,&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>encounter</td>
<td>[n=24]</td>
<td>[n=44]</td>
<td>[n=18]</td>
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</tr>
<tr>
<td>Bisphosphonate</td>
<td>8 (33%)</td>
<td>2 (5%)</td>
<td>4 (22%)</td>
<td>0.004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>treatment ever (n)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data expressed as n (%) and median (25<sup>th</sup>, 75<sup>th</sup> percentile).

(between group p-values adjusted for multiple testing using False Discovery Rate adjustment)

<sup>a</sup>SMA1 vs. SMA2 between group difference adjusted p<0.05;  
<sup>b</sup>SMA1 vs. SMA3 between group difference adjusted p<0.05;  
<sup>c</sup>SMA2 vs. SMA3 between group difference adjusted p<0.05
Table 2: Bone Health Outcomes

<table>
<thead>
<tr>
<th></th>
<th>SMA 1 (most severe)</th>
<th>SMA 2 (intermediate)</th>
<th>SMA 3 (mild)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>24</td>
<td>44</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Patients ≥1 fracture</td>
<td>11 (46%)</td>
<td>12 (27%)</td>
<td>8 (44%)</td>
<td>0.22</td>
</tr>
<tr>
<td>Age (y) at 1&lt;sup&gt;st&lt;/sup&gt; reported fracture*</td>
<td>3.0 (1.9, 6.0)</td>
<td>6.6 (3.3, 11.1)</td>
<td>10.4 (9.2, 11.5)</td>
<td>0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Femur fracture/Total fracture (%)</td>
<td>13/22 (59%)</td>
<td>7/19 (37%)</td>
<td>5/12 (42%)</td>
<td>0.36</td>
</tr>
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Baseline aBMD Z-scores

<table>
<thead>
<tr>
<th></th>
<th>LS aBMD</th>
<th>WB aBMD</th>
<th>LDF BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>3.9 (2.8, 4.8)</td>
<td>4.5 (4.0, 4.9)</td>
<td>4.8 (4.1, 6.2)</td>
</tr>
<tr>
<td>Z-score</td>
<td>-4.7 (-5.7, -3.6)</td>
<td>-2.9 (-4.3, -2.6)</td>
<td>-4.6 (-5.0, -4.3)</td>
</tr>
<tr>
<td>[n=14]</td>
<td>[n=13]</td>
<td>[n=22]</td>
<td>[n=13]</td>
</tr>
<tr>
<td></td>
<td>5.2 (4.0, 5.7)</td>
<td>5.4 (4.6, 6.8)</td>
<td>5.5 (4.3, 7.4)</td>
</tr>
<tr>
<td>Z-score</td>
<td>-2.5 (-3.3, -0.7)</td>
<td>-1.8 (-2.6, -0.2)</td>
<td>-3.5 (-4.3, -2.7)</td>
</tr>
<tr>
<td>[n=22]</td>
<td>[n=22]</td>
<td>[n=22]</td>
<td>[n=22]</td>
</tr>
<tr>
<td></td>
<td>7.5 (4.3, 8.5)</td>
<td>7.6 (4.8, 8.7)</td>
<td>8.9 (4.3, 13.8)</td>
</tr>
<tr>
<td>Z-score</td>
<td>-0.2 (-1.8, 0.2)</td>
<td>-1.9 (-2.9, -1.3)</td>
<td>-2.8 (-3.7, -1.1)</td>
</tr>
<tr>
<td>[n=13]</td>
<td>[n=13]</td>
<td>[n=13]</td>
<td>[n=13]</td>
</tr>
</tbody>
</table>

Data expressed as n (%) and median (25<sup>th</sup>, 75<sup>th</sup> percentile). *Summary statistics only for those patients with a reported fracture.

Between group p-values adjusted for multiple testing using False Discovery Rate adjustment)

<sup>a</sup>SMA1 vs. SMA2 between group difference adjusted p<0.05; <sup>b</sup>SMA1 vs. SMA3 between group difference adjusted p<0.05; <sup>c</sup>SMA2 vs. SMA3 between group difference adjusted p<0.05
Figure A.1 - A.4: Change in areal bone mineral density over time by skeletal site.

BMDZ: Areal Bone Mineral Density Z-score.