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Evolution of bone mineral density, bone metabolism and fragility fractures in Spinal Muscular Atrophy (SMA) types 2 and 3

Giovanni Baranello[a,b,*], Silvia Vai[c], Francesca Broggi[c], Riccardo Masson[a], Maria Teresa Arnoldi[a], Riccardo Zanin[a], Chiara Mastella[d], Maria Luisa Bianchi[c]

[a] *Developmental Neurology Unit, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy*

[b] *Dubowitz Neuromuscular Centre, UCL Great Ormond Street Institute of Child Health, NIHR Great Ormond Street Hospital Biomedical Research Centre, London, United Kingdom*

[c] *Experimental Laboratory for Children's Bone Metabolism, Research Bone Metabolism Unit, Istituto Auxologico Italiano IRCCS, Milan, Italy*

[d] *SAPRE-UONPIA, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy*

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Abstract

With recent advances in the treatment of Spinal Muscular Atrophy (SMA), there is a strong need to increase knowledge on the involvement of organs and systems outside the central nervous system. We investigated bone metabolism, bone mineral density (BMD) and fractures, and their possible correlation with age and motor capacities. Thirty-two children with SMA (27 type 2, 5 type 3), mean age 40 ± 32.3 months, underwent two evaluations at an 18-month interval (V1 and V2). Twelve of these children also underwent a third evaluation at month 36 (V3). Diet, bone metabolism, BMD, X-rays, and motor function (by the Hammersmith Functional Motor Scale Expanded – HFMSE – and the Upper Limb Module – ULM) were assessed. At V1, 25-OH vitamin D_3 (25OH D) therapy was started, and dietary calcium intake adjusted according to the recommended dietary allowance. Low 25OH D levels and asymptomatic vertebral fractures were mainly observed at V1. At all visits, bone resorption markers were higher than normal. At V2 and V3, decreased BMD was observed. Higher spine BMD values at follow-up were associated with HFMSE score >12 at baseline ($p<0.03$). This study suggests that even young children with SMA are at risk of severe bone fragility. Further investigations of the molecular mechanisms leading to altered bone metabolism in SMA could help identify novel therapeutic targets and establish better guidelines for bone fragility management.

Keywords: Spinal Muscular Atrophy; Bone density; Bone metabolism; Bone turnover markers; Fragility fractures; Children.

1. Introduction

Spinal Muscular Atrophy (SMA) is caused by homozygous deletion and/or mutation of the survival motor neuron 1 (SMN1) gene on chromosome 5q13, leading to degeneration of lower motor neurons [1]. An increasing body of preclinical and anecdotal clinical studies suggest that SMA can be considered as a multi-system disorder, due to the ubiquity of the SMN protein [2–4]. Four types of SMA are currently recognized, with types 1–3 having a childhood onset. Infants with SMA type 1 are most severely affected, showing generalized muscle weakness, hypotonia, and inability to sit unsupported since early infancy, with death occurring within the first 2 years of life in the absence of proactive nutritional and respiratory support. Children with SMA type 2, of intermediate severity, are able to sit independently but never to walk. Children with SMA type 3, a milder form, present with proximal weakness and abnormal gait in childhood, have a variable disease progression and near normal life expectancy [1]. Immobilization typically affects bone mass, density and strength and facilitates bone fragility. For this reason, all children with diseases characterized by low muscular

* Corresponding author at: The Dubowitz Neuromuscular Centre, UCL NIHR GOSH Biomedical, Research Centre, Great Ormond Street Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.

E-mail addresses: giovanni.baranello@istituto-besta.it, g.baranello@ucl.ac.uk (G. Baranello).

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Table 1
Patient characteristics.

	Visit 1	Visit 2	*P*
No. of patients with visit 1 and 2	32	32	
Sex	*F* = 15; *M* = 17	*F* = 15; *M* = 17	
Age (mos.)	40 ± 32.3	58.8 ± 32.8	
SMA type	27 type II; 5 type III	27 type II; 5 type III	
Weight (Kg)	13.1 ± 4.4	16.2 ± 5.4	$p<0.001$ V2 vs. V1
Height (cm)	96.2 ± 19.9	104.2 ± 16.5	$p<0.002$ V2 vs. V1
BMI (kg/cm^2)	14.2 ± 2.5	15 ± 2.3	
Tanner stage	I (30) II (2)	I (28) II (4)	
No. of patients on Salbutamol	20	20	
Time between visits (mos.)	–	16 ± 2	

	Visit 1	Visit 2	Visit 3	*P*
No. of patients with visit 1, 2 and 3	12	12	12	
Sex	*F* = 7; *M* = 5	*F* = 7; *M* = 5	*F* = 7; *M* = 5	
Age (mos.)	35.2 ± 26.6	50.7 ± 26.2	70.2 ± 26.2	
SMA type	9 type II; 3 type III	9 type II; 3 type III	9 type II; 3 type III	
Weight (Kg)	13.1 ± 5.7	15.7 ± 5	18.2 ± 6.1	$p<0.001$ V2 vs. V1 $p<0.01$ V3 vs. V2
Height (cm)	92.7 ± 15.9	108.9 ± 13.1	110.6 ± 12.1	$p<0.003$ V2 vs. V1 $p<0.005$ V3 vs. V2
BMI	14.9 ± 3.1	13.3 ± 2.9	14.9 ± 3.3	
Tanner stage	I (12)	I (10) II (2)	I (10) II (2)	
No. of patients on Salbutamol	7	7	7	
Time between visits (mos.)	–	16 ± 2 vs. visit 1	18 ± 4 vs. visit 2	

F: female; M: male; Mos: months; BMI: body mass index.

mass/force and reduced motility, including those with SMA, are at high risk for pathologically low bone mass and fractures [2,5,6]. Data from mouse models have demonstrated a direct interaction of SMN protein with a peptide called osteoclast-stimulatory factor, leading to altered bone remodeling and impaired bone mineralization in SMA type 1 [7]. Data on the natural history of bone involvement and markers of bone metabolism, and on their correlations with level of motor abilities in SMA are still limited. As an approved treatment for SMA is now commercially available in several countries [8], and other therapeutic options are in advanced phases of clinical development [9], there is a strong need to fill the gap in the knowledge on the effects of the disease on different organs and systems, including bone, and the possible efficacy of therapies on the resultant comorbidities. In a previous cross-sectional study we investigated bone metabolism, bone mineral density (BMD) and presence of fractures in a cohort of 30 children with SMA types 2 and 3, analyzing the correlations with age and motor function status. Our data showed that children with SMA types 2 and 3 can have low 25OH D levels, high levels of parathyroid hormone (PTH), increased bone resorption markers, and increased incidence of undiagnosed vertebral fractures [2].

The aim of the present study was to investigate the evolution of bone metabolism, BMD and fractures, and their possible correlation with age and motor function levels, in the same cohort of children with SMA types 2 and 3 (with 1 case excluded and 3 cases added).

2. Patients and methods

2.1. Subjects

This prospective study enrolled 32 children affected by SMA types 2 or 3 followed at the Developmental Neurology Unit of the Fondazione IRCCS Istituto Neurologico Carlo Besta and the SAPRE Centre in Milan (Italy). Twenty-nine of these children were already included in our previous study. All 32 children underwent at least two evaluations (baseline, V1, and after 18 months, V2); 12 of them had an additional follow-up evaluation (after 36 months, V3), while the remaining 20 were either lost at follow up ($n = 5$) or excluded for clinical reasons (4 had several hospitalizations and were no longer available for further evaluations, 11 started administration of nusinersen or entered SMA clinical trials). The enrolment criteria were: genetically proven diagnosis of SMA types 2 or 3; age 2–9 years; no previous spinal surgery; ability to correctly lie on the dual X-ray absorptiometry (DXA) scan table for bone density evaluation.

Out of the 32 enrolled children, 27 had SMA type 2 and 5 SMA type 3 (4 still ambulant). All children were prepubertal at V1 and remained prepubertal throughout the study (see Tanner stage in Table 1). Twenty children were routinely treated with oral salbutamol (2 mg 3 times a day), in accordance with preliminary evidence of clinical and molecular efficacy [10,11]. None of the children received the FDA and EMA approved intrathecal treatment (nusinersen) or other investigational products for SMA.

Clinical data and fracture history (site, date and type of intervention) were collected (Table 1). All fractures were documented by X-rays.

All patients were able to eat (none had percutaneous endoscopic gastrostomy). Dietary calcium intake was estimated by a dietician using a food diary.

At V1, no patient was receiving vitamin D supplementation. Calcifediol (25-OH D) was then prescribed according to the child's weight (0.8 mcg/kg/day), and dietary calcium intake was adjusted to the RDA [12].

Dietary evaluation, laboratory tests, DXA scans, and X-rays, were performed at the Experimental Laboratory for Children's Bone Metabolism Research, Istituto Auxologico Italiano IRCCS, Milan (Italy).

The study was conducted as part of the standard of care clinical management at the recruiting sites and according to the Good Clinical Practice rules. An informed consent for all exams and procedures was signed by the parents.

2.2. Anthropometry

Weight was measured with an electric scale to the nearest 0.1 kg. Standing height was measured with a stadiometer. For patients unable to stand up, a measuring board was used, with a standardized procedure: the child was helped to lie supine (with legs straight and well-aligned with the body, and ankles as close together as possible) and the footboard-headboard distance was accurately measured. Body mass index (BMI, kg/m^2) was calculated. Tanner's pubertal stage was evaluated by an endocrinologist.

2.3. Laboratory analyses

Blood samples were collected in the early morning after an overnight fast. Bone metabolism was evaluated with the following tests: serum calcium, phosphate, alkaline phosphatase (ALP), bone specific alkaline phosphatase (BSAP), measured with standard laboratory methods; serum osteocalcin (OC), with radioimmunological assay (RIA, Technogenetics, Milano, Italy); PTH with immunoradiometric assay (IRMA; DiaSorin Inc, Stillwater, MN, USA); 25OH D with RIA (DiaSorin); C-terminal telopeptide (CTx) with electro-chemical luminescence (ECLIA; Roche Diagnostic, GMBH, Mannheim, Germany).

The values of calciotropic hormones and bone turnover markers were compared with our laboratory's reference values for sex- and age-matched healthy Italian children.

2.4. DXA scans

DXA was performed at lumbar spine (L2–L4) using a Hologic Discovery Horizon A scanner (Hologic, Inc, Bedford, MA). Data are expressed as absolute values and Z-scores (i.e., the number of standard deviations that a patient's BMD differs from the average of a healthy control population of the same ethnicity, sex and age). Considering the known problems of DXA use in the study of growing children,[1] we used a standard adjustment that uses an "approximate bone volume", on the assumption that the lumbar vertebral bodies are cylindrical. This adjustment gives a measure called "bone mineral apparent density" (BMAD = BMC/[approximate vertebral body volume]; mg/cm^3). The Z-scores were calculated with respect to the values of our reference sample of age-matched healthy Italian boys and girls, adjusted with the same corrections.

Moreover, if a patient's height Z-score was <-1, the BMAD Z-score was also corrected for the height Z-score, according to the ISCD indication for the measurement of pediatric BMD [13].

2.5. X-rays

The presence of vertebral fractures was investigated by lateral X-rays of thoracic and lumbar spine, and bone age was estimated by hand and wrist X-rays.

2.6. Motor function assessment

Motor function was assessed by the Hammersmith Functional Motor Scale Expanded (HFMSE) and the Upper Limb Module (ULM).

The HFMSE assesses motor function (lying, rolling, sitting, crawling, attaining standing, walking, running and jumping), with higher values indicating higher function abilities. Each item scores 2 for unaided, 1 for assistance and 0 for inability. A total score is calculated by summing the scores of the individual items. The total score can range from 0 (all activities failed) to 66 (all activities achieved unaided). The HFMSE shows good test–retest reliability and correlation with other clinical measures [14].

The ULM was specifically developed to assess upper limb function in non-ambulant SMA patients, including young low-functioning children. It includes 9 items, scored on a 3-point scale using the following criteria: 2 = normal, achieves goal without any assistance; 1 = modified method but achieves goal independently of physical assistance from another person; 0 = unable to achieve independently. A total score is calculated by summing the scores of the individual items, and can range from 0 (all activities failed) to 18 (all activities achieved unaided) [15].

[1] With DXA, the BMD is calculated by dividing the bone mineral content (BMC, mg) by the area (cm^2) of the projection surface of bone, thus being only an "areal density" (mg/cm^2) and not a "volumetric density" (mg/cm^3). For mathematical reasons, the areal BMD overestimates the true (volumetric) density of larger bones and underestimates that of smaller bones. Studying a growing skeleton, in which both bone mass and bone volume increase along with changes in body size, it is important to overcome this error using a suitable approximation of the bone volume.

Table 2
Bone metabolism data.

	Visit 1	Visit 2	*P*	Normal range
No. of patients with visit 1 and 2	32	32		
calcium (mg/dL)	9.7 ± 0.4	9.7 ± 0.4	NS	8.4–10.4
phosphate (mg/dL)	5.1 ± 0.5	5.2 ± 0.5	NS	3.5–5.7
PTH (ng/L)	26.5 ± 10.5	29.5 ± 14.1	NS	20–64
25OH D (mcg/L)	30.7 ± 16.3	29.2 ± 15	NS	20–70
osteocalcin (mcg/L)	61.8 ± 18.8	60.7 ± 20	NS	20–60
alkaline phosphatase (U/L)	146.6 ± 30.9	139.9 ± 31.7	NS	82–302
BSAP (mcg/L)	46.1 ± 13.7	43.1 ± 15.6	NS	20–62
CTx (ng/L)	1246.8 ± 254.5	1250.7 ± 379.2	NS	90–420

	Visit 1	Visit 2	Visit 3	*P*
No. of patients with visit 1,2 and 3	12	12	12	
calcium (mg/dL)	9.6 ± 0.4	9.6 ± 0.2	9.7 ± 0.3	NS
phosphate (mg/dL)	5.2 ± 0.5	5.2 ± 0.3	5.3 ± 0.2	NS
PTH (ng/L)	32.6 ± 20.1	26.1 ± 11.3	22.5 ± 8.9	<0.05 V1 vs. V3
25OH D (mcg/L)	27.2 ± 16.5	26.2 ± 13.3	39.8 ± 12.6	<0.05 V1 vs. V3
osteocalcin (mcg/L)	60.2 ± 22.2	66.1 ± 13.9	74.6 ± 22.8	NS
alkaline phosphatase (U/L)	146.0 ± 32.1	136.6 ± 27.1	142.1 ± 23.1	=0.05 V2 vs. V3
BSAP (mcg/L)	38.2 ± 15.1	37.8 ± 14.2	56.1 ± 16.1	<0.02 V2 vs. V3
CTx (ng/L)	1370.3 ± 399.5	1338.6 ± 270.3	1368 ± 315.3	NS

PTH: parathyroid hormone; 25OH D: 25-hydroxyvitamin D; BSAP: bone specific alkaline phosphatase; CTx: C-terminal telopeptide; NS: not significant.

2.7. Data analysis

Data are expressed as the mean ± SD. The normality of all variables was evaluated with the Shapiro–Wilkinson test to choose the appropriate statistical tests.

Correlation between bone markers and BMC and BMD values at each time (V1, V2, V3) were evaluated by Pearson test or Spearman test. Statistical significance was defined as $p<0.05$, two-sided.

Comparison of data at V1 and V2 was performed with one-sample *t*-test. The longitudinal comparison of data at V1, V2, V3 was performed by variance analysis with one-way ANOVA (or non-parametric test), using Tukey or Dunn test as post-hoc analysis.

3. Results

Patients' characteristics and bone metabolism data are shown in Tables 1 and 2, respectively (V1, V2 for all 32 children; V3 for 12/32 children).

3.1. Laboratory tests

3.1.1. Whole group of 32 children with V1 and V2

Serum calcium, phosphate and ALP were within normal range for age in all children at both visits. PTH levels were slightly above normal range (i.e., >64 ng/L) in only 2 children (6.2%) at V1 and in 1 child (3.1%) at V2. The levels of 25-OH vitamin D were low (below 20 mcg/L) in 10 children (31.2%) at V1 and in 6 (18.8%) at V2; in the other cases, the 25-OH vitamin D values were within the range of normality.

The bone formation markers (BSAP, OC) were in the expected range for age in most cases: BSAP was slightly elevated in only 3 children (9.3%) at V1 and V2, and OC was slightly elevated in 11 children (34.3%) at V1 and in 9 (28.1%) at V2. The bone resorption marker (CTx) was higher than normal in 18 children (56.2%) at V1 and in 22 (68.7%) at V2.

3.1.2. Subgroup of 12 children with V1, V2 and V3

Serum calcium, phosphate and ALP were within normal range for age in all 12 children at all visits. PTH levels were above 64 ng/L in 2 children (17%) at V1, 1 child (8%) at V2, and none at V3. The levels of 25-OH vitamin D were below 20 mcg/L in 5 children (42%) at V1, 3 (25%) at V2, and none at V3; with these exceptions, the 25-OH vitamin D values were within the low range of normality.

BSAP was slightly elevated in 8 children (67%) at V1, 5 (42%) at V2, and 3 (25%) at V3. OC was slightly elevated in 3 children (25%) at V1, 7 (58%) at V2, and 4 (33%) at V3. CTx was higher than normal in 9 children (75%) at V1 and V2, and in 11 (92%) at V3.

3.2. Bone densitometry

Considering the whole group of 32 children, bone age was consistent with chronological age in all children but two, who had a slightly delayed bone age (25°–50° percentile).

The lumbar spine BMAD Z-scores of all children are reported in Table 3. The percentages of children with normal or low BMAD Z-scores at each visit are shown in Fig. 1. One child had a vertebral fracture and BMAD <−2, and could thus be defined as having "osteoporosis", according to the ISCD 2013 consensus [13].

The BMAD Z-score showed a decrease over time: in the whole group of 32 children, 5 had a Z-score <−2 at V1 and 12 at V2. In the subgroup of 12 children who had 3 visits,

Table 3
Lumbar spine bone density.

	Visit 1	Visit 2	P
No. of patients with visits 1 and 2	32	32	
BMD	0.355 ± 0.09	0.383 ± 0.08	NS
BMAD	0.199 ± 0.10	0.213 ± 0.10	NS
Z-score	−0.73 ± 1.5	−1.8 ± 0.9	p <0.02

No. of patients with visits 1, 2 and 3	Visit 1	Visit 2	Visit 3	P
	12	12	12	
BMD	0.361 ± 0.10	0.387 ± 0.08	0.401 ± 0.13	NS
BMAD	0.201 ± 0.15	0.213 ± 0.17	0.223 ± 0.18	NS
Z-score	−0.37 ± 1.5	−1.67 ± −1.01	−2.46 ± 0.88	p<0.01 V3 vs V1

BMD: bone mineral density; BMAD bone mineral apparent density; NS: not significant.

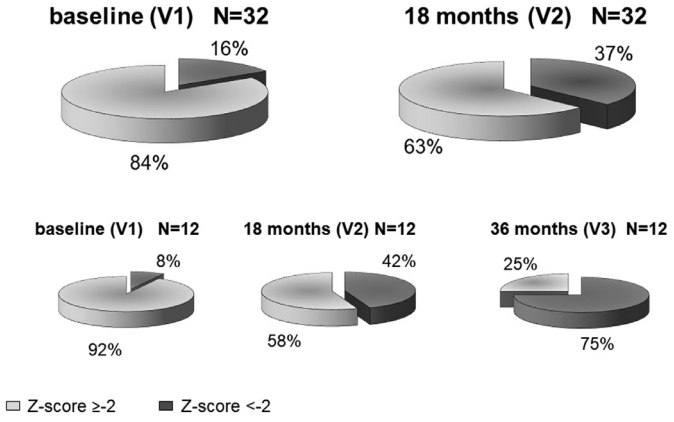

Fig. 1. This figure shows the evolution of spine BMAD Z-scores for children having visits 1, 2 (n = 32) and 1, 2, 3 (n = 12).
BMAD = bone mineral apparent density.

the BMAD Z-score was <−2 in 1 child at V1, 5 at V2, and 9 at V3.

3.3. Fractures

3.3.1. Whole group of 32 children with V1 and V2

At V1, the clinical histories reported 4 peripheral fractures in 4 children, and no vertebral fractures. On spine X-rays, we discovered 7 undiagnosed vertebral fractures (6 dorsal, 1 lumbar) in 3 children (1 child with 1 fracture, 1 with 2 fractures, and 1 with 4 fractures). At V2, 2 patients reported 2 incident peripheral fractures and X-rays did not reveal any incident vertebral fractures. The patients with vertebral fractures had a slightly lower BMAD than those without vertebral fractures, but the difference was not statistically significant. The difference in bone turnover markers between the groups with or without fractures was also not significant.

3.3.2. Subgroup of 12 children with V1, V2 and V3

At V1, no child reported peripheral fractures; X-rays revealed 3 undiagnosed vertebral fractures (2 dorsal, 1 lumbar) in 2 children. At V2, there was 1 incident peripheral fracture in 1 child, and no vertebral fractures. At V3, there were 4 incident peripheral fractures in 4 children, and no incident vertebral fractures.

3.4. Motor function assessment

Mean HFMSE score was 17 at V1 and 20 at V2 in the whole group. In the subgroup with longer follow-up, the mean score was 23 at V1 and 29 at V3.

Mean ULM score was 10 at V1 and V2 in the whole group, and 14 at V1 and 11 at V3 in the subgroup with longer follow-up.

Data are shown in Table 4.

3.5. Correlations

Table 5 shows the correlation data. A significant inverse correlation was found between PTH and 25-OH D levels, and as expected, higher PTH levels were observed in the cases with lower 25-OH D levels.

Bone formation markers (OC and BSAP) were significantly correlated with the resorption marker CTx. A significant correlation was found between either BMC or BMAD and PTH, as well as between 25-OH D and BMAD (in the whole group only), with lower 25-OH D levels associated with lower values of lumbar spine BMAD.

No correlation was found between motor function assessment scores and BMC or BMAD, but higher spine BMAD values at follow-up were associated with HFMSE score >12 at baseline (p<0.03).

No significant correlation was observed between age and spine BMC or BMAD.

4. Discussion

In SMA patients, longitudinal data on the evolution of comorbidities and involvement of other systems and organs secondary to the deficiency of the SMN protein have become highly demanded in recent years due to the availability of the intrathecal treatment nusinersen and the clinical development of other systemic approaches [8,9]. While intrathecal administration of nusinersen principally targets motor neurons thus proving to be effective in alleviating motor and bulbar impairment in treated patients [16,17], it is still debated if other systems and organs can be similarly benefited from the treatment, or if symptoms or signs of

Table 4
Motor function assessment.

	Visit 1	Visit 2	P
No. of patients with tests at visits 1 and 2	29	29	
ULM	10.5 ± 5.9	10.5 ± 5.9	NS
HFMSE	17.4 ± 19.6	20.3 ± 19.8	NS

	Visit 1	Visit 2	Visit 3	P
No. of patients with tests at visits 1,2 and 3	9	9	9	
ULM	14.9 ± 6.3	11.1 ± 6.2	10.7 ± 5.2	$p<0.06$ vs. V1
HFMSE	23.0 ± 26.1	22.5 ± 21.4	28.8 ± 24.8	NS

ULM: Upper Limb Module; HFMSE: Hammersmith Functional Motor Scale Expanded; NS: not significant.

Table 5
Correlations.

Patients with visits 1 and 2 ($n=32$)	R	P
PTH vs. 25-OH D	−0.47	<0.002
BSAP vs. CTx	0.37	<0.002
OC vs. CTx	0.57	<0.001
25OH D vs. BMAD	0.32	<0.05
PTH vs. BMC	0.47	<0.003
PTH vs. BMD	0.49	<0.001
Patients with visits 1, 2 and 3 ($n=12$)	R	P
PTH vs. 25-OH D	−0.40	<0.002
BSAP vs. CTx	0.32	<0.05
OC vs. CTx	0.40	<0.002
25OH D vs. BMAD	0.29	NS
PTH vs. BMC	0.40	<0.002
PTH vs. BMD	0.41	<0.002

n: number; PTH: parathyroid hormone; 25OH D: 25-hydroxyvitamin D; BSAP: bone specific alkaline phosphatase; CTx: C-terminal telopeptide; OC: osteocalcin; BMAD: bone mineral apparent density; BMC: bone mineral content; BMD: bone mineral density; NS: not significant.

dysfunction in other systems and organs may even appear over time notwithstanding the treatment [3].

Only very few studies have evaluated bone density in children and adolescents affected by SMA. In a retrospective study, Kathry et al. found lower BMD in young SMA patients than age-matched controls [18]; conversely, Kinali et al. [19] found that younger SMA patients (below 10 years of age) had normal values of total body BMD, and only older patients showed decreased values. However, no corrections for body size were reported by the authors in the latter paper.

In the present prospective study, we performed a comprehensive set of exams and assessments to investigate the evolution of BMD, bone turnover and metabolism markers, and the occurrence of fragility fractures in a cohort of 32 young patients with SMA types 2 and 3, followed for an average of 18 months, with a subgroup of 12 patients followed for 36 months.

The choice to only enroll prepubertal children, who were expected to remain prepubertal throughout the study, was made in order to have a homogeneous sample and to avoid the interference of puberty on bone turnover markers and bone density. An important aspect of our study is that all the measurements and tests were performed with the same protocol, same instruments, same laboratory kits, at the same center.

In our previous cross-sectional study, we found low levels of 25-OH vitamin D in approximately 37% of cases [2]. In the present longitudinal follow-up study, 31% of the children showed abnormally low values of 25-OH vitamin D at the first assessment, with abnormally increased values of PTH in 6% (2 cases). Our findings highlight the importance of correcting vitamin D deficiency to avoid an abnormal increase in bone resorption [20] due to increased PTH secretion. It is noteworthy that in our patients the 25-OH vitamin D values tended to ameliorate or even return to normal over time, as documented in those with a longer follow-up after starting vitamin D supplementation.

The normalization of vitamin D levels corrected the moderately increased PTH values observed at V1 in 2 patients. However, in chronic diseases, normal values of 25-OH D are necessary but not sufficient to normalize BMD levels, considering the effect of many other factors (i.e., genetics, reduced mobility, diet, concomitant medications).

The percentage of patients with low lumbar spine BMAD Z-scores (<−2) increased over time: in particular, it reached 75% at V3 in the subgroup with a longer follow-up (Table 3).

According to some retrospective studies, fragility fractures seem more frequent in children with SMA than in healthy children of comparable age, but there are no prospective published studies. A few retrospective studies reported that peripheral fractures (femur, lower leg, and upper arm) were relatively frequent [21–23]. Vertebral fractures were also reported. The fact that most fractures were probably due to low impact trauma indicates that serious bone fragility can be present in SMA. Our 36-month study confirms that fragility fractures are relatively frequent in children with SMA, especially in type 2. At baseline, 4 patients had a history of peripheral fractures, and 5 additional patients sustained further peripheral fractures during the study. Although there was a significant number of undiagnosed vertebral fractures at baseline, no further vertebral fractures were subsequently detected. The patients with vertebral fractures had a slightly

lower BMAD than those without vertebral fractures, but the difference was not statistically significant.

Regarding the bone turnover markers, there was no significant difference between the groups with or without fractures. In our SMA patients, the bone formation markers were within the expected range (or mildly increased), suggesting that bone formation was essentially adequate for age. By contrast, the persistently high levels of CTx (a bone resorption marker) suggest the presence of excess bone resorption in SMA. The increase of BSAP observed between V1 and V3 in the subgroup with longer follow-up could be expected, considering that bone formation markers increase with age not only in healthy but also in chronically ill growing subjects, but could not overcome the excess bone resorption.

As expected in a chronic disease affecting bone metabolism, no correlation between age and spine BMC or BMAD was observed. This is in contrast with a recent retrospective study on a cohort of SMA 1 ($N=24$), 2 ($N=44$), and 3 ($N=17$) by Wassermann et al. [6] who found that lumbar spine areal bone mineral density Z-scores significantly increased with age, irrespective of SMA subtype. However, the Wasserman's study cannot be easily compared with our study, as its design was retrospective and the study included patients up to 18 years; additionally, 24/85 (28.2%) cases were affected by the most severe form of SMA, while SMA type 1 patients were not included in our study.

Longitudinal data on the correlation between bone status and motor function assessments are limited. In clinical practice, it is important to counsel families appropriately on the management of different aspects of the disease and personalize the interventions according to the clinical and functional status of the patient; furthermore, more objective outcome measures and biomarkers to monitor the evolution of the disease and the effect of treatments are highly needed. It is important to note that, although no correlation was found between motor function scores and BMC or BMAD, we found that higher values of spine BMD at follow-up were associated with HFMSE score >12 at baseline. This points out that high functioning children [24] with SMA 2 and 3 might be at lower risk of osteopenia and osteoporosis, thus confirming the role of physical activity and muscular strength on bone accrual in this young population [2,25,26]; however, the fact that reduced BMD has also been detected in children with higher motor function scores, and that spine BMAD Z-score worsened over time while HFMSE scores did not significantly change, further suggests that other factors than unloading, reduced mobility and neurogenic muscular atrophy may contribute to bone alterations in SMA. Similar conclusions can be outlined by the retrospective study by Wassermann et al. [6] in which patients with SMA1 and SMA2 had initially lower BMD than those with SMA3 in a different region than lumbar spine, i.e., the lateral distal femur, but SMA 3 patients showed subsequent decreases in BMD with time.

Animal data further support the hypothesis of a primary bone remodeling defect in SMA. An osteoporotic bone phenotype in SMA mice (increased numbers of osteoclasts, thin cortices and decreased number of trabeculae) was described by Jiang et al. [26] and by Shanmugarajan et al. [27]. The latter authors identified an up-regulation of signaling molecules, such as osteoclast stimulatory factor (OSF), and increased kinase activity in preosteoclast cells responsible for enhanced rate of osteoclast differentiation and bone-resorbing activity. An interaction of the SMN protein with OSF was described by Kurihara et al. [7], who suggested that the study of this interaction might offer new insights into the signaling mechanisms involved in the increased bone resorption, and consequent bone fragility, in SMA.

Further explorations of these mechanisms are necessary to confirm the hypothesis that spinal motor neuron degeneration and low physical activity cannot entirely explain the altered bone metabolism and increased bone fragility observed in SMA patients.

5. Conclusions

Our main findings – low 25-OH D levels and the presence of undiagnosed asymptomatic vertebral fractures also in very young patients, along with decreased BMAD and increased bone resorption markers over time – strongly suggest that even young children with SMA are at risk of developing severe bone fragility. A deeper investigation of the molecular mechanisms leading to altered bone metabolism in SMA could help to identify novel therapeutic targets and to establish better guidelines for the management of bone fragility in SMA. The long term follow-up of patients treated with the approved intrathecal antisense oligonucleotides and the comparison with new systemic treatments currently under clinical development will provide further evidence on the possibility to counteract and/or recover bone abnormalities in SMA patients.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2019.06.001.

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