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Skeletal effects and growth in children with chronic kidney disease: a 5-year prospective study

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Abstract

This study was designed to follow the evolving process of growth, bone modeling and remodeling in children with chronic kidney disease (CKD) who are at risk of developing CKD-mineral bone disorder (CKD-MBD). Fifteen patients, 4-15 years, were included with a median glomerular filtration rate of 46 (range 12–74) mL/min/1.73 m^2. Growth, bone mineral density (BMD) and markers of bone and mineral metabolism were investigated over a 5-year period. The median height standard deviation score was –0.65 at start and 0.1 after 5 years, with a range from –1.7 to 1.7, which implies that growth was acceptable. Total body, femoral neck, and lumbar spine BMD increased over the study period ($p<0.0001$). None had total body BMD Z-scores and lumbar spine Z-scores below –2.0 at follow-up. Most bone markers were within the reference intervals, but the formation markers alkaline phosphatase and type I procollagen intact amino-terminal propeptide (PINP) were slightly increased in about one-third of the patients after 5 years. Eleven out of 15 CKD patients had increased parathyroid hormone levels at baseline and 10 patients after 5 years. Taken together, this is the first 5-year longitudinal study of skeletal effects, growth and bone turnover in children with CKD. Growth and BMD Z-scores were well preserved on a group basis; however, these parameters varied significantly on an individual basis. We suggest, therefore, that it is difficult to state an overall recommendation and growth, bone mass, and markers of bone and mineral metabolism should be monitored and treated individually in CKD children.

Keywords: Bone metabolism markers, Bone mineral density, Dual-energy X-ray absorptiometry, Growth, Renal osteodystrophy

Introduction

Children and adolescents with chronic kidney disease (CKD) are at risk of developing CKD-mineral bone disorder (CKD-MBD) [1] with long-term consequences, such as growth retardation, bone deformities, low peak bone mass, and, subsequently, fragility fractures in the future [2–4]. High-bone turnover disease, secondary to hyperparathyroidism, seems to predominate during childhood while adynamic bone disease affects approximately 25% of those treated with dialysis [5, 6]. Factors of importance for skeletal development include genetics, mechanical loading, activity level, longitudinal growth and puberty, vitamin D, hormones, cytokines, and nutritional status. Peak bone mass is achieved during early adulthood and serves as the "bone bank" for the remainder of life. The interpretation of bone mineral measurements is more complex in children than in adults since children are growing individuals [7]. The current guidelines for clinical care of CKD-MBD focus on controlling secondary hyperparathyroidism, restriction of dietary phosphate, pharmacological therapy with phosphate binders and vitamin D analogues [8].

Only few studies have been published about bone health in children with CKD [9–13]. Most studies are cross-sectional and they have, therefore, many limitations since they only provide a single measurement of the investigated subjects, which does not fully reflect the growing individual and a constantly developing bone. Prospective studies are therefore warranted in order to follow the evolving process of growth, bone modeling and remodeling in children with CKD.

This study was designed to investigate the development of growth, bone mineral density (BMD) and markers of bone turnover over a 5-year period. Results from the first 3 years of this longitudinal prospective study of bone health in children with CKD have been published [14, 15].

Materials and methods

Subjects

Patients were recruited from the Queen Silvia Children's Hospital, Gothenburg, Sweden, after informed consent from the children and their parents. Included patients had a reduced glomerular filtration rate (GFR) below the reference interval 90–140 mL/min/1.73 m^2. The mean follow-up time was 5.2 years, minimum 4.6 years and maximum 6.0 years, during the years 2001-2008. The local research ethics committee of Gothenburg University, Sweden, approved this study (no. Ö374-01). The study group, at baseline, was comprised of 15 patients with CKD, 5 males and 10 females, median age 10.2 years (4.2–15.0 years) of age at inclusion and at follow-up, median 15.0 years (9.3–21.0 years). Table 1 shows clinical information for each patient. GFR was calculated according to the formula by Schwartz et al. [16], and the included patients had a median GFR of 46 (range 12–74) mL/min/1.73 m^2. Patients were categorized in groups in accordance with the Kidney Disease Outcomes Quality Initiative classification [17], CKD stage 2 (n = 3), stage 3 (n = 7), stage 4 (n = 2), and stage 5 (n = 3). Median GFR did not change significantly during the study period, 52 mL/min/1.73 m^2 after 5 years, but six patients underwent kidney transplantation during the course of the study (0.7, 0.8, 1.0, 3.3, 4.5, and 4.5 years after study start). Conservative treatment was given when indicated with sodium bicarbonate, calcium carbonate and alphacalcidol but no patients received aluminum containing phosphate binders. Antihypertensive treatment was given when indicated. A total of five patients received treatment with recombinant human growth hormone (GH).

Assessment of bone mass

BMD and bone mineral content (BMC) for total body, total hip, and lumbar spine ($L_1 – L_4$) were assessed by the Lunar DPX IQ (pencil beam), (GE Lunar Corp., Madison, WI, USA).

Dual-energy X-ray absorptiometry (DXA) measures bone in 2 dimensions and this areal bone mineral density, and not true volumetric bone mineral density, is referred to BMD (g/cm^2) in this study. The calculated Z-scores were age-and gender-specific. Lean body mass and fat mass were also measured. In the DXA and laser (DXL) Calscan technique, BMD is measured by using DXA in combination with laser measurements of the total heel thickness. This technology reduces the uncertainty related to the variable composition of soft tissue in adults [18]. For calcaneal BMD, the DXL Calscan (Demetech AB, Täby, Sweden) has been used for diagnosis of osteoporosis in adults and is in conjunction with measurements by axial DXA technology [19, 20]. The original DXL Calscan for adults has been modified for use in pediatric patients and has been shown to measure BMD with high accuracy [21]. The DXL Calscan pediatric version includes a function, which makes it possible to measure the calcaneal height. This height, together with the BMD value, provides an opportunity to calculate the volumetric bone mineral apparent density (BMAD), which could be valuable when measuring bones of different sizes as, e.g., for growing individuals [21]. The left foot of each child was measured. Regular hand X-ray was used to assess signs of rickets.

Biochemical determinations

A detailed description of the biochemical methods applied has been reported elsewhere [22]. Serum type I procollagen intact amino-terminal propeptide (PINP) was determined by radioimmunoassay (RIA) (Orion Diagnostica, Oulunsalo, Finland). Serum total alkaline phosphatase (ALP) was measured by a kinetic assay with 1.0 M diethanolamine buffer (pH 9.8), 1.0 mM $MgCl_2$ and 10 mM p-nitrophenylphosphate. The relation between the ALP activity units kat and U are 1.0 µkat/L corresponds to 60 U/L. The serum osteoclast-derived tartrate-resistant acid phosphatase isoform 5b (TRACP5b) was determined by a solid-phase immunofixed enzyme activity assay (SBA Sciences, Oulu, Finland). Serum parathyroid

hormone (PTH) was determined by a two-site immunoradiometric assay (Nichols Institute, San Clemente, CA, USA). Osteoprotegerin (OPG) is an anti-resorptive cytokine and a key-regulator of osteoclastogenesis and activity. Serum OPG was measured by a sandwich enzyme-linked immunosorbent assay (Immundiagnostik AG, Bensheim, Germany). Pediatric age- and gender-specific reference intervals are reported elsewhere for PTH, OPG, total ALP, PINP and TRACP5b [22]. Serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were determined by ^{125}I RIAs (DiaSorin, Stillwater, MN, USA).

Statistical analysis

All calculations were performed with the SAS software release 9.2 (SAS Institute, Cary, NC, USA). Spearman's rank correlation coefficient was used for nonparametric correlation analysis and Wilcoxon signed rank test was used to test for differences between study entry and after 5 years. The level of significance was set to $p < 0.05$.

Results

Auxological data are presented in Table 2, which shows that growth was adequate in the study group. Growth was regarded as acceptable when the height standard deviation score (SDS) was above –1.5 SDS, which was found in 12 out of the 15 patients in reference to their estimated target height. Four were treated with GH among these 12 patients. Out of the three patients that had poor growth, one patient received GH treatment. At the end of this 5-year follow-up study their height was –1.8, –1.1 and –2.8 SDS. The median height SDS for the entire group increased from –0.6 to 0.1 during the study period (Table 2). The median height SDS minus the mid-parental (i.e., mean height of both parents) height SDS was –0.7 at study entry and 0.1 after 5 years. Body composition data was obtained from the DXA measurements, which showed that the total body fat percentage increased; however, the body

mass index (BMI) SDS was unchanged during 5 years. Total lean mass increased significantly during the study period (Table 2). None of the patients had any radiological signs of rickets. Two traumatic fractures, one uncomplicated green-stick fracture in distal radius and one distal ring finger fracture, occurred during the course of the study.

Bone mass

Data from the DXA and DXL Calscan BMD and BMC measurements are shown in Table 3. Total body BMD (TBBMD) and total body BMC, lumbar spine BMD and BMC and total hip BMD and BMC increased over the study period (Table 3). The median Z-scores for TBBMD and lumbar spine did not change significantly over the study period. However, nine out of 15 patients (60%) decreased in their TBBMD Z-score over the study period, whereas 6 patients (40%) increased their TBBMD Z-score. The TBBMD Z-scores showed high variability among the individual CKD patients and no apparent pattern was found on the group level. No patient had a TBBMD Z-score below –1.5 after, at least, 5-years kidney disease duration (Fig. 1). Five out of 15 patients (33%) decreased in their BMD $L_1 – L_4$, Z-score over the study period, whereas 9 patients (60%) increased their BMD $L_1 – L_4$, Z-score, and one had the same Z-score. No patient had a BMD $L_1 – L_4$ Z-score below –1.5 after, at least, 5-years CKD disease duration (Fig. 1).

The patient (a boy, 13 years at baseline and within CKD stage 3) with the lowest TBBMD and $L_1 – L_4$ Z-scores was diagnosed with multicystic kidney and a pelvoureteral junction obstruction on the other side and he was not transplanted or on dialysis during the study period. The observed catch-up in BMD $L_1 – L_4$ Z-score between 3 and 5 years (Fig. 1) after inclusion was parallel to his pubertal growth spurt. The patient (a girl, 14 years and within CKD stage 4 at baseline) with the highest TBBMD and $L_1 – L_4$ Z-scores (1.3 and 1.2,

respectively) after 5 years was diagnosed with chronic glomerulonephritis (Fig. 1). This girl was transplanted after 3 years.

Biochemical markers of bone and mineral metabolism

Results of the biochemical markers of bone and mineral metabolism are presented in Table 4. Eleven of 15 patients, at baseline, and ten after 5 years, had increased PTH levels. Almost all patients had 25-hydroxyvitamin D levels within the reference interval, although some had concentrations in the lower quartile, and most patients had normal 1,25-dihydroxyvitamin D levels. Bone formation, as measured by total ALP and PINP, were slightly elevated in about 1/3 of the patients. The bone resorption marker TRACP5b levels were within the reference interval during the entire study period. The OPG levels were normal at baseline and after 5 years for all patients.

Discussion

This is the first longitudinal study over a 5-year period of skeletal effects, growth and bone turnover in children with a full spectrum of CKD severity. Significant developments have emerged over the recent decades preventing and/or correcting growth retardation as a consequence of improving dialysis procedures, renal transplantation, in addition to GH treatment of children and adolescents with CKD, causing significant impact on final height [23]. A significant factor, when reporting growth in children, is the genetic potential height. In the present study, the median height SDS minus the mid-parental height SDS was 0.1 after 5 years. Thus, the longitudinal growth and height SDS was acceptable during this 5-year follow-up study for most patients, which is in conjunction with the findings reported by Waller et al. [24]. Furthermore, it was recently concluded that avoiding the development of

significant bone disease through strict control of PTH levels permits good growth, however, the optimal ranges for PTH in childhood CKD has to be investigated [25].

There is no consensus on the best method to measure the degree of bone involvement in CKD-MBD. Recently, Weber and Mehls [26] described the limitations of DXA in children with CKD because this population can have poor growth which results in an artificial underestimation of BMD. We found that our patients had an acceptable growth, thus our DXA results are not limited to the confounding factor of poor growth. The BMD Z-scores were well preserved on a group basis; however, it was demonstrated that these BMD parameters can vary significantly on an individual basis over the years (Fig. 1).

There is an overall concern that CKD children can develop growth retardation and low mass with low Z-scores at all sites, which contradicts our positive findings of preserved Z-scores. We interpret these bone mass findings as positive results despite the rather severe disease spectrum of kidney disorders in this young population. We also measured bone mass by DXL that mostly measures trabecular bone in the calcaneus which has been shown to correlate well with whole body DXA measurements [27]. During this 5-year follow-up study, we could not detect a significant decrease in BMAD (measurement independent of size) due CKD as one might except, though this BMAD was unchanged which is a positive finding which reflect the trabecular portion of bone mass. Wetzsteon et al. [13] used peripheral quantitative computer tomography, which distinguishes cortical and trabecular bone, and reported recently elevated trabecular BMD Z-scores in younger CKD children. Our results support the findings of Waller et al. [24] who reported a normal mean BMD; however, others have found less encouraging results. Groothoff et al. [11] found less encouraging results with mean lumbar spine and femoral neck BMD Z-scores of –2.1 and –1.8, respectively; however, Ziolkowska et al. [9] described mean Z-scores in the range of –1.5 to –0.3. Another study by

Pluskiewicz et al. [10] reported a mean total body Z-score of –1.4. Bakr [12] observed osteopenia in 62% of the children with predialysis CKD.

Biochemical markers of bone turnover are of potential value as early indicators of diagnostic and/or monitoring of CKD-MBD since they reflect bone remodeling. It is, in general, more difficult to make an accurate classification of low- and high-bone turnover disease in pediatric patients in comparison with adults, because younger individuals have wider reference intervals than adults. Most bone markers were within the reference intervals, which indicate a normal bone remodeling in concurrence with the observed BMD Z-score values. The circulating levels of osteocalcin and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) are elevated in the majority of CKD children because reduced renal clearance inevitably leads to increased levels [15]. Although used in studies of CKD patients [13], the clinical usefulness of osteocalcin and CTX is uncertain in patients with CKD, since they are cleared by the kidneys. CTX and osteocalcin were therefore not analyzed in this study. Bone ALP and TRACP5b may have additional utility in kidney disease since their clearance from the circulation is independent of renal function.

The low number of patients is a limitation of our study. CKD is uncommon during childhood; however, we were able to recruit almost all CKD patients, who we considered to be representative patients with various CKD stages, within our hospital region. We found no significant associations between CKD stages, disease duration, and transplantation, with the BMD Z-scores; nevertheless, an extended subgroup analysis would have been preferable. We lacked matching controls in this study but there is currently no consensus on the optimal approach for control-matching BMD and BMC for factors such as body size, pubertal stage, skeletal maturity, lean mass, and fat mass. We did not measure the true volumetric BMD with peripheral quantitative computer tomography (pQCT) or perform bone histomorphometry, which would have been preferable [7]. However, the calculated calcaneal BMAD, which

reflects a 3D approach to investigate bone structure, was unchanged during the study period suggesting that the true density remained the same in trabecular bone.

In summary, our study is the first investigating skeletal effects, growth and bone turnover in CKD children over a 5-year period. We interpret our results as positive findings in general for CKD children on the group level because growth was acceptable in relation to target height, and BMD Z-scores were well preserved and none had TB Z-scores below –1.5 after at least 5 years with CKD. However, an individual approach is of great importance and children with low Z-scores, poor growth, and defective bone and mineral metabolism, should be monitored carefully and more often than children demonstrating normal values. Bone ALP (formation marker) and TRACP5b (resorption marker) may have clinical utility in kidney disease since their clearance from the circulation is independent of the renal function. Extended long-term studies, 10 – 20 years, are warranted in pediatric CKD patients which will be a future challenge.

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Conflict of interest statement: All authors have no conflicts of interest.

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Table 1 Clinical information at study entry

Patient no.	Gender	Age (years)	Diagnosis	Transplanted (years after study entry)	GFR (mL/min/ 1.73 m^2)	Urinary protein*	Hb (g/L)	Albumin (g/L)	PTH (ng/L)	25(OH)D (µg/L)	GH therapy	EPO therapy (IE/week)	Steroids (mg/day)
1	Female	12	Bartter's syndrome	No	71	0	115	41	99	18	No	No	No
2	Female	12	Juvenile nephronophthisis	4.5	39	0	107	44	133	20	Yes	No	No
3	Female	7	Cystinosis	4.5	39	3	124	32	28	15	Yes	No	No
4	Female	14	Kidney dysplasia	3.3	19	2	104	41	227	17	No	No	No
5	Female	11	Pyelonephritic scarring	No	74	0	125	36	32	14	No	No	No
6	Female	15	Neonatal hypovolemia	0.7	12	2	130	50	172	34	No	No	No
7	Female	7	Neonatal hypovolemia	No	36	0	154	42	87	31	No	No	No
8	Male	6	Polycystic kidney disease	No	60	0	115	37	52	26	No	No	No
9	Male	13	Multicystic kidney dysplasia	No	52	0	124	44	59	18	No	No	No
10	Female	10	Juvenile nephronophthisis	No	49	0	93	38	202	12	Yes	No	No
11	Male	10	Obstructive uropathy	No	28	1	102	38	99	26	Yes	No	No
12	Male	11	Obstructive uropathy	No	46	0	146	41	53	27	Yes	No	No
13	Male	4	Obstructive uropathy	1.0	Dialysis	0	101	32	86	40	No	1500	No
14	Female	6	Interstitial nephritis	0.8	Dialysis	3	90	32	82	5	No	2250	5
15	Female	9	Kidney dysplasia	No	50	1	131	43	41	19	No	No	No

25OHD 25-hydroxyvitamin D, *EPO* erythropoietin, *GFR* glomerular filtration rate, *GH* growth hormone, *Hb* hemoglobin, *PTH* parathyroid hormone,

* Arbitrary scale from 0 to 4.

Table 2 Auxological data for the investigated children with CKD

	Study entry n = 15	After 5 years n = 15
Height (cm)	130.6 (99.0–164.0)	158.5 (129.5–175.3)**
Height SDS	–0.6 (–2.0 to 1.3)	0.1 (–1.7 to 1.7)
Weight (kg)	27.0 (14.7–63.5)	49.0 (25.8–86.0)**
Weight SDS	–0.5 (–3.1 to 2.3)	–0.2 (–2.7 to 2.7)
BMI (kg/m^2)	16.1 (12.9–25.5)	19.5 (15.4–34.5)**
BMI SDS	0.0 (–2.6 to 2.5)	0.1 (–2.2 to 3.2)
Total fat tissue (%)	19.4 (5.4–48.6)	23.6 (9.0–55.0)*
Total lean mass (kg)	21.9 (11.3–39.1)	33.2 (21.3–55.0)**

SDS standard deviation score, *BMI* body mass index

Values are given as median, with minimum and maximum values in parentheses.

* $p < 0.05$, ** $p < 0.001$, Wilcoxon signed rank test.

Table 3 Bone mass data for the investigated children with CKD

	Study entry n = 15	After 5 years n = 15
TBBMD (g/cm^2)	0.91 (0.73–1.19)	1.04 (0.84–1.19)**
TBBMD, Z-score	0.7 (–1.0 to 1.3)	–0.2 (–1.5 to 1.3)
TBBMC (g)	1168 (479–2511)	1998 (928–2935)**
BMD $L_1 – L_4$ (g/cm^2)	0.72 (0.46–1.14)	1.02 (0.91–1.82)**
BMD $L_1 – L_4$, Z-score	0.3 (–1.8 to 1.5)	0.3 (–1.5 to 1.2)
Total hip BMD (g/cm^2)	0.80 (0.49–1.07)	0.96 (0.80–1.17)**
Calcaneal BMD (g/cm^2)	0.30 (0.21–0.43)	0.39 (0.26–0.51)*
Calcaneal BMC (g)	0.22 (0.16–0.32)	0.30 (0.20–0.37)**
Calcaneal BMAD (mg/cm^3)	89 (64–119)	89 (67–134)

BMD bone mineral density, *TBBMD* total body BMD, *BMC* bone mineral content, *BMAD* bone mineral apparent density. For all calcaneal measurements, n = 14.

Values are given as median, with minimum and maximum values in parentheses.

* $p < 0.01$, ** $p < 0.001$, Wilcoxon signed rank test.

Table 4

Biochemical markers of bone and mineral metabolism in children with CKD in comparison with age- and gender-adjusted reference intervals*

	Study entry				After 5 years			
	Median (min – max)	Patients below the reference interval	Patients within the reference interval	Patients above the reference interval	Median (min – max)	Patients below the reference interval	Patients within the reference interval	Patients above the reference interval
Total ALP (µkat/L)	10.5 (6.2 – 18.4)	0	15	0	7.2 (1.8 – 18.8)	0	11	4
PINP (µg/L)	607 (210 – 1145)	0	13	2	478 (54 – 1270)	0	10	5
TRACP5b (U/L)	10.8 (6.8 – 27.7)	0	15	0	10.5 (4.9 – 25.0)	0	15	0
OPG (pmol/L)	3.2 (2.0 – 4.6)	0	15	0	3.1 (1.2 – 4.6)	0	15	0
PTH (ng/L)	86 (28 – 227)	0	4	11	69 (19 – 1440)	0	5	10
25-OH vitamin D (µg/L)	19 (5 – 40)	1	14	0	19 (10 – 38)	0	15	0
1,25-$(OH)_2$ vitamin D (ng/L)	32 (13 – 97)	2	10	3	45 (32 – 96)	0	14	1

ALP alkaline phosphatase, *PINP* type I procollagen intact amino-terminal propeptide, *TRACP5b* tartrate-resistant acid phosphatase isoform 5b, *OPG* osteoprotegerin, *PTH* parathyroid hormone

* Reported pediatric age- and gender-specific reference intervals for markers of bone and mineral metabolism were used in comparison with our study group, see *Subjects and methods* for details.

Figure legend

Figure 1. TBBMD Z-score (top) and lumbar spine BMD (L_1-L_4) Z-score (bottom) for children with CKD over the study period, n = 15. One patient (4 years of age at entry) lacked Z-scores initially since these were not available at the time for children younger than 5 years for the used Lunar DPX IQ. Lines partially marked in red designates the time after kidney transplantation.

Figure 1 TOP

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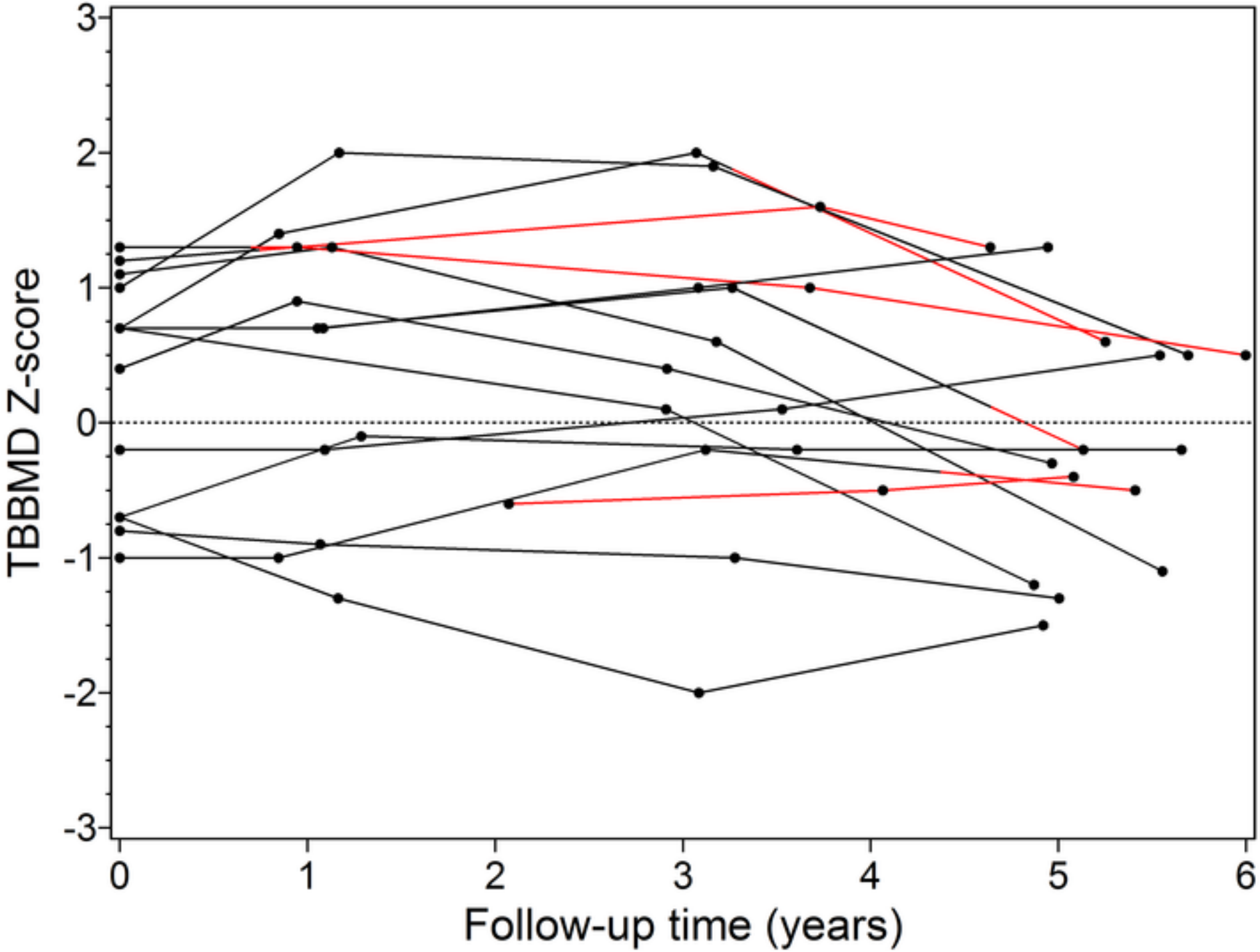

Figure 1 BOTTOM
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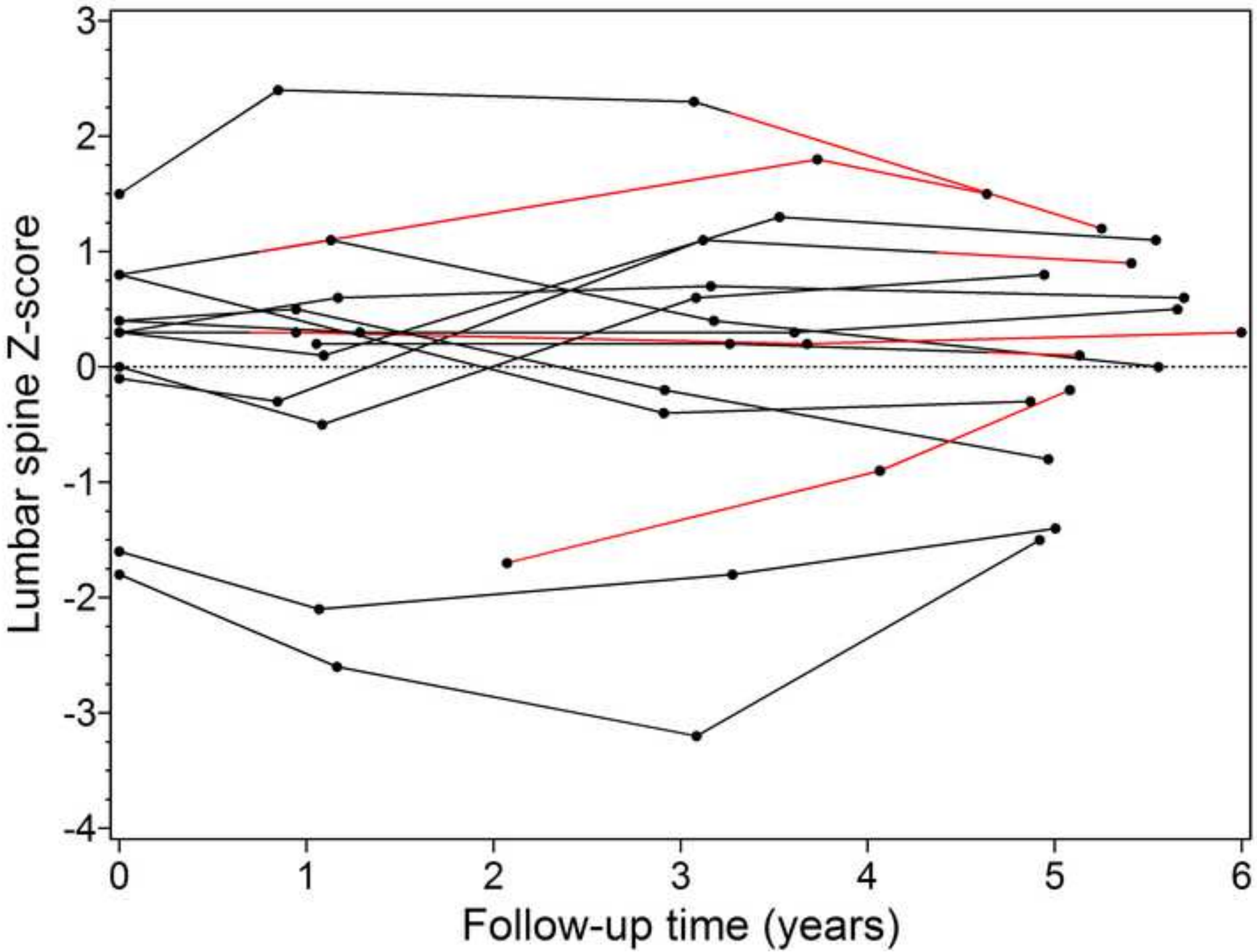

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