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Original Paper

Bone Mineral Density in Children with Acute Lymphoblastic Leukaemia

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Bone mineral density (BMD) may be negatively affected by the disease or its treatment in patients with acute lymphoblastic leukaemia (ALL). Therefore, we evaluated lumbar spine and total body BMD and bone metabolism in children with ALL at diagnosis, during treatment with chemotherapy and 1 year after completion of treatment. 32 children (21 boys and 11 girls) participated in the study. 14 children started the study at diagnosis and 18 during or after the treatment period. Lumbar spine and total body BMD were measured with dual energy X-ray absorptiometry, and expressed as standard deviation scores (SDS). Blood samples were obtained to assess bone metabolism. 3 of 14 children had low lumbar spine BMD (<−2 S.D.) at diagnosis. All children had normal total body BMD. Markers of bone turnover were depressed. Total body BMD SDS decreased significantly during the first year of treatment ($P<0.001$). Lumbar spine BMD SDS did not change significantly. Parameters of bone turnover increased to normal during the treatment period. Parathyroid hormone had increased significantly after 1 year ($P<0.05$). Mineral homeostasis was disturbed in some patients during treatment. 4 of 9 patients had low total body BMD and 1 patient low lumbar spine BMD one year after completion of treatment. All patients had normal biochemical results at that time. In conclusion, lumbar spine BMD and bone turnover were decreased in some patients at diagnosis. Total body BMD decreased significantly during treatment and was low in 4 of the 9 patients 1 year after completion of the treatment. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: bone mineral density, children, acute lymphoblastic leukaemia, chemotherapy
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INTRODUCTION

SKELETAL ABNORMALITIES and bone pain are known to occur in childhood acute lymphoblastic leukaemia (ALL) [1]. Fractures and osteoporosis have been described in association with ALL [2–5]. One study showed that at diagnosis 13% of 40 children with ALL showed radiographic osteopenia [2]. One case report described osteopenia and vertebral fractures after induction treatment in an adolescent patient with ALL [3]. Disturbances in mineral metabolism and low bone mass of the radius, measured with single photon absorptiometry, were reported during therapy in children with ALL [4]. Survivors of childhood ALL who had received cranial irradiation had osteopenia whilst non-irradiated survivors had normal bone mass [5]. Nussey and colleagues showed that adult survivors of childhood ALL with untreated growth hormone deficiency had reduced bone mineral density (BMD) whilst patients without growth hormone deficiency or patients who received growth hormone treatment had normal BMD [6]. The objective of the present study was to evaluate BMD and markers of bone metabolism of children with ALL at diagnosis, during treatment with chemotherapy and without cranial irradiation, and 1 year after cessation of treatment.

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PATIENTS AND METHODS

Patients

32 (21 boys and 11 girls) participated in the study. Between June 1994 and November 1995 all 14 newly diagnosed patients with ALL, older than 4 years were enrolled consecutively in the study. 22 patients under treatment or 1 year after cessation of treatment for ALL without a relapse were asked to participate in the study of whom 4 refused. The mean age of the patients at the time they started the study was 7.9 years (standard deviation (S.D.) 4.0). 8 children were pubertal during the study period. One girl suffered from Down's syndrome. None of the other children had dysmorphic characteristics. All children were treated according to protocol ALL-8 of the Dutch Childhood Leukaemia Study Group. None of them received cranial irradiation. This protocol resembles the Berlin–Frankfurt–Münster (BFM)-ALL 90 study [7]. 9 patients were in the standard, 19 in the medium and 4 in the high-risk group [7]. Chemotherapy involved vincristine, prednisone or dexamethasone, daunorubicin, L-asparaginase, methotrexate, cytosine-arabinoside, cyclophosfamide, 6-mercaptopurine, doxorubicin, 6-thioguanine and in the high-risk group additional vindesine, ifosfamide and etoposide. Maintenance therapy included low-dose methotrexate, 6-mercaptopurine and in some patients L-asparaginase. Treatment was completed in 2 years. 3 patients were excluded from further study because of relapse.

The points of time of measurements were at diagnosis (t_0), after treatment for 6 months ($t_{\frac{1}{2}}$), 1 year (t_1) and 2 years (t_2), and 1 year after cessation of treatment (t_3).

14 children had BMD measurements at t_0. Of these one was also measured at $t_{\frac{1}{2}}$, t_1, t_2 and t_3, 3 were also measured at $t_{\frac{1}{2}}$, t_1 and t_2, 4 at $t_{\frac{1}{2}}$ and t_1, 1 at $t_{\frac{1}{2}}$, 4 at t_1 and 1 at t_1 and t_2. 18 other children began measurements during and after treatment. 2 were measured at $t_{\frac{1}{2}}$, of whom one was also measured at t_1 and one at t_1 and t_2. 3 started measurements at t_1, of these one was also measured at t_2 and 1 at t_2 and t_3. 7 were measured at t_2, of whom one was also measured at t_3. 6 were measured once at t_3.

Measurements

BMD (g/cm^2) of lumbar spine and total body was measured by Dual Energy X-ray Absorptiometry (DXA, Lunar DPXL/PED, Madison, Wisconsin, U.S.A.). The results were compared with healthy age- and sex-matched controls of the same population and expressed as standard deviation scores (SDS) [8]. DXA total body measurement also gives estimates of body composition as lean tissue mass (g), fat mass (g) and bone mineral content (g). Lean tissue mass, fat mass and percentage of body fat were compared with our age- and sex-matched Dutch reference values and expressed as SDS [9].

Height was assessed with a fixed stadiometer, compared with Dutch reference values and expressed as SDS [10].

Blood samples were obtained in the morning on the same day as BMD measurements. In these samples, assessments were performed of calcium, phosphate, parathyroid hormone (PTH), osteocalcin and procollagen type I C-terminal propeptide (PICP), carboxy-terminal telopeptide of type I collagen (ICTP), insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein 3 (IGFBP-3). Osteocalcin, intact PTH were measured by radioimmunoassay (Incstar Corporation, Stillwater, U.S.A.). For measurement of PICP and ICTP radioimmunoassay kits of Orion Diagnostica, Espoo, Finland were used. For prepubertal children, our own reference values of osteocalcin, PICP and ICTP were used, based on, respectively, 25, 82 and 88 samples. Reference values for the older children were taken from other studies which used the same assays [11–13]. Assessment of IGF-1 and IGFBP-3 was performed with radioimmunoassay kits of Med-Genix Diagnostics, Fleurus, Belgium and Diagnostic System Laboratories, Webster, Texas, U.S.A. IGF-I and IGFBP-3 age- and sex-specific reference values were based on 600 samples of healthy Dutch children [14]. Samples for assessment of osteocalcin, PICP, ICTP, vitamin D and PTH were missed in 4 patients and of IGF-I and IGFBP-3 in 3 patients at diagnosis. Samples for these assessments were missed at $t_{\frac{1}{2}}$ in 2 patients, at t_1 and t_2 in one patient and at t_3 in 4 patients.

Statistics

Comparison of the mean SDS from the expected zero was tested with one sample *t*-test. We tested the changes during time with paired *t*-tests. Two groups of variables with a normal distribution were compared with a two-sample *t*-test. One-way analysis of variance was used to compare the three risk groups.

RESULTS

Bone mineral density

At diagnosis the mean SDS of lumbar spine BMD was −0.67 (S.D. 1.3) and of total body BMD 0.02 (S.D. 1.3), both not significantly different from zero. However, 3 of the 14 children had lumbar spine BMD SDS below −2. None of the children had total body BMD SDS below −2. During the first 6 months lumbar spine BMD SDS remained stable, but total body BMD SDS decreased significantly (mean change ($\triangle$) −0.59, $P<0.001$) (Figure 1). The mean change in total body BMD SDS between t_0 and t_1 was −1.10 (S.D. 0.69), significantly lower than zero ($P<0.001$). Both mean BMD SDS of lumbar spine and total body at $t_{\frac{1}{2}}$ were significantly below zero ($P<0.02$ for lumbar spine and $P<0.05$ for total body). This was also the case for mean total body BMD SDS at t_1 ($P<0.01$) and t_2 ($P<0.02$).

At t_3 1 of the 9 children had lumbar spine BMD SDS below −2 and 4 children had total body BMD SDS below −2.

There was no difference in BMD SDS between the sexes or between prepubertal and pubertal patients. At diagnosis BMD SDS did not vary significantly between the standard-, medium- and high-risk group. The decrease in total body BMD SDS did not differ between the three risk groups.

5 patients (16%) had a fracture at diagnosis or during the treatment period. One girl with Down's syndrome had multiple thoracal vertebral impression fractures at diagnosis. She started the study at t_1. At that time her lumbar spine and total body BMD SDS were both −3.0. Fractures of the humerus, os cuneiforme, os metatarsalis V and forearm occurred in the other 4 patients after trauma during treatment. One of them had both lumbar spine and total body BMD SDS below −2 and one had total body BMD SDS below −2. The BMDs were measured within 1 year from the time of fracture.

Height and body composition

At diagnosis, height SDS was not significantly different from zero; only 1 patient had height SDS below −2. Height

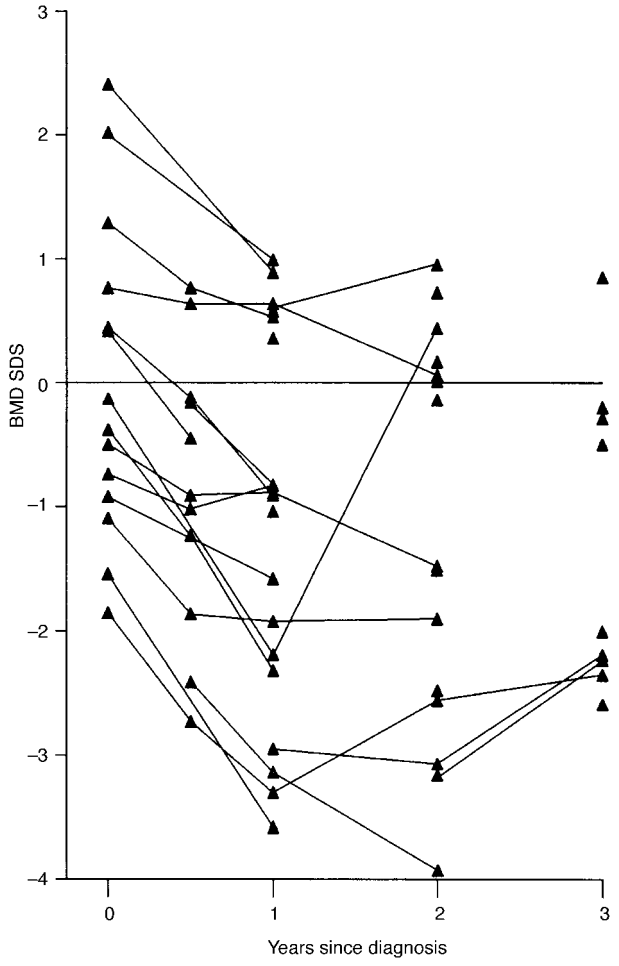

Figure 1. Standard deviation scores (SDS) of total body bone mineral density (BMD) before, during and 1 year after cessation of treatment in children with acute lymphoblastic leukaemia. The lines connect measurements within 1 patient.

SDS decreased during the first 6 months (mean △ −0.33, $P<0.001$). From $t_{\frac{1}{2}}$ to t_3 height SDS did not change significantly. At t_3, 2 patients had height SDS below −2. Only at time point t_1, actual height SDS correlated significantly with lumbar spine and total body BMD SDS ($r=0.68$, $P<0.01$ for lumbar spine and $r=0.51$, $P<0.05$ for total body BMD). △ height SDS after 1 year of treatment had no correlation with △ total body BMD SDS after the same period ($r=0.09$).

At diagnosis, mean lean tissue mass SDS was significantly lower than zero (−0.74, $P<0.01$). Fat mass and percentage fat SDS did not differ significantly from zero. Lean tissue mass, fat mass and percentage fat SDS did not change significantly during treatment.

Biochemical markers of bone turnover and growth factors

The results of the biochemical bone markers are shown in Table 1. At diagnosis mean osteocalcin and PICP, both parameters of bone formation, and ICTP, a marker of bone resorption, were significantly lower compared with controls ($P<0.001$ for osteocalcin and ICTP, $P<0.05$ for PICP). Osteocalcin was below the normal range (less than −2 S.D.) in 5, PICP in one and ICTP in none of the 10 children. All three biochemical parameters had increased significantly after 6 months of treatment and remained stable thereafter. All children had normal values of osteocalcin, PICP and ICTP at $t_{\frac{1}{2}}$, t_1, t_2 and t_3 and the means did not differ significantly from the means of controls.

6 of the 14 patients had low alkaline phosphatase at diagnosis. After 1 year, alkaline phosphatase had increased significantly. During and after treatment none of the patients had low alkaline phosphatase.

At diagnosis, 2 of the 14 patients had hypocalcaemia, 2 had hyperphosphataemia, 2 had low PTH, 1 had high PTH, 1 had low 25 hydroxyvitamin D and 2 had low 1,25 dihydroxyvitamin D. During the 2 years of treatment, 7 of 25 patients had decreased serum calcium, 2 had increased phosphate, 2 had high PTH, 4 had low 25 hydroxyvitamin D and 2 had low 1,25 dihydroxyvitamin D in at least one measurement. Mean PTH, 1,25 dihydroxyvitamin D and calcium had increased significantly after 1 year ($P<0.05$ for PTH and 1,25 dihydroxyvitamin D, $P<0.02$ for calcium). Phosphate and 25 hydroxyvitamin D did not change significantly. At t_3, 1 year after treatment, all had normal serum calcium, phosphate, PTH and 1,25 dihydroxyvitamin D. 2 patients had low 25 hydroxyvitamin D.

At diagnosis, 5 of 11 patients had low IGF-I (IGF-I SDS below −2). IGFBP-3 was normal in all patients. IGF-I SDS and IGFBP-3 increased significantly during the first 6 months (both $P<0.01$). During the 2 years of treatment, 5 of 20 patients had low IGF-I in at least one measurement. At t_3, all had normal IGF-I and IGFBP-3.

At diagnosis, 1,25 dihydroxyvitamin D correlated positively with lumbar spine BMD SDS ($P<0.05$), total body BMD SDS ($P<0.01$) and IGF-I levels ($P<0.02$). During and after treatment no correlation was observed. Other biochemical parameters had no correlation with lumbar spine or total body BMD SDS Changes of any of the biochemical markers of bone metabolism did not relate to the decrease of total body BMD.

DISCUSSION

At diagnosis, 3 of 14 (21%) children with ALL had a low lumbar spine BMD. Markers of bone turnover were reduced. Total body BMD decreased during the first year of treatment, suggesting a negative effect of chemotherapy or other factors like decreased physical activity on cortical bone.

At diagnosis of ALL, biochemical markers indicated suppression of bone turnover by the disease. This is in agreement with the findings of Halton and colleagues [2], who reported low osteocalcin and low urinary deoxypyridinoline, a parameter of bone resorption, in children with ALL at diagnosis. Biopsy specimens of iliac bone showed abnormalities in bone mineralisation in 3 of 9 children [2]. Leukaemic infiltration of the bone marrow and marrow expansion have been mentioned as pathogenic factors of osteoporosis in ALL [3]. This may have caused the low lumbar spine BMD and suppression of bone turnover found in some patients. Lumbar spine has a high content of trabecular bone, which contains a high proportion of bone marrow. Trabecular bone is metabolically more active than cortical bone.

Lumbar spine and total body BMD SDS seemed to be negatively affected by low levels of 1,25 dihydroxyvitamin D at diagnosis in agreement with the findings of Halton and colleagues [2]. Reduced production of 1,25 dihydroxyvitamin D is related to low IGF-I levels [15].

During treatment, markers of bone turnover increased to normal levels. However, hypocalcaemia, hyperparathyroidism

Table 1. *Mean (S.D.) Biochemical parameters at diagnosis and during treatment of ALL*

	At diagnosis	0.5 year	1 year	2 years	3 years	Reference values
Osteocalcin (μg/l)	4.8 (3.4)	15.6 (4.5)†	15.7 (5.4)‡	12.3 (7.2)	15.3 (8.5)	4–20
PICP (μg/l)	144 (77)	387 (129)†	295 (118)‡	263 (64)	263 (64)	77–626
ICTP (μg/l)	9.6 (2.2)	18.5 (4.0)*	12.1 (3.3)	11.0 (3.9)	12.7 (3.5)	6–19
Alkaline phosphatase (U/l)	92 (40)	144 (38)	171 (71)†	198 (59)	172 (57)	80–225
PTH (ng/l)	15.2	18.8	23.7‡	36.3	27.0	10–55
25 OH-vitamin D (nmol/l)	115 (67)	60 (26)	79 (36)	63 (30)	56 (32)	31–129
1,25 diOH-vitamin D (pmol/l)	76 (30)	84 (34)	141 (35)‡	132 (38)	124 (22)	40–140

*$P<0.001$, †$P<0.01$, ‡$P<0.05$ significant increase compared with baseline. PICP, carboxy terminal propeptide of type I collagen; ICTP, cross-linked telopeptide of type I collagen; PTH, parathyroid hormone; 25-OH-vitamin D, 25-hydroxyvitamin D; 1,25-diOH-vitamin D, 1,25-dihydroxyvitamin D. The median is given of PTH because of a skewed distribution. The mean within patient change from baseline was tested until 1 year, thereafter the change compared with previous measurement was tested.

and low 25 hydroxyvitamin D were demonstrated in some patients. Atkinson and colleagues [4] also showed alterations in calcium and vitamin D metabolism during treatment for ALL in children. Approximately 50% of the patients demonstrated hypocalcaemia and hypomagnesemia and 12 of the 13 patients had low 1,25 dihydroxyvitamin D levels [2]. The increase of PTH which we observed during the first year of treatment might be involved in the decrease in total body BMD, 80% of which consists of cortical bone [16]. Hyperparathyroidism negatively affects cortical bone [17]. Vitamin D preparations suppress PTH secretion by both raising serum calcium and inhibiting PTH gene transcription [18]. Therapy with vitamin D might prevent or diminish the decrease of total body BMD and may prevent fractures of cortical bone.

Methotrexate and corticosteroids both can influence calcium metabolism. Osteoporotic fractures secondary to high-doses of methotrexate in children with ALL have been described [19]. Osteoporosis has been reported in patients treated with low doses of methotrexate for psoriasis and rheumatoid arthritis [20]. In rats, low-dose methotrexate caused a decrease in trabecular bone mass and decreased osteoblast activity, whilst cortical bone mass was not affected [21]. It is unknown if high-dose methotrexate influences cortical bone. In the protocol of the present study, high-dose methotrexate was given during the first months of treatment when the decrease in total body BMD was observed. During maintenance treatment low-dose methotrexate was prescribed.

Corticosteroids mainly affect trabecular bone [22], present in the vertebrae. Since, in the present study, total body BMD, mainly cortical bone, decreased and not lumbar spine BMD, corticosteroids may not be the causal factor. In the protocol, corticosteroids are prescribed during the first 6 months of treatment. Corticosteroids may have caused the decrease in height SDS observed during the first 6 months. Corticosteroids negatively affect growth. The reduction in height might have influenced the decrease in total body BMD, although there was no correlation between change in height SDS and the change in total body BMD SDS after 1 year. It is not known if other cytotoxic drugs affect bone metabolism.

A decrease in physical activity during the treatment period could negatively influence BMD. Physical activity was not estimated in our study. Physical activity of the patient may decrease during treatment, but lean tissue mass, mainly muscle mass, remains stable. Gilsanz and colleagues [5] reported normal spine BMD in survivors of ALL who did not receive cranial irradiation 6 months to 8 years after completion of treatment. The present study is in agreement with this. Normal BMD of the femoral neck, mainly cortical bone, was found in adult survivors of childhood ALL without growth hormone deficiency [6].

In conclusion, lumbar spine BMD and bone turnover were decreased in some patients at diagnosis. Total body BMD decreased during the first year of treatment in children with ALL. Bone turnover increased to normal during the treatment period. One year after cessation of treatment 4 of 9 children still had a low total body BMD. Further studies are needed to evaluate the long-term effect on BMD in these patients and how to prevent a decrease of BMD.

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