

CLINICAL STUDY

Bone mineral density and bone turnover in young adult survivors of childhood acute lymphoblastic leukaemia

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Abstract

Objective: Treatment for childhood leukaemia induces many risk factors for development of decreased bone mineral density (BMD). Physical activity is also known to affect BMD. The aim was to study BMD and markers of bone turnover in a well-defined group of survivors of acute lymphoblastic leukaemia (ALL) who had all reached final height as well as peak bone mass, taking both previous treatment and physical activity into consideration.

Design: All patients treated for ALL before the onset of puberty in the region of western Sweden, between 1973 and 1985, in first remission were included. Thirty-five out of forty-seven patients aged 20–32 years participated. Nineteen patients had received cranial radiotherapy, and the median follow-up time was 20 years.

Methods: BMD was assessed using dual-energy X-ray absorptiometry (DEXA). Serum concentrations of markers of bone turnover were analysed. Physical performance was measured using a performance exercise capacity stress test.

Results: BMD was slightly reduced in lumbar spine (-0.4 SD), but not in femoral neck or total body. BMD in femoral neck was correlated to physical performance and dose of corticosteroid, but no correlation was found with spontaneous growth hormone (GH) secretion. Markers of bone turnover were also correlated to physical performance, but not to GH secretion.

Conclusions: Physical fitness seems to be the most important factor in developing and preserving normal bone mineral density in ALL patients. We propose that lifestyle education promoting physical activity is encouraged from an early point in time for these patients.

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Introduction

Osteoporosis is a condition of clinical importance, since 50% of women and 25% of men over the age of 50 will sustain a fragility fracture (1, 2). The incidence of osteoporosis has increased since the 1950s (3). Lifestyle changes affecting diet and physical activity have been proposed to play a major role in relation to this increasing problem (4, 5). Survivors of childhood acute lymphoblastic leukaemia (ALL) have previously been shown to have low bone mineral density (BMD) (6–12); however, this matter is still controversial with other studies demonstrating little or no such effect (13–16). Various possible causative factors for decreased BMD in these patients have been proposed: the disease itself, growth hormone (GH) and/or sex hormone deficiency, treatment with corticosteroids and

cytostatic drugs, low calcium intake, and reduced physical activity (14, 17, 18). The matter of decreased BMD is, however, somewhat controversial, since the patient populations included in previous studies have been heterogeneous, including children diagnosed both before and during puberty, and some assessments of BMD were performed long before attainment of peak bone mass (6, 7, 16). Since childhood ALL is the most common childhood malignancy with a 5-year survival rate of 75%, it is important to identify risk factors and elaborate prevention programmes for late effects for this increasing cohort of young adults (19). The aim was to study bone mineral density and bone markers in this well-defined group of survivors of ALL who had all reached final height as well as peak bone mass, taking both previous treatment and physical activity into consideration.

Patients and methods

For recruitment of participants in the study we reviewed records from all patients who had completed treatment for ALL before the onset of puberty in the region of western Sweden, between 1 January 1973 and 31 December 1985. For inclusion, patients had to be in first remission with a minimum follow-up time of 10 years from remission and a minimum age of 20. The oncological treatment had to have been concluded before the onset of puberty. These criteria were met by 47 young adults aged 20–32 years, of whom seven patients were lost to follow-up. Of the 40 patients contacted, 35 agreed to participate in the study. Thirty out of the thirty-five patients performed the performance exercise capacity stress test (18 had received cranial radiotherapy (CRT +) and 12 CRT –). The clinical details of the patients are given in Table 1. All patients were Caucasian and stated that they were in good health and were either employed or engaged in studies. Two females were on antidepressant therapy and eight were taking contraceptive pills. The oestrogen levels in the women were normal according to their present phase in the menstrual cycle. All men had normal testosterone levels. Clinically and biochemically, all patients were euthyroid, with normal free thyroxine and thyrotrophin levels. One patient was euthyroid on thyroxine replacement after total thyroidectomy due to thyroid adenoma. In a previous report on this group of patients, we found that 50% of patients who received prophylactic cranial radiotherapy (CRT +) had a maximum growth hormone level (GH_{max}; attained in a 24-h spontaneous GH profile) below the cut off-level (3.3 µg/l) for GH treatment (20). No patient had received treatment with growth hormone at the time of the study. Final height was in the expected range for midparental height in 34/35 patients. Patients who had not received cranial irradiation (CRT –) had GH_{max} levels within the normal range (20). In these patients low GH secretion was correlated with increased body fat, especially trunk fat, reduced fat-free mass and a tendency for an unfavourable lipid profile (21).

Treatment

The patients had been treated on the basis of three subsequent protocols from the Swedish childhood

leukaemia group (22). For all protocols, the total treatment time was three years. All patients had been treated with intrathecal injections of methotrexate, and nineteen, 10 men and 9 women, had received cranial irradiation (CRT +) with a dose of 18–24 Gy. The remaining sixteen patients, 7 men and 9 women, who had not received cranial irradiation (CRT –), were treated with high-dose methotrexate (500 mg/m² on three occasions) as prophylaxis to prevent recurrence in the central nervous system. All patients received prednisolone 60 mg/m²/24 h for 6 weeks initially. In addition, 6 patients also received 1-week courses of prednisolone 60 mg/m²/24 h on 6–8 occasions and one patient received dexamethasone (10 mg/m² for 4 weeks). Twenty patients had received anthracyclines (doxorubicin) in a total dose of 120 mg/m². Six patients had received 180–400 mg/m² and one patient had received doxorubicin 125 mg/m² and cerubidin 100 mg/m². Eight patients had not received anthracyclines at all. The mean age was somewhat higher and the follow-up time longer in the CRT + than in the CRT – group ($P < 0.01$), owing to a greater proportion of patients treated with radiotherapy early rather than late in the inclusion period of the study.

Informed consent was obtained from all participants. The study was approved by the ethics committee at the Sahlgrenska Academy of Göteborg University.

Methods

Dual-energy X-ray absorptiometry (DEXA) DEXA was performed using a LUNAR-DXP-IQ (Scanexport Medical, Helsingborg, Sweden). The system uses a constant-potential X-ray source and K-edge filter to achieve a congruent beam of stable dual-energy radiation. Whole body bone mineral density (BMD^{total}, excluding the head) and bone mineral density of the femoral neck (BMD^{fem.neck}) and lumbar spine (BMD^{lumbar}) scans were obtained. Reference values were obtained from the manufacturer and expressed in age- and gender-matched z-scores.

Definition of osteoporosis The World Health Organization (WHO) defined osteoporosis operationally as DEXA BMD 2.5 standard deviations (s.d.) below the mean for young adults at the time of peak bone mass,

Table 1 Clinical background of the treatment groups. Data are presented as medians with range in parenthesis.

	Men			Women		
	CRT +	CRT –	<i>P</i> value	CRT +	CRT –	<i>P</i> value
Age at diagnosis (years)	4.5 (2.0–7.0)	3.0 (1.0–5.0)	0.13	5.0 (1.0–6.0)	6.0 (3.0–8.0)	0.19
Age at follow-up (years)	27.0 (22.0–32.0)	21.0 (20.0–24.0)	0.001*	28.0 (23.0–32.0)	23.0 (21.0–29.0)	0.006*
Height (cm)	179.8 (170.5–187.0)	184.0 (180.8–194.0)	0.024*	161.7 (152.5–167.4)	170.7 (157.0–178.9)	0.009*
BMI (kg/m ²)	26.2 (22.3–28.6)	21.4 (18.7–23.8)	0.002*	23.4 (20.2–29.1)	23.6 (20.6–28.9)	0.90

* $P < 0.05$.

and osteopenia as a DEXA BMD of above -2.5 s.d. but below -1 s.d. for young adults at the time of peak bone mass (23).

Bone mineral apparent density (BMAD) As BMD measurements are influenced by bone size, we also calculated bone mineral apparent density (BMAD), using the DEXA-derived bone area and other skeletal measurements to estimate bone volume (24–26). BMAD of the lumbar spine ($\text{BMAD}^{\text{lumbar}}$) was calculated by the following formula: $\text{BMD}^{\text{lumbar}}/(\pi \times (\text{diameter}/2)^2 \times \text{height of the vertebrae})$ (25, 26). The BMAD of the femoral neck ($\text{BMAD}^{\text{fem.neck}}$) was calculated by the formula: $\text{BMC}^{\text{fem.neck}}/\text{projected area}^2$ where $\text{BMC}^{\text{fem.neck}}$ = bone mineral content of femoral neck (24).

Markers of bone turnover Blood samples were collected after an overnight fast. Serum was then stored at -20°C until analysis. The analyses of bone markers were performed at the accredited laboratory of Sahlgrenska University Hospital. The serum concentrations of osteocalcin, a marker of bone formation, were determined by radioimmunoassay (Schering/CIS Bio International, Gif-sur-Yvette Cedex, France). The sensitivity of the test was $0.5 \mu\text{g/l}$. The concentrations of carboxyterminal propeptide of type I procollagen (PICP), a marker of bone formation, were determined by radioimmunoassay (Orion Diagnostica, Espoo, Finland). The sensitivity of the test was $5 \mu\text{g/l}$. The concentrations of carboxyterminal telopeptide of type I collagen (ICTP), a marker of bone resorption, were determined by radioimmunoassay (Orion Diagnostica). The sensitivity of the test was $0.7 \mu\text{g/l}$. Concentrations of skeletal alkaline phosphatase (ALP-skeletal), a marker of bone formation, were determined with immunoradiometric assay (Immunotech, Marseille, France). The sensitivity of the test was $2.0 \mu\text{g/l}$.

Physical activity score The patients described their physical activity by responding to a questionnaire. Only physical activities during leisure time were reported. The activities were separated into three groups: less than one physical activity/week (0), one or two physical activities/week (1), three or more physical activities/week (2).

Physical performance Physical performance was investigated with a performance exercise capacity stress test. Twenty-three patients were examined with an exercise treadmill test according to Bruce and McDonough (27). Seven patients were examined using a cycle ergometer stress test, with fixed steady state increments of 25 W every three min. The tests were all terminated when the patient experienced maximal stress. The rate of energy expenditure was expressed in metabolic equivalents, METs. The metabolic equivalent (MET) is a unit of basal oxygen uptake equal to approxi-

mately $3.5 \text{ ml O}_2/\text{kg}/\text{min}$. This value is the oxygen required to maintain life in the resting state. Thus a subject exercising at 10 METs would have a volumetric rate of oxygen uptake of $35 \text{ ml O}_2/\text{kg}/\text{min}$ (28, 29).

Statistical methods

Data are presented as medians with range in parentheses, unless otherwise stated. Correlations between variables (body mass index (BMI), sex, GH_{max} , dose of corticosteroids, physical performance (METs) and physical activity vs bone mineral density variables and bone markers were tested using Pitman's non-parametric test (30). Statistical significance between groups was calculated using the Mann–Whitney–Wilcoxon Sum Rank test. For all statistical tests a P -value of <0.05 was considered significant, using two-tailed tests.

Results

Bone mineral density

The median z-score $\text{BMD}^{\text{total}}$ was 0.4 s.d. (-1.1 to 2.4) in the group as a whole. The median z-score $\text{BMD}^{\text{fem.neck}}$ was -0.1 s.d. (-2.2 to 2.2) and the $\text{BMD}^{\text{lumbar}}$ -0.4 s.d. (-2.2 to 2.6). No patient could be defined as having osteoporosis. There was one patient who fulfilled the WHO criteria for osteopenia in $\text{BMD}^{\text{total}}$, four patients with osteopenia in the femoral neck and eight patients with osteopenia in the lumbar spine. The results of the DEXA measurements are listed in Table 2. There were no differences between the CRT + and CRT – patients in BMD in any of the measured sites. There was a positive correlation between $\text{BMD}^{\text{fem.neck}}$ and physical performance (METs), ($r = 0.380$, $P = 0.0355$), and a negative correlation between $\text{BMD}^{\text{fem.neck}}$ and dose of corticosteroids ($r = -0.336$, $P = 0.0446$). There were no correlations between $\text{BMD}^{\text{total}}$, $\text{BMD}^{\text{lumbar}}$ or $\text{BMD}^{\text{fem.neck}}$ and growth hormone secretion. $\text{BMD}^{\text{total}}$ and $\text{BMD}^{\text{lumbar}}$ did not correlate to corticosteroid dose, reported physical activity or physical performance.

Bone mineral apparent density (BMAD)

The results of the BMAD calculations are listed in Table 2. There was no difference in BMAD between the CRT + and CRT – patients in $\text{BMD}^{\text{total}}$, $\text{BMD}^{\text{lumbar}}$ or $\text{BMD}^{\text{fem.neck}}$.

Markers of bone turnover

The results are summarized in Table 2. No differences were found in any of the markers of bone turnover between CRT + and CRT – patients. There was a positive correlation between physical performance (METs) and ALP-skeletal ($r = 0.36$, $P = 0.04$), osteocalcin ($r = 0.50$, $P = 0.006$), PICP ($r = 0.42$, $P = 0.01$) and

Table 2 Results of bone mineral measurements and markers of bone turnover. Data are presented as medians with range in parenthesis.

	Men			Women		
	CRT +	CRT –	P value	CRT +	CRT –	P value
BMD ^{total} (z-score)	0.6 (–1.0–1.9)	0.1 (–1.1–2.0)	>0.1	0.0 (–0.2–2.4)	0.4 (0.0–2.2)	>0.1
BMD ^{lumbar} (z-score)	–1.0 (–1.5–1.1)	–0.7 (–2.3–1.5)	>0.1	–0.4 (–1.2–2.6)	0.3 (–0.7–1.8)	>0.1
BMD ^{fem.neck} (z-score)	–0.4 (–1.5–1.5)	0.1 (–2.2–2.2)	>0.1	–0.2 (–1.8–2.2)	–0.1 (–0.8–2.1)	>0.1
BMAD ^{lumbar} (g/cm ³)	0.34 (0.29–0.38)	0.34 (0.24–0.39)	>0.1	0.36 (0.34–0.48)	0.38 (0.34–0.42)	>0.1
BMAD ^{fem.neck} (g/cm ³)	0.18 (0.16–0.25)	0.20 (0.12–0.25)	>0.1	0.20 (0.16–0.28)	0.19 (0.17–0.27)	>0.1
ALP-skeletal (µg/l)	14.7 (9.8–28.0)	22.8 (10.4–28.2)	>0.1	8.3 (5.1–10.4)	10.3 (7.7–14.5)	0.07
Osteocalcin (µg/l)	12.4 (4.2–18.8)	16.2 (10.2–18.8)	>0.1	5.5 (1.5–14.3)	10.3 (5.9–15.7)	0.08
PICP (µg/l)	152 (107–342)	180 (138–239)	>0.1	136 (86–364)	125 (77–353)	>0.1
ICTP (µg/l)	3.8 (2.5–4.6)	6.6 (2.3–8.7)	>0.1	3.0 (2.4–4.5)	3.5 (2.9–5.1)	0.07
Physical performance (METs)	11.1 (7.0–14.5)	13.5 (13.5–15.0)	0.02*	10.4 (9.0–13.5)	13.1 (11.0–13.5)	>0.1

* $P < 0.05$.

ICTP ($r = 0.353$, $P = 0.04$) (Fig. 1). There was no correlation between any of the markers of bone turnover and GH_{max}, reported physical activity or dose of corticosteroids.

Physical performance

The CRT + men had lower exercise capacity than the CRT – men, 11.1 (7.0–14.5) and 13.5 (13.5–15.0) METs respectively ($P = 0.02$). There were no differences

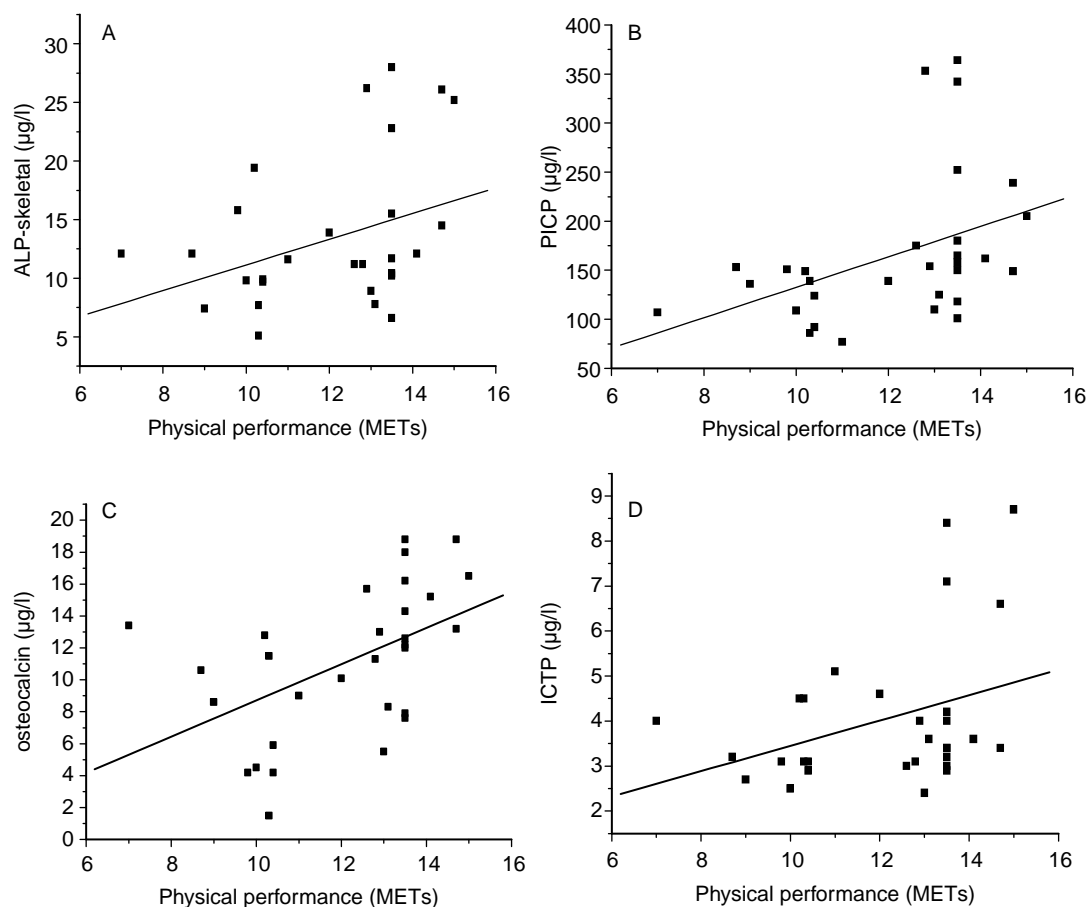


Figure 1 Correlations between markers of bone turnover and physical performance. Correlation between physical performance (METs) and (A) skeletal alkaline phosphatase (ALP-skeletal) ($r = 0.36$, $P = 0.04$), (B) carboxyterminal propeptide of type I procollagen (PICP) ($r = 0.42$, $P = 0.01$), (C) osteocalcin ($r = 0.50$, $P = 0.006$) and (D) carboxyterminal telopeptide of type I collagen (ICTP) ($r = 0.35$, $P = 0.04$).

among the women, 10.4 (9.0–13.5) and 13.1 (11.0–13.5) METs in the CRT + and CRT – group respectively ($P > 0.1$). In addition, men with growth hormone deficiency (GHD) had significantly lower exercise capacity than the non-GHD men, 11.0 (7.0–14.1) and 13.5 (10.2–15.0) METs respectively ($P = 0.04$). However, exercise capacity failed to reach significant correlation to GH_{max} on the 24-h profile ($r = 0.35$, $P = 0.06$).

Physical activity score

There were no differences in reported frequency of leisure physical activity between the CRT + (6/3/1 patients in category 0/1/2 respectively) and CRT – men (3/2/2 patients in category 0/1/2 respectively) nor between the CRT + (4/4/1 patients in category 0/1/2, respectively) and CRT – women (4/2/3 patients in category 0/1/2 respectively) ($P > 0.1$). There was no correlation between reported physical activity score and bone mineral density at any site or in markers of bone turnover. There was no correlation between physical activity score and physical performance.

Discussion

In the present study we could not show a clear impact on BMD in a group of young patients treated for childhood ALL at a median of 20 years previously. We did not find a correlation with BMD or markers of bone turnover and spontaneous secretion of GH, while we did find a correlation with exercise capacity, suggesting that physical fitness is a very important means of preserving bone mineral density in these patients.

There are conflicting reports on bone mineral density in long-term survivors of childhood ALL with both low (6, 8–11, 31) and normal (14–16) BMD. In many studies, the reduced BMD measured using DEXA in cranially irradiated patients (CRT +) can be explained by reduced height, since BMD is a measurement of area and patients with childhood onset GHD usually have reduced height which, in turn, affects their BMD values negatively. However, in this study the loss of height in the CRT + patients was comparatively small: median -0.8 s.d. (20). Low BMD has been shown in adults with GHD. It tends to be more severe in patients with childhood-onset GHD. This is probably attributable to insufficient accumulation of bone mass during childhood and puberty rather than later loss of bone mass (32, 33). Low bone mass has been shown to be less frequent with increasing age in patients with GHD (34). Furthermore, in a meta-analysis, treatment with GH has not been shown to improve BMD in this patient group (35). In our study we found no correlation between BMD or markers of bone turnover and spontaneous growth hormone secretion. Our

hypothesis that a gradually developing GH deficiency would affect accretion of bone mass during puberty was not verified. These findings may be attributable to the limited number of patients in the study. However, they are in accordance with Brennan *et al.*, who found reduced bone mineral density in patients treated for ALL, but no correlation to growth hormone status measured using provocative tests (9). Hypothetically, our findings could be caused by frequent physical activity during adolescence leading to a good peak bone mass despite diminishing secretion of growth hormone. Decreased bone turnover has previously been demonstrated in patients with GHD in some (36, 37) but not all (38) studies. Neither this study nor the study of Brennan *et al.* found a correlation between growth hormone and markers of bone turnover (9). More studies will be needed to evaluate the effect of GHD on bone turnover. Osteoporosis is a well-known complication of long-term use of glucocorticoids (39). The mechanisms involved are enhancement of bone resorption and decreased bone formation (40, 41). In the treatment of ALL, the glucocorticoid dose is very high, but the duration of treatment is relatively short.

However, we did find a correlation between high doses of corticosteroids and lower BMD in femoral neck. This is in accord with the findings of Mandel *et al.*, who found a tendency towards lower BMD in femoral neck in patients who had received high doses of corticosteroids but no correlation between CRT and BMD (14). However, we did not find a correlation between markers of bone turnover and previous corticosteroid treatment. This may be due to the fact that markers of bone turnover indicate the actual state of bone turnover. Effects of varying corticosteroid dose on bone turnover were not detectable at the time of the study, possibly due to the long observation time.

High dose methotrexate treatment has been reported by Mandel *et al.* to lead to low BMD in follow-up (14). Methotrexate osteopathy has also been found in children with ALL and in infants with intracranial tumours treated with high doses of methotrexate (42, 43). The doses of methotrexate administered to the patients in our study were low compared with the doses currently used (1.5 g/m^2 total compared with 40 g/m^2 total for the current standard intensive treatment protocol). More studies of BMD in patients receiving high doses of methotrexate in the currently used protocols will be needed in order to evaluate the impact of methotrexate on BMD. In this study, physical performance correlated to BMD in the femoral neck. The role of physical performance was also studied by Warner and colleagues who found a correlation between higher oxygen uptake and higher bone mineral content in lumbar spine and hip in children and adolescents treated for ALL (7). We found higher bone turnover in patients with high physical performance. High physical activity as reported by patients has been reported to be associated with high BMD in

healthy individuals (44, 45). The effects of physical activity on bone turnover are well known (46, 47). The finding that self-reported leisure time physical activity did not correlate to bone mineral density measurements or markers of bone turnover in this study may indicate that this subjective estimation fails adequately to reflect the physical activity of these patients. However, Tillmann *et al.* found that reduced BMD correlated with physical activity measured using both questionnaire and accelerometer in children treated for ALL with chemotherapy, median 4.5 years after discontinuation of therapy (18). Overall, we found, after a median follow-up of 20 years, slightly reduced bone mineral density in lumbar spine, but not in femoral neck or total body, in a group of young adults treated for ALL in early childhood. For these long-term survivors we found that bone mineral density in femoral neck was correlated to physical performance and dose of corticosteroid, but no correlation was found with spontaneous growth hormone secretion. Bone turnover was also correlated with physical performance, but failed to correlate to GH secretion.

In conclusion, the treatment given for ALL with chemotherapy, corticosteroids and cranial irradiation had little impact on bone mineral density in long-term survivors 20 to 30 years old. Physical fitness seems to be the most important factor for developing and preserving normal bone mineral density in young adult survivors of ALL. We propose that lifestyle education promoting physical activity is encouraged from an early point in time for these patients.

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