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Bone loss after childhood acute lymphoblastic leukaemia: an observational study with and without growth hormone therapy

Short title: Bone loss in GH deficient ALL patients

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Abstract

OBJECTIVE: Bone mineral density (BMD) in survivors of acute lymphoblastic leukaemia (ALL) seems to vary with time, type of treatments and GH status. We aimed to evaluate BMD in ALL patients with growth hormone deficiency (GHD), with and without GH therapy.

DESIGN: Case-control study.

METHODS: 44 (21 women) GHD patients (median 25 years), treated with cranial radiotherapy (18-24 Gy) and chemotherapy and matched population controls were examined for BMD with DXA (Dual-energy X-ray absorptiometry). Two subgroups; with (0.5 mg/day) (n=16) and without GH therapy (n=13), and matched controls, were followed for 5 and 8 years, respectively.

RESULTS: At baseline, no significant differences in BMD or Z-scores at femoral neck and L2-L4 were recorded (all $P > 0.3$). After another 8 years with GHD, Z-scores at femoral neck had decreased significantly compared to baseline (0.0 to -0.5; $P < 0.03$), and became lower at femoral neck ($P = 0.05$), and at L2-L4 ($P < 0.03$), compared to controls. After 5 years of GH therapy only female ALL patients had a significantly lower femoral neck Z-scores ($P = 0.03$). The female ALL patients reached an IGF-I level of -0.7 SD and in men the level was +0.05 SD.

CONCLUSIONS: On average 25 years since diagnosis GH deficient ALL patients experienced a significant decrease in Z-scores at femoral neck and if Z-scores continuous to decrease there is a premature risk for osteoporosis. GH therapy was not shown to have a clear beneficial effect on BMD. Whether higher GH doses, particularly in women, will improve Z-scores needs further investigation.

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common pediatric malignancy (1) and its treatment includes many known risk factors for low bone mineral density (BMD).

High doses of glucocorticosteroids and methotrexat (MTX), included in nearly all ALL treatment regimes, can at least temporarily affect bone formation (2,3). Further, boys with radiation to the testes need adequate testosterone supplementation and patients treated with cranial radiotherapy (CRT) are at risk for particularly growth hormone deficiency (GHD) (4,5). Adults with CO GHD, due to hypothalamic-pituitary disease, have shown reduced BMD and bone mineral content (BMC) (6) and discontinuation of GH therapy before attainment of peak bone mass (PBM) is suggested as the main cause of low bone mass (7). After GH replacement beneficial effect on BMD and BMC (8,9), together with increased markers of bone turnover have been shown (8,9).

As patients with CO GHD are shorter (5) their bones have smaller width and will also be thinner i.e. volume corrections, using bone mineral apparent density (BMAD) is therefore preferable (10). There are conflicting results regarding long-term BMD in former ALL patient where six studies have shown low BMD, 4-24 years after 18-30 Gy of CRT (11-16) and two studies showed normal BMD, 4 to 20 years after 18-24 Gy (17,18). In these studies GH status has only been taken into account in subgroups of patients (12,14-17) and often with GH tests not always optimal to unmask radiation induced GHD (13,16). In a recent large study normal BMD was recorded 10 years after diagnosis, with the exception of low BMD in a subgroup of patients treated with high doses of MTX (18). Thus, BMD status is not definitely settled in adult ALL patients, and seems to vary with time since treatment, type of treatment and with GH status. Further, properly matched controls have previously not been included and neither have physical activity nor calcium intake or serum levels of thyroid and sex steroids been carefully addressed.

One aim of the present study was to evaluate BMD, using dual energy X-ray absorptiometry (DXA) and with volume correction using BMAD, and markers of bone turn over in a representative cohort of adults with CO GHD, treated with CRT and chemotherapy for ALL. Another aim was to evaluate the effect of 5 years of GH therapy on BMD in a subgroup of GH deficient ALL patients and to compare them to an untreated GH deficient ALL group after 8 years. Comparisons were also made with

matched population controls at baseline, and to the same population controls after 5 and 8 years of follow up.

Subjects and methods

Patients

A consecutive series of 58 surviving patients treated for childhood ALL with chemotherapy and CRT with a median dose of 24 Gy (18-24) during 1971-1992 at the Children's Hospital Lund, Sweden and ≥ 18 years of age were invited to participate in a study in year 2000 (5). In the period 1971-1981 all patients diagnosed with ALL received CRT. In the latter period (1982-1992) children belonging to the lower risk groups received no CRT. All patients had received chemotherapy according to the common protocols of the Nordic countries and of the Swedish Child Leukemia Group; details presented in Table 1 and all patients were in first remission. Excluded and included patients are shown in Figure 1 and Table 1. Eighteen (8 women) of them were offered GH therapy and 15 patients completed the present 5 year study. The GH dose (Humatrope, Eli Lilly and Company, Indianapolis, USA) was 0.5 mg/day (0.2-0.8) in women and men given by subcutaneous injections. The GH dose was titrated against the response of serum IGF-I, aiming at a ± 0 IGF-I level. Another subgroup of 13 patients (4 women) was not offered GH therapy but had regular contact with a doctor or nurse during 8 years of follow up. The main reasons to be included in the GH-therapy group were: willing to accept GH therapy, living close to the hospital, and able to participate in a long-term study. The remaining 16 patients were excluded due to reasons shown in figure 1.

Four patients had previously received GH treatment in childhood, but not for at least 5 years. All females had spontaneous and regular menstrual cycles except for one patient with primary amenorrhea and 5 patients were using estrogen contraceptives. Ten of the 12 males who had received radiation to the testes were properly substituted with testosterone as injections (n=7), tablets (n=1) or patches (n=2). Two patients subjected to testis radiation had no androgen substitution; one had not been properly irradiated due to an undescended testis and had normal testosterone levels, and the other was not sufficiently substituted. Three men and two women were smokers.

Control groups

Each patient was matched with a control subject (n=44) similar in age-, sex-, residence (rural/non-rural) and smoking habits who was randomly selected from a computerized population register described in detail elsewhere (5) and in Figure 1.

All baseline investigations, including DXA and physical activity in the patients and in the controls, were repeated after 5 and 8 years, respectively.

All participants gave written informed consent and the study was approved by the Ethic's Committee of Lund University.

BMC, BMD, BMAD and anthropometric measurements

BMC and BMD of the femoral neck, lumbar spine 2–4 (L2-L4) and Z-scores of femoral neck and L2–L4, standardized to sex, age, weight, and race/ethnicity, and body composition, i.e. fat mass and lean mass were assessed using two DXA machines as one broke during study (Lunar Expert XL [LEXL] and Lunar Prodigy [LP], Lunar Co., Madison, WI, USA). Both machines were calibrated with the same phantom. For BMD the coefficient of variation (CV) was 0.6% and 0.2%, respectively, for the LEXL and LP, the CV was 0.7% between machines. All investigations in both patients and controls at baseline were performed with Lunar Expert and at 5 and 8 years for both patients and controls were performed with Lunar Prodigy. The official position of the International Society for Clinical Densitometry states that the World Health Organization classifications (T-scores) for osteoporosis and osteopenia are not applicable to premenopausal women or men, and recommended instead that Z-scores be used (19). Body mass index (BMI) was defined as body weight (kg) divided by the square of height (meters). BMAD was applied and the following formulas for volume corrections were used: for the femoral neck; $BMAD = BMC \div \text{area}$ and for the spine; $BMAD = BMC \div \text{area}^{3/2}$ (10).

History of fractures, present calcium intake and physical exercise

All subjects were interviewed regarding number of previous fractures and present calcium intake. The degree of physical exercise during working time and spare time was assessed by self-rating questionnaire in which patients and controls classified their physical activity according to a four-grade scale: Grade 1; sedentary lifestyle e.g. reading watching television, 2; moderate exercise e.g. bicycling, working in the garden for at least four hours a week, 3; regular exercise e.g. swimming or playing tennis two or three

hours a week, 4; hard exercise e.g. running, skiing or swimming several times per week (20).

Hormone assays, test procedures for GH secretion and markers of bone turnover

Details of assay procedures for serum levels of IGF-I and testosterone and plasma levels of TSH, free T4, free T3, and estradiol have been presented elsewhere (5). All patients and 44 controls underwent a GHRH-arginine test (21) and 39 patients were also investigated with an insulin-tolerance test (ITT). Details of test procedures are shown elsewhere (5). GHD was defined as $< 3 \mu\text{g/L}$ to an ITT or $< 9 \mu\text{g/L}$ to the GHRH-Arginine test and with a cut-off point of $11.5 \mu\text{g/L}$ if $\text{BMI} < 25 \text{ kg/m}^2$, $8.0 \mu\text{g/L}$ if $\text{BMI} > 25 \text{ kg/m}^2$ and $< 30 \text{ kg/m}^2$ and $4.2 \mu\text{g/L}$ if $\text{BMI} > 30 \text{ kg/m}^2$ for this latter test (22). The ITT result determined the GHD classification when the GH response to the GHRH-arginine test was $> 9 \mu\text{g/L}$ (5). All patients in the GH-treated group and all but one in the non-GH-treated group, failed the ITT ($\text{GH}=4.6 \mu\text{g/L}$). This patient had a GH-response of $1 \mu\text{g/L}$ during the GHRH-Arginine-test together with an IGF-I of -3.0 SDS, at re-testing 7 years after baseline. Serum levels of crossLaps and osteocalcin were analysed with an immunometric Method (Elecsys 2010). The detection level for serum crossLaps was 10 ng/L and the CV total was 8.3% at a level of 350 ng/L and 5.2% at a level of 3000 ng/L . The detection level for serum osteocalcin was $0.5 \mu\text{g/L}$ and the CV total was 3.3% at a level of $20 \mu\text{g/L}$ and 3.4% at a level of $200 \mu\text{g/L}$.

Statistical analysis

Data are presented as median and range (min-max). Differences between 44 patients and 44 matched population controls, before and after 5 years of GH treatment and before and after 8 years without, were compared using the Wilcoxon's signed rank test for matched pairs. Bivariate correlations were assessed using the Spearman rank correlation coefficient. A P-value < 0.05 was regarded as statistically significant.

We used the SPSS version 15.0 for the statistical analysis.

Results

Anthropometric and hormone assessments in 44 patients in comparison with matched controls at baseline

Height was significantly reduced by 13 cm [(SD-1.25 (-2.0—0.49))] in the male patients and by 9 cm [(SD -1.6 (-2.0—1.1))] in the female patients (Table 2). BMI and FM were significantly increased in ALL women. LM was significantly decreased in both genders (Table 2).

Serum levels of IGF-I were significantly lower in both genders, to - 1.6 SD in ALL women and to - 1.25 SD in ALL men. The serum TSH levels were without significant differences between ALL women and men in comparison to controls (1.9 vs 2.0 mU/L, and 2.1 vs 1.9 mU/L, respectively; >0.3). Serum free T4 was significantly higher, but in the normal range in the ALL women, compared to controls (14 vs 12 pmol/L, P=0.04), but without difference in ALL men (14 vs 14 pmol/L, P=>0.3). There were no significant difference in free T3 levels in ALL women or men compared to controls (both P>0.3). Testosterone levels in men (15.5 vs 17.6 nmol/L) and estradiol levels in women (198 vs 193 pmol/L) were without significant difference compared to controls (both P>0.3).

BMAD, BMD and markers of bone turnover in 44 patients in comparison with 44 matched controls at baseline

No significant differences in BMAD, BMD, or in Z-scores at femoral neck or L2-L4 were recorded in former ALL patients of both genders (Table 2). When the male patients with normal testosterone secretion and thus isolated GHD (n = 12) were compared to those males with GHD and testosterone substitution (n= 10) the BMD measurements were without significant difference (data not shown).

No significant differences in the serum levels of osteocalcin and crossLaps (Table 2) or in calcium, albumin, phosphate, or PTH levels were recorded between patients and controls in either gender (data not shown).

History of previous fractures, daily calcium intake and physical exercise in 44 ALL patients in comparison with 44 matched population controls at baseline

No significant difference in the number of previous fractures or in present calcium intake was recorded in patients compared with controls of both genders (P>0.3).

Neither was there any difference in the degree of physical exercise during leisure time between patients and controls of both genders (P>0.3). However, during working time

both genders reported significantly higher degree of physical exercise compared to controls (P=0.04).

Correlations between disease-related factors to Z-scores and BMD among the 44 ALL patients at baseline

The cumulative dose of MTX (p.o) was negatively correlated to the Z-scores at L2-L4 ($r = -0.31$, $P=0.03$) (Figure 2).

The cumulative dose of corticosteroids, level of serum IGF-I, maximum GH response to GHRH-Arginine, dose of CRT, and time since diagnosis were without significant correlations to BMD or to Z-scores at L2-L4 and femoral neck (all; $P>0.3$) (data not shown).

Comparisons before and after 5 years of GH therapy in ALL patients and to the same matched population controls at baseline and after 5 years

No significant differences in BMD, BMAD at any site or to the Z-Score at L2-L4 were recorded in former ALL patients of both genders before compared to after 5 years of GH therapy, and with no differences in these measures in comparison to controls (Table 3A). The Z-score at femoral neck decreased, but not significantly after 5 years of GH therapy (-0.2 to -0.6; Figure 3A). and became significantly lower in ALL females, but not in males (Figure 3A).

Serum crossLaps levels in the whole patient group increased significantly after GH therapy, and became significantly higher than controls (Table 3A). No significant change in serum levels of osteocalcin was recorded after 5 years and these levels remained similar to controls after 5 years. A significant increase in serum IGF-I levels were recorded from - 2.5 SD to + 0.05 SD ($P=0.03$) and without differences in these levels compared to controls after 5 years. The IGF-I levels in males increased from -2.8 SD to + 0.05 SD and in females from -3.5 SD to - 0.7 SD. Serum levels of thyroid hormones, testosterone and estradiol were without difference compared to controls after 5 years (data not shown) (all; $P>0.3$).

Comparisons before and after 8 years without GH therapy in former ALL patients and to the same matched population controls at baseline and after 8 years

The serum IGF-I levels decreased further and became significantly lower after 8 years (-2.0 to -3.2 SD), corresponding to a decrease in serum IGF-I by -30% (-15 to -56%) (Table 3B).

Z-scores at femoral neck decreased significantly compared to baseline levels (0.0 to -0.5 SD; Figure 3B) and became borderline significantly lower compared to controls after 8 years (Table 3B). Analysis by gender in the untreated group was not possible due to the small number of women (n = 4).

Compared to controls no significant difference in BMD and BMAD at L2-L4 was recorded at baseline, but after 8 years BMD became significantly lower (Table 3B). Z-scores at L2-L4 were border line significantly lower at baseline but after 8 years levels became significantly lower compared to controls (Table 3B).

After 8 years the serum levels of crossLaps and osteocalcin decreased significantly in patients and these levels became significantly lower compared to controls after 8 years (Table 3B). Levels of thyroid hormones, estradiol and testosterone remained at similar levels as controls after 8 years (data not shown) (all; $P > 0.3$).

Differences in the change of Z-scores between GH-and non-GH-treated patients

The difference in the change of Z-scores between GH-treated and non-GH-treated patients were not significantly different ($P > 0.3$). The median net-difference for the GH treated vs non-GH-treated group for femoral Z-scores and for Z-scores at L2-L4 levels were; -0.20 vs -0.25 and -0.10 vs -0.25, respectively.

Discussion

On average 20 years since diagnosis and CRT treatment, former ALL patients had, in comparison to matched population controls, no difference in BMD, BMAD or expressed as standardized Z-scores, at femoral neck or L2-L4. However, after an additional 8 years with GHD, Z-scores at femoral neck had decreased significantly from 0.0 to -0.5 SD, and had together with Z-scores at L2-L4 become significantly lower compared to controls. In contrast, after 5 years of GH therapy only female ALL patients had a significantly lower femoral neck Z-scores. The present study is the first long-term prospective follow up study of CRT treated ALL patients and patients were followed 20, 25 and 28 years since diagnosis. In addition, it is also the first to include proper controls

and to control for variables as, smoking, physical activity, calcium intake and all other hormones of importance for bone health.

Twenty years after diagnosis the vast majority of the ALL patients were GH deficient (91%) and the rest GH insufficient. However, their heights were only somewhat reduced (-1.6 in women and -1.25 in men), which means that they probably had partial GHD during puberty and adolescence, although treatments with dexametasone and chemotherapeutic agents and the ALL disease itself could have halted growth. Recently, it was suggested that (23) in patients with hypothalamic-pituitary injury following cranial irradiation one cannot solely rely on the response to an ITT for the diagnosis isolated GHD, as a few of these patients had normal spontaneous GH secretion. In the present study at baseline, we recorded a low ITT ($<3.0 \mu\text{g/L}$) response in 3 patients with diagnosed GHD, together with an IGF-I SD level of > -2.0 SD and a response to the GHRH-arginine test $>9 \mu\text{g/L}$. However, after 8 years the non-GH treated group had < -2.0 SD in serum IGF-I, which clearly shows manifested GHD.

When exactly GHD was manifested in the ALL patients is difficult to know as the change from partial to more severely GHD is a continuum and is particularly evident after CRT (24). This was also illustrated by a further drop in serum IGF-I after 8 years from -2.0 to -3.2 SD. In parallel, a significant reduction in markers of bone turnover was also seen after 8 years, which coheres with the finding that patients with the most profound GHD also experienced the most depressed bone markers (25). This is in contrast to the matched population controls where all BMD, Z-scores (Figure 3A, B), bone markers, and IGF-I measurements were stable or increased.

It has been shown that both GH and IGF-I stimulate osteoblasts resulting in bone formation, but also bone resorption (26). After GH therapy several non-ALL studies report a temporary increase in osteocalcin with a peak after 1-3 years followed by a decrease (27,28). We recorded no difference in bone markers at baseline, which accords with some previous studies in ALL patients (14,15). After 5 years of GH therapy however, crossLaps levels increased and osteocalcin levels remained unchanged, which coheres with increased bone resorption, but without concurrent increase in bone formation. In the GH treated group there was a non-significant decrease in the Z-scores at femoral neck. However, there was a gender difference and in the ALL females, Z-scores at femoral neck were significantly reduced, but the serum IGF-I levels only increased to -

0.7 SD, which illustrates a low GH dose. In contrast, the ALL males had an increase to +0.05 SD in IGF-I and also received a better Z-score. There was however, no significant net-change in Z-scores between GH-treated and non-GH-treated patients, which means that the current GH regime did not show a clear positive effect on bone.

Hitherto, profound CO GHD patients have been GH treated during childhood and stopped GH therapy at final height. Studies in these patients have particularly been investigating if continuous GH therapy results in a progressive increase in BMD after epiphyseal closure and final height (8,9,29,30,31,32) and whether stopping GH therapy causes a negative change in BMD. The longest follow up in these patients is 2 years (9, 30, 31, 32) and 5 studies included a control group of non-GH treated patients who had been off GH therapy for 1 week to 5 years before enrolment (8, 9, 30, 31, 32). BMD increased between 5-8% on GH therapy and between 1-5% in the non-GH treated patients (8, 9, 30, 32) and in one study no difference between groups were recorded (31). One problem with these studies is the carry-over effect of previous GH therapy on BMD that will last for 1-2 years after stopping GH therapy (9). Further, higher GH doses were used and some even used pediatric doses (9, 30), and particularly females needed the higher doses (32), as they are less sensitive to standard GH doses (32, 33). GH deficient ALL patients are however, different from patients in the above mentioned studies, as most of the ALL patients are GH-naïve and have changed from partial GHD to insufficient and more severely GHD later in life. Thus, there are several reasons for the failed net-effect of the present GH therapy, 1) no recent GH therapy, 2) too low GH dose (females) 3) a start of GH therapy at 25 years of age might be somewhat late to improve BMD and PBM. However, the Z-scores only dropped to -0.6 SD, but it occurred when PBM is attained and interference with PBM is perhaps the most important determinant of lifelong skeletal health.

Our study has some limitations that potentially may impact the findings. We did not conduct a randomized placebo-controlled study and the population we studied was small and the follow up was different between the two groups. However, the baseline characteristics and treatment modalities were very similar and without substantial difference in treated or non-treated patients (Table 1). Further, the non-GH treated group was observed for 8 years which in fact may result in a worsening of BMD in comparison to the possible improvement that

could be seen within 5 years of GH therapy. At the start of the present study the intention was to treat a subgroup of the ALL patients as none hitherto has done so. Further, to give a physiological rather low GH dose aiming at a serum IGF-I level of ± 0 SD. The reason for this precaution was an ongoing discussion on the risk of second neoplasms in this patient population. At present, however, there seem to be a particular high risk for meningiomas, which seem to be connected to previous CRT (34).

The unexpected breakage of the DXA machine had probably no impact on the present results as the CV% between machines was very low (CV% 0.7), and that all patients together with their controls were scanned with the same DXA-machine at baseline, and after 5 and 8 years, respectively.

A further causative factor to low BMD is reduced physical activity, shown among ALL children (35). However, we have previously shown that adult ALL patients experience no difference in the degree of physical exercise during spare time, but during working time they reported significantly more physical exercise compared to their matched controls (5). The present study showed no change in these activities after an additional 5 and 8 years, respectively.

Another new finding, was the significant association between a low dose of MTX p.o. (2 700 mg/m²) and the Z-score at L2-L4, as previously only high-dose MTX (> 40 000 mg/m²), have been correlated to low BMD (18). Experimental studies show that MTX strongly inhibits osteoblast proliferation and causes a decrease in bone formation (36). The interpretation of the present results, 20 years after ALL diagnosis, is hardly an ongoing MTX effect, but mirrors a previous arrest of bone formation during periods of MTX therapy.

In conclusion, on average 20 years after ALL treatment with 24 Gy and a low dose of MTX, normal BMD and Z-scores was recorded in GH deficient ALL patients. Thereafter, Z-scores at femoral neck decreased significantly during the following 8 years, which means that if Z-scores continuous to decrease there is a premature risk for osteoporosis. The present GH therapy regime did not show a clear positive effect on bone formation. Besides GHD also yet unknown factors have to be considered. Whether higher GH doses particularly in women, will improve Z-scores needs further investigation.

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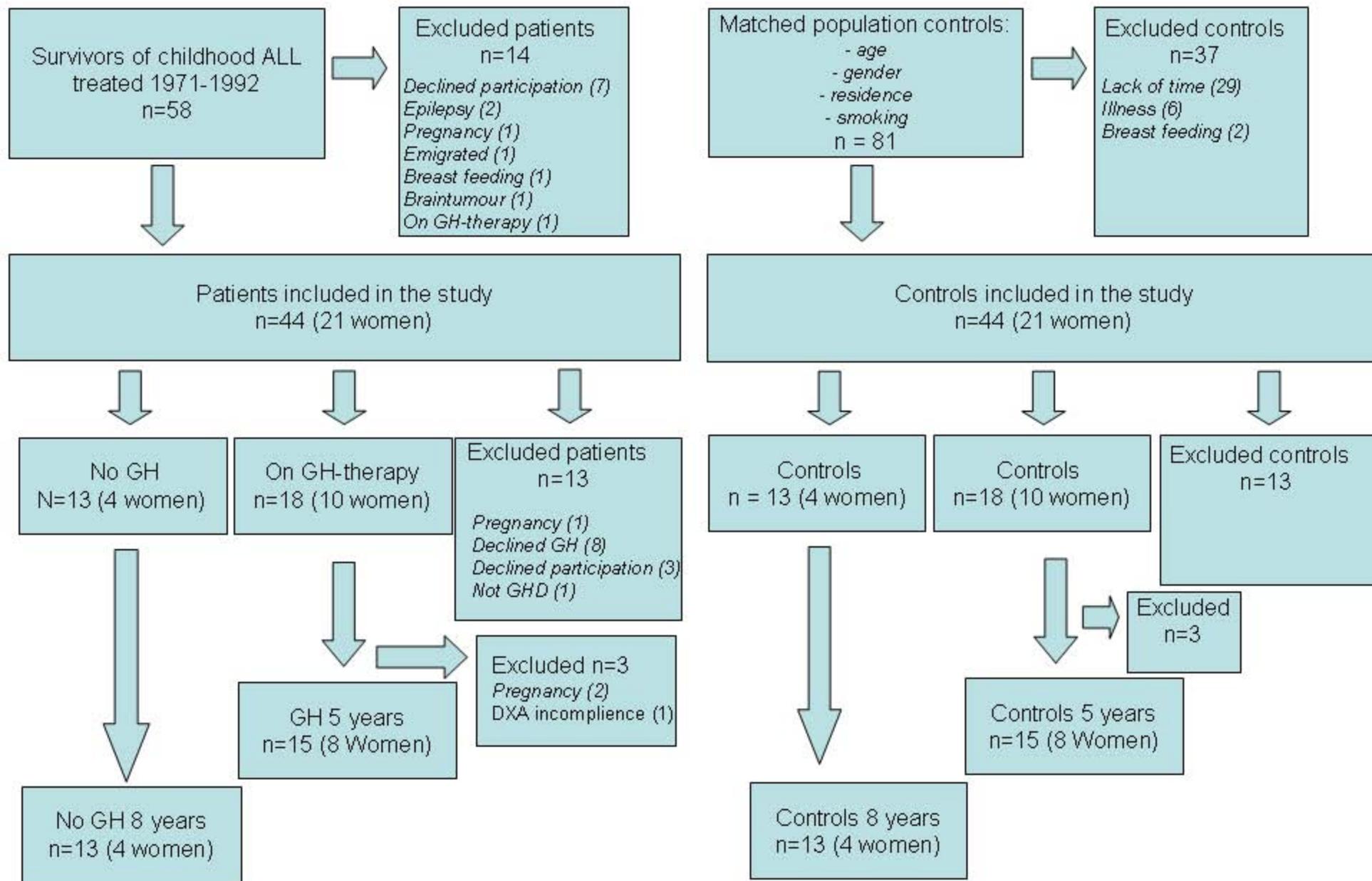
Legends to figures

Figure 1. Flow chart of excluded and included former ALL patients and matched population controls.

Figure 2. The cumulative dose of MTX p.o. was negatively correlated to the BMD Z-scores at L2-L4 ($r = -0.31$, $P=0.03$) among the 44 ALL patients

Figure 3. (A) BMD Z-score at femoral neck in 15 ALL patients before and after 5 years of GH therapy, in 15 matched population controls before and after 5 years, (B) in 13 ALL patients before and after 8 years without GH therapy and in 13 matched population controls before and after 8 years. Data are presented as median and range. P-values are referred to before and after 5 and 8 years, respectively.

Figure 1



Abbreviations: ALL, acute lymphoblastic leukaemia; GH, growth hormone; GHD, growth hormone deficiency

Figure 2

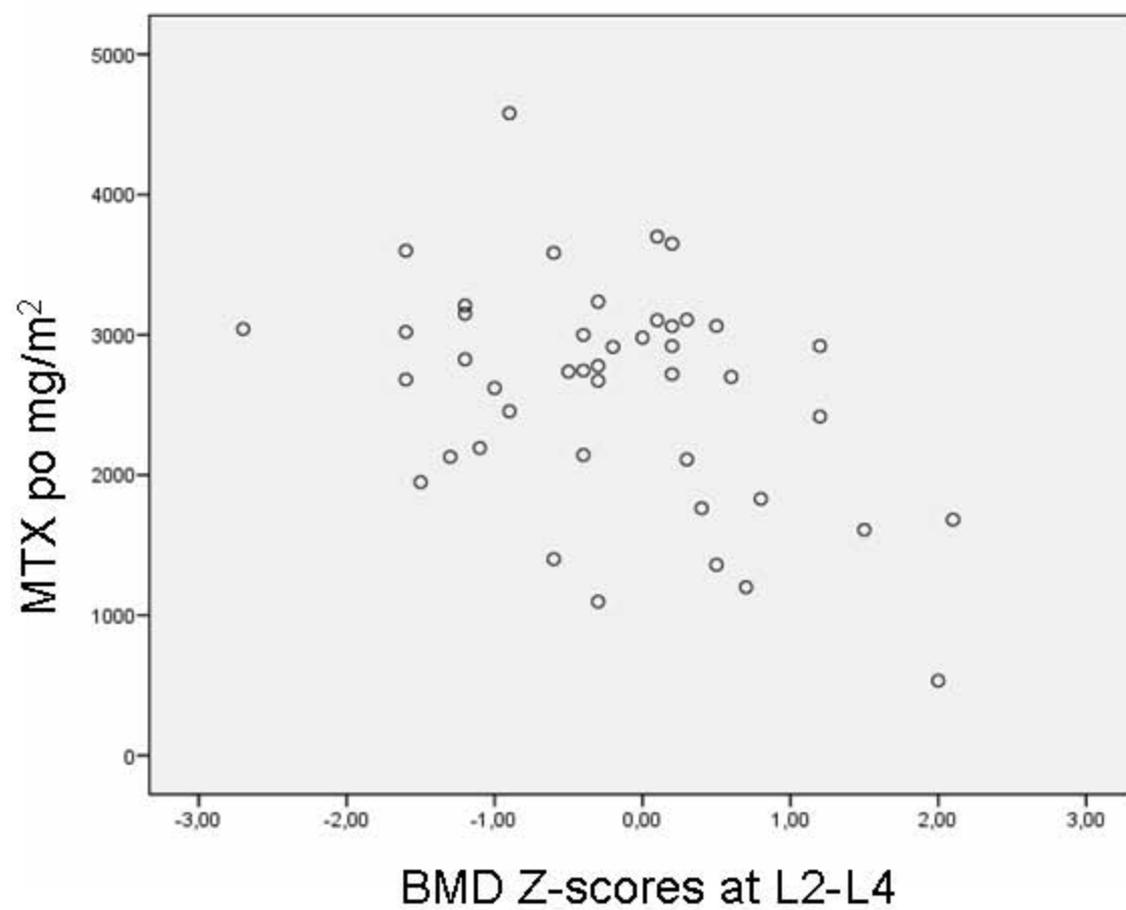


Figure 3

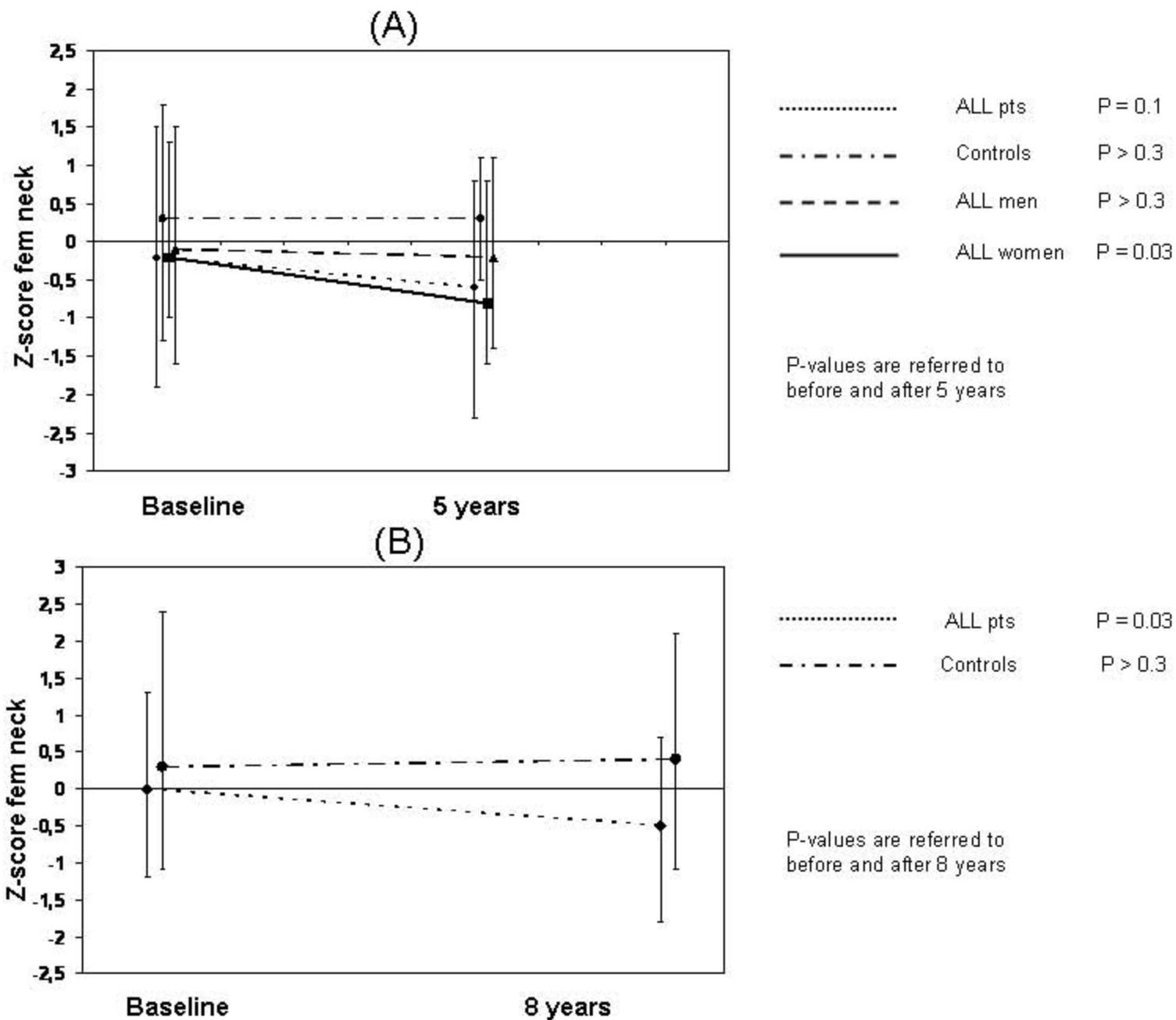


Table 1. Patients' baseline characteristics, medical history and GH status in 44 former ALL patients with and without GH treatment. Data are presented as median and range (min-max).

	5 years of GH therapy (n=15)	8 years of GHD (n=13)	Excluded patients (n=16)
Men/women (n)	7/8	9/4	7/9
Age at baseline (yr)	25 (22-32)	25 (19-32)	25 (20-32)
Height (cm)	161 (149-181)	170 (148-186)	162 (150-192)
Weight (kg)	66 (44-98)	79 (53-121)	74 (50-127)
Age at diagnosis (years)	3.9 (1-17)	4.2 (2-9)	6.3 (2-13)
Years since CRT (years)	21 (8-27)	19 (9-27)	19 (9-27)
Target dose CRT (Gy)	24 (18-24)	24 (24-25)	24 (18-30)
Anthracycline dose (mg/m²)	120 (80-540)	120 (40-540)	120 (60-540)
MTX dose it (mg/ m²)	60 (12-144)	72 (12-204)	110 (60-324)
MTX dose iv (mg/ m²)	3000 (3000-4000)	1763 (30-4000)	3000 (3000-4000)
MTX dose po (mg/ m²)	2672 (533-4200)	2723 (1097-3900)	2687 (893-3600)
Prednisolone dose (mg/m²)	2508 (1600-6682)	2410 (1540-6573)	2514 (1610-6432)
Decadron dose (mg/m²)	370 (296-390)	325 (200-400)	340 (300-400)
Standard/intermediate/high-risk group (n)	11/0/5	10/0/3	8/1/6
Spinal radiation (n)/ target dose (Gy)	2/23	2/23	1/14
Testes-radiation (n)/ target dose (Gy)	4/24	5/24	3/24
Peak GH during ITT (µg/L)	0.4 (0.4-2.4)	0.2 (0.04-4.6 [*])	0.4 (0.2-3.8)
IGF-1 (µg/L)	124 (75-264)	142 (81-329)	134 (78-256)
GH during childhood	1	2	1
Oral contraception	2	1	2
BMD Z-score Femoral neck	-0.2 (-1.6-1.5)	0.0 (-1.5-1.3)	0.4 (-2.0-1.4)
BMD Z-score L2-L4	-0.4 (-1.6-1.5)	-0.2 (-1.4-2.1)	-0.3 (-2.7-2.0)

Abbreviations: GH, growth hormone; ALL, acute lymphoblastic leukaemia; CRT, Cranial radiotherapy; Gy, grey; MTX, methotrexate; It, intrathecal; iv, intravenously; po, per os; ITT, insulintolerance test; IGF-I, insulin growth factor I

* One patient responded with 4.6 µg/L but had < 1 during GHRH-Arginine-test and IGF-I – 3.0 SD.

Table 2. Anthropometric measurements, body composition, bone mineral density (BMD), z-scores using DXA and serum hormone levels at baseline in 21 former ALL women and 23 former ALL men and matched population controls. Data are presented as median and range (min-max).

	Patients Women Median (range) N=21	Controls Women Median (range) N=21	P	Patients Men Median (range) N=23	Controls Men Median (range) N=23	P
Age (yr)	26 (22-33)	26 (22-33)	--	25 (20-32)	25 (20-32)	--
Height (cm)	158 (145-180)	167 (159-174)	0.001	172 (160-192)	185 (168-200)	0.002
Height (SD)	-1.6 (-2.0—1.1)	0.0 (-0.5-0.7)	0.001	-1.25 (-2.0—0.49)	0.2 (-0.7-0.5)	0.002
BMI (kg/m²)	25.9 (19.7-33.3)	21.3 (18.4-33.8)	0.02	23.9 (19.2-37.4)	23.0 (20.3-34.5)	0.1
Weight (kg)	68 (44-81)	61 (52-94)	>0.3	79 (53-127)	80 (65-109)	>0.3
Fatmass (kg)	27.5 (12.3-42.3)	16.5 (11.1-46.2)	<0.0001	19.6 (38.0-59.5)	13.6 (69.9-35.4)	0.07
Fatmass (%)	42 (28-53)	30 (21-49)	0.001	28 (7-47)	17 (8-39)	0.01
Leanmass (kg)	33.2 (28.1-44.7)	38.0 (33.7-47.7)	0.01	40 (32-57)	47 (38-62)	0.02
Leanmass (%)	43 (28-53)	64 (46-73)	0.001	67 (49-84)	76 (57-83)	0.01
IGF-I (µg/L)	137 (81-289)	196 (107-338)	0.03	159 (110-329)	191 (110-341)	0.04
BMAD femoral neck (g/cm³)	0.21 (0.18-0.28)	0.22 (0.17-0.28)	>0.3	0.20 (0.14-0.26)	0.20 (0.15-0.29)	>0.3
BMD femoral neck (g/cm²)	0.99 (0.82-1.15)	1.01 (0.76-1.29)	0.3	1.05 (0.78-1.32)	1.10 (0.87-1.35)	>0.3
Z-score femoral neck (SD)	-0.20 (-1.5-1.4)	0.30 (-1.5-2.4)	>0.3	0.00 (-2.0-1.5)	0.30 (-1.4-2.0)	>0.3
BMAD L2-L4 (g/cm³)	0.19 (0.15-0.24)	0.18 (0.15-0.21)	0.2	0.18 (0.14-0.21)	0.18 (0.12-0.20)	>0.3
BMD L2-L4 (g/cm²)	1.15 (1.0-1.48)	1.19 (1.0-1.52)	0.3	1.23 (0.84-1.53)	1.27 (0.92-1.45)	0.3
Z-score L2-L4 (SD)	-0.30 (-1.6-2.1)	0.00 (-1.4-2.1)	>0.3	0.10 (-2.7-2.09)	0.00 (-2.8-1.7)	0.3
S-Osteocalcin (µg/L)	23 (15-51)	31 (17-52)	0.2	40 (18-61)	40 (25-113)	>0.3
S-CrossLaps (ng/L)	422 (233-827)	482 (274-1001)	>0.3	668 (211-1085)	706 (432-1489)	>0.3

Abbreviations: ALL, acute lymphoblastic leukaemia, SD, standard deviation score; IGF-I, insulin growth factor

Table 3. A. Bone mineral density (BMD) and biochemical assays in 15 former ALL patients (8 women) at baseline and after 5 years of GH treatment and matched population controls at baseline and after 5 years. Data are presented as median and range (min-max).

A.

	Patients baseline Median (range) N=15	Controls baseline Median (range) N=15	Patients after 5 yrs GH treatment N=15	Controls after 5 yrs Median (range) N=15	P Patients' baseline vs controls at baseline	P Patients' baseline vs 5 yrs of GH treatment	P Patients' 5 yrs of GH treatment vs controls 5 yrs after baseline
Age (yr)	25 (22-32)	25 (22-32)	31 (27-38)	31 (27-38)	-	-	-
BMI (kg/m²)	23.4 (19.6-37.4)	22 (18.4-34.5)	25.6 (20-39.9)	23.3 (18.9-40.1)	>0.3	0.003	0.2
BMAD femoral neck (g/cm³)	0.21 (0.17-0.26)	0.22 (0.17-0.29)	0.19 (0.17-0.24)	0.23 (0.18-0.30)	>0.3	>0.3	>0.3
BMD Femoral neck (g/cm²)	0.99 (0.78-1.27)	1.03(0.86-1.33)	0.90 (0.75-1.19)	1.06 (0.89-1.34)	0.2	0.3	>0.3
Z-score Femoral neck (SD)	-0.2 (-1.6-1.5)	0.3 (-0.9-2.0)	-0.6 (-2.3-1.1)	0.3 (-0.9-2.0)	0.2	0.1	>0.3
BMAD L2-L4 (g/cm³)	0.19 (0.15-0.25)	0.19 (0.15-0.23)	0.18 (0.15-0.22)	0.18 (0.15-0.21)	>0.3	>0.3	>0.3
BMD L2-L4 (g/cm²)	1.14 (0.97-1.47)	1.23 (1.05-1.52)	1.13 (1.02-1.26)	1.22 (0.99-1.5)	0.2	>0.3	>0.3
Z-score L2-L4 (SD)	-0.4 (-1.6-2.1)	0.1 (-1.5-2.1)	-0.3 (-2.1-1.3)	-0.2 (-2.3-1.8)	>0.3	>0.3	>0.3
CrossLaps (µg/L)	436 (211-937)	518 (274-1001)	453 (152-1559)	417 (26-711)	>0.3	0.04	0.02
Osteocalcin (ng/L)	30 (21-58)	30 (17-51)	27 (18-106)	25.5 (11-428)	>0.3	>0.3	>0.3
IGF-I (µg/L)	124 (75-264)	139 (99-250)	176 (110-301)	135 (73-253)	0.05	0.03	0.09
IGF-I SD	-2.5 (-3.2-0.7)	0.3 (-1.3-1.5)	0.05 (-0.8-1.0)	0.0 (-1.5-1.8)	0.05	0.03	>0.3

Abbreviations: ALL, acute lymphoblastic leukaemia; GHD, growth hormone deficiency; IGF-I, insulin growth factor I

Table 3. B. Bone mineral density (BMD) and biochemical assays in 13 former ALL patients (4 women) at baseline and after 8 years since confirmed GHD, and matched population controls at baseline and after 8 years. Data are presented as median and range (min-max).

B.

	Patients baseline Median (range) N=13	Controls baseline Median (range) N=13	Patients after 8 yrs Median (range) N=13	Controls after 8 yrs Median (range) N=13	p Patients baseline vs controls baseline	p Patients baseline vs patients 8 yrs of GHD	p Patients 8 yrs of GHD vs controls after 8 yrs
Age (yr)	25 (19-32)	25 (19-32)	33 (27-40)	33 (27-40)	-	-	-
BMI (kg/m²)	25.3 (19.2-37.4)	21.5 (18.7-26.7)	29.5 (21-39.5)	24.3 (21-32)	0.04	0.01	0.03
BMAD femoral neck (g/cm³)	0.21 (0.17-0.26)	0.22 (0.19-0.27)	0.19 (0.17-0.26)	0.23 (0.20-0.28)	0.3	>0.3	>0.3
BMD femoral neck (g/cm²)	1.13 (0.82-1.33)	1.14 (0.92-1.34)	1.02 (0.80-1.30)	1.16 (0.95-1.35)	>0.3	0.2	0.2
Z-score femoral neck (SD)	0.0 (-1.5-1.3)	0.3 (-1.4-2.4)	-0.5 (-1.8-0.7)	0.4 (-1.1-1.8)	0.3	0.03	0.05
BMAD L2-L4 (g/cm³)	0.18 (0.14-0.24)	0.19 (0.15-0.25)	0.17 (0.14-0.23)	0.20 (0.16-0.27)	>0.3	>0.3	>0.3
BMD L2-L4 (g/cm²)	1.23 (1.01-1.52)	1.31 (1.13-1.57)	1.17 (1.00-1.32)	1.33 (1.14-1.56)	0.1	0.3	0.02
Z-score L2-L4 (SD)	-0.2 (-1.4-2.1)	0.8 (-1.3-2.0)	-0.5 (-1.7-1.1)	1.0 (-1.5-1.9)	0.05	0.2	0.03
CrossLaps (ng/L)	631 (233-1085)	599 (274-1268)	374 (144-698)	401 (196-983)	>0.3	0.001	0.03
Osteocalcin (µg/L)	39 (15-61)	33 (17-45)	20 (17-38)	21 (16-34)	>0.3	0.004	0.02
IGF-I (µg/L)	142 (81-329)	175 (107-338)	128 (52-150)	167 (105-304)	0.09	0.005	0.01
Difference (%) IGF-I	---	---	30 (-15-56)	4.3 (-0.7-48)	-	-	0.04
IGF-I SD	-2.0 (-3.2-0.5)	0.2 (-1.4-1.7)	-3.2 (-4.2- -1.2)	-0.6 (-1.5-1.0)	0.03	0.005	0.002

Abbreviations: ALL, acute lymphoblastic leukaemia; GHD, growth hormone deficiency; IGF-I, insulin growth factor I