ABSTRACT. Objectives. Abnormalities in bone mineral density (BMD), body composition, and bone metabolism have been reported in children who were treated for acute lymphoblastic leukemia (ALL) during and after completion of therapy. However, these studies are cross-sectional, and no longitudinal data are available in a large group of patients after completion of therapy. In the present study, 1-year longitudinal changes in BMD, body composition, and bone metabolism were evaluated in children with ALL during the first 3 years after completion of therapy. In the present study, 1-year longitudinal changes in BMD, body composition, and bone metabolism were evaluated in children with ALL during the first 3 years after completion of therapy without cranial irradiation.

Methods. BMD of total body (TB; g/cm²), areal and apparent volumetric lumbar spine (L2–L4), lean body mass, and percentage of body fat were measured by dual-energy x-ray absorptiometry in 37 children (median age: 7.9 years; range: 4.7–20.6 years) who were treated for ALL at a median age of 3.3 years (range: 1.1–16.6 years), after a median time of 2.2 years after the completion of treatment, and after a 1-year follow-up period. Two control subjects ($n = 74$) who were matched for gender, age, and pubertal stage were also longitudinally investigated for body composition for 1 year. Usual serum biochemical markers of calcium metabolism and bone turnover were measured in patients during the study period.

Results. A slight decrease in TB BMD was found after a median time of 2.2 years after the completion of therapy for ALL in childhood. Patients showed a significantly lower median TB BMD when evaluated <1.5 years as compared with those at $\geq 1.5$ years since completion of therapy. At the time of first evaluation, the percentage of body fat mass was significantly higher and patients were physically less active than their matched control subjects. Although, as expected, during the 1 year of follow-up both groups showed an annual increment in their BMD measurements, a significantly higher increase in TB BMD was observed in patients in comparison with control subjects. During this same period, the increase in the percentage of body fat mass was slightly lower in ALL patients as compared with control subjects. At the end of the follow-up year, BMD, body-composition parameters, and physical activity of ALL patients were similar to those observed in matched control subjects. Serum biochemical markers of bone turnover were normal at both evaluations.

Conclusions. A significant increase in TB BMD and a tendency to a lesser increase in percentage of body fat mass were observed during the study period in ALL patients as compared with chronological age-, gender-, and pubertal stage–matched control subjects. These findings suggest a positive effect of long-term completion therapy and increase in physical activity on BMD, body composition, and bone metabolism in patients who have been treated for ALL.

ABBREVIATIONS. ALL, acute lymphoblastic leukemia; BMD, bone mineral density; SDS, SD score; TB, total body; LS, lumbar spine; PTH, parathyroid hormone; IRMA, immunoradiometric assay.
able for patients after completion of therapy. The aim of this study was to determine BMD, bone metabolism, and body composition in children with ALL during the first 3 years after completion of therapy without cranial irradiation and to evaluate their 1-year longitudinal changes.

METHODS

Patients

All patients who received a diagnosis of ALL in the Hematology Unit of the Robert Debre Hospital (Paris, France) between 1995 and 1997 and who met the following criteria were eligible for the study: (1) 3 to 21 years of age; (2) white origin; (3) 0 to 3 years after cessation of ALL therapy; and (4) no relapse, second neoplasm, or bone marrow transplant. Exclusion criteria were (1) cranial irradiation, (2) pregnancy, and (3) chronic diseases or any treatment associated with altered bone metabolism. Among the potential study subjects (n = 56), 19 declined to participate and 37 (17 girls, 20 boys) were enrolled for the study. No difference was found between participating and nonparticipating subjects with respect to age, gender, or length of time since the completion of treatment. Patients were studied at a first evaluation point (n = 37) and a second evaluation point 1 year later (n = 34). Three patients were eliminated from the second evaluation because of relapse (n = 1) or moving to another country (n = 2).

Patients were treated according to the Protocols of the Child Leukemia Cooperative group from EORTC 58881,1 which included systemic administration of prednisolone, vincristine, and daunorubicin; l-asparaginase; 6-mercaptopurine; cytarabine; cyclophosphamide; and 10 intrathecal methotrexate injections during the intensive phase of treatment. Four patients received a supplementary administration of intravenous methotrexate (6 × 5 g/m²). All patients received prednisone 60 mg/m² for 28 days and 9 days of decreasing dose (1785 mg) during the induction therapy. For standard-risk patients (low and intermediate risk: n = 35), the reinduction period consisted in 10 mg/m² prednisone with 12 days of progressive decrease (246 mg). In the reinduction period, high-risk patients (n = 2) received 10 mg/m²/dexmethasone for 5 days and then three prednisone pulses of 100 mg/m² (1500 mg) for 5 days, alternating with three dexmethasone pulses for 5 days (350 mg). Treatment was completed in ~2 years. No patients were submitted to cranial irradiation.

Two control subjects who were matched for gender, age (within ±6 months), and pubertal stage were randomly identified for each patient from a large, healthy group of white children (n = 266) who were longitudinally investigated for BMD and body composition for 1 year at the Robert Debre hospital. At both evaluations (initial visit and 1-year follow-up visit), in addition to the BMD (total body [TB] and lumbar spine [LS]), all subjects (patients and control subjects) were evaluated for age, weight, height, pubertal status, calcium intake, and physical activity. Bone age and usual biochemical markers of calcium metabolism and bone turnover were measured in patients (fasting blood samples obtained in the morning). Biochemical markers of bone turnover were assessed by serum osteocalcin and bone alkaline phosphatase, specific parameters of bone formation, and by serum CrossLaps as a marker of bone resorption. No blood samples were taken in the control population, which was evaluated only for BMD and body composition. Biochemical data were compared with that from normal children with no history of chronic disease or any current disease or drug therapy (unpublished data).

The study protocol was reviewed and approved by the faculty ethics committee. It was explained to each subject and his or her parents who signed a written consent for participation.

Clinical Assessment

Height, weight, and growth velocity (cm/year) were expressed as z-score (SDS) for gender and chronological age.29 Weight for height was expressed as BMI (kg/m² = weight/height²) in SDS for gender and chronological age.30 Pubertal development was scored according to Tanner stage.31 Bone age was determined under blind conditions by 1 investigator (J.L.) according to the method of Greulich and Pyle.32

Questionnaires

Questionnaires were administered to determine current dietary calcium intake, physical activity, and history of fractures in the previous 5 years for all subjects. Calcium dietary intake (mg/day) was assessed by a semiquantitative food questionnaire.25 Weekly physical activity was determined according to 3 categories: (1) no sports at all, (2) physical educational classes only with an average of 3 hours/week, and (3) physical educational classes and extracurricular organized sports. History of fracture incidence rate was compared with the incidence rate in our healthy control group during the same period, i.e., the 4 years before the first evaluation (which corresponded to the median duration since ALL diagnosis in the patient group).

BMD Measurements

BMD (bone mineral content divided by bone area) measurements of LS (L2–L4) and TB were performed using dual-energy x-ray absorptiometry (Lunar DPXL, Madison, WI). To correct for bone size, we calculated apparent volumetric BMD of LS with the model apparent volumetric BMD LS = BMC/AV/A (volume = AV/A).34 Dual-energy x-ray absorptiometry of TB also estimates body composition as lean tissue mass (g) and percentage of fat mass. The results were compared with those from the matched control subjects. The coefficient of variation (CV) was 1% and 1.64% for L2–L4 BMD and for TB BMD, respectively. For lean tissue mass and percentage fat mass, the CVs were 1% and 4%, respectively.

Biochemical Parameters

Fasting blood samples of all patients were obtained for the assessment of calcium, phosphorus, magnesium, alkaline phosphatase, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, parathyroid hormone (PTH), osteocalcin, bone alkaline phosphatase, and CrossLaps. Samples were stored at −20°C until assayed. Serum PTH was measured using the immunoradiometric assay (IRMA) with a kit from Nichols Institute Diagnostics (Paris, France). Serum 25-OH vitamin D was measured using a radioimmunologic assay, kit from Nichols Institute Diagnostics. Serum 1,25-OH-D was measured using a radioimmunologic assay commercial kit 1–25 (OH2) Vit D RIAC from Brahms (Saint-Ouen, France). Osteocalcin was measured using the IRMA Osteo-Riact kit from Schering (Gif-sur-Yvette, France). Bone alkaline phosphatase was measured using the IRMA kit Tandem-R Ostase (Beckman Coulter, Paris, France). Serum CrossLaps was measured using an immunoenzymometric technique with the Serum CrossLaps kit from Nordic Biosciences Diagnostics (Herlev, Denmark). The mean intra- and interassay CVs of osteocalcin, bone alkaline phosphatase, and CrossLaps were <3% and <7%, <7% and <9%, and <5% and <8%, respectively.

Statistical Analysis

Results are expressed as median (25th–75th percentiles) for quantitative variables and number (percentages) for qualitative variables. Statistical differences between first and second visit within control and patient groups were assessed by nonparametric paired tests (Wilcoxon paired test). Statistical differences between control subjects and patients were assessed by conditional logistic regression. SDS for serum markers of bone turnover were calculated from normative data in 550 healthy children (unpublished data). Relationships between BMD (LS and TB) and body composition (lean body mass and percentage of body fat mass), calcium intake, physical activity, age at diagnosis of ALL, time elapsed since the end of treatment, and patients with or without supplement intravenous methotrexate adjusted for age and gender were studied by median regression. All tests were 2-tailed. Statistical analyses were performed using the SAS 8.2 (SAS Inc, Cary, NC) and Stata 7.0 (Stata Corp, College Station, TX) software packages, with P < .05 considered as statistically significant.

RESULTS

Clinical Characteristics

The clinical characteristics of the study population are indicated in Table 1. The 37 patients had received
Values are expressed as median (25th; 75th percentiles).

| TABLE 1. Clinical Characteristics of the Study Population at Baseline and During the 1-Year Follow-up |
|--------------------------------------------------------|------------------|------------------|------------------|------------------|
|                                                      | ALL Group         | Control Group    |
|                                                      | (17 Girls/20 Boys)| (34 Girls/40 Boys)|              |
|                                                       | (n = 37)          | (n = 34)         | (n = 74)         | (n = 74)         |
| Chronological age, y                                  | 7.9 (6.2; 9.8)    | 8.9 (7.3; 11.0)  | 7.9 (6.5; 10.2)  | 9.1 (7.6; 11.7)  |
| Pubertal stage                                        |                  |                  |                  |                  |
| 1                                                     | 29               | 25               | 58               | 56               |
| 2                                                     | 2                | 1                | 4                | 2                |
| 3                                                     | 2                | 2                | 4                | 4                |
| 4                                                     | 6                | 6                | 12               | 12               |
| Bone age, y                                          | 7.6 (6.5; 10.5)  | 8.5 (7.8; 11.0)  | —                | —                |
| Weight (SDS)                                         | 1.2 (0.3; 1.7)   | 0.9 (0.0; 2.1)   | 0.8 (0.3; 1.8)   | 0.9 (0.3; 2.1)   |
| Height (SDS)                                         | 0.6 (0.0; 1.2)   | 0.4 (-0.3; 0.8)* | 0.8 (0.1; 1.5)   | 0.8 (0.1; 1.6)*  |
| BMI (SDS)                                            | 0.9 (-0.1; 1.7)  | 0.7 (-0.1; 1.8)  | 0.3 (-0.4; 1.1)  | 0.4 (-0.1; 1.3)  |
| Growth velocity (SDS)                                | 1.0 (0.0; 2.0)   | -0.1 (-0.9; 0.7) | —                | 0.0 (-0.9; 1.2)  |
| Categorizes of physical activity, n (%)              |                  |                  |                  |                  |
| No sports at all                                      | 3 (8)†           | 3 (4)†           | 2 (3)            |                  |
| Physical educational classes only with an average of 3 h/wk | 17 (46)         | 10 (29)         | 20 (27)         | 12 (16)         |
| Physical educational classes and extracurricular organized sports | 17 (46)         | 24 (71)         | 51 (69)         | 60 (81)         |
| No. of individuals with fractures (%)                 | 8 (22)           | 8 (24)          | 6 (8)            | 8 (11)          |
| No. of fractures                                      | 9                | 9               | 6                | 9                |
| Induced/spontaneous                                   | 4/5             | 4/5             | 0/6             | 0/9             |
| Calcium intake, mg/J                                  | 796 (528; 1152)  | 697 (424; 1018)  | 866 (642; 1034)  | 717 (511; 1018)  |
| Calcium intake >800 mg/J, n (%)                       | 17 (49)         | 16 (48)         | 43 (58)         | 27 (40)         |

Values are expressed as median (25th; 75th percentiles).

* P = .03 patients versus control subjects.
† P = .02 patients versus control subjects.

A diagnosis of ALL at a median age of 3.3 years (range: 1.1–16.6 years) and were enrolled in the study after a median period of 2.2 years (range: 0.1–3.1 years) after completion of treatment. Overall, the patient group was physically less active (P = .02), and their mean BMI (SDS) was slightly higher (P = .08) than that of healthy control subjects at the first evaluation. These differences were not found at the second evaluation after 1 year of follow-up.

At the end of the study, the fracture rate observed for the previous 5 years was higher (2 times higher) in patients than in healthy control subjects. In the ALL group, 9 fractures occurred in 8 of the 37 patients (male, n = 2; female, n = 6; 22% of subjects). Five fractures were discovered at the time of ALL diagnosis (location of fractures in the extremities, n = 3; compression of the vertebrae, n = 2), and 4 fractures (extremities) occurred during treatment (n = 1) or after discontinuation of therapy (n = 3). In the control group, 9 fractures occurred in 8 of the 74 patients (male, n = 6; female, n = 2; 11% of subjects), 6 fractures occurred before the first evaluation, and 3 fractures occurred during the follow-up period. For control subjects, all fractures were located in the extremities.

**BMD and Body Composition**

As shown in Table 2, the median BMD (g/cm²) of TB in ALL patients was slightly reduced (P = .06) at baseline, but no difference from control subjects was found at the 1-year follow-up evaluation (P = .23). When the time elapsed since the end of treatment was considered, the median BMD of TB was significantly reduced in patients who were evaluated <1.5 years as compared with those at ≥1.5 years since completion of therapy, either at baseline (P = .03) or after the 1 year follow-up (P = .008). Although the median areal BMD (g/cm²) of LS in ALL patients was significantly lower at baseline (P = .04) and slightly reduced at the 1-year follow-up evaluation (P = .06) in comparison with control subjects (who were matched for age, pubertal stage, and gender), the apparent volumetric BMD of LS was also reduced in patients as compared with control subjects, but this did not reach significance at either evaluation. When we corrected BMD for bone age instead of chronological age, similar patterns were seen (data not shown). No correlation was found between LS BMD measurements and time elapsed since the end of treatment. The median lean body mass was similar for both groups in both evaluations. The median percentage of body fat mass was significantly higher in patients than in control subjects at baseline (P = .05), but the difference was no longer significant at the 1-year follow-up evaluation (P = .94). No correlation was found between the body-composition measurements and time elapsed since the end of treatment.

As expected, both groups showed an annual increment in their BMD measurements. However, at the 1-year follow-up evaluation, TB BMD (but not LS BMD) demonstrated a significantly higher increase (P = .01) in ALL patients as compared with control subjects, and percentage of body fat mass showed a slightly lower increase (P = .08) in ALL patients as compared with control subjects (Fig 1).

None of the subjects had BMD values ≤-2 SDS or TB BMD ≤-1 SDS. As shown in Table 3, no difference was found in either BMD measurements or
biochemical markers of bone turnover in patients with and without a history of fractures.

In patients, no relation was found at baseline between BMD (LS and TB) and body composition (lean body mass and percentage of body fat mass) and calcium intake, physical activity, age at diagnosis of ALL, or presence or absence of the intravenous methotrexate supplement. Male subjects showed lower LS...
areal BMD than female subjects (this was seen across all ages and pubertal stages; \( P = .001 \)). However, this gender difference was not observed when apparent volumetric BMD of LS or TB BMD was analyzed.

**Bone Metabolism**

Serum levels of calcium, phosphate, alkaline phosphatase, 25 OHD, 1-25 OHD, and PTH were within the normal ranges in patients (Table 3). As shown in Table 4, serum osteocalcin, bone alkaline phosphatase, and CrossLaps levels were normal at both baseline and 1-year follow-up evaluations.

**DISCUSSION**

In the present study, a slight decrease in TB BMD was found 2.2 years after completion of therapy of ALL in childhood. At that time, percentage of body fat mass was significantly higher and patients were physically less active than their matched control subjects. After a 1-year follow-up period, a significantly higher increase in TB BMD was observed in patients than in healthy age-, gender-, and pubertal stage-matched control subjects. Moreover, during this period, the increase of percentage of body fat mass was slightly lower in ALL patients as compared with control subjects. However, after 1 year of follow-up, BMD and body-composition parameters of ALL patients and their physical activity were similar to those observed in matched control subjects. Serum markers of bone turnover were normal at both evaluations for patients.

Previous longitudinal studies performed at diagnosis and during treatment in childhood ALL have found normal\(^7\) or low\(^9,10,12\) LS BMD at diagnosis. Recently, a reduced bone volume and trabecular thickness, especially in children younger than 10 years, was demonstrated by bone histomorphometry in children with newly diagnosed ALL.\(^8\) During treatment and even without the use of cranial irradiation as part of their treatment (prophylactic treatment of the central nervous system), a significant reduction in BMD was found in these patients in comparison with baseline values.\(^6,7,9,11,12\)

There are several cross-sectional studies in which BMD status has been assessed in long-term survivors of childhood ALL. Studies on patients who were treated with cranial irradiation have shown significantly reduced BMD in TB as well as in LS, even >8 years after completion of treatment.\(^16,18-22\) Defects in the hypothalamic-pituitary axis leading to abnormalities in growth hormone and gonadotropin secretion are probable causes, leading to disturbed bone metabolism.\(^36\) The effect of chemotherapy alone on BMD is much less clear; normal\(^12,14,24,25\) or reduced BMD\(^8,23\) have been reported. After completion of treatment with chemotherapy alone, no decrease in either areal or volumetric BMD for LS and TB was found 9.6 years (mean) after completion of treatment in 23 patients aged 17 years,\(^24\) whereas a significant decrease in volumetric LS BMD was demonstrated 5 years (mean) after completion of treatment in 28 patients aged 10.7 years.\(^23\) As is the case in our study (although only for areal BMD), this was associated with male gender and current level of physical activity.\(^23\) Male gender as a risk factor for decreased BMD after completion of treatment was also shown in 2 other studies in which cranial irradiation was conducted.\(^17,22\) A gender difference of sensitivity to cytotoxic gonadal damage and/or to glucocorticoids was hypothesized to be a probable explanation.\(^23\)

As is the case for normal children,\(^37-39\) physical activity level was also shown to be an important determinant of BMD and of body fat mass in these patients.\(^23,40\) Excessive overweight after childhood ALL has been reported frequently.\(^26,27\) It has been postulated that the increase in body fat mass was related to multifactorial causes such as cranial irradiation (thus partly resulting from growth hormone insufficiency), to glucocorticoids (as part of treatment protocols), to increased energy intake and to the reduced energy expenditure resulting from decreased physical activity.\(^19,26,28,40-42\) Only 2 studies have investigated long-term body composition 5 to 9.6 years (mean) after completion of treatment with chemotherapy alone. A significantly higher percentage of body fat mass was found after 5 years in only a subgroup of patients who received intravenous high-dose methotrexate.\(^23,24\) As is the case in our study, the difference in BMI was not significant. However, our study demonstrated a significantly higher percentage of body fat mass in patients 2.2 years after completion of therapy that was not found later during the follow-up period.

Concern has also been expressed regarding the detrimental effects of methotrexate and glucocorticoid on bone\(^5,13,43\) as well as the harmful effects of the disease process itself. At diagnosis, children with ALL have reduced bone alkaline phosphatase, insulin-like growth factor 1 (IGF-1) and IGF binding protein 3 (IGFBP-3), all of which suggest low bone turnover resulting from the disease.\(^4,5,7,44-46\) Bone alkaline phosphatase remained low throughout the treatment with an increase of bone resorption markers, probably as a result of the effects of methotrexate and corticosteroid treatment. These effects occurred independent of circulating IGF-1 and IGFBP-3, which were restored to normal values during treatment.\(^4,5,7,43,44,46\) However, it has been seen in a small

| TABLE 4. Serum Markers of Bone Formation (Osteocalcin and Bone Alkaline Phosphatase) and Bone Resorption (CrossLaps) Expressed as z Scores in Patients Who Were Treated for ALL During the 2.2 to 3.2 Years After Completion of Treatment During 1-Year Follow-up |
|----------------------|----------------------|
| **Baseline** | **At 1-Year Follow-up** |
| Osteocalcin (SDS) | 0.4 (−0.5; 1.1) | 0.2 (−0.4; 1.2) |
| Bone alkaline phosphatase (SDS) | −0.4 (−0.8; 0.3) | −0.2 (−0.8; 0.2) |
| CrossLaps (SDS) | −0.1 (−0.7; 0.6) | −0.3 (−1.2; 0.3) |

Values are expressed as median (25th; 75th percentiles).
study on a limited number of patients that bone alkaline phosphatase increased to normal levels during the first month after cessation of therapy. In our study, all serum markers of bone turnover were normal after completion of treatment.

Moreover, no factors such as age at diagnosis of ALL, higher methotrexate dosage, calcium intake, or physical activity were identified as contributing to impairment of body composition and/or bone metabolism. Except for TB BMD measurements, no relationship was found between body-composition parameters and follow-up time after completion of treatment. The number of patients who were investigated in our study was small; however, this was compensated for by the fact that the study population was very homogeneous regarding malignancy (only 2 patients in a high-risk group) and treatment schedule.

In conclusion, a significant increase in TB BMD and a tendency to a lesser increase in percentage of body fat mass were seen during the study period in patients as compared with control subjects who were matched for chronological age, gender, and pubertal stage. Moreover, as seen after a 1-year follow-up period, body composition, biochemical markers of bone turnover, and physical activity all recovered to normal levels at 3.2 years (median) after the end of treatment. These findings were seen across ages and independent of gender and pubertal status, suggesting a positive effect of long-term completion therapy as well as increase in physical activity on BMD, body composition, and bone metabolism in patients who have been treated for ALL.

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Improvement in Bone Mineral Density and Body Composition in Survivors of Childhood Acute Lymphoblastic Leukemia: A 1-Year Prospective Study
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