Bone mineral density in adult survivors of childhood acute leukemia: impact of hematopoietic stem cell transplantation and other treatment modalities

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Femoral and lumbar bone mineral densities (BMDs) were measured in 159 adults enrolled in the Leucémies de l'Enfant et de l'Adolescent program, a French prospective multicentric cohort of childhood leukemia survivors. BMDs were expressed as Z-scores, and multivariate linear regression analyses were used to construct association models with potential risk factors. Mean age at evaluation and follow-up was 23 and 14.7 years, respectively. In the whole cohort, mean femoral Z-score was −0.19 ± 0.08. Two factors were associated with lower femoral BMD transplantation (−0.49 ± 0.15 vs −0.04 ± 0.10 in the chemotherapy group; *P* = .006) and female sex (−0.34 ± 0.10 vs −0.03 ± 0.13; *P* = .03). Among patients who received a transplant, the only significant risk factor was hypogonadism (−0.88 ± 0.16 vs −0.10 ± 0.23; *P* = .04). A slight reduction in lumbar BMD (mean Z-score, −0.37 ± 0.08) was detected in the whole cohort without difference between the transplantation and chemotherapy groups. Among patients who received a transplant, younger age at transplantation was correlated with a low lumbar BMD (*P* = .03). We conclude that adults who had received only chemotherapy for childhood leukemia have a slight reduction in their lumbar BMD and a normal femoral BMD. Patients who received a transplant with gonadal deficiency have a reduced femoral BMD which might increase the fracture risk later in life. (*Blood*. 2011; 118(6):1481-1489)

Introduction

During the past decades, the survival rates after childhood acute leukemia (AL) have improved substantially. Childhood AL is now a curable disease in 80% of patients with acute lymphoblastic leukemia (ALL) and in 50% of patients with acute myeloblastic leukemia (AML). Consequently, more emphasis is placed on the long-term side effects of this disease and its treatment.

Reduced bone mineral density (BMD) has been largely reported at diagnosis and during treatment of AL. Its cause is most probably multifactorial; the disease process itself, steroid therapy, intensive chemotherapy, lesion of endocrine organs that control bone accretion, immunosuppressive agents after HSCT, poor nutrition, and decreased physical activity may contribute to these abnormalities.[1-4]

However, it is still controversial if survivors of childhood AL maintain low BMD after the end of treatment. The question is whether decreased bone mineralization during a period of illness will be restored or whether inappropriate bone mass will persist during adulthood. Some studies have already described long-term AL survivors with normal[5-9] as well as reduced[10-18] BMD. Most of those studies were limited by their low power because of limited sample size. They were also heterogeneous, reporting bone mass assessment performed before or after attainment of peak bone mass in both AL and other cancer survivors.[19] Moreover, reports of BMD after childhood HSCT are rare and are limited to few patients.

In the study presented here, we aimed to determine in a group of 159 adults surviving childhood AL whether this disease and its therapy may have a lasting effect on bone density later in life and which (if any) AL-related factors, patient characteristics, or treatment modalities (HSCT especially) correlate with reduced BMD in this population.

Methods

Patients

This prospective study was designed to assess BMD in young adults included in the Leucémies de l'Enfant et de l'Adolescent program. This French multicentric program was created in 2003 to evaluate prospectively the long-term health status, quality of life, and socioeconomic status of childhood leukemia survivors who were treated from 1980 to now in 2 geographic areas (PACA-Corse and Lorraine). Details of the whole program have been previously described.[20]

In 2007 and 2008, assessment of BMD by dual-energy x-ray absorptiometry (DXA) was systematically proposed to all adults with a new health status evaluation by the Leucémies de l'Enfant et de l'Adolescent program. Among 220 eligible patients, 159 (72.3%) underwent a DXA scan and are the subjects of this report. All patients have signed informed consent in accordance with the Declaration of Helsinki. This study was approved by the French National Clinical Research Program and the French National Cancer Institute.

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BMD assessment

According to the 2007 International Society for Clinical Densitometry official positions for BMD reporting in women before menopause and in men younger than age 50,[21] BMD was measured by DXA scan at the lumbar spine (LS; vertebrae L1 through L4) and at the left femoral neck (FN). The results were expressed as the number of SDs from normal values of sex-, age-, and ethnicity-matched controls (Z-score). A low BMD for age was defined as a Z-score of −2 or lower at 1 of the 2 sites.

Hormonal status

Growth hormone (GH) deficiency (GHD) was detected by measuring plasma levels of insulinlike growth factor I and GH peak response to ≥ 2 stimulation tests per patient. These tests were insulin tolerance tests, GH-releasing hormone infusion tests, or propranol-glucagon tests. GHD was diagnosed when peak GH levels after stimulation were inferior to 20 mUI/L (or 10 ng/mL). The tests were performed ≥ 6 months away from any antileukemic treatment. Evaluation was done only for patients with decreased height growth velocity.

Hypogonadism was defined by low testosterone (males) or estradiol (females) serum level and classified as hypergonadotrophic or hypogonadotrophic according to values of luteinizing hormone and follicle-stimulating hormone. Evaluation was done for patients who underwent stem cell transplantation or who had pubertal delay.

Evaluation of corticotherapy

For each patient, we collected the doses of prednisone and dexamethasone received as part of conventional therapy and during the posttransplantation period. With these data, the total dose of corticosteroid in equivalent of prednisone received by each patient was calculated with the use of the following formula: total dose of corticosteroids = dose of prednisone + (dose of dexamethasone × 6.67) in mg/m^2 (Table 1).

Statistical methods

Statistical analysis was performed with SPSS Version 15.0 (SPSS Inc) and SAS Version 9.1 (SAS Institute). Qualitative data were expressed as counts and percentages, and quantitative variables were expressed as mean ± SEM. In the univariate analysis, χ^2 and Fisher exact tests were used to compare qualitative variables, whereas quantitative variables were compared with the Student test, the Mann-Whitney test, or ANOVA.

The effect of patient, disease, and treatment-related variables on BMD Z-scores was evaluated in the whole population and also separately for patients who received HSCT ("HSCT group") or not ("chemotherapy group"). Potential confounding factors were the following: sex, initial diagnosis (ALL or AML), age at diagnosis, follow-up duration, steroid therapy, and HSCT. Age at HSCT, type of graft, use of total body irradiation (TBI), steroid therapy after transplantation, and transplantation-related complications such as significant graft-versus-host disease (GVHD; ie, grade ≥ 2 acute GVHD or chronic extensive GVHD), hypogonadism, and GHD were evaluated only in the HSCT group. Conversely, CNS radiation was only evaluated in the chemotherapy group because only 2 patients who received a transplant also received CNS radiation. Multiple linear regression was used to construct association models of FN and LS BMD Z-scores (dependent variable) with the different explanatory variables listed earlier. Each model was given with its standardized β coefficient and significance of the association set as $P < .05$.

Results

Eligible patients and comparison between participants and nonparticipants

Among 220 eligible patients, 159 (72.3%) underwent a DXA scan. To evaluate potential bias into the study cohort we compared patient, disease, and treatment-related characteristics among participants (n = 159) and nonparticipants (n = 61, who had not been evaluated by DXA scan at the time of the study). No statistically significant difference was observed between the 159 included patients and the 61 remaining patients for sex, type of leukemia, age at diagnosis, duration of follow-up, type of treatment (chemotherapy alone, chemotherapy and CNS irradiation, chemotherapy and HSCT), and treatment-related late endocrine complications.

When patients who only received a transplant were considered, participants and nonparticipants were not statistically different for age and disease status at transplantation (first complete remission vs more advanced), type of transplantation (allograft vs autograft, donor type), conditioning regimen (TBI or not), and occurrence of GVHD or late endocrine complications.

Studied cohort: clinical characteristics and treatment modalities

Between January 2007 and December 2008, 159 patients were studied (Table 1); 49.7% of the participants were males. The mean age at diagnosis was 8.33 ± 0.38 years, and the mean follow-up duration from diagnosis to DXA scan was 14.66 ± 0.44 years.

One hundred thirty patients (81.8%) were treated for ALL and 29 (18.2%) for AML. Twenty-nine patients (18.2%) had experienced relapse. They were treated according to various French multicentric protocols (ie, French Acute Lymphoblastic Leukaemia, European Organisation for Research and Treatment of Cancer, Leucemie Aigue Myeloblastique Enfant, ELAM), depending on the period of the treatment and the type of leukemia.[22-25] Most of the patients had received corticosteroids (137 patients; 86.2%). Among them, all had received prednisone and 95 had received dexamethasone. The mean total dose of steroid, expressed in mg/m^2, was 4534 ± 229 mg/m^2. Fifty-four of our patients (34%) had received HSCT (HSCT group), whereas 105 patients (66%) had not (chemotherapy group).

In the chemotherapy group, 96 patients (91.4%) had received corticosteroids (mean total dose: 4488 ± 224 mg/m^2). Twenty-eight patients (26.7%) had received a CNS irradiation, and 1 patient had received a testis irradiation on the basis of the underlying disease status and the protocol in use at the time of leukemia treatment. CNS irradiation dose was 18 Gy in 22 cases and 24 Gy in the 6 others.

In the HSCT group the proportion of patients with AML (n = 20; 37%) and the percentage of patients who experienced relapse (n = 25; 46.3%) were higher. Forty-one patients who received a transplant (75.9%) had received corticosteroids (mean total dose: 4622 ± 517 mg/m^2). Steroids were given as part of chemotherapy regimen in 34 patients and after transplantation in 23 patients. One patient had received a testis irradiation before HSCT and 2 others had received cranial irradiation. Mean age at transplantation was 10.4 ± 0.74 years and allograft accounted for 66.7% of the transplantations, distributed as follows: matched related donors (n = 25; 69.4%), matched unrelated donors (n = 4; 11.1%), mismatched related donors (n = 2; 5.6%), and cord blood (n = 5; 13.9%). Two patients had received 2 HSCTs because they had relapsed after the first one. TBI was used as part of the conditioning regimen in 38 of patients who received an allograft or autograft (70.4%) and was administered fractionated, usually as 2 Gy twice daily during 3 days for a total dose of 12 Gy with lung shielding at 8 Gy.

Among allograft recipients 23 (42.6%) had developed GVHD; 20 of them experienced an acute GVHD (16 of a grade ≥ 2) and 13 of them a chronic GVHD (only 1 in an extensive form). Nineteen patients who experienced GVHD required a treatment for it.

Table 1. Patients and treatment characteristics

	All patients (n = 159)	Chemotherapy group (n = 105)	HSCT group (n = 54)	*P*
Patient and disease related characteristics				
Sex				
Female, n (%)	80 (50.3)	57 (54.3)	23 (42.6)	.18
Male, n (%)	79 (49.7)	48 (45.7)	31 (57.4)	
Initial diagnosis				
ALL, n (%)	130 (81.8)	96 (91.4)	34 (63)	< .0001
AML, n (%)	29 (18.2)	9 (8.6)	20 (37)	
Age at diagnosis, y, mean ± SEM	8.33 ± 0.38	8.03 ± 0.44	8.9 ± 0.72	.30
Age at HSCT, y, mean ± SEM			10.4 ± 0.74	NA
Relapse, n (%)	29(18.2)	4 (3.8)	25 (46.3)	<.0001
Follow-up, y, mean ± SEM	14.66 ± 0.44	14.83 ± 0.54	14.34 ± 0.74	.60
Age at DXA scan, y, mean ± SEM	23.05 ± 0.38	22.99 ± 0.47	23.17 ± 0.66	.82
Treatment modalities				
Corticotherapy				
Yes, n (%)	137 (86.2)	96 (91.4)	41 (75.9)	.007
Prednisone, n (%)	137 (86.2)	96 (91.4)	41 (75.9)	.007
Dexamethasone, n (%)	95 (59.7)	70 (66.7)	25 (46.3)	.01
Mean ± SD total dose of steroids, mg/m^2*	4534 ± 229	4488 ± 224	4622 ± 517	.81
Cranial radiation, n (%)	30 (18.9)	28 (26.7)	2 (3.7)	<.0001
TBI, n (%)			38 (70.4)	NA
Type of graft				
Allograft, n (%)			36 (66.7)	NA
Autograft, n (%)			18 (33.3)	
Posttransplantation steroid therapy				
Yes, n (%)			23 (42.6)	NA
Mean ± SD dose, mg/m^2			444 ± 111	NA
Treatment-related complications				
GVH disease, n (%)			23 (42.6)	NA
Acute GVHD, n (%)			20 (37.1)	
Chronic GVHD, n (%)			13 (24.1)	
Significant GVHD, n (%)†			17 (31.5)	
Treatment for GVHD, n (%)			19 (35.2)	
Hypogonadism				
Yes, n (%)	30 (18.9)	2 (1.9)	28 (51.9)	<.0001
Compensated hypogonadism, n (%)	18 (11.3)	1 (0.9)	17 (31.5)	<.999
Uncompensated hypogonadism, n (%)	12 (7.5)	1 (0.9)	11 (20.4)	
GHD, n (%)	6 (3.8)	1 (1)	5 (9.3)	.02

*Mean total dose of steroids is dose of prednisone + (dose of dexamethasone × 6.67) in mg/m^2.
†Significant GVHD indicates acute GVHD grades II, III, or IV or chronic extensive GVHD.

Hormonal status

Thirty patients (18.9%) had hypogonadism, all except 2 after HSCT, and 18 of them received hormonal replacement. Six patients (11.3%) had GHD, all except 1 after HSCT. Only 1 patient received GH replacement therapy.

Bone density measurements

The BMD values of the 2 regions, expressed in Z-scores, are shown in Table 2.

FN BMD. FN BMD was slightly reduced compared with age- and sex-matched normal values in the overall study population (mean FN Z-score, −0.19 ± 0.08) with 5 patients (3.2%) having a low FN BMD for age (ie, Z-score ≤ −2DS). A transplantation history was significantly associated with a lower FN BMD in both univariate analysis (mean FN Z-score, −0.49 ± 0.15 for the HSCT group vs −0.04 ± 0.10 for the chemotherapy group; $P = .009$; Table 3) and multivariate analysis ($P = .006$; Table 4). Female sex was also a significant predictor of low FN BMD in multivariate analysis ($P = .031$ Table 4). We did not detect any influence of other variables such as initial diagnosis, age at diagnosis, follow-up duration, use of corticosteroids, type of steroid, and total steroid dose.

In the chemotherapy group, FN BMD was normal (mean FN Z-score, −0.04 ± 0.10) with no increase in the incidence of low BMD for age (1.9%). None of the studied covariables (including CNS radiation) was found significant. A trend toward a lower FN BMD was observed among older patients at diagnosis ($P = .04$), but this difference did not remain significant anymore in the multivariate analysis (Table 4).

Patients in the HSCT group had a significantly reduced FN BMD (mean FN Z-score, −0.49 ± 0.15) compared with normal values and with patients who did not receive a transplant with a slight increase in patients with a low BMD for age (5.8%). In univariate analysis, female sex and gonadal deficiency were the

Table 2. BMD results at FN and LS

	All patients	Chemotherapy group	HSCT group	*P*
FN Z-score				
Mean ± SEM	−0.19 ± 0.08	−0.04 ± 0.10	−0.49 ± 0.15	.009
n ≤ 2DS (%)	5 (3.2)	2 (1.9)	3 (5.8)	.33
LS Z-score				
Mean ± SEM	−0.37 ± 0.08	−0.39 ± 0.11	−0.33 ± 0.13	.74
n ≤ 2DS (%)	6 (3.8)	5 (4.8)	1 (1.9)	.66

Table 3. Univariate analysis: FN BMD results

	All patients		Chemotherapy group		HSCT group	
	Mean Z-score ± SEM	Univariable *P*	Mean Z-score ± SEM	Univariable *P*	Mean Z-score ± SEM	Univariable *P*
Patient and disease characteristics						
Sex						
Female	−0.34 ± 0.10	.07	−0.13 ± 0.12	.28	−0.87 ± 0.14	.03
Male	−0.03 ± 0.13		0.08 ± 0.16		−0.22 ± 0.23	
Initial diagnosis						
ALL	−0.15 ± 0.09	.33	−0.02 ± 0.10	.71	−0.53 ± 0.19	.78
AML	−0.36 ± 0.19		−0.18 ± 0.31		−0.44 ± 0.25	
Age at diagnosis	NA	.53	NA	.04	NA	.09
Age at HSCT					NA	.09
Follow-up	NA	.46	NA	.30	NA	.79
Treatment modalities						
Corticotherapy						
No	−0.39 ± 0.15	.33	−0.18 ± 0.31	.66	−0.54 ± 0.15	.87
Yes	−0.16 ± 0.09		−0.02 ± 0.10		−0.48 ± 0.20	
Dexamethasone						
No	−0.21 ± 0.13	.87	−0.01 ± 0.15	.88	−0.43 ± 0.22	.66
Yes	−0.18 ± 0.11		−0.05 ± 0.13		−0.57 ± 0.19	
HSCT						
No	−0.04 ± 0.10	.009				
Yes	−0.49 ± 0.15					
TBI						
No					−0.53 ± 0.18	.87
Yes					−0.48 ± 0.20	
Type of graft						
Allograft					−0.41 ± 0.21	.43
Autograft					−0.66 ± 0.20	
Posttransplantation steroid therapy						
No					−0.55 ± 0.14	.69
Yes					−0.41 ± 0.32	
Transplantation-related complications						
Significant GVHD*						
No					−0.37 ± 0.18	.22
Yes					−0.78 ± 0.25	
Hypogonadism						
No					−0.10 ± 0.23	
Compensated					−0.59 ± 0.18	.004
Uncompensated					−1.37 ± 0.26	
GHD						
No					−0.45 ± 0.16	.27
Yes					−1.08 ± 0.42	

*Significant GVHD indicates acute GVHD grades II, III, or IV or chronic extensive GVHD.

2 factors that influenced FN BMD (Table 3). Mean Z-score was −0.87 ± 0.14 for females and −0.22 ± 0.23 for males ($P = .03$). Mean Z-score was −0.88 ± 0.16 for hypogonadic patients compared with −0.10 ± 0.23 for the others ($P = .02$). The negative influence of gonadal deficiency was more important in case of uncompensed gonadal deficiency but was also present in patients receiving hormonal replacement at the time of the evaluation (mean FN Z-score, −1.37 ± 0.26 in patients with uncompensed gonadal deficiency vs −0.59 ± 0.18 in patients with hormone replacement vs −0.10 ± 0.23 in nonhypogonadic patients; $P = .004$).

We did not detect any influence of other variables such as type of graft, TBI, occurrence of GVHD after transplantation steroid therapy or GHD (Table 3). After multivariate analysis, gonadal deficiency was the only factor significantly associated with low FN BMD ($P = .004$; Table 4).

LS BMD. LS BMD was reduced compared with age- and sex-matched normal values in the overall studied population (mean LS Z-score, −0.37 ± 0.08) with a slight increase in low BMD for age (3.8%). We did not find any influence of patient and disease characteristics, treatment modalities, and treatment-related complications on LS BMD in both univariate and multivariate analyses, taking into account the same factors as described earlier (Tables 4 and 5).

In the HSCT group, younger patients at HSCT have a greater LS BMD sequel than the others in univariate analysis ($P = .02$; Table 5), as well as in multivariate analysis, including the same covariates described earlier ($P = .03$; Table 4). Other factors studied did not influence LS BMD in univariate or multivariate analysis in both the HSCT and chemotherapy groups (Tables 4 and 5).

History of fractures

Only 6 fractures were reported: 4 in the HSCT group and 2 in the chemotherapy group. Patients with a history of fracture had a significantly lower FN BMD (FN Z-score, −1.26 ± 0.34 vs

Table 4. Multivariate analysis

	LS		FN	
	β-coefficient	*P*	β-coefficient	*P*
All patients				
Sex	−0.01	.91	0.18	.03
Initial diagnosis	0.05	.78	0.14	.40
Age at diagnosis	0.06	.57	−0.005	.96
Follow-up	0.04	.69	0.06	.56
Corticotherapy	0.19	.23	0.14	.35
HSCT	0.05	.56	−0.24	.006
Chemotherapy group				
Sex	−0.11	.3	0.12	.26
Initial diagnosis	−0.06	.58	−0.04	.67
Age at diagnosis	−0.09	.52	−0.18	.19
Follow-up	0.01	.94	0.01	.94
CNS radiation	0.001	.99	−0.06	.62
HSCT group				
Sex	0.13	.39	0.15	.31
Age at HSCT	0.38	.03	0.3	.07
Follow-up	0.11	.50	0.14	.39
TBI	0.23	.12	0.14	.31
Significant GVHD*	−0.11	.41	−0.15	.25
Hypogonadism	−0.17	.30	−0.32	.04

*Significant GVHD indicates acute GVHD grades II, III, or IV or chronic extensive GVHD.

-0.10 ± 0.09; $P = .008$). No difference in LS BMD was identified (Table 6).

Discussion

Under healthy conditions, BMD increases dramatically during childhood and adolescence until peak bone mass is reached at the beginning of adulthood. Bone mass in young adults is an important determinant of long-term bone health, which correlates with the risk of involutional osteoporotic fractures.[26-28] Our study shows that most survivors of childhood AL do not sustain significant long-term impairment of BMD. However, a subset of patients has lower than expected BMD for age, which may be related to specific aspects of their treatment and its consequences.

Chemotherapy group

Patients who did not receive a transplant have apparently normal FN BMD and a slight reduction of LS BMD. Unlike some prior studies, we were unable to detect any subgroup at risk for BMD involvement in this population.

Twenty years ago, Gilsanz et al[29] used quantitative computed tomography (QCT) to determine BMD in 43 childhood ALL survivors and first reported decreased BMD and severe osteopenia exclusively in the 29 patients who received cranial irradiation. Later, many studies also implicated cranial irradiation as the main risk factor for decreased BMD in survivors of childhood AL.[11-15] Indeed, cranial irradiation has a dose-dependent effect on the hypothalamic-pituitary axis and can induce GHD,[10-12,17] which is known to impair bone accrual.[30-33] Thus, Nussey et al[33] found in a cohort of 39 ALL long-term survivors who had received CNS irradiation a reduced DXA BMD only in the 14 patients who had untreated GHD during the growth period. Our patients were all treated after the early 1980s, within the "modern era" of leukemia therapy. Therefore, they benefited from decreased dose and frequency of cranial irradiation: only 6 of our patients who received CNS irradiation received a 24-Gy dose, and all except 1 patient had normal GH function. This might explain the lack of effect of CNS irradiation on BMD as previously reported by Mandel et al[6] who found normal DXA BMD in 106 patients with ALL who received cranial irradiation of 18 Gy after 1985.

Another potential cause of impaired bone mineralization after childhood AL might be prolonged corticosteroid treatment and high-dose methotrexate. Indeed, the negative effect of corticosteroids on bone metabolism is well known.[34,35] Corticosteroids have been proposed to decrease the lifespan and the activity of osteoblasts and to increase bone resorption. Methotrexate osteopathy has also been largely reported in inflammatory diseases and in malignancies, but it usually is reverted spontaneously when methotrexate was stopped.[13,35-37] Because our patients were treated according to different protocols, our study could not allow us to assess precisely the effect of each individual chemotherapy component on BMD. However, we did not detect a more pronounced BMD involvement in patients with ALL (who received steroids and methotrexate as a part of their treatment) compared with patients with AML in both univariate and multivariate analysis. Similarly, the use of steroids, whatever the type (prednisone only or associated with dexamethasone) did not influence BMD. This is in agreement with several studies in ALL long-term survivors which did not find any influence on BMD of dose[4,6,9,12,17] and type[7,9] (prednisone or dexamethasone) of steroid used.

At the spine, the detected slight reduction of BMD has no clear explanation. It might result from a particularly high sensibility of trabecular bone to metabolic factors (calcium and vitamin D deficiency especially) and from a direct effect of the disease itself. Indeed, the effect of various factors secreted by leukemic cells (osteoblast-inhibiting factor, parathyroid hormone-related peptide) on trabecular bone and the destruction of spongiosa caused by the leukemic infiltration and the repeated expansions of the bone marrow spaces have been reported in AL at diagnosis and during treatment.[1,2,4]

HSCT group

HSCT is now an established therapy for several hematologic malignancies. As the cohort of surviving patients treated with HSCT grows, recognition of long-term transplantation-related complications increases. To our knowledge, the present study is the first one to report DXA measurements at the femur and the spine of > 50 adult patients treated with BMT for childhood AL. Although ~ 25 studies have been published describing the features of bone loss consequent to HSCT in adulthood,[38] reports on BMD after HSCT in childhood remain rare, limited by the small sizes of the studied cohorts and the heterogeneity of age at evaluation.[39-43]

The previously published DXA BMD measurements in long-term survivors of childhood HSCT found a reduced BMD at different sites.[43] Thus, Bhatia et al[39] and Nysom et al[40] reported a mean total body Z-score at −0.5 and −0.54 in 10 and 25 HSC transplant recipients at a mean of 3.3 and 7.5 years after HSCT, respectively. Similarly, Daniels et al[41] found mean Z-scores ranging between −0.7 and −0.9 at FN, LS, and total body in 15 HSC transplant recipients 6.3 years after transplantation, and Kaste et al[42] reported a mean LS Z-score at −0.89 with the use of QCT in 48 HSC transplant recipients 5 years after transplantation. In agreement with these data, we found reduced BMDs 14 years after transplantation. Moreover, as reported in adult cohorts, we found a more preferential femoral bone loss. Interestingly, the incidence of severe osteopenia was finally low with only

Table 5. Univariate analysis: LS BMD results

	All patients		Chemotherapy group		HSCT group	
	Mean Z-score ± SEM	Univariable *P*	Mean Z-score ± SEM	Univariable *P*	Mean Z-score ± SEM	Univariable *P*
Patient and disease characteristics						
Sex						
Female	−0.35 ± 0.11	.89	−0.28 ± 0.13	.26	−0.55 ± 0.19	.16
Male	−0.38 ± 0.13		−0.51 ± 0.17		−0.17 ± 0.18	
Initial diagnosis						
ALL	−0.32 ± 0.09	.27	−0.36 ± 0.11	.47	−0.20 ± 0.16	.24
AML	−0.56 ± 0.22		−0.65 ± 0.52		−0.53 ± 0.24	
Age at diagnosis	NA	.72	NA	.34	NA	.07
Age at HSCT					NA	.02
Follow-up	NA	.93	NA	.57	NA	.49
Treatment modalities						
Corticotherapy						
No	−0.71 ± 0.24	.10	−0.65 ± 0.52	.47	−0.75 ± 0.24	.07
Yes	−0.31 ± 0.09		−0.36 ± 0.11		−0.19 ± 0.15	
Dexamethasone						
No	−0.44 ± 0.15	.45	−0.39 ± 0.23	.97	−0.50 ± 0.19	.16
Yes	−0.31 ± 0.10		−0.38 ± 0.11		−0.12 ± 0.18	
HSCT						
No	−0.39 ± 0.11	.74				
Yes	−0.33 ± 0.13					
TBI						
No					−0.40 ± 0.24	.23
Yes					−0.22 ± 0.16	
Type of graft						
Allograft					−0.36 ± 0.18	.71
Autograft					−0.26 ± 0.20	
Posttransplantation steroid therapy						
No					−0.55 ± 0.14	.69
Yes					−0.41 ± 0.32	
Transplantation-related complications						
Significant GVHD*						
No					−0.26 ± 0.17	.48
Yes					−0.47 ± 0.20	
Hypogonadism						
No					−0.16 ± 0.19	
Compensated					−0.38 ± 0.25	.37
Uncompensated					−0.65 ± 0.27	
GHD						
No					−0.32 ± 0.14	.88
Yes					−0.40 ± 0.32	

*Significant GVHD indicates acute GVHD grades II, III, or IV or chronic extensive GVHD.

3 patients (5.8%) having a Z-score ≤ 2DS at the FN and 1 patients (1.9%) at the LS. Unfortunately, we do not have any information on bone density before transplantation or any serial measurement after transplantation to determine when the decrease in BMD occurred in the posttransplantation period. In adult reports, an early stage of rapid demineralization (within 6-12 months) at all skeletal sites but more pronounced at the femur is followed by an improvement in lumbar BMD, whereas bone loss at the FN persisted longer (48-120 months) and might be irreversible.[38] The underlying mechanism responsible for these differences is unclear.

Table 6. BMD and history of fractures

	Patients with fractures (n = 6)	Patients without fractures (n = 153)	*P*
FN Z-score			
Mean ± SEM	−1.26 ± 0.34	−0.10 ± 0.09	.008
n ≤ 2DS (%)	1 (16.7)	4 (2.8)	.19
LS Z-score			
Mean ± SEM	−0.48 ± 0.29	−0.35 ± 0.09	.77
n ≤ 2DS (%)	0 (0.0)	6 (4.2)	< .999

Bone loss after HSCT is a multifactorial disorder. There is consistent evidence that major risk factors include myeloablative conditioning regimens and their induced hormonal deficiencies, immunosuppressive therapy after HSCT, reduced mobility and sun exposure, reduced calcium intake, and secondary hyperparathyroidism. In addition, altered kidney, liver, and bowel functions (especially because of GVH disease) might result in reduced absorption and abnormal metabolism of calcium and vitamin D.[38] Other potential mechanisms of bone loss after transplantation have been reported. Early decline in the production of growth factors and osteoclast activation by increased systemic or local cytokine production immediately after transplantation might play an important role in early demineralization, but the cytokine production and its influence on bone might decrease with time. Moreover, an alteration in the balance between the receptor activator of the NF-κB and

osteoprotegerin have been reported in various conditions, including HSCT, but the exact contribution of impaired osteoprotegerin production in bone loss after HSCT remains far from clear.[44-46] Finally, the HSCT procedure itself has been reported to cause severe and persistent quantitative and qualitative impairment of the osteoblastic precursors within the stromal stem cell compartment, suggesting that the inability to regenerate a normal osteogenic cell compartment may partly explain the reason for persistent bone damage after HSCT.[36,46]

Sex hormones play a crucial role in the attainment of peak bone mass and in the maintenance of the bone mass in adulthood with estrogens inhibiting osteoclast activity and promoting osteoclast apoptosis and with androgens affecting directly osteoblast differentiation or at least acting on bone after being converted to estrogens by aromatization.[32] Thus, gonadal failure secondary to the myeloablative conditioning regimen has been reported to be one of the main related factors of bone loss after HSCT in adult patients,[44,45,47,48] as well as in 48 patients who received HSCT during childhood for various disorders.[42] According to those previous report, gonadal deficiency was significantly associated with lower FN BMD in our HSCT group in univariate analysis (even more in case of uncompensated gonadal deficiency but also in patients receiving hormonal replacement at the time of the evaluation) and was the only factor significantly associated with low FN BMD after multivariate analysis.

TBI-related GHD is also a potential risk factor of bone loss after HSCT, as previously suggested by Nysom et al.[40] In our study, there was a trend toward lower FN BMD in the patients who experienced GHD after transplantation, but this difference did not reach the threshold of significance, perhaps because of the very low sample size (4 patients).

In addition to the myeloablative conditioning regimen, immunosuppressive agents used after HSCT might also accelerate bone loss. Thus, several studies performed in adult patients have pointed out the relationship between severe GVH disease, its treatment (corticosteroids and cyclosporin A) and more severe bone loss.[45-47,49] Indeed, in addition to the well-known negative effect of corticosteroids on bone, calcineurin inhibitors have been reported to induce accelerated cortical bone loss after solid-organ and BM transplantation with an involvement dependent on the duration of exposure. In our study, patients who experienced significant GVH disease (acute GVHD grade ≥ 2 or chronic extensive GVHD) seemed to have a lower FN BMD than the others, but this difference was not statistically significant. This lack of significance might be explained by the low incidence of chronic extensive GVHD in our cohort (only 1 patient).

In our HSCT group, children who were younger at the time of transplantation had a significant higher risk of low LS BMD during adulthood in both univariate ($P = .02$) and multivariate ($P = .03$) analyses. This finding is consistent with previous data of Bhatia et al[39] who reported a decrease in total body Z-score assessed by DXA scan with decreasing age at transplantation in 10 childhood recipients of a BM transplant. However, this detected reduction of BMD might be related with a more impaired growth in patients who received a transplant at a younger age. Indeed, because DXA scanning is available worldwide, easily reproducible, and a low irradiant, it is recognized to be the standard method to evaluate BMD and to diagnose osteopenia or osteoporosis (according to the WHO criteria), and it has been used in most of the studies in AL survivors. However, its main limitation is that it provides a surfacic and not a volumetric measurement of bone mineral content. Therefore, DXA assessments of BMD do not take into account the thickness of the bone and might be influenced by skeletal size (height). For this reason, reduced bone size after HSCT might partly explain the lower BMD detected in our patients who received a transplant (especially in those who received a transplant at a younger age). QTC, which provides a volumetric assessment of BMD (and therefore avoids the influence of height on BMD measurements) and in addition differentiates trabecular from cortical bone, providing additional information about bone health, is therefore useful in children and has also been used in childhood AL survivors (who are at risk of short stature). Interestingly, the few QTC-based studies found a reduced BMD in high-dose radiated patients,[17,29] GH-deficient patients,[15] and patients who received a transplant[42] identically to DXA-based studies, confirming that the results of these later (and our results) reflect a significant reduction in bone mass rather than misinterpretation because of the limitations imposed by radiologic techniques.

Finally, we reported bone loss after HSCT in both allo- and auto-HSC transplant recipients. In some previous studies, bone loss after allo-HSCT seemed to be greater than after autologous HSCT,[47] probably because of a prolonged and greater cytokine release after transplantation and a more important use of immunosuppressive agents in the allogenic setting. The lack of statistical difference in BMD between our auto- and allo-HSC transplant recipients might be explained by the use of identical conditioning regimens in both types of transplantations and consequently by a same risk of gonadal failure.

In conclusion, 15 years after diagnosis, adult patients treated for childhood AL within the modern era of chemotherapy have a normal FN BMD associated with only a slight reduction in their mean LS BMD. Despite this reduction, no increase in the incidence of low BMD for age was detected at this site; therefore, this reduction might not have clinical consequence. However, our numbers remain too small to state this with confidence.

On the other hand, HSCT recipients with gonadal deficiency have a reduced mean FN Z-score with an increase in low BMD for age at this site (5.8%). However this detected reduction remains small, and its clinical significance is therefore uncertain. It is nevertheless interesting to note that, according to previous reports which showed that low bone density at the FN is the strongest predictor of hip fracture,[50] lower FN Z-score is correlated to occurrence of fractures in our study ($P = .008$).

These findings underscore the importance of bone mass measurements in the HSCT survivors' routine long-term follow-up, as well as the benefit of the early diagnosis and prolonged treatment of medical conditions such as gonadal failure or GHD. Any secondary cause of osteoporosis should also be identified and treated: patients should avoid smoking, limit intake of caffeine and carbonate beverages, assure adequate dietary intake of calcium and vitamin D (ie, to achieve a serum 25-hydroxyvitamin D concentration of ≥ 20 ng/mL), and establish a weight-bearing exercise regimen. Further studies should be conducted to determine whether these interventions could prevent bone loss and reduce the fracture risk in these patients.

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Authorship

Contribution: M.L.M. and G.M. designed the study, reviewed all medical records, analyzed data, and wrote the manuscript; P.A., M.-C.S., and J.B. participated in study design and in data analysis; V.B., M.P., C.G., A.C., N.S., P.C., P.B., and H.C. enrolled patients and revised the manuscript; V.V. performed statistical analysis; and B.P. contributed to data collection.

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