Osteoporos Int (2000) 11:344–353

Osteoporosis International

Original Article

Bone Loss in Long-Term Survivors after Transplantation of Hematopoietic Stem Cells: A Prospective Study

C. Schulte[1], D. W. Beelen[2] and U. W. Schaefer[2], K. Mann[1]

[1]Division of Endocrinology, Department of Medicine, and [2]Department of Bone Marrow Transplantation, University of Essen, Essen, Germany

Abstract. Organ transplantation is associated with relevant bone loss. Bone loss of up to 20% of pretransplant bone mineral density (BMD) values within the first year after kidney, liver, heart and lung transplantation has been reported. Patients undergoing transplantation of hematopoietic stem cells provide an interesting model to study transplantation-induced bone loss, especially because most patients do not have pre-existing bone disease. A longitudinal study was performed in 81 patients undergoing bone marrow or peripheral blood stem cell transplantation. BMD was determined by dual-energy X-ray absorptiometry before transplantation, at discharge from the hospital, and at 6 and 12 months after transplantation in all 81 patients. In 35 patients BMD was re-evaluated 24 months after transplantation. Vitamin D and parathyroid hormone, bone alkaline phosphatase as a marker of bone formation, and N-terminal telopeptide of type I collagen as a marker of bone resorption were assessed before transplantation and in the short-term follow-up 14 and 28 days after transplantation. The majority of patients (72%) showed normal BMD before transplantation. However, lower BMD was observed in patients who had received high-dose cytoreductive chemotherapy before transplantation compared with those who had received no chemotherapy or only hydroxyurea. Despite supplementation with elemental calcium (1000 mg/day) and vitamin D (1000 IU/day), the mean rate of bone loss during the first year was 7.2 ± 6.3% at the lumbar spine, 11.9 ± 8.1% at the femoral neck and 3.8 ± 2.5% at the total body compartment. Evaluation of the pattern of bone loss during the first year demonstrated that the amount of bone loss was largest within the first 40 days after transplantation and small during the second half of the first year after transplantation. The majority of patients showed vitamin D deficiency and secondary hyperparathyroidism. Bone formation was normal before and after transplantation, whereas bone resorption was dramatically increased before and after transplantation. Exposure to glucocorticoids was associated with higher bone loss at spine and femoral neck but not at the total body compartment. Our data demonstrate rapid bone loss in patients undergoing transplantation of hematopoietic stem cells. Bone turnover is characterized by biochemical uncoupling of bone resorption and bone formation, changes interestingly pre-existing before transplantation. The observed alterations in bone mass and metabolism emphasize the importance of clinical trials with antiresorptive agents to prevent and treat post-transplantation osteoporosis in this group of patients.

Keywords: Bone loss; Bone resorption; Organ transplantation, Osteoporosis

Correspondence and offprint requests to: Dr med. C. Schulte, Division of Endocrinology, Department of Internal Medicine, University of Essen, Hufelandstrasse 55, D-45122 Essen, Germany. Tel: +49 201 723 3233. Fax: +49 201 268988. e-mail: claudia.schulte@uni-essen.de

Introduction

Transplantation of hematopoietic stem cells either as bone marrow or peripheral stem cell transplantation [1,2] has dramatically improved the survival prospects for suitable patients with hematological malignancies such as chronic myeloid leukemia [3], acute leukemia [4] and some forms of lymphoma, especially non-Hodgkin lymphoma [5,6]. However, patients have to be treated

with conditioning regimens including high-dose cytoreductive chemotherapy and total-body irradiation before transplantation. Cure can only be achieved at the price of considerable morbidity and mortality, with an increasing number of long-term complications after transplantation such as bone disease with bone loss, osteoporotic fractures [7] and avascular necrosis of bone [8].

Organ transplantation in general is followed by bone loss and increased incidence of fractures [9–11], for example after kidney [12,13], liver [14,15], heart [16–18] and lung transplantation [19,20]. Rapid bone loss with a decrease in spinal bone mineral density (BMD) as high as 6.8 ± 5.6% at 6 months and 8.8 ± 7.0% 18 months after renal transplantation [12] is seen, especially in the first year after transplantation. Besides immobilization after transplantation and the use of immunosuppressive regimens, pre-existing osteoporosis and/or osteomalacia [21,22] are risk factors for occurrence of fractures.

Data on the temporal sequence of bone loss in patients after BMT are sparse [7]. In an effort to elucidate the etiology and pathogenesis of bone loss in these patients, a prospective longitudinal study of BMD and indices of mineral metabolism after transplantation of hematopoietic stem cells was initiated. We report here the analysis of 81 long-term survivors (survival longer than one year) after transplantation of hematopoietic stem cells.

Patients and Methods

Study Population

We consecutively enrolled 170 patients (age range 18–58 years) elected to undergo bone marrow or peripheral blood stem cell transplantation in the Bone Marrow Transplant Department of the University Hospital of Essen between August 1995 and December 1997. Exclusion criteria were disorders known to affect bone and mineral metabolism (primary hyperparathyroidism, multiple myeloma, thyrotoxicosis and serum creatinine above 2.5 mg/dl before transplantation). Bone marrow transplantation (60%) or peripheral blood stem cell transplantation (40%) was performed because of chronic myeloid leukemia (CML) in 48 patients, acute myeloid leukemia (AML) in 18 patients, non-Hodgkin lymphoma (NHL) in 6 patients, acute lymphoblastic leukemia (ALL) in 4 patients, myelodysplastic syndrome in 2 patients and osteomyelofibrosis, osteomyelosclerosis and severe aplastic anemia in 1case each.

During the first year after transplantation 69 patients died and 20 patients were lost during follow-up; thus 81 patients were followed for at least 12 months. Analysis was restricted to these 81 patients. So far 35 of these 81 patients have been reassessed 24 months after transplantation; two patients died during the second year of follow-up.

Study Design

BMD was determined at the lumbar spine, left hip and total body. BMD measurements were done 10–20 days before transplantation, when patients were still outpatients, at discharge from the transplant unit (61 ± 27 days after transplantation), and at 6, 12 and 24 months after transplantation. Blood and urine for biochemical analysis of markers of bone metabolism were taken 10–20 days before transplantation and every 14 days after transplantation until discharge from the transplant unit. A questionnaire about physical activity, diseases predisposing for the development of osteoporosis, family and social history was filled in before transplantation. After transplantation all patients received 1 g of calcium (as the carbonate salt) and 1000 IU vitamin D once per day. Women were treated with estrogen (1.25 mg of estrogen equivalent) and gestagen after transplantation, if not contraindicated.

The hospital ethics committee approved the study protocol and the patients gave informed consent for the procedures.

Transplantation Procedure and Immunosuppressive Regimen

Before transplantation patients received different cytoreductive chemotherapies for their underlying hematologic disease. For the chronic phase of CML standard therapy includes hydroxyurea or busulfan and human interferon-alpha [23], and for AML [3], ALL [24,25] and non-Hodgkin lymphoma [26] various well-known and previously described approaches of induction polychemotherapy. Ninety percent of the patients with CML in chronic phase had received therapy with hydroxyurea until transplantation; patients under combination therapy with interferon-alpha stopped interferon medication when elected to undergo transplantation [27]. These patients are hereafter referred to as the low-toxicity-chemotherapy group.

Patients with CML with excessive blasts and all patients with AML, ALL and NHL had received different combination chemotherapies with higher doses of cytoreductive medication. These patients are hereafter referred to as the high-toxicity-chemotherapy group.

The different standard preparative regimens for conditioning therapy and for graft-versus-host disease (GvHD) prophylaxis have been described in detail previously [2,28]. In the majority of cases fractionated total-body irradiation with a total dose of 10–12 Gy and cyclophosphamide (60 mg/kg per day on two consecutive days) or busulfan (4 mg/kg per day on 4 consecutive days) was part of the conditioning therapy prior to transplantation. Isolation and decontamination was started before conditioning therapy; GvHD prophylaxis with cyclosporin A and methotrexate was started on day –1. Intravenous administration of the graft was performed within 24–48 h after the last course of

cyclophosphamide, transfusing the donor cells (for allogeneic transplantation) or the patient's cells (for autologous transplantation) using either bone marrow cells (BMT) or peripheral blood stem cells (PBSCT). In patients with an HLA-identical related donor without GvHD, cyclosporine was tapered and stopped at day 90 post-transplantation; in patients with an HLA-mismatched or unrelated donor, after 1 year. Patients with acute GvHD were typically treated with cyclosporin A plus corticosteroids, starting with 2 mg/kg body weight and dose escalation or reduction depending on the response of GvHD symptoms. Other immunosuppressive agents used for treatment of acute GvHD were monoclonal antibody OKT3 or antithymocyte globulin. Parenteral nutrition was started on day –1 with glucose 40%, amino acids 10% and was completed by lipids 20% starting on day 5. Water and fat-soluble vitamins supplemented nutrition therapy; 200 IU of ergocalciferol (vitamin D_2) was included. Energy intake was approximately 147 kj/kg body weight per day.

Chronic GvHD, occurring within the first year after transplantation and typically not before day 100, was treated with prednisolone (1–2 mg/kg per day) in combination with cyclosporin A and mycophenolate mofetil (MMF). In patients receiving steroids, the cumulative steroid dose (expressed in prednisolone equivalents) was calculated with the help of the patient's medical record and recorded at discharge and at 6, 12 and 24 months after transplantation. The average steroid dose per day was calculated by dividing the total cumulative steroid dose by the number of days. Other medications such as cyclosporin A and MMF were documented.

Bone Mineral Density Assessment

BMD was measured by Dual-energy X-ray absorptiometry (DXA) (Lunar DPX-L, Madison, WI) at the lumbar spine (second, third and fourth vertebrae) and at the proximal left femur (femoral neck, trochanter, Ward's triangle). A single DXA machine was used for all measurements.

BMD results were expressed as the number of standard deviations from normal values of young healthy sex-matched controls (*T*-score) and from normal values of age- and sex-matched controls (*Z*-score). Normal values were supplied by Lunar Corporation (Madison, WI). These data were obtained from a group of 2588 white women and men age 20–79 years in Europe and the United States. According to the WHO definition [29], normal BMD was defined as a *T*-score > –1 SD, osteopenia as a *T*-score at the spine < –1 and > –2.5 SD and osteoporosis as a *T*-score at the spine <–2.5 SD of the mean of young, healthy controls.

A quality assurance test was performed daily with a standard block of tissue-equivalent material with three bone-simulating chambers of known bone mineral content and weekly with an anthropomorphic spine phantom (Hologic).

Assessment of Total Body Composition

Total body composition was measured by (DXA) (Lunar DPX-L, Madison, WI) as a total-body scan, with the body compartments of fat mass, lean mass (muscle, fluid, connective tissue, etc.) and bone mineral. Data were expressed in kilograms fat mass and kilograms lean mass, percent fat mass being the fat mass related to total body weight in percentage.

Biochemical Assessment of Bone Metabolism

Urine (not the first morning urine) and a venous blood sample were taken between 0800 and 1000 hours. The subjects had no dietary restrictions. Blood was immediately centrifuged, and serum, plasma und urine were stored at –30 °C until assay performance.

Serum concentrations of *25(OH)-vitamin D* (normal range 10–55 ng/ml) were measured by a radioimmunoassay using an antibody with specificity to 25(OH)-vitamin D (Incstar, Stillwater, MN). Plasma concentrations of *parathyroid hormone (PTH (1–84))* (normal range 10–60 ng/ml) were measured by a radioimmunoassay using an antibody with specificity to human parathyroid hormone (Nicols Institute, San Juan Capistrano, CA). Serum concentrations of *bone specific alkaline phosphatase (BAP)* (normal range 8–24 U/l) were measured by an enzyme-linked immunoassay using a monoclonal anti-BAP antibody (Alkphase Metra, Mountain View, CA). The intra- and interassay coefficients of variation (CV) were less than 10%; the sensitivity was 2 U/L.

Urinary concentrations of *N-terminal-telopeptide of type I collagen (NTX)* (normal range 5–60 mM/mM creatinine) were measured by an enzyme-linked immunoassay (Osteomark, Ostex Inc., USA) using a monoclonal antibody directed against the N-telopeptide-to-helix intermolecular cross-linking domain of type I collagen isolated from human urine. This monoclonal antibody does not recognize free cross-links [30]. The intra- and interassay CVs were less than 9%; the sensitivity was 25 nmol/l. Urinary concentration of *free desoxypyridinoline (free D-Pyr)* (normal range 1.5–6.1 mM/mM creatinine) as a marker of bone resorption was measured by an ELISA using a monoclonal antibody with less than 2.5% cross-reactivity with free Pyr (Pyrilinks-D, Metra, Mountain View, CA) and 10% with cross-linked peptides. The intra- and interassay CVs were 8% and 9% respectively; the sensitivity was 25 nmol/l.

Plasma concentrations of *transferrin* (normal values 230–430 mg/dl) were measured by nephelometry using a nephelometer analyzer (Behring, Marburg, Germany). Intra-assay CV was 2.6%, interassay CV, 2.7%.

All ELISAs were performed on a 96-well plate reader. All data obtained from urinary assays were standardized to the urinary creatinine concentration measured by a standard colorimetric method (Boehringer, Mannheim, Germany).

Statistical Analysis

Data are presented as mean ± 1 standard deviation of the mean. Statistical analysis was carried out using the JMP program (SAS Institute, Cary, NC).

For the transformation of BMD changes in annualized data, differences between consecutive values were transformed to annualized rates by subtracting the given value from the previous value and dividing by the number of days between measurements and multiplying by 365 days. Student's grouped *t*-test was used to compare differences between groups, and the paired *t*-test was used to compare changes after transplantation. Multiple linear regression analysis was used to evaluate the relationships between the variables.

Results

Study Population

Patient characteristics of the 81 patients who were followed for at least 1 year after transplantation (see Patients and Methods) are summarized as follows: length of the hospital stay was 48 ± 22 days, age was 37 ± 10 years (range 18–55 years), body mass index was 25.5 ± 4 kg/m^2, 59% of patients were male, 41% were female. Eighty percent of the patients received the most frequently used conditioning therapy including total-body irradiation plus cyclophosphamide. Analysis of the two large populations of 18 patients with AML and 48 patients with CML revealed no difference concerning length of hospital stay, age, sex, type of conditioning therapy, irradiation dose, HLA match, grip strength and body mass index. All patients with AML, but only 2 patients with CML, had received high-toxicity chemotherapy. Transferrin levels before transplantation were significantly lower in AML compared with CML patients (232 ± 35 vs.329 ± 44 mg/dl; $p < 0.05$). This phenomenon is probably related to the different chemotherapy regimens preceding AML and CML.

Bone Mineral Density Before Transplantation

Mean pretransplant BMD, expressed in grams per square centimeter and *T*- and *Z*-scores, was normal before transplantation at all sites of measurement (Table 1). As summarized in Fig. 1a, 72% of the patients showed normal pretransplant BMD, 24% osteopenia and 4% osteoporosis at the lumbar spine. The mean lumbar spine *T*-score (men vs women: –0.3 ± 1.5 vs –0.2 ± 1.2, NS) and femoral neck *T*-score (men vs women: 0.3 ± 1.3 vs 0.1 ± 1.2, NS) were comparable in men and women. The mean spinal and femoral *T*-scores were higher in CML patients (*T*-score spine: –0.2 ± 1.3; *T*-score femoral neck: 0.3 ± 1.1) compared with AML (*T*-score spine: –0.4 ± 1.6; *T*-score femoral neck: 0.1 ± 1.6) or NHL patients (*T*-score spine: –0.9 ± 1.1; *T*-score femoral neck: –0.8 ± 1.1). The difference reached significance at the lumbar spine and femoral neck between CML and NHL ($p < 0.05$). The low-toxicity-chemotherapy group had a higher mean BMD than the high-toxicity-chemotherapy group at the femoral neck (*T*-score: 0.5 ± 1.0 vs –0.3 ± 1.3, $p = 0.02$) and Ward's triangle (0.7 ± 1.2 vs –0.2 ± 1.3, $p = 0.02$), but not at the lumbar spine, total body and BMC.

Table 1. Follow-up of bone mineral density and anthropometric data before and after transplantation of hematopoietic stem cells

	Before TX	Discharge after TX	6 months after TX	12 months after TX	24 months after TX
No. of patients	81	75	77	78	35
BMD spine (g/cm^2)	1.2 ± 0.1 (**)	1.1 ± 0.1 (††)	1.1 ± 0.1 (NS)	1.1 ± 0.1	1 ± 0.4
T-score spine	–0.2 ± 1.1	–0.6 ± 1.1	–0.9 ± 1.2	–0.9 ± 1.2	–1 ± 1.1
BMD femoral neck (g/cm^2)	1.1 ± 0.1 (**)	1.0 ± 0.2 (††)	0.9 ± 0.2 (NS)	0.9 ± 0.2	0.9 ± 0.2
T-score femoral neck	0.2 ± 1.2	–0.5 ± 1.4	–0.9 ± 1.4	–1.1 ± 1.3	–1.4 ± 1.2
BMD Ward's triangle (g/cm^2)	1.0 ± 0.2 (**)	0.9 ± 0.2 (††)	0.8 ± 0.2 (NS)	0.8 ± 0.2	0.8 ± 0.2
T-score Ward's triangle	0.4 ± 1.4	–0.2 ± 1.2	–0.7 ± 1.5	–1 ± 1.4	–1.4 ± 1.5
BMD trochanter (g/cm^2)	1.0 ± 1.8 (**)	0.9 ± 0.2 (††)	0.8 ± 0.2 (NS)	0.8 ± 0.1	0.7 ± 0.1
T-score trochanter	0.8 ± 1.4	0.1 ± 1.3	–0.4 ± 1.2	–0.5 ± 1.1	–1.3 ± 1.3
BMC (mg)	3139 ± 595	3007 ± 575	2982 ± 534	2955 ± 560	2749 ± 557
BMD total body (g/cm^2)	1.2 ± 0.1 (*)	1.2 ± 0.1 (NS)	1.2 ± 0.1 (§)	1.2 ± 0.1	1.1 ± 0.1
T-score total body	0.6 ± 1.1	0.3 ± 1.2	0.3 ± 1.2	0 ± 1.1	–0.4 ± 1.0
Total body (kg)	74.3 ± 14.7	68.1 ± 11.7	73 ± 13.3	72.6 ± 12.9	66.8 ± 12.5
Fat (kg)	24.7 ± 14.8	24.6 ± 8.3	26.9 ± 9.1	24.8 ± 8.3	21.9 ± 7
Muscle (kg)	51.1 ± 12	44.6 ± 9.4	45.4 ± 9.5	47.3 ± 9.8	44.8 ± 9
BMI (kg/m^2)	25.5 ± 4	23.5 ± 3.4	25 ± 3.9	24.8 ± 3.5	23.4 ± 3.4

BMD results are presented in absolute numbers (g/cm^2) and *T*-scores (± 1 standard deviation).
TX, transplantation.
P-values are given in parentheses: **, †† = $p < 0.001$; *,†,§= $p < 0.01$.
*for the differences between the pretransplant BMD result and the BMD result at discharge.
†for the differences between the BMD result at discharge and the BMD result 6 months after transplantation.
§for the difference between the BMD result 6 months and 12 months after transplantation.

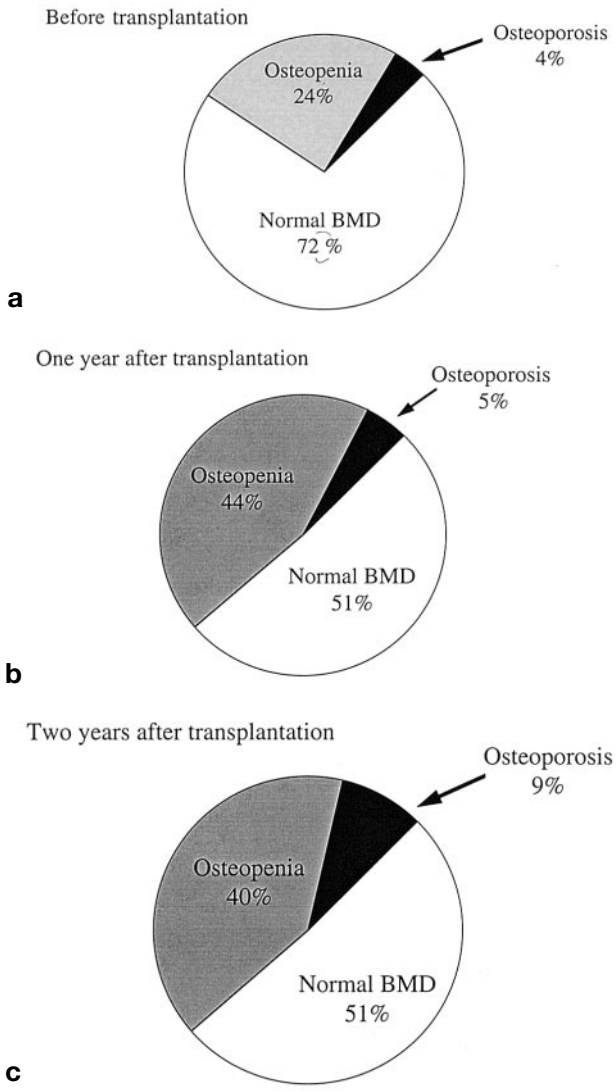

Fig. 1. Prevalence of spinal osteoporosis before transplantation (**a**), and 1 year (**b**) and 2 years (**c**) after transplantation. Definition of spinal osteoporosis according to the WHO definition [29]: Normal BMD, >–1 SD compared with young, healthy controls; osteopenia, <–1 >–2.5 SD compared with young, healthy controls; osteoporosis, <–2.5 SD compared with young, healthy controls.

Indices of Bone Turnover before Transplantation

Data for markers of bone turnover are summarized in Fig. 2. Vitamin D levels were in the lower normal range (11 ± 9 ng/ml) and parathyroid hormone levels in the upper normal range (45 ± 21 ng/ml), indicating pre-existing vitamin D deficiency with consecutive secondary hyperparathyroidism. BAP as a marker of bone formation was within normal limits (16 ± 8 U/l), while the markers of bone resorption NTX (151 ± 100 mM/mM creatinine) and DPD (13 ± 8 mM/mM creatinine) showed a dramatic increase before the transplantation procedure had even started. The high-toxicity-chemotherapy and low-toxicity-chemotherapy groups did not differ in pretransplant vitamin D levels (10.8 ± 11.2 vs

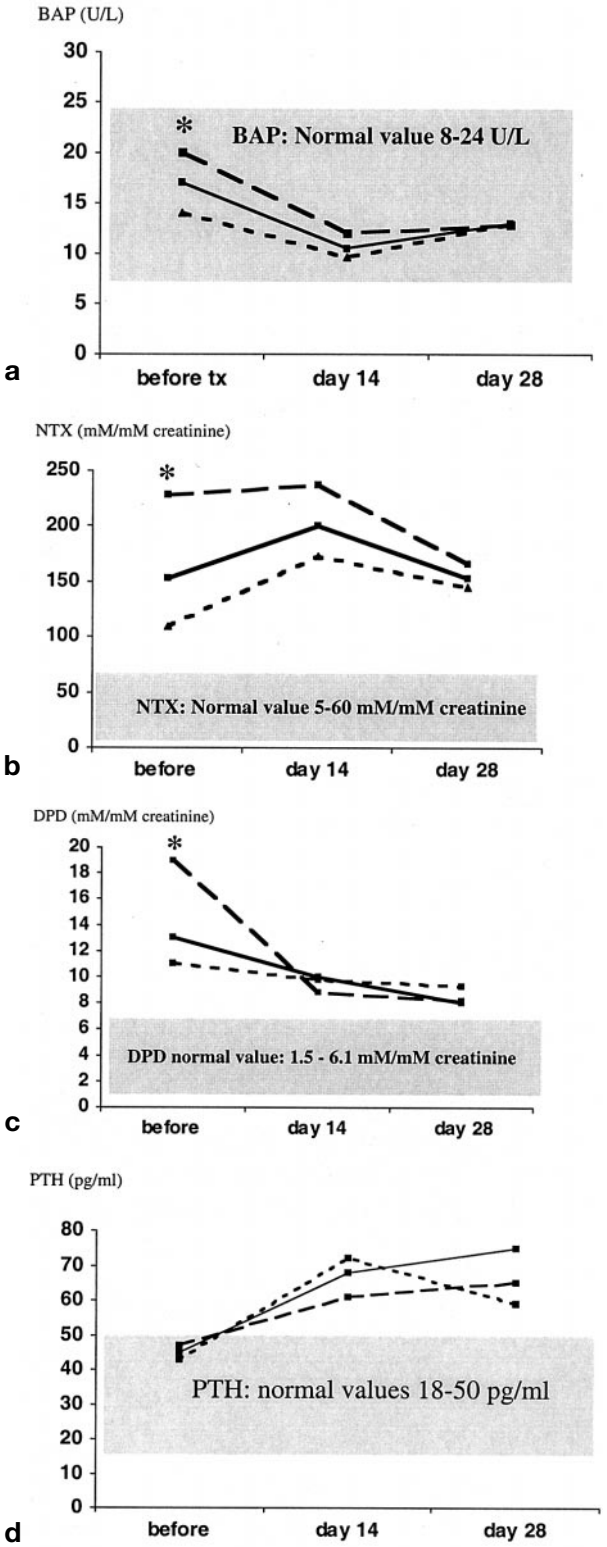

Fig. 2. a–d. Markers of bone metabolism during short-term follow-up after transplantation. **a** N-telopeptide of type I collagen (NTX). **b** Free desoxypyridinoline (DPD). **c** Bone alkaline phosphatase (BAP). **d** Parathyroid hormone (PTH). Before transplantation bone resorption and bone formation were significantly (*p <–0.01) more increased in patients who had received high-dose chemotherapy (*broken lines*) compared with patients who had received low-dose chemotherapy (*dotted lines*). Data for all patients are shown *as continuous lines.*

12.5 ± 10.4 ng/ml) or parathyroid hormone levels (45 ± 23 vs 43 ± 22 ng/ml), but did differ in levels of BAP (20.3 ± 11 vs 14.1 ± 3.9 U/l, $p = 0.01$), NTX (181 ± 110 vs 109 ± 68 mM/mM creatinine, $p = 0.01$) and DPD (17.7 ± 9 vs 11.4 ± 5 mM/mM creatinine, $p = 0.01$). In summary, patients in the high-toxicity-chemotherapy group showed a more pronounced increase in bone resorption compared with bone formation.

Indices of Bone Turnover after Transplantation

As described above, pretransplant bone metabolism was characterized by severely unbalanced bone turnover, with a significant rise in bone resorption compared with bone formation. Fig. 2 a–d summarize the assessment of bone metabolism in the short term after transplantation. While BAP as a bone formation marker (Fig. 2a) ranged within normal limits, NTX and DPD as bone resorption markers (Fig, 2b, 2c) were increased far above normal limits at all points in time. Bone resorption increased to its highest levels 14 days after transplantation, while bone formation fell to its lowest levels, but there was no statistical significance for the time dependent changes in any marker.

25(OH)-vitamin D levels decreased (day 28: 8 ± 3 ng/ml), although all patients received vitamin D supplementation with their parenteral nutrition (200 IU vitamin D_2 every day). With decreasing vitamin D levels parathyroid hormone levels increased, highest levels being found at day 14 after transplantation (Fig. 2d). Sixty percent of all patients showed parathyroid hormone levels above normal (>60 pg/ml) at day 14 after transplantation.

Bone Loss after Transplantation

BMD was determined again at discharge from the transplant unit, at 6 and 12 months after transplantation in all 81 patients and at 24 months after transplantation in 35 patients. Absolute BMD values are summarized in Table 1; rates of bone loss during the first and second years are presented in Figs 3 and 4. In the 35 patients observed for 2 years BMD showed dramatic changes during the first year compared with the second year (Fig. 3). Therefore the pattern of bone loss within in the first year was analyzed in greater detail in all 81 patients observed for at least 1 year. Figure 4 shows the cumulative amount of bone loss compared with baseline at discharge from the hospital and at 6 and 12 months after transplantation. Bone loss at the spine and femoral neck, the regions with higher amount of trabecular bone and higher metabolic activity, was more pronounced early after transplantation, while relevant bone loss at the total-body compartment still occurred during the second half-year after transplantation.

Comparing three time intervals during first year (interval I, between baseline and discharge from the hospital; interval II, between discharge from the hospital and month 6 after transplantation; interval III, between month 6 to month 12 after transplantation), the differences regarding bone loss between time interval I and III were highly significant ($p < 0.0001$) at all sites of measurement. The differences between time interval II and interval I were significant at the spine ($p = 0.0001$) and femoral neck ($p = 0.0007$) but not at the total body. The differences between interval II and III were significant at the total body ($p = 0.0001$) but not at the spine and femoral neck.

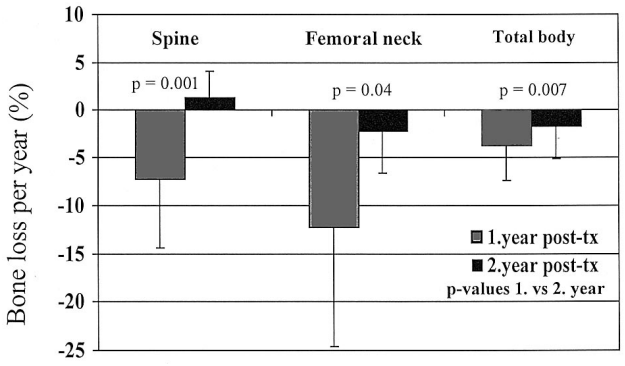

Fig. 3. Bone loss at different sites of measurement 1 and 2 years after transplantation. Analysis of data in the 35 patients observed 2 years after transplantation showed that bone loss was significantly higher during the first than during the second year at all sites of measurement (see *p*-values within the figure). Bone loss during the first year was significantly higher at the femoral neck than at the spine and the total body (spine vs femoral neck, $p = 0.0001$; spine vs total body, $p = 0.0001$; total body vs femoral neck, $p = 0.0001$). Bone loss during the second year was significantly lower at the spine than at the femoral neck ($p = 0.05$) and at the total body ($p = 0.006$).

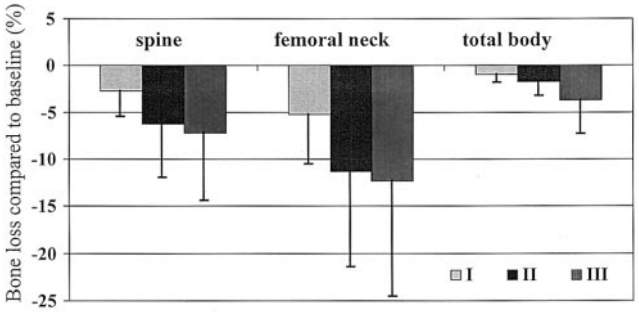

Fig. 4. Sequential bone loss during first year after transplantation. *I*, cumulative bone loss from baseline until discharge from the transplant unit; *II* cumulative bone loss from baseline until month 6 after transplantation; *III* cumulative bone loss from baseline until month 12 after transplantation.

Influence of Corticosteroids on BMD and Bone Metabolism on Bone Loss

Corticosteroid use is always described as associated with bone loss after organ transplantation, but we could not show a clear dose–effect relationship between bone loss

after transplantation and the cumulative steroid dose applied. This was true at all points of time – at discharge as well as 6 months and 12 months after BMT.

During the first year after transplantation, only 10 patients had not received any steroids, while 71 patients had received steroids with an average dose of 34 ± 25 mg prednisolone equivalent per day. The difference in bone loss between steroid and no-steroid patients was significant for the spine (–1.1 ± 6.3% vs –8.1 ± 5.9%, $p = 0.0027$) and the femoral neck (–5.4 ± 7% vs –13 ± 9%, $p = 0.05$), but not for the total-body compartment (–3.3 ± 2.7% vs –3.8 ± 2.5 %, NS).

During the second year after transplantation, 7 of the 35 patients reassessed after 2 years had not received steroids, while 28 patients had received steroids with an average dose of 12 ± 10 mg/day. The difference between steroid and no-steroid patients did not reach statistical significance at either the spine (–0.76 ± 6.6% vs –5.1 ± 4.8%, NS), the femoral neck (–3.9 ± 4% vs –0.19 ± 2.8%, NS) or the total-body compartment (–2.11 ± 3.2% vs –0.44 ± 1.1%, NS).

Markers of bone metabolism before and at day 14 and 28 after transplantation did not differ between steroid and non-steroid groups. Even if the small number of patients who did not use steroids affected the results, the data emphasize that the disturbance in bone metabolism must be influenced by other factors besides corticosteroids.

Influence of Type of Transplantation on Bone Loss

Of the 81 patients, 5 (6%) were managed with autologous transplantation, 21 patients received a transplant from a nonrelated donor, 17 (21%) from a donor with HLA identity, and 4 (5%) from a donor with HLA mismatch; 55 patients received the transplant from a related donor, 45 (55%) with HLA identity and 10 (12%) with HLA mismatch.

The comparison of the 5 patients who had received an autologous transplant (no steroids during the first year after transplantation) and the 76 patients who had received an allogeneic transplant (mean daily steroid dose of 32 ± 25 mg/day) revealed significant differences in bone loss during the first year after transplantation at the spine and femoral neck. The post-autologous group lost significantly less bone mass than the post-allogeneic group at the spine (–0.83 ± 5%/year vs –7.6 ± 6.2%/year, $p = 0.02$), at the femoral neck (–0.49 ± 2.9 %/year vs –13 ± 9.3 %/year, $p = 0.02$) and at Ward's triangle (0.97 ± 3.5%/year vs –8.5 ± 5.56 %/year, $p = 0.006$), but not at the total body (–4.1 ± 2 %/year vs –3.66 ± 2.5%/year), $p = 0.034$).

Comparison of the 21 patients with un-related donors and the 53 patients with related donors (mean daily steroid dose 41 ± 28 mg/day vs 28 ± 24 mg/day, $p = 0.06$) revealed no significant difference in bone loss during first year after transplantation at any site.

Identification of a Risk Profile for High Bone Loss in the Different Time Periods

To further analyze the specific risk factors for high bone loss in the different time intervals, we compared bone loss in relation to age, sex and the type of chemotherapy before transplantation. There was no correlation between age and amount of bone loss at any site of measurement or time interval. Bone loss did not show gender-dependent loss at any site of measurement or time interval. Bone loss in high-toxicity-chemotherapy patients did not differ from that in the low-toxicity-chemotherapy at any site of measurement or time interval.

Occurrence of Fractures and Aseptic Bone Necrosis During Time of Observation

One patient suffered from a vertebral fracture 18 months after transplantation. The patient was male, and the fracture occurred at age 20 years; he had received 43 g of prednisolone equivalent during the first year and 5.8 g during the second year after transplantation.

Three patients suffered from aseptic bone necrosis. Patient 1 (female, 38 years, cumulative prednisolone during first year 8.5 g, during second year 0 g), had necrosis of the right hip 20 months after transplantation receiving a total endoprosthesis; in patient 2 (female, 27 years, cumulative prednisolone during first year 12 g, during second year 1.6 g) a hip necrosis occurred 5 months after transplantation and in patient 3 (male, 34 years, cumulative prednisolone during first year 5.6 g, during second year 5 g) necrosis of the knee occurred 4 months after transplantation.

Discussion

This prospective study in hematopoietic stem cell recipients documents that the hematologic disorders treated by transplantation of hematopoietic stem cells themselves are associated with substantial bone loss and increased bone turnover. Pretransplant BMD values differed according to the underlying disease, with lower BMD in patients with acute leukemias and NHL compared with CML, and there was a dramatic increase in bone resorption seen before transplantation. The post-transplant period was characterized by rapid bone loss, which did not differ between men and women or old and young, and occurred despite supplementation with vitamin D and calcium in all patients and estrogen in most women. Bone loss was lower in the small subgroup of 5 patients after autologous transplantation compared with allogeneic transplantation. The temporal pattern of bone loss showed a dramatic bone loss preferentially during the first 2 months after transplantation and little bone loss later than 6 months after transplantation. Rapid bone loss during first post-transplant year was most pronounced at the level of spine and femoral neck (sites

with a predominance of trabecular bone) and significantly lower at the total body, characterizing spine and hip as the rapid-response metabolic bone compartment. During the second year after transplantation partial recovery of spinal bone mass was seen while femoral and total-body bone mass continued to decrease.

There are only sparse data about the incidence of osteoporosis after BMT [7,31,32] and the temporal sequence of bone loss after transplantation of hematopoietic stem cells [7]. Kelly et al. [31] and Bhatia et al. [32] demonstrated in cross-sectional studies a small collective reduction in bone mass after BMT. Ebeling et al. [7] provided cross-sectional data on 83 patients and furthermore prospective data in 39 patients followed for a median of 30 months after transplantation. In their study post-allogenic BMT bone loss correlated best with the cumulative prednisolone dose, with a rate of bone loss of 4% per 10 g prednisolone at the spine and 9% per 10 g prednisolone at the femoral neck.

The observation that bone loss after transplantation is greatest during the first year and especially the first months after transplantation is in general agreement with the findings after heart [16,17,33], kidney [12] and liver [34] transplantation. We also demonstrated partial reversibility of the process at the spine, but not at the other compartments, during the second year after transplantation – a phenomenon which has also been observed in other transplant situations [35,36].

Bone metabolism in the pretransplant and early post-transplant period was characterized by vitamin D deficiency, a slight increase in parathyroid hormone in response to the falling vitamin D levels, normal bone formation and a marked increase in bone resorption. The observed pattern of bone metabolism differs from data after other organ transplantation with regard to the amount and temporal sequence of changes. Firstly, such excessive increase in bone resorption has not been observed after other types of transplantation. This increase in bone resorption is not a result of cyclosporine medication, which is not in use for the first weeks after transplantation, or steroids, which do not influence bone resorption to such an intense degree. Therefore we have to hypothesize that the dramatic increase in bone resorption is the result of the acute catabolic reaction to the tremendous amount of toxins applied. Secondly, the dramatic increase in bone resorption was not only observed after transplantation, but already at baseline. The pretransplant difference in levels of bone resorption markers between the low- and the high-toxicity chemotherapy groups disappeared rapidly after transplantation. We hypothesize that patients with low-toxicity chemotherapy go through a phase of rapid bone loss with highly toxic conditioning therapy as part of the transplantation procedure, while patients after high-toxicity chemotherapy had already gone through this phase of rapid bone loss with chemotherapy. Further studies need to be performed to determine the influence of chemotherapy itself on bone metabolism and bone loss. Bone formation is influenced in different directions by the different immunosuppressants, with an increase under the influence of cyclosporin A and a decrease under steroids. The minor changes in bone alkaline phosphatase observed do not allow an interpretation of the relevance of different influences. Rises in parathyroid hormone levels are partly a result of the vitamin D deficiency, but further studies are needed to illuminate in detail the regulation of parathyroid hormone release in the situation of acute illness.

Osteoporosis after organ transplantation is often summarized as a steroid-induced bone loss with consecutive osteoporosis. While the association between steroid therapy, bone loss and vertebral fractures seems to be well established [11,37–39], it is less clear whether the steroid medication itself or the underlying disease treated by steroids is the reason for bone loss. Bone loss may be due to coexisting independent factors that influence BMD, such as the severity of the disease [40,41] and the inflammatory process being treated [42]. In many studies prospectively following bone loss under influence of steroids, a clear relationship between the applied steroid dose and the amount of bone loss is missing, e.g., in patients with inflammatory bowel disease [43–45]. This also proves to be true in organ transplantation, in which a clear dose –effect relationship is lacking [19,46]. Our data revealed a significant difference in the amount of bone loss between patients with and without glucocorticoid exposure only at the lumbar spine and femoral neck during the first year, but not at the total-body compartment.

Although bone loss is seen associated with the transplantation, multiple treatment conditions to which patients are subjected shortly after transplantation could not be proven as predictors for bone loss and increased bone turnover. In liver transplantation, menopause, cholestatic liver disease [34], duration of immobilization after transplantation [46] and chronic alcohol abuse were named as the principal contributing factors for osteoporosis. In heart transplant recipients, Sambrook et al. [17] found osteocalcin to be the only predictor of lumbar spine bone loss, while age, corticosteroid and cyclosporin A dose, lean body mass and body mass index were not suitable as predictors of bone loss. Ebeling et al. [7] showed that post-allogenic BMT bone loss correlated best with the cumulative prednisolone dose (4% per 10 g prednisolone at the spine and 9% per 10 g prednisolone at the femoral neck). In our series neither sex, age, body mass index before or after transplantation, loss of muscle or fat mass nor cumulative corticosteroid dose were useful predictors for the amount of bone loss. The only factor favorably influencing bone loss was receipt of an autologous transplant. Thus, there is as yet no established risk panel for bone loss after transplantation of hematopoietic stem cells, but the rapid bone loss urges the establishment of therapeutic and preventive strategies for these patients. Preventing bone loss associated with transplantation rather than treating osteoporosis is more appealing because the increased risk of fracture can be avoided and the microarchitecture of bone can be preserved. The supplementation of transplant patients with calcium and genuine vitamin D is accepted, but our

data confirm previous studies showing that while bone loss can be slowed it is not stopped [18]. Treatment with calcitonin in addition to calcium supplementation could also not prevent bone loss after allogeneic bone marrow transplantation [47]. Bisphosphonates are especially useful for preventing bone loss in situations with biochemical proof of increased bone resorption such as the perimenopause [48,49] and use of corticosteroids [50,51]. Those patients with the highest increase in bone resorption suffer most from bone loss without therapy; however, these patients show best response to therapy with antiresorptive agents [52]. Further studies need to be performed to determine the role of early intervention with highly potent bisphosphonates after transplantation to prevent rapid bone loss after transplantation. Such therapy should consequently preserve the normal microarchitecture of bone and reduce long-term fracture risk in these patients.

Acknowledgement. This study was supported by a research grant from the University of Essen (IFORES 1996 to C.S.).

References

1. Runde V, Schaefer UW. Stellenwert der Transplantation hämatopoetischer Stammzellen in der Behandlung von Leukämien. Internist 1996;37:1030–9.
2. Schaefer UW, Beelen DW. Bone marrow transplantation. Freiburg: Karger, 1996.
3. Savage DG, Goldman JM. Allografting for chronic myeloid leukemia. Curr Opin Hematol 1997;4:369–76.
4. Zittoun RA, Mandelli F, Willemze R, de Witte T, Labar B, Resegotti L, et al. Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Leukemia Cooperative Groups. N Engl J Med 1995;332:217–23.
5. Dann EF, Daugerthy CK, Larson RA. Allogeneic bone marrow transplantation for relapsed and refractory Hodgkin's disease and non-Hodgkin's lymphoma. Bone Marrow Transplant 1997;20:369–74.
6. Verdonck LF, Dekker AW, Lokhorst HM, Petersen EJ, Nieuwenhuis HK. Allogeneic versus autologous bone marrow transplantation for refractory and recurrent low grade non-Hodgkin's lymphoma. Blood 1997;90:4201–5.
7. Ebeling PR, Thomas DM, Erbas B, Hopper JL, Szer J, Grigg AP. Mechanisms of bone loss following allogeneic and autologous hematopoietic stem cell transplantation. J Bone Miner Res 1999;14:342–50.
8. Socié G, Cahn JY, Carmelo J, Vernant JP, Jouet JP, Ifrah N, et al. Avascular necrosis of bone after allogeneic bone marrow transplantation: analysis of risk factors for 4388 patients by the Société Francaise de Greffe de Moelle (SFGM). Br J Haematol 1997;97:865–70.
9. Rodino MA, Shane E. Osteoporosis after organ transplantation. Am J Med 1998;104:459–69.
10. Katz IA, Epstein S. Perspectives: posttransplantation bone disease. J Bone Miner Res 1992;7:123–6.
11. Epstein S, Shane E, Bilezikian JP. Organ transplantation and osteoporosis. Curr Opin Rheumatol 1995;7:255–61.
12. Julian BA, Laskow DA, Dubovsky J, Dubovsky EV, Curtis JJ, Quarles LD. Rapid loss of vertebral density after renal transplantation. N Engl J Med 1991;325:544–50.
13. Grotz WH, Mundinger FA, Gugel B, Exner V, Kirste G, Schollmeyer PJ. Bone fracture and osteodensiometry with dual energy X-ray absorptiometry in kidney transplant recipients. Transplantation 1994;58:912–5.
14. Meys E, Fontanges E, Fourcade N, Thomasson A, Pouyet M, Delmas PD. Bone loss after orthotopic liver transplantation. Am J Med 1994;97:445–50.
15. Hawkins FG, Leon M, Larrodera L, Garcia-Garcia I, Lopez MB, Valero MA, et al. Bone loss and turnover in patients with liver transplantation. Hepato-Gastroenterology 1994;41:158–61.
16. Shane E, Rivas M, McMahon DJ, Staron RB, Silverberg SJ, Seibel MJ, et al. Bone loss and turnover after cardiac transplantation. J Clin Endocrinol Metab 1997;82:1497–506.
17. Sambrook PN, Kelly PJ, Keogh AM, Macdonald P, Spratt P, Freund J, et al. Bone loss after heart transplantation: a prospective study. J Heart Lung Transplant 1994;13:116–21.
18. Cleemput JV, Daenen W, Geusens P, Dequeker J, Werf vd F, Vanhaecke J. Prevention of bone loss in cardiac transplant recipients. Transplantation 1996;61:1495–9.
19. Aris RM, Neuringer IP, Weiner MA, Egan TM, Ontjes D. Severe osteoporosis before and after lung transplantation. Chest 1996;109:1176–83.
20. Aringer M, Kiener HP, Koeller MD, Artemiou O, Zuckermann A, Wieselthaler G, et al. High turnover bone disease following lung transplantation. Bone 1998;23**:**485–8.
21. Hay JE. Bone disease in cholestatic liver disease. Gastroenterology 1995;108:276–83.
22. McCaughan GW, Feller RB. Osteoporosis in chronic liver disease: pathogenesis, risk factors, and management. Dig Dis Sci 1994;12:223–31.
23. Goldman JM. Chronic myeloid leukemia. Curr Opin Hematol 1997;4:277–85.
24. Pui CH, Evans WE. Acute lymphoblastic leukemia. N Engl J Med 1998;339:605–15.
25. Pawson R, Mufti GJ, Pagliuca A. Management of adult T-cell leukemia/lymphoma. Br J Haematol 1998;100:453–8.
26. Davis TA, Horning SJ. Advances in the mangement of Hodkin's and non-Hodgkin's lymphoma. Curr Opin Hematol 1998;5**:**259–63.
27. Beelen DW, Graeven U, Elmaagacli AH, Niederle N, Kloke O, Opalka B, et al. Prolonged administration of interferon-alpha in patients with chronic-phase Philadelphia chromosome-positive chronic myelogenous leukemia before allogeneic bone marrow transplantation may adversely affect transplant outcome. Blood 1995;85:2981–90.
28. Klingebiel T, Schlegel PG. GVHD: overview on pathophysiology, incidence, clinical and biological features. Bone Marrow Transplant 1998;21(Suppl 2)**:**S45–9.
29. Kanis JA, Group WS. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. Osteoporos Int 1994;4:368–81.
30. Hanson DA, Weis MAE, Bollen A-M, Maslan SL, Singer FR, Eyre DR. A specific immunoassay for monitoring human bone resorption: quantitation of type I collagen cross-linked N-telopeptides in urine. J Bone Miner Res 1992;7:1251–8.
31. Kelly PJ, Atkinson K, Ward RL, Sambrook PN, Biggs JC, Eisman JA. Reduced bone mineral density in men and women with allogenic bone marrow transplantation. Transplant 1990;50:881–3.
32. Bhatia S, Ramsay NKC, Weisdorf D, Griffiths H, Robison LL. Bone mineral density in patients undergoing bone marrow transplantation. Bone Marrow Transplantation 1998;22:87-90.
33. Sambrook PN, Kelly PJ, Fontana D, Nguyen T, Keogh A, Macdonald P, et al. Mechanism of rapid bone loss following cardiac transplantation. Osteoporos Int 1994;4:273–6.
34. Porayko MK, Wiesner RH, Krom RAF, Dickson ER, Beaver S, Schwerman L. Bone disease in liver transplant recipients: incidence, timing, and risk factors. Transplant Proc 1991; 23:1462–5.
35. Muchmore JS, Cooper DKC, Schlegel VJ, Zudhi N. Loss of vertebral bone density in heart transplant patients. Transplant Proc 1991;23:1184–5.
36. Henderson NK, Sambrook PN, Kelly PJ. Bone mineral loss and recovery after cardiac transplantation. Lancet 1995;II:905.

37. Hochberg MC. Recommendations for the prevention and treatment of glucocorticoid-induced osteoporosis. Arthritis Rheum 1996;39:1791–801.
38. Eastell R. Management of corticosteroid-induced osteoporosis. J Intern Med 1995;237:439–47.
39. Peat ID, Healy S, Reid DM, Ralston SH. Steroid induced osteoporosis: an opportunity for prevention? Ann Rheum Dis 1995;54:66–8.
40. Hall GM, Spector TD, Delmas PD. Markers of bone metabolism in postmenopausal women with rheumatoid arthritis. Arthritis Rheum 1995;38:902–6.
41. Pearce G, Tabensky A, Delmas PD, Baker HW, Seeman E. Corticosteroid-induced bone loss in men. J Clin Endocrinol Metab 1998;83:801–6.
42. Lin C-L, Moniz C, Chambers TJ, Chow JWM. Colitis causes bone loss in rats through suppression of bone formation. Gastroenterology 1996;111:1263–71.
43. Roux C, Abitbol V, Chaussade S, Kolta S, Guillemant S, Dougados M, et al. Bone loss in patients with inflammatory bowel disease: a prospective study. Osteoporos Int 1995;5:156–60.
44. Bjarnason I, Macpherson A, Mackintosh C, Buxton-Thomas M, Forgacs I, Moniz C. Reduced bone density in patients with bowel disease. Gut 1997;40:228–33.
45. Schulte C, Dignass AU, Mann K, Goebell H. Reduced bone mineral density and unbalanced bone metabolism in patients with inflammatory bowel disease. Inflammatory Bowel Dis 1998; 4:268–75.
46. McDonald JA, Dunstan CR, Dilworth P, Sherbon K, Sheil AGR, Evans RA, et al. Bone loss after liver transplantation. Hepatology 1991;14:613–9.
47. Valimaki MJ, Kimmunen K, Volin L, Tahtela R, Loyttyniemi E, Laitinen K, et al. A prospective study of bone loss and turnover after allogeneic bone marrow transplantation: effect of calcium supplementation with or without calcitonin. Bone Marrow Transplant 1999;23**:**355–61.
48. Hosking D, Chilvers CED, Christiansen C, Ravn P, Wasnich R, Ross P, et al. Prevention of bone loss with alendronate in postmenopausal women under 60 years of age. N Engl J Med 1998;338:485–92.
49. McClung M, Clemmesen B, Daifotis A, Gilchrist NL, Eisman J, Weinstein RS, et al. Alendronate prevents postmenopausal bone loss in women without osteoporosis. Ann Intern Med 1998; 128:253–61.
50. Adachi JD, Bensen WG, Brown J, Hanley D, Hodsman A, Josse R, et al. Intermittent etidronate therapy to prevent corticosteroid-induced osteoporosis. N Engl J Med 1997;337:382–7.
51. Roux C, Oriente P, Laan R, Hughes RA, Ittner J, Goemaere S, et al. Randomized trial of effect of cyclical etidronate in the prevention of corticosteroid-induced bone loss. J Clin Endocrinol Metab 1998;83:1128–33.
52. Rosen CJ, Chesnut CHI, Mallinek NJS. The predictive value of biochemical markers of bone turnover for bone mineral density in early postmenopausal women treated with hormone replacement or calcium supplementation. J Clini Endocrinol Metab 1997; 82:1904–10.

Received for publication 30 March 1999
Accepted in revised form 12 October 1999