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Post-transplant complications

Recovery of bone mass and normalization of bone turnover in long-term survivors of allogeneic bone marrow transplantation

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Summary:

Osteoporotic fractures are potential long-term complications of bone marrow transplantation (BMT). We previously reported that bone mineral density (BMD) of patients undergoing allogeneic BMT decreased by 6% to 9% during the first 6 months after BMT and that bone turnover rate was still increased 1 year after BMT. BMT patients do not need lifelong immunosuppressive treatment, which should offer favorable circumstances for the recovery of BMD. Thus, 27 (14 women, 13 men) of 29 long-term survivors of our previous study were invited to a follow-up study at a median of 75 months after BMT. From 12 months after BMT the BMD of the lumbar spine had increased by 2.4% ($P = 0.002$). The respective changes in femoral sites were +4.1% in the femoral neck ($P = 0.087$), 4.0% in the trochanter ($P = 0.095$), +4.7% in Ward's triangle ($P = 0.072$) and +1.4% in the total hip ($P = 0.23$). The markers of bone formation, serum osteocalcin and type I procollagen aminoterminal propeptide (PINP) had returned to control levels, but out of the markers of bone resorption the mean level of serum type I carboxyterminal telopeptide (ICTP) was 41% higher ($P = 0.0001$) and that of urinary type I collagen N-terminal telopeptide/creatinine (NTx) 41% lower ($P = 0.0002$) in patients than in controls. The mean serum 25-hydroxyvitamin D [25(OH)D] was 33% lower in patients ($P = 0.0002$), most of whom had hypovitaminosis D [serum 25(OH)D $\leqslant$37 nmol/l]. Except for two, males had serum testosterone level lower than before BMT and four men had hypogonadism. In conclusion, in long-term survivors of allogeneic BMT BMD recovers and bone turnover state normalizes as compared to the situation 1 year after BMT. More attention should be paid to the vitamin D status of all recipients and to possible hypogonadism of male patients.
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Osteoporosis and osteoporotic fractures are potential long-term complications of not only solid organ transplants[1] but also of the bone marrow.[2,3] In our prospective study of 44 patients undergoing allogeneic bone marrow transplantation (BMT) bone mineral density (BMD) decreased by 6% in the lumbar spine and by 7% to 9% in the three femoral sites during the first 6 post-transplant months; no significant further decline occurred between 6 and 12 months.[2] Shortly after BMT bone loss was explained by uncoupling between increased bone resorption and decreased bone formation.[2] At the end of 1-year follow-up both resorption and formation markers were elevated, indicating accelerated bone turnover in survivors.[2]

Unlike transplant recipients of solid organs, recipients of hemopoietic transplants do not need lifelong immunosuppression, and glucocorticoids and cyclosporine A can usually be stopped. This fact, together with a relatively young age of BMT recipients offer the bone favourable circumstances to recover, the magnitude of which has not been addressed in previous studies. Thus, we invited the long-term survivors of our original patient population to a follow-up study, in which their BMD and bone turnover state were re-evaluated at a median of 6 years after BMT. The values were compared to those obtained at the end of the previous 1-year follow-up, and biochemical data were compared to those produced by sex-matched controls. The vitamin D status of the patients was also evaluated.

Patients and methods

Patients

Twenty-nine of 44 patients who had received an allogeneic bone marrow transplant and taken part in our previous study were still alive and were invited to the follow-up study. Twenty-seven (13 men and 14 women) agreed to participate. Before bone marrow transplantation (BMT) patients had been conditioned with cyclophosphamide (CY)

60 mg/kg body weight (BW) intravenously on 2 consecutive days, and with total body irradiation 12 Gy (lungs 10 Gy) in six fractions of 2 Gy over 5 days; two patients received busulfan 4 mg/kg BW daily for 4 days and then CY as above. Patients used cyclosporine A (CsA) and methylprednisolone (MP) for prevention and treatment of graft-versus-host disease (GVHD). CsA was started the day before BMT at a dose of 3 mg/kg BW intravenously daily, switched 2 to 3 weeks later to an oral dose of 3–4 mg/kg BW daily which was continued for 1 year after BMT. Thereafter, CsA was tapered off over a couple of months. MP was started orally 14 days after BMT at a dose of 0.5 mg/kg BW for a week, then the dose was doubled for 2 weeks, and thereafter halved every third week and stopped by day +110 after BMT. Acute GVHD was treated with MP starting with a dose of 10 mg/kg BW. The daily dose of MP was halved every third day until the dose was approximately 1 mg/kg BW and it was thereafter tapered off individually. Chronic GVHD was treated with a low daily dose of MP alone or in combination with CsA or thalidomide.

Study design

Patients came to the follow-up study after an overnight fast. Blood was sampled for the determination of serum ionized calcium, creatinine, type I procollagen aminoterminal propeptide (PINP), type I collagen carboxyterminal telopeptide (ICTP), osteocalcin (Osca), 25-hydroxyvitamin D [25(OH)D], and testosterone in men and estradiol in women. Urine specimens were collected as a 2-h second morning void for the determination of type I collagen N-terminal telopeptide /creatinine (NTx). BMD of the lumbar spine and the femoral sites was measured. X-rays of the spine were taken to evaluate vertebral fractures. The results were compared to the values of the same 27 patients in our previous study, in which BMD was measured before and 6 and 12 months after BMT and the biochemical evaluation was performed before and 1 week, 3 weeks, 6 weeks, 3 months, 6 months and 12 months after BMT. In comparison, a group of 28 healthy sex-matched persons was studied for biochemical parameters. The study was approved by the Ethics Committee of the Department of Medicine, University of Helsinki.

BMD measurement

BMD of the lumbar spine (lumbar vertebrae L1–L4) and of the femoral sites (femoral neck, trochanter, Ward's triangle and total hip) was measured by dual energy X-ray absorptiometry (DXA) using a Hologic QDR-1000 densitometer (Hologic, Waltham, MA, USA). Precision of the method (coefficient of variation) was 0.9% at the lumbar spine, and 1.2% at the femoral neck. Bone density was expressed as grams per cm^2 and as standardized T score analysis, which compares individual bone density determinations to those of a young, normal population of the same gender. According to the criteria defined by the WHO, T scores more than 2.5 s.d. below the mean of the young, normal population represent osteoporosis, whereas T scores between −1.0 and −2.5 represent osteopenia.[4] The manufacturer's database was used in calculations of T scores.

Assays

Intact PINP and ICTP were determined by RIAs (Orion Diagnostica, Oulunsalo, Finland). The intra- and interassay CVs for these assays ranged from 2 to 9%. Serum osteocalcin was measured by an IRMA recognizing intact osteocalcin and N-Mid-fragment of the peptide (CIS Bio International, Gif-Sur-Yvette, France) with intra- and interassay CVs of 1.5 to 4%. Urinary NTx was measured by an automated CIA (Vitros ECi, Ortho Clinical Diagnostics, Amersham, UK) with a sensitivity of 5 nmol/l and the intra- and interassay CVs ranging from 2 to 10%; the measured values were proportioned to urinary creatinine excretion. Serum 25(OH)D concentration was measured by an RIA after acetonitrile extraction (DiaSorin, Stillwater, MN, USA). The sensitivity of the method was 5 nmol/l, and the intra- and interassay CVs ranged from 6 to 9%. Serum testosterone was assayed by an automated CIA (Chiron Diagnostics, Medfield, USA) with intra- and interassay CVs ranging from 4% to 7%. Serum estradiol was measured by an RIA (Orion Diagnostica) with a sensitivity of 0.02 nmol/l and intra- and interassay CVs ranging from 3 to 12%. For determination of serum ionized calcium blood samples were centrifuged immediately after being drawn, and the serum was analyzed with an ion selective analyzer (Microlyte; Kone Inc, Helsinki, Finland) within a few hours of blood collection (intra-assay CV 1.6%). Serum and urine creatinine were determined by routine methods.

Life habits

Patients answered a questionnaire where their smoking habits, alcohol consumption, calcium intake, medications, medical and fracture history were asked.

Statistics

The data with normal distributions are expressed as means with s.d.s, otherwise as medians with ranges. In the comparisons between the study groups, normally distributed variables were studied using two group *t*-tests, and those not-normally distributed were tested with the Mann–Whitney rank sum test. Paired data were compared using the paired *t*-test (normally distributed data) or Wilcoxon signed-rank test (non-normal data). Spearman rank order correlations were calculated between the doses of MP and CsA and changes in BMD. All analyses were done using NCSS 2000 software (NCSS Statistical Software, Kaysville, UT, USA).

Results

Patient characteristics are presented in Table 1. The patients were older [44(9); mean (s.d.)] years than biochemical controls [35(12)] years ($P = 0.005$). The median time from BMT to the follow-up study was 75 (54–96) (median with range) months. All patients except two had been able to

Table 1 Characteristics (mean (SD) or median with range) of the patients

Number	27
Age (years)	44 (9)
F/M	14/13
Weight (kg)	72 (18)
Height (cm)	170 (7)
Disease	
ALL	2
AML	12
Burkitt's lymphoma	1
CML	10
MDS	2
Time from BMT (months)	75 (54–96)
Duration of ERT (months)	67 (52–96)
Number of smokers/ex-smokers/never smokers	2/12/13
Duration of smoking smokers/ex-smokers (years)	22 (18)/14 (10)
Alcohol intake of those using it (g/week; $n = 13$)	10 (7)
Calcium intake (mg/day)	1100 (600)
Cumulative dose of MP (g)	
at 12 months after BMT	4.4 (2.6)
from 12 months to follow-up	2.0 (3.5)
at follow-up	6.3 (5.0)
Cumulative dose of CsA (g)	
at 12 months after BMT	67.1 (20.8)
from 12 months to follow-up	8.8 (31.7)
at follow-up	75.8 (34.1)

F = female; M = male; ERT = estrogen replacement therapy; MP = methylprednisolone, CsA = cyclosporine A.

Table 2 Bone mineral density (g/cm^2) (mean (SD)) in patients

	Before BMT	*At 12 months*	*At follow-up*
L1–L4			
All	1.015 (0.160)	0.977 (0.156)[c]	1.000 (0.114)[a]
Men	1.071 (0.167)	1.019 (0.182)[b]	1.035 (0.102)
Women	0.963 (0.140)	0.941 (0.126)	0.968 (0.117)
Femoral neck			
All	0.906 (0.142)	0.826 (0.155)[d]	0.860 (0.130)[c]
Men	0.966 (0.111)	0.851 (0.077)[b]	0.899 (0.093)[b]
Women	0.859 (0.149)	0.791 (0.119)[c]	0.805 (0.121)
Trochanter			
All	0.774 (0.128)	0.695 (0.103)[d]	0.723 (0.116)[b]
Men	0.834 (0.118)	0.744 (0.077)[c]	0.788 (0.095)
Women	0.722 (0.117)	0.654 (0.108)[c]	0.667 (0.105)[b]
Ward's triangle			
All	0.730 (0.152)	0.641 (0.111)[d]	0.671 (0.138)[c]
Men	0.786 (0.125)	0.681 (0.071)[b]	0.718 (0.100)[b]
Women	0.682 (0.161)	0.607 (0.128)[c]	0.630 (0.156)
Total hip			
All	1.050 (0.150)	0.969 (0.144)[d]	0.983 (0.152)[c]
Men	1.123 (0.123)	1.044 (0.126)[c]	1.063 (0.133)
Women	0.987 (0.146)	0.910 (0.133)[d]	0.915 (0.136)[c]

[a]$P = 0.002$ compared to 12 months, [b]$P < 0.05$, [c]$P < 0.01$, [d]$P = 0.000$ compared to before BMT.

stop CsA medication about 1 year after BMT, and at the time of the follow-up study only one patient was still using it. Twelve patients needed methylprednisolone later than 12 months after BMT and four were still using it at the time of the follow-up examinations. All female patients used estrogen replacement therapy; the median duration of treatment was 67 months. Two female patients were receiving bisphosphonate therapy due to prolonged MP treatment. One man had been receiving testosterone for 7 months due to impotence.

BMD

The BMD values are shown in Table 2. From 12 months after BMT to the follow-up study the BMD increased at all five measurement sites, even though statistically significantly so only at the lumbar spine. The mean increases were from 0.977 g/cm^2 to 1.000 g/cm^2 in the lumbar spine (+2.4%) ($P = 0.002$), from 0.826 g/cm^2 to 0.860 g/cm^2 in the femoral neck (+4.1%) ($P = 0.087$), from 0.695 g/cm^2 to 0.723 g/cm^2 in the trochanter (+4.0%) ($P = 0.095$), from 0.641 g/cm^2 to 0.671 g/cm^2 in Ward's triangle (+4.7%) ($P = 0.072$) and from 0.969 g/cm^2 to 0.983 g/cm^2 in the total hip (+1.4%) ($P = 0.23$). The trends for increases in BMD were similar for both genders (Table 2).

Table 3 shows the numbers of the patients who fulfilled the WHO criteria for osteopenia and osteoporosis.[4] One year after BMT 12 patients had had osteopenia and one patient osteoporosis in the lumbar spine, but at the time of the follow-up study only seven patients had osteopenia and nobody osteoporosis. From 1 year after BMT to the follow-up study one patient's vertebral osteoporosis had improved to osteopenia, seven patients' osteopenia had disappeared, but one patient's normal vertebral BMD had worsened to becoming osteopenic. By the follow-up study 12 patients had reached the same level of lumbar spine BMD as they had had before BMT. One year after BMT 11 patients had had osteopenia and one osteoporosis at the femoral neck. By the follow-up study seven of these patients still had osteopenia and three patients with previously normal BMD had worsened to osteopenia. The single patient still had osteoporosis. The number of patients reaching the pre-BMT level of BMD were seven in the femoral neck, eight in the trochanter, nine in Ward's triangle and nine in the total hip. No patient experienced a new vertebral fracture from 12 months after BMT to the follow-up study. Two patients (one man and one woman) had a distal radius fracture and one man had an ankle fracture. One female patient and one male patient had had unilateral and one man bilateral aseptic osteonecrosis of the femoral head.

To study the effect of treatment with MP or CsA on changes in BMD we divided the patients into those who needed MP or CsA for more than 1 year ($n = 12$) and into those who had been able to stop using them earlier than at 1 year ($n = 15$) (Table 4). The groups differed significantly with respect to total MP dose at follow-up ($P = 0.0006$) and tended to do so even at 1 year after BMT. CsA doses were similar at each time point. No statistically significant differences existed between the groups in the recovery of BMD (= the mean of individual per cent changes from 1 year after BMT to the follow-up). However, in terms of total changes in BMD from the pre-BMT values to follow-up, the long-term treatment group experienced more severe bone loss than did the short-term treatment group at the femoral neck ($P = 0.04$), Ward's triangle ($P = 0.04$) and the total hip ($P = 0.03$) (Table 4). Except for the trochanter, these total changes in BMD at the four other measurement

Table 3 Number of patients (% of assessable patients) fulfilling the WHO criteria for osteopenia and osteoporosis at each time point

	Lumbar spine			*Femoral neck*		
Time	*Before BMT*	*12 months after BMT*	*Follow-up*	*Before BMT*	*12 months after BMT*	*Follow-up*
Osteopenia	10	12	7	7	11	10
Osteoporosis	1	1	0	0	1	1
Total	11 (41)	13 (50)	7 (26)	7 (27)	12 (48)	11 (41)

Table 4 Percent changes in BMD [mean (SD)] from 1 year after BMT to follow-up (=recovery) and from pre-BMT values to follow-up (=total change) as well as total MP and CsA doses 1 year after BMT and at follow-up in patients with 1 year or less (=short-term) and more than 1 year (=long-term) treatment with MP and/or CsA

	Short-term (n = 15)	*Long-term (n = 12)*	*P value*
L1–L4			
recovery	4.1 (8.0)	2.7 (4.4)	0.62
total change	2.5 (8.5)	−3.1 (7.2)	0.08
Femoral neck			
recovery	4.4 (8.3)	2.7 (11.7)	0.45
total change	−1.9 (11.1)	−10.5 (7.9)	0.04
Trochanter			
recovery	3.7 (7.9)	2.1 (10.0)	0.67
total change	−3.3 (7.0)	−9.6 (11.8)	0.11
Ward's triangle			
recovery	5.9 (11.3)	2.0 (14.0)	0.46
total change	−2.1 (15.1)	−13.6 (12.0)	0.04
Total hip			
recovery	5.2 (6.4)	0.1 (6.7)	0.38
total change	−2.6 (6.4)	−10.1 (7.9)	0.03
Total MP dose			
1 year (g)	3.6 (2.6)	5.4 (2.5)	0.05
follow-up	3.6 (2.6)	9.7 (5.3)	0.0006
Total CsA dose			
1 year (g)	69.7 (16.8)	63.9 (25.3)	0.48
follow-up	71.3 (17.3)	81.5 (48.0)	0.30

sites correlated significantly with the total doses of both MP ($r = 0.46–0.52$; $P = 0.006–0.015$) and CsA ($r = 0.47–0.58$; $P = 0.003–0.012$) at follow-up.

Biochemical measurements

Results of the biochemical measurements are presented in Table 5. Serum PINP was similar in patients and controls, but its mean level had decreased by 30% ($P = 0.024$) from that seen at 12 months after BMT, when it was significantly higher than in the present controls ($P = 0.031$). Another marker of bone formation, osteocalcin, was also similar in patients and controls; it was not followed in the primary study. The mean level of serum ICTP was 41% higher in patients than in controls ($P = 0.0001$), but had decreased by 43% ($P = 0.018$) from that obtained 12 months after BMT. In contrast, another marker of bone resorption, urinary NTx was 41% lower in patients than in controls ($P = 0.0002$). Both female and male patients had lower urinary NTx [33.1 (24.5) nmol/mmol crea ($P = 0.036$) and 28.9 (15.1) nmol/mmol crea ($P = 0.001$)] and higher ICTP [3.41 (0.81) μg/l ($P = 0.005$) and 4.56 (2.62) μg/l ($P = 0.007$)] values than sex-matched controls [46.1 (17.7) nmol/mmol crea and 59.7 (24.7) nmol/mmol crea; 2.65 (0.53) μg/l and 2.92 (0.82) μg/l, respectively]. The mean serum 25(OH)D was 33% ($P = 0.0002$) lower in patients than in controls. Twenty-four of 27 patients and 11 of 28 controls had hypovitaminosis D [25(OH)D $\leqslant$37 nmol/l]. The mean serum ionized calcium had decreased from 1.28 mmol/l at 12 months after BMT to 1.22 mmol/l at follow-up ($P = 0.0001$). The mean serum estradiol level in the female patients was 0.23 nmol/l which was easily compatible with the estrogen replacement therapy that they were all using. The mean testosterone level in male patients was low normal [11.7 (4.3) nmol/l; normal range 9–34 nmol/l] and it was 34% lower than before BMT ($P = 0.006$) and 33% lower than 1 year after BMT ($P = 0.005$). Four of 13 men had serum testosterone levels below the reference range, and 11 men had a level which was lower than before BMT. The four hypogonadal men did not have a lower BMD than the eugonadal men (data not shown) but they belonged to the group which needed MP or CsA for less than 1 year, and consequently, experienced less bone loss. At the time of the follow-up study no patient had a creatinine value above the reference limit ($<$115 μmol/l).

Discussion

In this study of the long-term effects of allogeneic BMT on bone mass and turnover we demonstrated that in the average 75 months after BMT the BMD of the survivors had improved by 2.4% at the lumbar spine and up to 4.7% at the femoral sites as compared to the situation 12 months after BMT. Except for serum ICTP which remained high, the markers of bone turnover had either normalized or were even lower than in controls, as was the case for urinary NTx. Hypovitaminosis D was common in all patients as was the tendency to low testosterone values in male participants.

In previous studies it has been demonstrated that after BMT the major bone loss takes place within the first 6 months.[2,3,5] Glucocorticoids and CsA are thought to be the major reasons for bone loss after organ transplants.[6] The observation that no significant bone loss occurs after autologous hemopoietic stem cell transplantation when immunosuppressive therapy is not needed, supports this theory.[5,7] Bone loss of allogeneic BMT has been related to

Table 5 Values for biochemical parameters [mean (SD)] in patients and controls

	Before BMT	*6 weeks*	*6 months*	*12 months*	*Follow-up*	*Controls*
PINP (μg/l)	53.5 (26.6)	26.5 (36.9)	92.1 (62.2)	61.6 (30.0)[a]	43.2 (26.8)[c]	43.0 (18.5)
ICTP (μg/l)	6.32 (4.25)	11.0 (8.36)	8.13 (3.29)	6.85 (3.31)[b]	3.93 (1.99)[d,e]	2.78 (0.69)
Osca (μmol/l)					20.7 (9.3)	22.1 (7.7)
NTx/creat (nmol/mmol)					31.1 (20.2)[f]	52.5 (22.0)
25(OH)D (nmol/l)					27.4 (7.4)[f]	40.6 (10.6)
Ca-ion (mmol/l)	1.27 (0.05)	1.25 (0.04)	1.27 (0.05)	1.28 (0.06)	1.22 (0.05)[g]	
Crea (μmol/l)	87 (15)	87 (18)	92 (19)	94 (22)	86 (10)	
Testo (nmol/l)	17.8 (6.2)	9.55 (8.4)	22.4 (8.2)	17.4 (4.0)	11.7 (4.3)[h,i]	
Estradiol (nmol/l)					0.23 (0.19)	

PINP = type I procollagen aminoterminal propeptide; ICTP = type I collagen carboxyterminal telopeptide; Osca = osteocalcin; NTx = urine type I collagen aminoterminal telopeptide/creatinine; 25(OH)D = 25-hydroxyvitamin D; Ca-ion = ionized calcium; Crea = creatinine; Testo = testosterone.
[a]$P = 0.031$ compared to controls; [b]$P = 0.000$ compared to controls; [c]$P = 0.024$ compared to 12 months; [d]$P = 0.018$ compared to 12 months; [e]$P = 0.0001$ compared to controls; [f]$P = 0.0002$ compared to controls; [g]$P = 0.0001$ compared to 12 months, [h]$P = 0.006$ compared to before BMT; [i]$P = 0.005$ compared to 12 months.

the cumulative doses of glucocorticoids and to duration of exposure to CsA.[3] In the present study the total changes in BMD between BMT and the follow-up also correlated significantly with the total doses of both MP and CsA. It remains, however, open which one – MP and/or CsA or GVHD with associated events the treatment for which the drugs are designed – is most detrimental to the bone. Most obviously the patients who had continued MP and/or CsA for more than 1 year after BMT (Table 4) were those with the most severe GVHD. Also, their bone state recovered between 1 year after BMT and follow-up but remained much worse than in short-term users, indicating that the most severe damage to the bone had happened during the first year. Interestingly, there was only a tendency to higher MP doses in long-term users at 1 year; the CsA doses were similar for both groups.

The present results imply that in most patients the lumbar spine BMD returns to the same level as it was before BMT. In fact, this trend for recovery was already seen in our previous study from 6 to 12 months after BMT.[2] Instead, the BMD of the femoral neck recovers to a lesser degree than that of the lumbar spine. Noteworthy is that recovery at all skeletal sites was similar for both genders despite the fact that all females received estrogen replacement therapy, while all males were without testosterone supplementation, although some of them were hypogonadal and nearly all had serum testosterone levels lower than before BMT. This point emphasizes the role of stopping or reducing glucocorticoids and CsA in the recovery of the bone state, besides other contributing factors such as improved mobilization.

One year after BMT all the biochemical markers pointed to accelerated bone turnover, with both increased resorption and enhanced formation.[2] This fitted with being primarily due to CsA which was still being used by all the patients in that phase.[6] In the present follow-up study all the markers of bone formation had returned to normal on an average of 6 years after BMT. Interestingly, the behavior of the two markers of bone resorption dissociated from each other. Serum ICTP was still elevated but urinary NTx was even lower than in controls; the latter finding was not explained by differences in renal function which was also normal in patients. Furthermore, the differences in resorption markers between patients and controls existed in both sexes despite the facts that estrogen replacement therapy was used by all the female patients, but none of the men used testosterone replacement therapy although some were hypogodanal. Two enzymatic pathways are involved in osteoclastic bone resorption, one involving cathepsin K and releasing NTx and type I collagen C-terminal telopeptide (CTx) from bone collagen, and the other involving matrix metalloproteinases and releasing ICTP but not NTx or CTx.[8–10] Thus, urinary NTx measures cathepsin K-mediated resorption and serum ICTP matrix metalloproteinases-mediated resorption of osteoclasts. The observed dissociation between the two markers could be due to a continuing increase in matrix metalloproteinase-mediated resorption together, even with reduced activity of cathepsin K.

Bisphosphonates have been proven to prevent postmenopausal and corticosteroid-induced bone loss.[11–14] They have also been used to treat bone metastases of certain malignancies.[15] In one study pamidronate appeared to lessen bone loss after cardiac transplantation.[16] During treatment with bisphosphonates cathepsin K-mediated markers of bone resorption such as NT_X and CT_X are decreased in serum and urine.[17,18] Instead, serum ICTP is continuously elevated, which might mean that bisphosphonates do not reduce metalloproteinase-mediated bone resorption.[18] However, *in vitro* bisphosphonates have been shown to directly inhibit most metalloproteinases.[19] Whether treatment with bisphosphonates is capable of improving the bone state of such patients as the ones in this study with high ICTP but low NTx levels, remains to be clarified in further investigations.

Serum 25(OH)D levels were low in nearly all patients but also a remarkable number of controls had hypovitaminosis D when studied in April to July. Hypovitaminosis D was defined as a serum level of 25(OH)D, at which serum parathyroid hormone concentration has started to increase in cross-sectional studies.[20] In part, the finding reflects the poor vitamin D status of the Finnish population;[21] when studied in October, 40% of middle-aged healthy Finnish men and women had hypovitaminosis D. In the present patients the vitamin D levels were, however,

significantly lower than in controls. Schulte *et al*[5] pointed out that serum 25(OH)D levels were low even before BMT and were further decreased 4 weeks afterwards when the PTH levels rose. This happened despite vitamin D supplementation (200 IU vitamin D_2 daily). Possibly as a sign of the deterioration in vitamin D status the mean level of serum ionized calcium had decreased in our patients between 12 months after BMT and the present evaluation; unfortunately, we did not measure serum PTH concentrations. Hypovitaminosis D could be one reason for poor recovery of the femoral neck BMD in our patients. Taken together, all these findings indicate that the vitamin D status of BMT patients should be actively improved.

In our previous study the mean serum testosterone level decreased by 57% 6 weeks after BMT but returned to baseline by 6 months.[2] In one study of 10 men a mean of 3 years after allogeneic bone marrow transplantation, all had normal serum total testosterone levels, and only two had a low free testosterone index. However, as a sign of subclinical hypogonadism their serum LH response to stimulation by GnRH was exaggerated as compared to the age-matched control group.[22] Out of the present 13 male patients studied at a median of 6 years after BMT, all except two had a serum testosterone level lower than before BMT, and four had hypogonadism. How we should relate to testosterone replacement therapy in men with still normal, even though lower testosterone levels than measured before is an open question, but a general agreement exists that truly hypogonadal male transplant recipients should be treated with testosterone.[23] The benefit of this treatment was documented in a very recent study of 18 men with hypogonadism due to different organic diseases, in which 2 years' replacement therapy increased the lumbar spine BMD by 7.7% and that of the trochanter by 4.0%.[24] In the present study the bone state was no worse in the four hypogonadal men than in the eugonadal men but most of them were short-term users of MP and CsA.

In conclusion, in long-term survivors of allogeneic bone marrow transplantation bone mineral density recovers and bone turnover state normalizes as compared to the situation 1 year after BMT. More attention should be paid to the vitamin D status of all recipients and to possible hypogonadism of male patients. An interesting dissociation in the behavior of serum ICTP and urinary NTx will be elucidated by further studies.

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