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Original Article

The Role of Cytokines in the Changes in Bone Turnover Following Bone Marrow Transplantation

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Abstract. Osteoporosis is a common disease among patients undergoing transplantation and a loss of bone mass is usually detected after bone marrow transplantation (BMT), particularly during the immediate post-BMT period. Post-BMT bone loss is primarily related to gonadal dysfunction and immunosuppression. Cytokines, especially interleukin 6, play an important role in the pathogenesis of postmenopausal osteoporosis. However, the pathogenetic role of cytokines in post-BMT bone loss is unknown and data on the changes of cytokines in accordance with bone turnover markers are scarce. The aim of this study was to assess the relationship between bone turnover markers and cytokines, which are regularly sampled at peripheral blood and bone marrow before and after allogeneic BMT. This prospective study included two analyses. The first was a study of 46 BMT recipients (M/F 28/18), examining the relationship between bone turnover markers and serum cytokines that were measured before and at 1 week, 2 weeks, 3 weeks, 4 weeks and 3 months after BMT. Serum intact parathyroid hormone was measured before BMT and at 3 weeks after BMT and its relation to other cytokines and bone turnover markers was evaluated. The second analysis was a study of 14 (M/F 9/5) of 46 patients in whom bone marrow plasma cytokines [interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α)] were measured at 3 weeks after BMT. The relationship between bone marrow plasma cytokines and bone turnover markers was studied because bone marrow is the microenvironment where the real changes in bone turnover occur. Serum type I collagen carboxyterminal telopeptide (ICTP), a bone resorption marker, increased progressively until 4 weeks (peak) after BMT and then decreased thereafter. Serum osteocalcin, a bone formation marker, decreased progressively until 3 weeks after BMT and then increased thereafter. Serum IL-6 increased until 2 weeks after BMT and declined thereafter. Serum TNF-*a* increased until 3 weeks after BMT and declined thereafter. There was a significant positive correlation between serum ICTP and bone marrow IL-6 levels at 3 weeks after BMT, when a marked change in bone metabolism occurs following BMT. However, a correlation between bone turnover markers and bone marrow TNF-aor peripheral blood cytokines was not found. At 3 months after BMT, there was a significant negative correlation between the mean daily steroid dose and the serum osteocalcin level ($r = -0.43$, $p < 0.05$). The correlation between the mean daily steroid dose and serum ICTP was also significant ($r = 0.41$, $p < 0.05$). Our data suggest that the progressive increase in bone resorption during the immediate post-BMT period is related to both steroid dose and the increase in bone marrow IL-6, which is a potent stimulator of bone resorption in vivo.

Keywords: Bone marrow transplantation; Bone turnover markers; Cytokinesl; Osteoporosis

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Introduction

Transplantation procedures have improved in recent decades, resulting in a great increase in the life expectancy of the recipients. Therefore, increasing importance is now being placed upon the long-term complications of therapy. One important target organ of the adverse effects of transplantation is bone [1]. It is well known that recipients of heart, kidney and liver transplants have an increased risk of osteoporosis. However, studies in bone marrow transplantation (BMT) are scarce. Although BMT recipients are usually younger compared with other organ transplant recipients, a loss of bone mass after BMT is usually detected. Post-BMT bone loss is related to the use of immunosuppressants and gonadal dysfunction secondary to myeloablative therapy and/or total body irradiation (TBI) [2]. The rapid impairment of bone formation and the increase in bone resorption, as mirrored by biochemical markers, might play a role in bone loss, particularly during the immediate post-BMT period [3].

Interleukin 6 (IL-6) plays a pathogenetic role in postmenopausal osteoporosis [4,5]. Several studies in vitro and in vivo have shown that IL-6 is an essential mediator of bone loss associated with menopause and also plays a pathogenetic role in the abnormal bone resorption associated with multiple myeloma, Paget's disease and rheumatoid arthritis [6–8]. However, the relation between cytokines and post-BMT bone loss is unknown and data on the changes of cytokines relative to bone turnover markers are scarce.

In view of the fact that our hospital is a reference center for BMT, we decided to conduct a prospective study. The aim of this study was to analyze the relationship between the changes in cytokines and bone turnover during the immediate post-BMT period, when a significant change in bone metabolism occurs.

Subjects and Methods

Subjects and Sample Collection

We screened 205 patients with various hematologic diseases who received BMT between October, 1998 and August, 1999 at St Mary's hospital in Korea. We excluded from this group all autologous bone marrow transplants and prospectively investigated 46 patients (28 men, 18 women) undergoing allogeneic BMT. Among the study group (mean age 32 ± 7.9 years), the proportion of patients who received TBI (10–13.2 Gy) as a conditioning regimen was 52%. In order to prevent graft versus-host disease (GVHD), intravenous cyclosporin A (5 mg/kg per day 1 day before BMT and 3 mg/kg per day until the 20th day after BMT) was administered to the allogeneic BMT patients. Thereafter, oral cyclosporin A at 6 mg/kg per day was begun and continued in a tapering dose for 6–12 months. The underlying hematologic diseases of the 46 patients were leukemia (n = 41), severe aplastic anemia (n = 3) and myelodysplastic syndrome (n = 2).

In all patients, blood was sampled in order to determine serum levels of calcium, phosphorus, creatinine, total alkaline phosphatase, bone turnover markers [osteocalcin, serum type I collagen carboxyterminal telopeptide (ICTP)] and cytokines [IL-6, tumor necrosis factor alpha (TNF-α)] before BMT, and at 1, 2, 3, 4 weeks and 3 months after BMT. The measurement of intact parathyroid hormone (PTH) was performed before BMT and at 3 weeks after BMT. The correlation between the serum levels of bone turnover markers (osteocalcin, ICTP) and cytokines (IL-6, TNF-α) was examined.

Additionally, the concentration of interleukin-6 and TNF-α in the bone marrow plasma in 14 of 46 patients was measured via bone marrow aspiration which was performed 3 weeks after BMT in order to confirm the engraftment of donor bone marrow cells. Thus, the correlation between bone marrow plasma levels of cytokines and serum levels of bone turnover markers could be examined. In order to measure the biochemical markers and cytokines, blood samples were taken between 0700 and 0900 hours following an overnight fast. Following centrifugation (1500 g) for 10 min, aliquots of serum were stored at –20 °C until analysis.

This study was approved by the Institutional Review Board of St Mary's Hospital, and informed consent was obtained from all participants before enrollment in the study.

Assays

Serum calcium, phosphorus, creatinine and total alkaline phosphatase levels were determined using an autoanalyzer (747 Automatic Analyzer, Hitachi, Japan). Serum osteocalcin (N-tact osteo SP, Incstar, USA) and ICTP (Telopeptide ICTP, Orion Diagnostica, Finland) concentrations were determined in duplicate by radioimmunoassay. The maximum inter- and intra-assay coefficients of variation (CVs) for the range of concentrations evaluated were 7.7% and 5.4% for osteocalcin, and 10.7% and 3.6% for ICTP, respectively. IL-6 and TNF-a (ELISA kit, Hyundae Pharm. Research Institute, Korea) concentrations were measured in duplicate by enzyme-linked immunosorbent assays with detection limits of 4 and 7 pg/ml, respectively. The maximum inter- and intra-assay CVs for the range of concentrations evaluated were 8.3% and 6.2% for IL-6, and 8.2% and 7.0% for TNF-α, respectively. Intact PTH concentrations were measured using an immunoradiometric assay (Diagnostic Products, Los Angeles, CA) with an interassay CV of 5.1%.

Statistical Analysis

All values are given as the mean ± SEM. Statistical analysis was performed using the SPSS system. Comparisons between the pre-BMT and post-BMT

biochemical characteristics were undertaken using Student's *t*-test for paired data. Pearson's correlation coefficients were used to examine the bivariate correlations between the two variables. $p < 0.05$ was considered to represent a statistically significant difference.

Results

Changes in Bone Turnover Markers Following BMT

Following BMT, the marker for bone resorption, ICTP, progressively increased and reached its maximum at 4 weeks (13.5 ± 1.4 ng/ml; $p < 0.001$ vs baseline). Thereafter, it declined. The marker for bone formation, osteocalcin, decreased until 3 weeks after BMT (2.1 ± 0.4 ng/ml; $p < 0.001$ vs baseline) and increased thereafter (Table 1, Fig. 1). Serum levels of creatinine were in the normal range throughout the entire observation period.

Changes in Serum Cytokines and Intact PTH Following BMT

Serum IL-6 increased until 2 weeks after BMT (226.4 ± 64.8 pg/ml; $p < 0.05$ vs baseline) and declined thereafter. Serum TNF-aincreased until 3 weeks after BMT (146.6 ± 35.6 pg/ml; $p < 0.01$ vs baseline) and declined thereafter (Table 1, Fig. 2).

There were no significant differences observed between the serum intact PTH level before BMT (20.2 ± 2.8 pg/ml) and at 3 weeks after BMT (27.4 ± 4.3 pg/ml; $p = 0.10$). Before BMT and at 3 weeks after BMT there was no significant correlation between the serum intact PTH level and any cytokines.

The concentrations of IL-6 and TNF-α in the bone marrow plasma of 14 recipients at 3 weeks after BMTwere 4282.3 ± 3117 pg/ml and 85.1 ± 16.3 pg/ml, respectively.

Table 1. The changes in serum bone turnover markers, cytokines, intact PTH and creatinine levels, before and after allogeneic BMT

		Post-BMT				
	Pre-BMT	1 week	2 week	3 week	4 week	3 months
Serum OC (ng/ml)	5.4 ± 0.9	5.1 ± 0.8	4.8 ± 0.7	2.1 ± 0.4	7.4 ± 3.4	10.9 ± 1.3
Serum ICTP (ng/ml)	6.7 ± 0.6	10.2 ± 1.1	10.0 ± 0.9	11.0 ± 1.2	13.5 ± 1.4	13.4 ± 1.9
Serum IL-6 (pg/ml)	119.0 ± 77.4	180.4 ± 71.6*	226.4 ± 64.8*	187.6 ± 54.9*	NA	132.0 ± 47.1
Serum TNF-α (pg/ml)	37.7 ± 8.4	48.1 ± 10.8	85.6 ± 18.7	146.6 ± 35.6	NA	119.7 ± 32.4*
Serum iPTH (pg/ml)	20.2 ± 2.8	NA	NA	27.4 ± 4.3	NA	NA
Serum Cr (mg/dl)	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2

Data are given as mean (± SEM).
OC, osteocalcin; ICTP, serum collagen I carboxyterminal telopeptide; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; iPTH, intact parathyroid hormone; Cr, creatinine; NA, not assayed.
*$p < 0.05$, †$p < 0.01$ against basal value (pre-BMT).

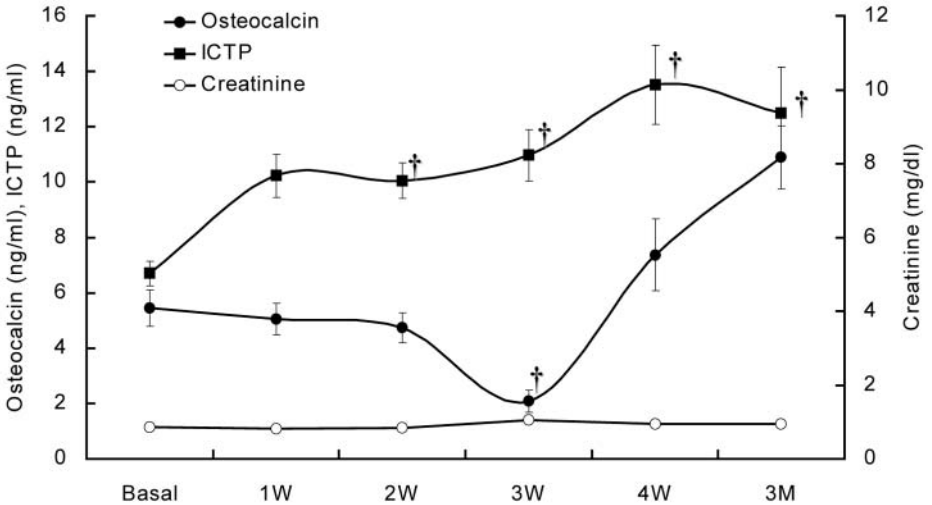

Fig. 1. The changes in serum bone turnover markers, before and after allogeneic BMT. Data are given as the mean (± SEM). †$p < 0.01$ against basal value. Following BMT, serum collagen I carboxyterminal telopeptide (ICTP), a marker of bone resorption, progressively increased, reaching a peak at 4 weeks and declining thereafter. Osteocalcin, a marker of bone formation, decreased, reaching its nadir at 3 weeks; thereafter, it increased until 6 months.

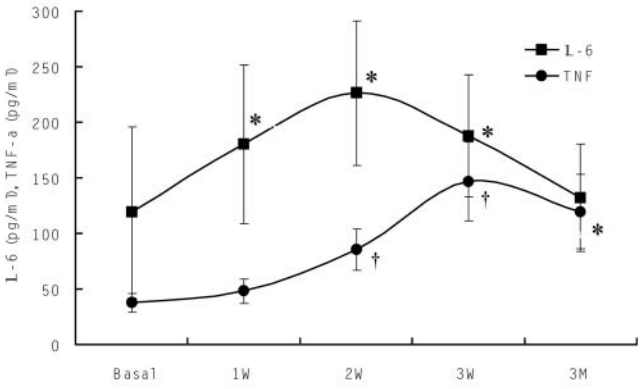

Fig. 2. The changes in serum cytokines in peripheral blood, before and after allogeneic BMT. Data are given as the mean (± SEM). *$p < 0.05$, †$p < 0.01$ against basal value. Following BMT, serum intrleukin 6 (IL-6) increased reaching a peak at 2 weeks and declined thereafter. Serum tumor necrosis factor alpha (TNF-α) increased, reaching a peak at 3 weeks and also declining thereafter.

Correlation Between Bone Turnover Markers and Serum Cytokines Following BMT

We examined the correlations between osteocalcin and IL-6, osteocalcin and TNF-α, ICTP and IL-6, ICTP and TNF-α before BMT, and at 1, 2, 3, 4 weeks and 3 months following BMT, respectively. However, there were no statistically significant correlations observed at any of the time points.

Correlation Between Serum Bone Turnover Markers and Bone Marrow Plasma Cytokines Following BMT

Cytokines in the bone marrow plasma of 14 recipients at 3 weeks after BMT were examined in order to determine the correlation with serum osteocalcin and ICTP, which were measured at the same time. The value of the correlation coefficient between bone marrow IL-6 and serum ICTP was 0.68 and was statistically significant ($p<0.01$). However, no significant correlation was observed between bone marrow IL-6 and serum osteocalcin (Fig. 3). The correlation between bone marrow TNF-α and serum bone turnover markers (osteocalcin and ICTP) was also not significant.

Changes in Bone Turnover Markers According to Steroid Administered Following BMT

The mean dose of prednisolone administered during the 3 months following BMT was 11.1 ± 1.4 mg/day. At 3 months after BMT there was a significant negative correlation between the mean daily steroid dose and the serum osteocalcin level ($r = -0.43$, $p < 0.05$). The correlation between the mean daily steroid dose and serum ICTP was also significant ($r = 0.41$, $p < 0.05$) (Fig. 4). We classified patients into two groups according to whether a mean dose of more than or less than 7.5 mg/day prednisolone that was administered during the 3 months following BMT. At 3 months after BMT the serum osteocalcin level was significantly lower in the high-dose steroid group (mean daily dose 14.2 ± 1.2 mg/day) than in the low-dose steroid group (mean daily dose 4.0 ± 0.9 mg/day; $p < 0.01$). The serum ICTP level was higher in the high-dose steroid group than in the low-dose steroid group, although without statistical significance.

We also examined the relation between steroid dose and bone turnover markers at 3 weeks after BMT. There was no correlation between mean daily steroid dose administered during 3 weeks (13.2 ± 2.3 mg/day) and the serum osteocalcin or ICTP at 3 weeks. At 3 weeks after BMT no significant difference was observed in bone turnover marker levels according to steroid dose administered until 3 weeks (high-dose vs low-dose steroid group: 23.9 ± 7.6 mg/day vs 5.5 ± 2.8 mg/day).

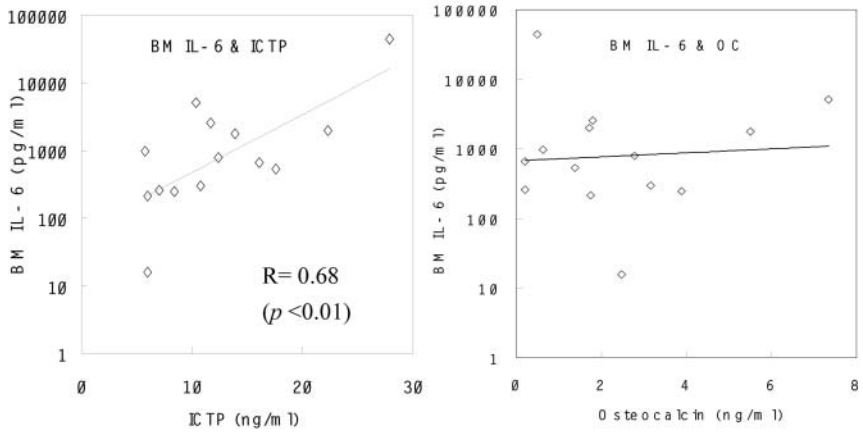

Fig. 3. The correlation between bone marrow plasma IL-6 and bone turnover markers at 3 weeks after BMT. There was a positive correlation between bone marrow IL-6 and the serum ICTP of peripheral blood that was statistically significant ($p < 0.01$). However, no correlation was observed between bone marrow IL-6 and serum osteocalcin.

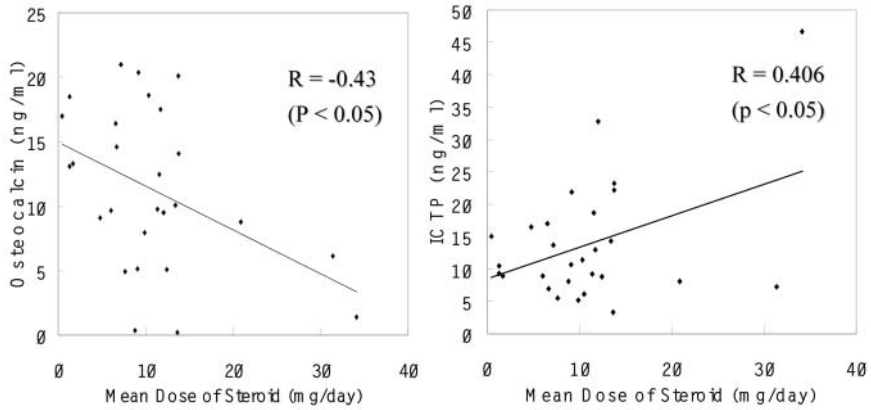

Fig. 4. The correlation between bone turnover markers and the daily steroid dose that was administered during the 3 months following BMT. There was a negative correlation between serum osteocalcin and the daily steroid dose that was statistically significant. A positive correlation between serum ICTP and the daily steroid dose was also statistically significant.

Table 2. The correlations between steroid dose and serum cytokines or bone turnover markers following BMT

	Serum OC		Serum ICTP		Serum IL-6		Serum TNF-α	
	3 W	3 M	3 W	3 M	3 W	3 M	3 W	3 M
Prednisolone[a] (3 W)	–0.10	0.06	–0.06	–0.09	0.02	–0.45*	–0.36*	–0.56
Prednisolone[a] (3 M)	–0.09	–0.43*	0.19	0.41*	0.08	0.28	0.10	0.07

Data are expressed as correlation factors between steroid dose and serum cytokines or bone turnover markers.
W, weeks; M, months; other abbreviations as in Table 1.
*$p < 0.05$, †$p < 0.01$.
[a]Prednisolone dose in this analysis is the mean daily dose administered until 3 weeks (13.2 ± 2.3 mg/day) or 3 months (11.1 ± 1.4 mg/day) after BMT.

Correlation Between Mean Daily Steroid Dose and Cytokines in Serum or Bone Marrow

There was a significant negative correlation between the mean daily steroid dose and serum TNF-α at 3 weeks ($r = –0.36$, $p < 0.05$), but no correlation between steroid dose and serum IL-6 (Table 2). Three months after BMT there was no correlation between steroid dose and serum IL-6 or TNF-α at 3 months. However, steroid dose administered during 3 weeks was significantly correlated with serum IL-6 ($r = –0.45$, $p < 0.05$) at 3 months or TNF-α ($r = –0.56$, $p < 0.01$) at 3 months (Table 2). No significant correlation was observed between steroid dose administered during 3 weeks and bone marrow cytokines 3 weeks after BMT.

Correlation Between Serum Cytokines and Bone Marrow Plasma Cytokines

No significant correlation was observed between bone marrow IL-6 and serum IL-6 at 3 weeks after BMT in 14 patients. However, there was a trend for a correlation between bone marrow TNF-α and serum TNF-α, but this was not significant ($r = 0.48$, $p = 0.08$). Because of the large variation in bone marrow IL-6 levels in our 14 patients, the difference between serum IL-6 and bone marrow IL-6 was not statistically significant. Similarly, no significant difference was observed between serum TNF-α and bone marrow TNF-α.

Discussion

Organ transplantation is now the treatment of choice for many patients with life-threatening chronic diseases. A new set of side effects unique to these groups of patients has been recognized and bone disease is one of these complications. It has been reported that 8–17% of bone loss develops during the year following renal transplantation and that one third of the recipients of cardiac or hepatic transplantation experience vertebral fracture [9–11]. However, little is known concerning the effects of myeloablative treatment followed by bone marrow transplantation (BMT) on bone mineral metabolism. According to the study by Castaneda et al. [12], 33% and 18% of BMT recipients had osteopenia and osteoporosis in the lumbar spine when examined at 33.6 months following BMT. We previously performed a prospective study on the short-term effects of BMT on bone mineral metabolism that showed lumbar spine BMD decreased by 2.2% and total proximal femoral BMD decreased by

6.2% 1 year after BMT [3]. The main causes of bone loss following BMT are the use of immunosuppressants and hypogonadism induced by TBI or chemotherapy [2]. Therefore, loss of bone mineral density is a relatively common finding in patients following BMT.

It is currently thought that bone-derived cytokines are the local effectors of the bone resorption induced by systemic hormonal changes [5]. However, the relation of cytokines to post-BMT bone loss is not well known and to our knowledge a prospective study on the changes in cytokines relative to bone turnover markers has not been performed. In addition, we measured cytokines not only in peripheral blood but also in bone marrow plasma, because bone marrow is the site where real changes in bone turnover occur. Our study demonstrates that bone resorption increased progressively and bone formation decreased during the period immediately following BMT, and bone marrow IL-6 level was related to the rapid increase in the marker for bone resorption (ICTP) following BMT. Our results concerning the change in bone turnover markers are the same as those of Carlson et al. [13] obtained during the first 12 weeks following BMT. These changes in bone formation and resorption play a possible role in post-BMT bone loss.

There are possible causes for the inhibition of osteoblast function, shown by the decrease in osteocalcin during the few weeks following BMT. One explanation is that a substantial number of osteoprogenitor cells are damaged by the myeloablative therapy [14]. The use of steroids which are administered for the treatment of GVHD or bronchiolitis obliterans with organizing pneumonia (BOOP) may be the other possible explanation for decreased osteoblastic activity following BMT [2]. We observed a negative correlation between the serum osteocalcin level and the mean daily dose of steroid that was administered during the 3 months after BMT. This may explain, in part, the decreased osteoblast function following BMT. It is well recognized that steroids have deleterious effects on bone mineral metabolism. Trabecular bone loss can occur from a dose of prednisolone as low as 7.5 mg per day. In this study, the mean daily dose of prednisolone administered during the first 3 months was 11.1 mg. That dose is sufficient to inhibit osteoblast activity. Although TNF-α or interleukin-1 (IL-1) is known to inhibit bone formation in in vitro studies [15], we found no correlation between the level of serum osteocalcin and TNF-α or IL-6 measured in the peripheral blood or bone marrow plasma.

Serum ICTP, a bone resorption marker, increased during the period immediately after BMT without a change in serum creatinine levels. In postmenopausal women it is generally agreed that IL-6 increases bone resorption and serum ICTP levels [5]. In this study, serum ICTP levels were correlated with IL-6 levels in bone marrow plasma but not with serum IL-6 levels. This suggests that the increase in bone resorption following BMT is influenced more by the change in IL-6 in bone marrow than by a systemic change in circulating IL-6. In addition to the role of cytokines, other possible causes of increased bone resorption following BMT include the use of immunosuppressants including steroid and cyclosporin, and hypogonadism following TBI and/or chemotherapy [13]. We also found a positive correlation between the level of serum ICTP and the mean daily dose of steroid used during the 3 months following BMT. Cyclosporine is known to produce high bone remodeling with bone resorption exceeding bone formation and can therefore induce osteopenia when applied in immunosuppressive doses [16]. However, because all patients included in our study were allogeneic BMT recipients and similar doses of cyclosporine were administered to all recipients according to a predetermined schedule, we did not confirm the effect of cyclosporine on bone metabolism and changes in cytokines after BMT.

In the case of post-BMT bone loss, we confirmed that the increase in bone resorption was significantly associated with the level of IL-6 in bone marrow, where cellular interactions between stromal cells and hematopoietic cells are continually occurring as part of the bone remodeling process [17]. To our knowledge, there has been only one study observing the change in bone turnover markers and cytokines following BMT and the correlation between them. In the study by Withold et al. [18] the enhancement of bone resorption after BMT was related to the increase in circulating IL-6. However, we did not observe a correlation between circulating IL-6 and bone resorption marker after BMT. It is difficult to discern why this discrepancy exists between the previous experiment and our study. However, the previous study was a cross-sectional study and did not observe the serial changes in bone turnover markers and cytokines during the period immediately after BMT, when the most significant change in bone metabolism occurs.

In this study, serum IL-6 seemed to be lower than bone marrow IL-6, whereas serum TNF-α seemed to be higher than bone marrow TNF-α, but the differences between serum and bone marrow cytokine levels in both IL-6 and TNF-α were not significant. The large variation in bone marrow cytokine levels and the small number of patients investigated (n = 14) might mean that more patients should be studied to find the true correlation. Until now no study has been conducted, to our knowledge on the relation between bone marrow cytokines and serum cytokines following BMT. We evaluated this relation and found no significant correlation. It is probable that serum cytokines originate from many tissues including not only bone marrow but also lymphocytes, monocytes, liver, kidney and lung, and that the contribution of bone marrow cytokines to serum cytokine levels could be less than expected.

It is generally known that, following BMT, the levels of circulating IL-6 increase during the aplastic phase in most patients and are associated with several complications, such as GVHD or infectious episodes [19]. IL-6 is produced by a variety of different cell types including T cells, B cells, monocytes, fibroblasts, keratinocytes and endothelial cells, and is involved in many aspects of the

inflammatory response [20]. It induces differentiation of T cells, B cells and macrophages, and stimulates the early stages of hematopoiesis, as well as the early stages of osteoclastogenesis [20]. We found that serum IL-6 and TNF-α increase during early post-BMT period and decline thereafter. These findings are similar to those of previous studies [19–21]. It also was found that the level of a bone formation marker (osteocalcin) decreased significantly and that of a bone resorption marker (ICTP) increased progressively during the early post-BMT period. The changes in serum cytokines were not significantly related to any bone turnover markers coincidentally examined following BMT. However, we found a significant relation between bone marrow IL-6 and the bone resorption marker. Thus, the development of an osteoclast lineage following BMT may be supported by bone-resorbing cytokines.

In this study, it is interesting that bone marrow IL-6 was correlated with serum ICTP and not with serum intact PTH. In vitro studies have demonstrated that IL-6 is produced in response to PTH by osteoblasts [22]. In humans with chronic PTH excess, IL-6 is known to play a role in PTH-induced bone resorption [23]. In our study, assuming that cytokine change is a primary biochemical event antecedent to the change in serum intact PTH following BMT, we think that no relation between IL-6 and intact PTH could be possible.

Our study implies that a rapid impairment of bone formation and an increase in bone resorption occurs during the immediate post-BMT period. Enhanced bone resorption following BMT is related to both the steroid dose and the increase in bone marrow IL-6, a potent stimulator of bone resorption in vivo.

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References

1. Keilholz U, Max R, Scheibenbogen C, et al. Endocrine function and bone metabolism 5 years after autologous bone marrow/blood-derived progenitor cell transplantation. Cancer 1997; 79:1617–22.
2. Ebeling PR, Thomas DM, Erbas B, et al. Mechanisms of bone loss following allogeneic and autologous hematopoietic stem cell transplantation. J Bone Miner Res 1999;14:342–50.
3. Kang MI, Lee WY, Oh KW, et al. The short-term changes of bone mineral metabolism following bone marrow transplantation. Bone 2000;26:275–9.
4. Jilka RL, Hangoe G, Girasole G, et al. Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 1992;257:88–91.
5. Manolagas SC, Jilka RL. Bone marrow, cytokines and bone remodeling. N Engl J Med 1995;332:305–11.
6. Klein B, Wijdenes J, Zhang X-G. Murine anti-interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia. Blood 1991;78:1198–204.
7. Kotake S, Sato K, Kim KJ. Interleukin-6 and soluble interleukin-6 receptors found in synovial fluids are responsible for inducing joint disruption in patients with rheumatoid arthritis. J Bone Miner Res 1994;9:S140.
8. Roodman GD, Kurihara N, Ohsaki Y. Interleukin 6: a potential autocrine/paracrine factor in Paget's disease of bone. J Clin Invest 1992;89:46–52.
9. Arnold JC, Hauser D, Ziegler, R et al. Bone disease after liver transplantation. Transplant Proc 1992;24:2709–10.
10. Julian BA, Lascow DA, Dubovsky J, et al. Rapid loss of vertebral mineral density after renal transplantation. N Engl J Med 1991;325:544–50.
11. Lee AH, Mull RL, Keenan GF, et al. Osteoporosis and bone morbidity in cardiac transplant recipients. Am J Med 1994;96:35–41.
12. Castaneda S, Carmona L, Carvajal I, et al. Reduction of bone mass in women after bone marrow transplantation. Calcif Tissue Int 1997;60:343–7.
13. Carlson K, Simonsson B, Ljunghall S. Acute effects of high-dose chemotherapy followed by bone marrow transplantation on serum markers of bone metabolism. Calcif Tissue Int 1994;55:408-11.
14. Kang MI, Oh KW, Lee WY, et al. The effects of bone marrow transplantation on osteoblastic differentiation of the human bone marrow-derived stromal cells. J Bone Miner Res 1999:S354.
15. Bertolini DR, Nedwin GE, Bringman TS, et al. Stimulation of bone resorption and inhibition of bone formation in vitro by human tumor necrosis factor. Nature 1986;319:516–8.
16. Aubia J, Masramon J, Serrano S, et al. Bone histology in renal transplant patients receiving cyclosporin. Lancet 1988;1:1048–9.
17. Shevde N, Anklesaria P, Greenberger JS, et al. Stromal cell-mediated stimulation of osteoclastogenesis. Proc Soc Exp Biol Med 1994;205:306–15.
18. Withold W, Wolf HH, Kollbach S, et al. Relationship between bone metabolism and plasma cytokine levels in patients at risk of post-transplantation bone disease after bone marrow transplantation. Eur J Clin Chem Clin Biochem 1996;34:295–9.
19. Ferra C, de Sanjose S, Gallardo D, et al. IL-6 and IL-8 levels in plasma during hematopoietic progenitor transplantation. Haematologica 1998;83:1082–7.
20. Pechumer H, Wilhelm M, Ziegler-Heitbrock HW. Interleukin-6 levels in febrile children during maximal aplasia after bone marrow transplantation are similar to those in children with normal hematopoiesis. Ann Hematol 1995;70:309–12.
21. Steffen M, Durken M, Pichlmeier U, et al. Serum interleukin-6 levels during bone marrow transplantation: impact on transplantation-related toxicity and engraftment. Bone Marrow Transplant 1996;18:301–7.
22. Lowik CW, van der Pluijm G, Bloys H, et al. Parathyroid hormone (PTH) and PTH-like protein (PLP) stimulate interleukin-6 production by osteogenic cells: a possible role of interleukin-6 in osteoclastogenesis. Biochem Biophys Res Commun 1989;162:1546–52.
23. Grey A, Mitnick MA, Shapses S, et al. Circulating levels of interleukin-6 and tumor necrosis factor-alpha are elevated in primary hyperparathyroidism and correlate with markers of bone resorption: a clinical research center study. J Clin Endocrinol Metab 1996;81:3450–4.

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