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Acute Effects of High-Dose Chemotherapy Followed by Bone Marrow Transplantation on Serum Markers of Bone Metabolism

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Abstract. There is an interplay between the cells in the bone marrow and the surrounding bone tissue, but little is known about the effects of myeloablative treatment followed by bone marrow transplantation on bone metabolism. We have therefore investigated 24 patients undergoing bone marrow transplantation (14 autologous, 10 allogeneic) for hematological malignancies. Serum concentrations of parathyroid hormone (PTH), albumin-modified calcium, and biomarkers for bone turnover—osteocalcin, bone alkaline phosphatase (B-ALP), and carboxyterminal cross-linked telopeptide of type I collagen (ICTP)—were measured. The samples were collected before myeloablative treatment, on the day of bone marrow infusion and 1, 2, 3, and 12 weeks thereafter. A serum PTH peak was consistently seen the day after total body irradiation, but no long-term effects on PTH/calcium homeostasis were observed. Bone formation as reflected by serum osteocalcin and B-ALP decreased, with nadir levels 2 to 3 weeks after marrow infusion. A simultaneous increase in bone resorption (increased S-ICTP) occurred. Pretreatment values were not completely regained 12 weeks after transplantation. The findings indicate that bone tissue is affected by myeloablative treatment, and the changes in biomarkers imply a net loss of bone over the study period.

Key words: Bone alkaline phosphatase — Bone marrow transplantation — Carboxyterminal cross-linked telopeptide of type I collagen — Osteocalcin — Parathyroid hormone.

The effects of myeloablative chemotherapy and bone marrow transplantation on bone metabolism are virtually unknown. Tools for such investigation have only recently been developed. Osteocalcin, bone alkaline phosphatase (B-ALP), and carboxyterminal cross-linked telopeptide of type I collagen (ICTP) are serum markers that reflect bone formation and resorption, and parathyroid hormone (PTH) influences the bone turnover rate. To our knowledge there has been only one report [1] on a transient decrease in serum osteocalcin after chemotherapy. In the present paper we describe changes in bone metabolism in patients undergoing bone marrow transplantation.

Patients and Methods

Twenty-four patients (median age 39 years, range 18–53) undergoing bone marrow transplantation for acute myelogenous leukemia (11), acute lymphoblastic leukemia (1), chronic myelogenous leukemia (5), and multiple myeloma (7) were studied. Myeloablative therapy was given before bone marrow infusion, as summarized in Table 1. The day of transplantation (day 0), before marrow infusion, all patients except one were given hydrocortisone 100 mg I.V. Except for this dose, seven patients received no glucocorticoids during the investigation, whereas the remaining 17 were given glucocorticoids either the week before transplantation as part of myeloablative treatment or as antiemetics (13) or following transplantation because of complications (4). In 14 patients, autologous (ABMT) and in 10 patients allogeneic bone marrow transplantation (BMT) was performed. Cyclosporin was initiated on the day before transplantation (day −1) in BMT patients, as prophylaxis against graft-versus-host disease (GVHD).

Serum Sampling and Storage

Sera were collected before the start of ablative treatment, day 0 before marrow infusion (and before hydrocortisone was given), and 1, 2, 3, and 12 weeks after bone marrow transplantation, and was stored at −20° until analyzed in sequence. Osteocalcin, B-ALP, total activity of alkaline phosphatases (T-ALP), ICTP, and PTH were assayed in thawed sera. Serum creatinine, calcium, albumin, and phosphate were determined in fresh blood samples.

Radioimmunoassay kits were used to measure the serum concentrations of osteocalcin (CEA Oris, France) (reference range 3–13 μg/liter); B-ALP (Boehringer Mannheim GmbH, Diagnostica, Germany) (reference range 0.5–1.9 μkat/liter); ICTP (Farmos Diagnostica, Finland) (reference range 1.8–6.0 μg/liter age 30–60 years); and PTH (Allégro Intact PTH immunoassay, Nichols Institute, San Juan Capistrano, CA, USA) (reference range 12–55 ng/liter).

The serum concentrations (reference range) of creatinine (64–106 μmol/liter), albumin-modified calcium (2.20–2.60 mmol/liter), albumin (42–55 g/liter), phosphate (0.76–1.44 mmol/liter) and T-ALP (0.8–4.8 μkat/liter) were measured as part of the clinical routine at the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden.

Statistics

Analysis of variance for repeated measures according to Fisher PLSD with a significance level of 95% was performed. For comparison of changes in different variables, correlation analysis was performed.

Results

In patients treated with glucocorticoids the week before marrow infusion, the S-osteocalcin level decreased significantly during the period between start of myeloablative treatment

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Table 1. Myeloablative regimens prior to bone marrow transplantation in 24 patients being treated for acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and multiple myeloma (MM)

Diagnosis (n)	Regimen	Dose, administration, route	Day
ALL (1)	Teniposide	200 mg/m^2 I.V.	−6
	Vincristine	1.5 mg/m^2 (max 2 mg) I.V.	−6
	Daunorubicin	30 mg/m^2 I.V.	−6
	Prednisone	100 mg/m^2 po	−6 and −5
	Cytarabine	500 mg/m^2 I.V.	−6 to −2
	Cyclophosphamide	40 mg/kg I.V.	−4 and −3
	TBI	7.5 Gy (0.15 Gy/min)	−1
AML (2)	Cyclophosphamide	60 mg/kg I.V.	−5 and −4
	TBI	7.5 Gy (0.15 Gy/min)	−1
AML (9)	Busulfan	1 mg/kg × 4 po	−8 to −5
CML (2)	Cyclophosphamide	60 mg/kg I.V.	−4 to −3
CML (3)	Busulfan	1 mg/kg × 4 po	−6 to −5
	Cyclophosphamide	60 mg/kg I.V.	−4 to −3
	TBI	7.5 Gy (0.15 Gy/min)	−1
MM (7)	Cyclophosphamide	400 mg/m^2 I.V.	−9
	Methylprednisolone	1 g × 2 I.V.	−4 to −1
	Melphalan	140 mg/m^2 I.V.	−3
	TBI	7.5 Gy (0.15 Gy/min)	−1

TBI = total body irradiation

and the day of bone marrow infusion. The level then remained lowered 3 weeks thereafter even though no further glucocorticoids were given. In patients who did not receive glucocorticoids, except for 100 mg hydrocortisone I.V. on day 0, a slow, progressive nonsignificant decrease in S-osteocalcin was noted over the studied period (Fig. 1). At the final measurement 12 weeks after transplantation, the basal value was not regained.

B-ALP, but not T-ALP, in serum decreased during and after myeloablative treatment. The B-ALP level was not influenced by glucocorticoid treatment. Nadir values were noted 2 weeks after transplantation. Normal values were regained after 12 weeks (Fig. 2).

S-ICTP increased after transplantation, and remained elevated after 12 weeks (Fig. 3). No association was found between changes in S-creatinine and S-ICTP from before to 0, 1, 2, and 3 weeks after transplantation, whereas changes in S-creatinine from before to 12 weeks after transplantation correlated with changes in S-ICTP during the same period ($r = 0.77$).

The serum concentrations of osteocalcin, B-ALP, and ICTP showed similar changes in ABMT and BMT patients, and in patients treated with or without total body irradiation (TBI). A serum PTH peak was consistently seen in TBI-treated patients on day 0, i.e., the day after irradiation, together with a nonsignificant decrease in S-calcium. No changes in the S-phosphate concentration occurred and no long-term persistent effects on serum PTH were observed (Fig. 4a). In non-TBI-treated patients there were no significant changes in serum calcium or PTH (Fig. 4b).

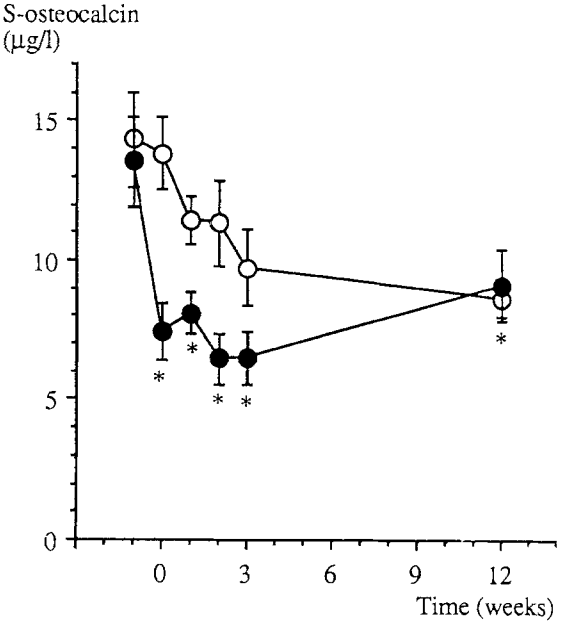

Fig. 1. S-osteocalcin before and after bone marrow transplantation in 17 patients who received (—●—) and 7 patients who did not receive (—○—) glucocorticoids. Bars represent SEM. *Significant change according to analysis of variance with significance level 95%.

Discussion

We have found that serum osteocalcin and serum B-ALP, markers for osteoblastic activity and bone formation, are reduced by myeloablative therapy followed by bone marrow transplantation. A similar decrease in serum osteocalcin has been described by Køther et al [1], who noted a concomitant decrease in the serum phosphate concentrations, and hypothesized that the reduction in serum osteocalcin is secondary to renal phosphate losses. This was not corroborated in the present study.

Glucocorticoids inhibit osteocalcin formation [2]. The inhibition is dose dependent and is reversible within days of glucocorticoid termination [3, 4]. The rapid initial drop in serum osteocalcin concentration seen in patients given glucocorticoids as part of myeloablative therapy is consistent with a glucocorticoid effect. However, as the serum osteocalcin level decreased also in patients who had not received glucocorticoids, and as the reduction persisted for weeks after the termination of glucocorticoid treatment, this could

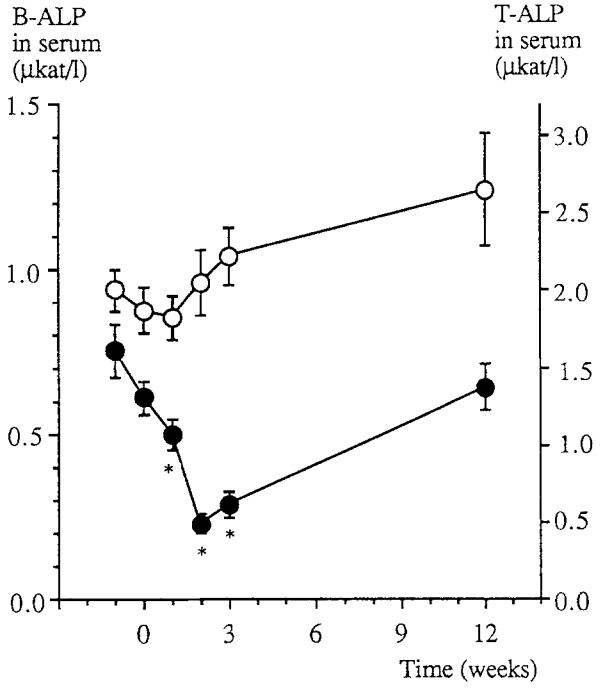

Fig. 2. B-ALP (—●—) and T-ALP (—○—) in serum before and after bone marrow transplantation. Bars represent SEM. *Significant change according to analysis of variance with significance level 95%.

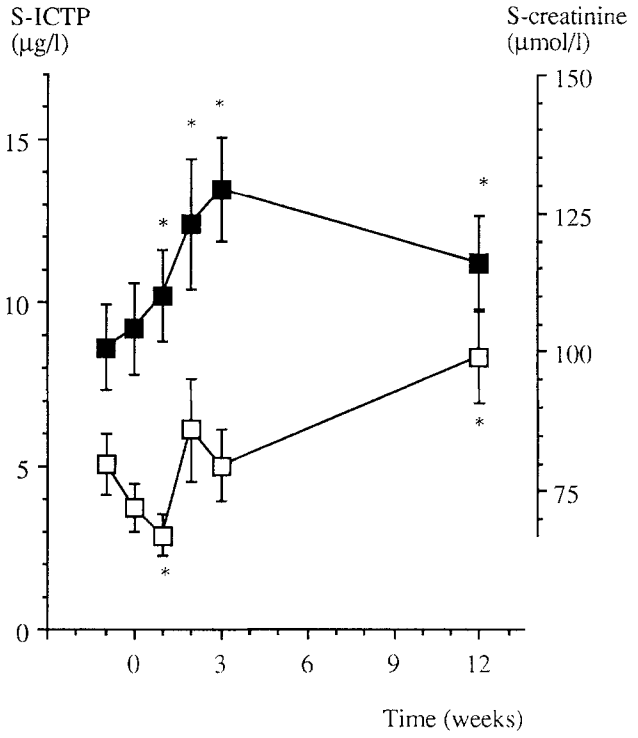

Fig. 3. S-ICTP (—■—) and S-creatinine (—□—) before and after bone marrow transplantation. Bars represent SEM. *Significant change according to analysis of variance with significance level 95%.

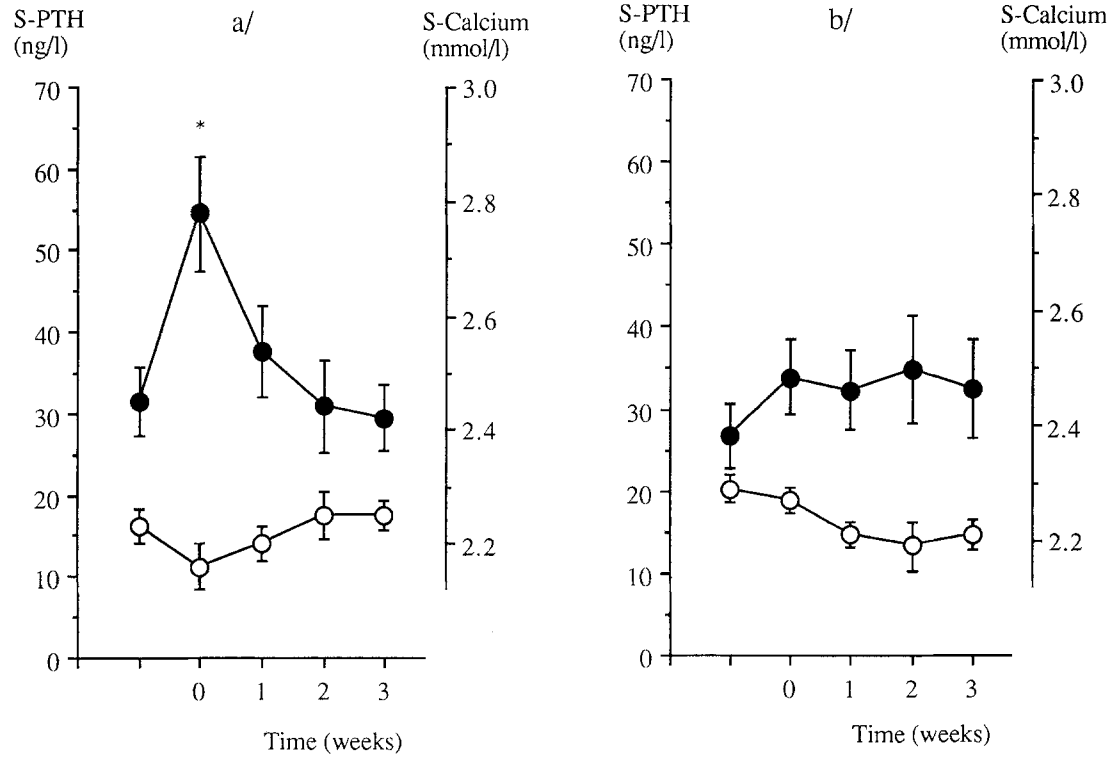

Fig. 4. S-PTH (—●—) and S-calcium (albumin modified) (—○—) before and after bone marrow transplantation. Bars represent SEM. *Significant change according to analysis of variance with significance level 95%. **(a)** Patients treated with TBI on day −1; **(b)** non-TBI-treated patients.

not have been the sole explanation. The fact that the serum B-ALP concentrations were also reduced, which are not affected by glucocorticoids [5], indicate that there is another explanation other than glucocorticoid treatment for changes in B-ALP and osteocalcin levels. One explanation is that a substantial part of the osteoprogenitor cells are damaged by the myeloablative therapy. The slow reconstitution is consistent with the remodeling cycle length. We suggest, however, that our findings point to an inhibition of osteoblast function. Tumor necrosis factor (TNF) and interleukin-1 (IL-1) are well-known inhibitors of B-ALP and osteocalcin secretion in human osteoblasts [6–10], and granulocyte-monocyte colony stimulating factor (GM-CSF) has also been shown to have inhibitory effects [11]. The myeloablative treatment and the bone marrow transplantation per se stimulate the release of these cytokines [12, 13].

Concomitantly with the reductions in serum osteocalcin and B-ALP, ICTP in serum increased during the first 3 weeks after bone marrow transplantation. S-ICTP is cleared through the kidneys [14, 15]. However, during this period, changes in serum ICTP levels were not associated with concomitantly increasing serum creatinine levels. Thus, the finding supports our hypothesis of cytokine-induced changes in bone metabolism, as both IL-1 and TNF are known to stimulate bone resorption (elevate S-ICTP) [7, 16]. Serum ICTP remained elevated after 12 weeks. The observed increase corresponded to a similar increase in serum creatinine. Thus, impaired renal clearance, and not only increased bone resorption, could have contributed to the raised serum levels.

Cyclosporin is known to enhance bone remodeling [17] and there is histological evidence for increased osteoblast and osteoclast activity in cyclosporin-treated patients [18]. However, no distinct differences were observed in serum markers of bone metabolism between patients who received cyclosporin and those who did not. It is, however, possible that a difference would have been noted with a longer follow-up.

A PTH peak in serum was consistently noted on the day after TBI. Severe illness may be associated with hypocalcemia and PTH elevations [19, 20]. As symptoms of acute irradiation damage almost invariably occur after TBI, it is likely that the acute stress caused by TBI may have similar effects.

Reduced bone mineral density is common after BMT [21]. Glucocorticoid treatment [22] and estrogen deficiency [23] are possible causes, whereas no relationship between cyclosporin therapy and changes in bone mineral density following transplantation has been seen [21]. The impairment of bone formation and increase in bone resorption, as mirrored by the biomarkers in the present study, might also play a role.

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