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BONE MASS AND TURNOVER AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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ACADEMIC DISSERTATION

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Allogeneic stem cell transplantation (SCT) is an important form of treatment for many haematological diseases. When acute complications, such as infections and graft-versus-host disease, are treatable, more attention is paid to the long-term complications of the procedure. The experience from solid organ transplantations shows that bone loss, osteoporosis and fractures are important complications of these procedures.

Consequently, the present study of patients with SCT was undertaken to answer the following questions: I What is the magnitude and timing of bone loss? II What is the mechanism of bone loss as examined by bone turnover markers and serum osteoprotegerin and RANKL measurements? III Can the bone status of SCT patients improve spontaneously? IV How and by what mechanism does allogeneic SCT performed in childhood affect peak bone mass in adolescence and young adulthood? V Can bone loss be prevented by calcium, vitamin D, calcitonin, sex steroid replacement therapy or bisphosphonates?

Altogether 187 patients with allogeneic SCT participated in the studies; 73 healthy individuals served as controls in biochemical measurements. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA). Bone turnover rate was assessed by measuring the markers of bone formation [bone-specific alkaline phosphatase (B-ALP), type I procollagen amino- (PINP) and carboxyterminal propeptides (PICP) and osteocalcin (OC)] and resorption [cross-linked carboxyterminal telopeptide of type I collagen (ICTP), C-terminal cross-linked telopeptide of type I collagen (CTX), aminoterminal telopeptide of type I collagen (NTX) and tartrate-resistant acid phosphatase 5 b (TRACP5b)].

During six months after SCT, BMD of the lumbar spine (LS) and the femoral neck (FN) reduced by 5.8 % and 7.0 %, respectively. During the next six months, the LS BMD slightly recovered but the FN BMD further decreased to 8.0%. Thirty-nine per cent of SCT patients had either osteopenia or osteoporosis at the LS before transplantation, and the number increased up to 47 % one year after SCT. At the FN, 25 % of patients had a reduced BMD at baseline and 58 % one year after SCT.

The markers of bone formation decreased during the first three months but returned to the pre-transplantation level by three months after SCT. The marker of metalloproteinase (MMP)-mediated bone resorption ICTP increased rapidly after SCT, also in patients who received bisphosphonates, and remained elevated several years after SCT. Instead the markers of cathepsin-K-mediated bone resorption (CTX, NTX) were suppressed by pamidronate therapy. Serum osteoprotegerin, but not sRANKL levels, was higher in patients than in controls. SRANKL levels were reduced with pamidronate therapy.

In the long-term adult survivors of SCT, BMD of the LS recovered almost to the baseline level on average six years after SCT. Also BMD of the FN recovered but remained reduced compared to the baseline.

On average one third of patients who have received a SCT during childhood had a reduced bone mass in adolescence and young adulthood. Female gender, hypogonadism, prepubertal status at the time of SCT, growth retardation and low BMI were risk factors for reduced peak bone mass.
Calcium either alone or with calcitonin was insufficient to prevent SCT-related bone loss. Also patients, who received a combination of calcium, vitamin D and sex steroid replacement therapy, had a bone loss equal to those who received no bone-protecting treatment. Instead, intravenous pamidronate prevented lumbar spine bone loss and significantly reduced, but did not totally abolish, it at the upper femur.

In conclusion, BMD of both the lumbar spine and the upper femur significantly decreases after SCT. At the LS bone loss spontaneously recovers. The major mechanisms behind SCT-associated bone loss are decreased bone formation and increased bone resorption; the inhibition of OPG production or excess of sRANKL do not seem to contribute to bone loss. Of the options to prevent bone loss studied, pamidronate was most efficient, but even it was incapable of totally abolishing bone loss at the hip. A reason for the relative inefficacy of pamidronate therapy might be continued MMP-related bone resorption, which cannot be prevented by bisphosphonates.
ABBREVIATIONS

AA= aplastic anaemia
ALL= acute lymphoblastic leukaemia
AML= acute myeloblastic leukaemia
ANOVA= analysis of variance
BMC= bone mineral content
BMD= bone mineral density
BMT= bone marrow transplantation
Bone ALP= bone-specific alkaline phosphatase
BSP= bone sialoprotein
BW= body weight
CLL= chronic lymphocytic leukaemia
CML= chronic myeloid leukaemia
Crea= creatinine
CTX= type I collagen carboxy-terminal telopeptide
CY= cyclophosphamide
CyA= cyclosporine A
25-OH-D= 25-hydroxyvitamin D
1,25-(OH)\textsubscript{2}-D= 1,25-dihydroxyvitamin D
24,25-(OH)\textsubscript{2}-D= 24,25-dihydroxyvitamin D
Dpy= deoxypyridinolene
DXA= dual energy X-ray absorptiometry
EGF= epidermal growth factor
EIА= enzyme immunoassay
ELISA= enzyme linked immunoabsorbent assay
FGF= fibroblast growth factor
FN= femoral neck
GH= growth hormone
GHL= galactosyl-hydroxylysine
Gla= γ-carboxyglutamic acid
GVHD= graft-versus-host disease
GY= Gray
HLA= human histocompatibility leukocyte antigen
ICTP= type I collagen carboxy-terminal telopeptide
IGF-I= insulin-like growth factor I
IL= interleukin
IRMA= immunoradiometric assay
LS= lumbar spine
MDS= myelodysplastic syndrome
MF= myelofibrosis
MP= methylprednisolone
MM= multiple myeloma
MMF= mycofenolate mofetil
MMP= matrix metalloproteinase
MTX= methotrexate
NHANES= national health and nutrition examination study
NTX= type I collagen aminoterminal telopeptide
OC= osteocalcin
OPG= osteoprotegerin
PICP= type I procollagen carboxy-terminal propeptide
PINP= type I procollagen aminoterminal propeptide
PTH= parathyroid hormone
PTHrP= PTH related peptide
Pyr= pyridinoline
QCT= quantitative computed tomography
RANK= receptor activator of NF-κB
RANKL= receptor activator of NF-κB ligand
RIA= radio immunoassay
SCT= stem cell transplantation
SD= standard deviation
SE= standard error
SPA= single photon absorptiometry
TBI= total body irradiation
TNF-α= tumour necrosis factor-α
TRACP5b= tartrate-resistant acid phosphatase 5b
TBMC= total body mineral content
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1 INTRODUCTION

Allogeneic stem cell transplantation (SCT) is an important treatment for many haematological malignancies and some other diseases. In Finland allogeneic SCTs are concentrated in Helsinki and Turku University Hospitals. At Helsinki University Central Hospital, the Division of Haematology of the Department of Medicine carries out about 70-90 transplantations per year and the Hospital of Children and Adolescents about 30 transplantations per year. When acute complications of these procedures, especially infections and graft-versus-host disease (GVHD), are now usually successfully managed, more attention is paid to the long-term complications of SCT. Five years after SCT, 50 % of those who have a non-related donor and even 80 % with sibling-donors are alive (Duncombe 1997).

It has been previously known that bone loss and osteoporosis are common after solid organ transplantations (Rodino and Shane 1998). These patients have an increased risk of fractures, especially painful vertebral fractures, the incidence of which can be even 50 % after cardiac or liver transplantation (Meyes et al 1994, Shane et al 1996).

When the present series of investigations was started there were only a few, cross-sectional studies showing that patients with SCT were also at risk of reduced bone mass (Kelly et al 1990, Castañeda et al 1997). No prospective studies were available.

Several factors in patients with SCT might affect bone metabolism. The haematological disease itself or infections associated both to the disease and its treatments enhance production of cytokines. Interleukins 1 and 6, in particular, are harmful to bones (Mundy 1996). High-dose chemotherapy and total body irradiation (TBI) lead to amenorrhoea and oestrogen deficiency in women (Kelly et al 1990, Boosma et al 2002). TBI can also result in the deficiency of growth hormone (GH), which is essential for bone growth in children and for maintaining bone mass in adults (Hovi et al 1990, Talvensaari et al 1994). Chemotherapy of the haematological disease can enhance bone loss (Epstein 1996). The most dangerous threats for bone health are immunosuppressive regimens, mainly cyclosporine A (CyA) and glucocorticoids, which are used to prevent and treat acute or chronic graft-versus-host disease (GVHD) (Epstein 1996).

Glucocorticoids both inhibit bone formation and enhance bone resorption (Lukert and Raiz 1990). CyA increases both bone formation and resorption, the latter more than the former with the net effect being bone loss (Epstein 1996, Movsowitz et al. 1988).

Osteoclast differentiation and activation are regulated at the local level by the relative expression of a recently found receptor activator of nuclear factor-κB
ligand (RANKL) and osteoprotegerin (OPG), which are produced by osteoblasts, bone marrow stromal cells and T-lymphocytes (Simmonet et al 1997). RANKL acts on osteoclast precursors and mature osteoclasts through its receptor RANK to increase osteoclast differentiation and activation. OPG instead functions as a decoy receptor able to neutralise both the cell-bound and soluble forms of RANKL (Lacey et al 1998).

Recent studies have shown that recipients of heart, kidney, liver and lung transplants benefit from bisphosphonate therapy with respect to their bone status (Reeves et al 1998, Krieg et al 2001, Grotz et al 2001, Henderson et al 2001, Shane et al 2004) but before the start of the present investigations bisphosphonates had not been tested in SCT recipients and only a few studies on recipients of other organ transplants were available.

The purpose of this study was to examine the magnitude, mechanisms, prevention and spontaneous recovery of bone loss in adult SCT recipients. Some of these aspects were also addressed in young adults who had received a SCT in childhood.
2 REVIEW OF LITERATURE

2.1 Bone functions and anatomy

2.1.1 Functions of bone

Bone is connective tissue, which together with cartilage builds up the skeletal system. It serves as a mechanical support and a site of muscle attachment. Muscles and the skeletal system together make locomotion possible. Furthermore, bone protects vital solid organs and bone marrow. Moreover, bone serves as the storehouse of the ions, especially calcium, phosphorus and magnesium (Baron 1996).

2.1.2 Bone macroscopic organisation

There are two types of bone. Trabecular or spongy bone, which makes up about 20 % of the skeleton, is found in flat bones, like the skull or the scapula, in vertebrae and in metaphyseal areas of long bones. Cortical or compact bone is found in the diaphyses of long bones. Trabecular bone is several times metabolically more active than cortical bone, and its function is mostly metabolic, whereas cortical bone takes care of support and protection (Baron 1996).

2.1.3 Bone microscopic organisation

Bone is composed of 70 % minerals and 30 % organic components. The main mineral is hydroxyapatite \([3\text{Ca}_3(\text{PO}_4)_2(\text{OH})_2]\), comprising 95 % of the mineral component. The rest is mostly calcium carbonate, citrate and magnesium. The most important organic constituent is type I collagen. In addition, there are noncollagenous proteins like osteocalcin, osteonectin and osteopontin. Two percent of the organic component is composed of different bone cells. In mature bone, the structural and functional basis is a multicellular unit or bone remodelling unit, that is, an osteon (Baron 1996). Total body calcium content is about 1000 g and it is mostly stored in bone as hydroxyapatite. Also 85 % of the body phosphorus and 50 % of the body magnesium reside in bone (Breslau 1996).

2.1.4 Osteoblasts

Osteoblasts originate from mesenchymal stem cells, which proliferate and differentiate into preosteoblasts and then to mature osteoblasts. They are responsible for bone formation. They synthesise collagen and other proteins. Osteoblasts line the layer of bone matrix, which they produce. They produce first osteoid tissue, which in 10 days calcifies into mature matrix. Toward the end of the
secreting period, osteoblasts become isolated into bone lacunae, and some osteoblasts become osteocytes (Puzas 1996, Boyle et al 2003).

2.1.5 Osteoclasts

Osteoclasts are responsible for bone resorption. They originate from haematopoietic mononuclear cells. Mononuclear osteoclast precursors circulate in the blood. At endosteal bone surfaces, they proliferate and fuse to form multinucleate cells. They secrete lysosomal and nonlysosomal enzymes and hydrogen ions. They mobilise hydroxyapatite crystals from type I collagen and degrade collagen. So calcium and inorganic phosphate are released from the degrading matrix into the circulation (Mundy 1996, Boyle et al 2003).

2.1.6 Osteocytes

Osteocytes are osteoblasts, which have become encased in calcified bone. Approximately 15 % of osteoblasts become osteocytes. Metabolic activity of osteocytes is low because they are encaded in mature bone and their nutrition comes only from small canaliculi (Puzas 1996).

2.2 Bone remodelling and its regulation

2.2.1 Bone remodelling

Bone growth in length is dependent upon the proliferation of cartilage cells in the growth plate followed by maturation of these cells and endochondral ossification. Growth in width is accomplished by the formation of bone at periosteal surfaces and by resorption at endosteal surfaces. When the epiphyses are closed at the age of about 15-18, bone growth in length ends. However, it is essential for bone strength and for the regulation of mineral metabolism that bone remodelling continues throughout life.

Figure 1 shows the bone remodelling cycle in trabecular bone. Bone remodelling constitutes the resorption of old bone and its replacement with new bone. This remodelling takes place by teams of cells called osteons. In trabecular bone, they are saucer-shaped Howship’s lacunae and in cortical bone cylindrical structures called Haversian units. The remodelling cycle comprises five phases. First in the activation phase osteoclasts are activated and wander to a resorption site. In the second phase they start to resorb old bone. They secrete enzymes and hydrochloride acid, which dissolve the mineral. At low pH cysteine proteinases, like cathepsin-K begin to digest bone matrix. When the pH rises matrix metalloproteinases (MMPs) complete the digestion (Evert et al 1998, Garnero et al,
In the third phase macrophage-like cells finish the resorption and prepare the site for bone forming cells. In the synthesis phase, osteoblasts fill the cavity with new bone, which mineralises, and a new osteon is completed. Normally bone formation and resorption are closely tied to each other. In trabecular bone, the remodelling cycle lasts about 100 days and in cortical bone about 200 days (Aurbach et al 1992b).

Remodelling is regulated by systemic hormones and local factors, which affect bone resorbing and forming cells. Uncoupling, which means imbalance between bone formation and resorption, leads to disorders in bone metabolism.

### 2.2.2 Sex steroids

Oestrogens play an important role in the regulation of bone remodelling both in males and females. In males they are formed from testosterone by extraglandular aromatase enzymes. In bone, oestrogens inhibit bone resorption, to a great extent through osteoblastic receptors by down-regulating the expression of bone resorbing cytokines, such as interleukins (IL)-1α, IL-1β, IL-6 and tumour necrosis factor-α (TNF-α). Lack of oestrogens leads to an increased number and activity of these cytokines. Consequently, especially in trabecular bone the interval between remodelling cycles shortens and at the osteon level both bone resorption and formation are accelerated, but resorption is quicker than formation (Kelly et al 1990, Brinhurst et al 1998, Riggs 2002).

Androgens may increase bone formation and by increasing muscle mass they increase the mechanical load on the bones and thus strengthen them (Brinhurst et al 1998).

### 2.2.3 Parathyroid hormone

Parathyroid hormone (PTH) is secreted by the parathyroid glands. Its main function is to keep the concentration of ionised calcium in blood and extra-cellular fluids constant. It activates the liberation of calcium and phosphate from bone. It stimulates the resorption of calcium and inhibits that of phosphate in the renal tubules. It also stimulates the renal synthesis of 1,25-dihydroxyvitamin D [1,25-(OH)2-D], and consequently, the intestinal absorption of calcium (Aurbach et al 1992a).

When constantly elevated in serum and extra-cellular fluids PTH is a strong stimulator of bone resorption leading to bone loss but when given intermittently in suitable doses it stimulates bone formation more than resorption thus increasing bone mass (Slovik et al 1986, Black et al 2005, Cosman et al 2005).
2.2.4 Vitamin D

Vitamins D is a fat-soluble, steroid-related family of molecules, which are both synthesised in the body and derived from diet. 7-dehydroxycholesterol is synthesised by the skin to cholecalciferol (vitamin D₃) with exposure to ultraviolet light. Cholecalciferol also comes from diet. Ergocalciferol (vitamin D₂) originates from plants. Vitamin D (both D₂ and D₃) needs two hydroxylations to become biologically active. The first hydroxylation takes place in the liver to form 25-hydroxyvitamin D (25-OH-D). 25-OH-D is further hydroxylated in the proximal tubules of the kidneys to 1,25-(OH)₂-D or calcitriol, which is the most active vitamin D metabolite. A part of 25-OH-D is hydroxylated to 24,25-dihydroxyvitamin D [24,25-(OH)₂-D] (Canalis 1996).

Vitamin D increases the absorption of calcium and phosphate in the gut and increases the resorption of calcium in the renal tubules. In the short term calcitriol inhibits osteoblast function, and increases osteoclastogenesis by up-regulating the synthesis of the receptor activator of nuclear factor-κB ligand (RANKL) and by down-regulating osteoprotegerin (OPG). Instead, in the long term it stimulates osteoblasts and the synthesis of osteocalcin (Brinhurst et al 1998, Canalis 1996).

2.2.5 Calcitonin

Calcitonin is secreted by C-cells of the thyroid gland. An increase in serum calcium stimulates calcitonin secretion. It decreases bone resorption by inhibiting osteoclasts through direct action on the osteoclastic calcitonin receptors (Delfos 1996).

2.2.6 Growth hormone and insulin-like growth factor –I

Growth hormone (GH) is a pituitary peptide hormone, which is needed to build up and maintain bone mass. GH deficiency in children leads to impaired growth but also to reduced bone mass (Shore et al 1980, Kaufman et al 1992, Saggese et al 1996). Also in adults GH is needed to maintain bone mass (Olney 2003). Partly GH acts by increasing the hepatic production of insulin-like growth factor –I (IGF-I), which is a potent stimulator of bone growth. It increases collagen synthesis and decreases its degradation (Canalis 1996, Olney 2003).
2.2.7 Glucocorticoids

Glucocorticoids are adrenal hormones, which decrease bone formation by inhibiting the differentiation of osteoblasts. They also increase bone resorption both directly and through secondary hyperparathyroidism by decreasing calcium absorption in the gut and by increasing calcium excretion into urine. They decrease type I collagen formation and increase the expression of collagen degrading enzymes, like matrix metalloproteinases (MMPs), in bone. They also impair the synthesis of skeletal growth factors (Lukert and Raisz 1990, Canalis 2003). The latest studies support the theory that the direct effects of glucocorticoids on the bone are more important than the secondary ones (Shaker and Lukert 2005). Bone loss induced by glucocorticoid treatment is dose-dependent and is observed within a few days after starting the therapy (Sambrook et al 1994a).

2.2.8 Local regulation of bone remodelling

Figure 2 summarises the local regulation of bone remodelling. Local factors, which are synthesised by skeletal, stromal, haematopoietic or immune cells, have a very important role in the regulation of bone remodelling. From the cytokine family IL-1α, IL-1β, IL-6 and TNF-α are potent stimulators of bone degradation and inhibitors of bone formation. Interferon-γ inhibits osteoclast function and bone resorption. Transformal growth factor-α (TGF-α) and epidermal growth factor (EGF) increase bone degradation. Granulocyte-macrophage (GM-CSF) and macrophage colony stimulating factors (M-CSF) enhance osteoclast differentiation. Bone morphogenic proteins (BMPs), TGF-β, 1,25-(OH)2-D, MMPs and bone lining cells are proposed to have a role in coupling bone remodelling. Prostaglandins have many effects on bone remodelling. Bone cells synthesise IGF-I and IGF-II, which enhance bone collagen and matrix synthesis. Also fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) act on bone. They stimulate collagen synthesis (Canalis 1996, Boyle et al 2003).

2.2.9 Osteoprotegerin and RANKL

Osteoclast differentiation and activation are regulated at the local level by the relative expression of RANKL and OPG, which are produced by osteoblasts, bone marrow stromal cells and T-cells (Simmonet et al 1997). RANKL acts on osteoclast precursors and mature osteoclasts through its receptor RANK to increase osteoclast differentiation and activation. OPG instead acts as a decoy receptor that is able to neutralise both the cell-bound and soluble forms of RAN-
The expression of RANKL and OPG are regulated by many factors, such as PTH, 1,25-(OH)$_2$-D, vitamin D$_3$ and oestrogens (Fohr et al 2003).

2.3. Bone life span

2.3.1 Accumulation

The accumulation of bone mass implies increases in bone volume and mineral content. It is a dynamic process, in which both bone resorption and formation are needed for the development of normal bone architecture and mass. Twin studies have shown that peak bone mass is determined mostly by genetic factors, which explain up to 80% of variance (Slemenda 1991).

However, the accumulation of 20% of the individual peak bone mass is affected by environmental factors, like nutrition, exercise, smoking and medications (Välimäki et al 1994, Lehtonen-Veromaa et al 2000). The adequate supply of calcium, phosphorus, magnesium and vitamin D is essential for skeletal growth during childhood and adolescence and to maintain bone mass in adulthood (Breslau 1996, Välimäki et al 2004).

Before puberty, girls and boys have an equal skeletal size and volumetric bone mineral density (Bonjour et al 1991). During the pubertal growth spurt, skeletal mass doubles (Riggs 2002). In males bone mass continues to increase until the age of 15-18, but in females the accumulation of skeletal mass dramatically slows down between the second and fourth year after menarche, usually before the age of 15 (Bonjour et al 1991). By the time of the epiphyseal plate closure, the volumetric BMD has reached about 90-95% of peak mass (Kröger et al 1993, Riggs 2002). The skeleton is then consolidated to its maximal BMD by continued periosteal apposition and by trabecular thickening by the end of the second decade (Theintz et al 1991). Due to a longer pubertal growth spurt and the effect of testosterone, which results in a greater bone size, males end up with 25% greater areal peak bone mass than females (Riggs 2002).

In addition to bone mass, bone strength depends on its structural (geometry, shape, micro architecture) and material (collagen quality, mineralisation) properties. Any disturbance in bone accumulation can lead to lowered peak bone mass and impaired bone quality and consequently, to an increased risk of osteoporosis and fractures (Aurbach et al 1992b).

2.3.2 Ageing

The velocity of bone loss increases during ageing. BMD begins to decrease in both sexes before 30-35 years of age, but before menopause in females and in
young adulthood in males the net loss is slow due to a slow remodelling rate. After menopause bone loss accelerates in women. Women lose about half of their bone mass by age 80 and men 25-30 %, respectively. Bone geometry changes only a little (in width) after growth stops (Aurbach et al 1992b, Seeman 2004). Thereafter bone formation decreases and to lesser extent bone resorption increases. Both males and females lose equal amounts of trabecular bone but in males thinning of trabeculae dominates whereas in females loss of connectivity is the main feature. Net cortical bone loss is less in males than in females because of greater periosteal apposition (Seeman 2004).

2.4 Measurement of bone mineral density

2.4.1 Techniques for measurement of bone mineral density

Conventional radiography shows bone loss when 30-50 % of bone mineral content has disappeared, thus being a very insensitive way of measuring BMD.

Single photon absorptiometry (SPA) utilises 125I (27 keV) coupled with a scintillation detector, which scans across the interested area, most commonly the peripheral sites of the tubular bones. Single energy X-ray absorptiometry uses an X-ray source in scanning appendicular sites. These techniques cannot be used to study the vertebrae or other bones surrounded by fat and muscles. In scanning these sites, dual photon absorptiometry (DPA) can be used. It utilises usually 153Gd, which emits photons at two energies (44 and 100 keV). This technique is expensive and the half-life of the isotope is short. Dual energy X-ray absorptiometry (DXA) uses X-rays instead of isotopes and has replaced DPA in clinical practice (Kanis 1994b).

In quantitative computed tomography (QCT) thin slices through the body are imaged, and the images are quantified to measure the volumetric BMD. This technique is very sensitive and it can separate trabecular and cortical bone, but it is expensive and the radiation exposure is high (Kanis 1994b). The latest techniques are based on microCT or magnetic resonance imaging and can give an image of the quality of the bone. Until now they have been used in studies but they are not yet widely used clinically (Chesnut et al 2005).

2.4.2 Dual energy X-ray absorptiometry

Because of a short scanning time (about 5 minutes), low radiation exposure (< 1 μSv), low precision error (0,5-2 %) and tolerable costs, DXA is today a widely-used method for the measurement of BMD (Kanis 1994a). In DXA an X-ray tube produces photons with two different energy levels, high and low, and thus
enables the evaluation of the areal densities of different tissues. The photons are detected after exiting from bone in the region of interest and measured by a computer, which converts them into determinations of bone mineral content (BMC). Areal BMD (grams/cm²) is counted by dividing BMC values of the projected areas of bone analysed (Genant et al 1989). Disadvantages of the method are that it does not take into account the three-dimensional bone size and shape, and does not separate trabecular and cortical bone (Kröger 1995). BMD values are compared to databases of healthy people of different ages and both sexes to calculate Z- and T-scores. The Z-score compares the measured value to those of people of the same age and gender and the T-score to the peak bone mass of the healthy individuals (20-40 years of age) of the same sex. One unit in these scores represents one standard deviation from the mean value of the reference population. According to WHO criteria, a T-score \( \leq -1 < -2.5 \) means osteopenia, and T-score \( \leq -2.5 \) means osteoporosis (Kanis 1994a).

2.5 Biochemical markers of bone metabolism

There is a panel of biochemical markers, which can be used to quantify bone metabolism. These assays measure in serum or in urine enzymes or matrix proteins synthesised by bone cells or the degradation products of bone matrix. They can be divided into those measuring bone formation and those quantifying bone degradation (Kanis 1994b).

2.5.1 Markers of bone formation

2.5.1.1 Alkaline phosphatase

The common form of alkaline phosphatase (ALP) is a cell-membrane-associated enzyme, which is expressed by the liver, bone, kidneys and placenta. In adults the liver and the bone are the major sources of ALP. In bone ALP is derived from osteoblasts and their precursors, and it has a role in bone mineralisation. ALP may increase the local concentration of inorganic phosphate, destroy local inhibitors of crystal growth, transport phosphate and act as a calcium-binding protein (Kanis 1994b, Calvo et al 1996). To be used as a marker of bone formation, the bone-specific isoform (bone ALP) has to be distinguished from the liver-specific-isoform. This can be done by using an immunoradiometric (IRMA) method, an ELISA, an immunoextraction or an electrophoresis (Behr and Barnett 1986, Magnusson 1998).
2.5.1.2 Osteocalcin

Human osteocalcin (OC) is a 49-residue polypeptide of γ-carboxyglutamic acid (Gla), which is synthesised by mature osteoblasts during the mineralisation phase of bone remodelling in a vitamin K-dependent carboxylation process. Vitamin D modulates the OC-gene (Stein et al 1996). OC is found also in other calcified tissues like dentin and calcified cartilage. In the bone it forms about one per cent of the organic matrix. OC is released into the circulation during the bone formation, and it is filtered in the kidneys (Eyre 1996). However, as OC is incorporated into the bone extracellular matrix, some of the circulating OC might originate from degrading bone. Thus circulating OC may rather reflect bone turnover than bone formation alone (Riggs et al 1986). OC can be measured by an IRMA method (Garnero et al 1992).

2.5.1.3 Type I collagen propeptides

Type I collagen is synthesised by osteoblasts from type I procollagen precursor proteins. These precursors have large extension domains at both ends. While type I collagen is synthesised, these type I aminoterminal and carboxyterminal propeptides, PINP and PICP respectively, are enzymatically removed and released into the circulation (Calvo 1996). While the bone is the major organ synthesising type I collagen, PINP and PICP reflect bone formation (Delmas 1992, Ebeling et al 1992, Calvo et al 1996). PINP and PICP are degraded in the liver and elevated serum levels have been measured in patients with chronic liver disease (Guanabens et al 1996). PINP and PICP can be measured by immunoassays (Melkko et al 1990, Melkko et al 1996).

2.5.2 Markers of bone degradation

2.5.2.1 Hydroxyproline

Collagen contains high amounts of hydroxyproline (HOP), and during its breakdown HOP is released into the blood. HOP has been traditionally used as a marker of bone degradation. It is not specific to bone collagen and this together with contributions from diet and the degradation of free amino acids in the liver makes it a very unspecific way to measure bone resorption (Eyre 1996).
2.5.2.2 Pyridinoline cross-links

Collagen molecules are held together by hydrogen bonds and pyridinium cross-links. When collagen degrades, these cross-links [pyridinoline (Pyr), deoxypyridinoline (Dpy)] are released into the circulation, and then excreted into urine. Because the bone is the major reservoir of the type I collagen, and turns over faster than most major connective tissues, pyridinolines in adult urine are mostly derived from bone and thus reflect bone resorption. Urinary Pyr and Dpy can be measured by immunoassays. There is a wide intra-individual variation, and results should be related to urine creatinine (Eyre 1996).

2.5.2.3 Hydroxylysine

Hydroxylysine is formed from lysine during the posttranslational phase of collagen synthesis and is incorporated into bone matrix as a component of collagen molecules. It occurs in two glycosylated forms, glycosyl-galactosyl-hydroxylysine and galactosyl-hydroxylysine (GHL). GHL is more bone-specific, and it is released into the circulation during collagen degradation. Both can be measured by an immunoassay in the urine (Fohr et al 2003).

2.5.2.4 Tartrate-resistant acid phosphatase

Tartrate-resistant acid phosphatase is an enzyme, the 5b-isofrom (TRACP 5b) of which is expressed in high amounts in osteoclasts. During bone degradation TRACP5b is released into the circulation. It has been shown to be elevated in patients with metabolic bone diseases. It seems to be a quite sensitive and specific marker of bone resorption. It reflects both cathepsin-K- and MMP-mediated bone degradation. During bisphosphonate therapy serumTRACP5b decreases. (Halleen et al 2001, Janckila et al 2001, Tähtelä et al 2005, Välimäki and Tähtelä 2005) TRACP5b can be measured in the serum by an immunoassay based on a highly characterised specific monoclonal antibody (Halleen et al 2000).

2.5.2.5 Cross-linked telopeptides of type I collagen

When type I collagen is degraded it is split into several fragments. Cathepsin-K and MMPs take part in this process. Two fragments have been characterised in the carboxyterminal end of the type I collagen. The first is a cross-linked carboxyterminal telopeptide of type I collagen (ICTP) and the second a C-terminal cross-linked telopeptide of type I collagen (CTX). ICTP is a larger molecule than CTX (Garnero 2003). There is a type I collagen aminoterminal telopeptide (INTP or NTX) in the aminoterminal end. It is thought that cathepsin-K relea-

2.5.2.6 Bone sialoprotein

Bone sialoprotein (BSP) is a glycoprotein produced by osteoblasts. It is glycosylated and incorporated into bone matrix during bone formation. It accounts for 10-15% of non-collagenous organic matrix. During bone degradation it is released and can be measured in serum by an immunoassay (Fohr et al 2003).

2.6 Stem cell transplantation

2.6.1 Autologous stem cell transplantation

Autologous stem cell transplantation (SCT) is an important treatment method of haematological malignancies and some other diseases. In autologous SCT, haematological stem cells are mobilised and harvested either from peripheral blood or sometimes from the bone marrow. Thereafter, the patient is conditioned with high-dose chemotherapy. Then the stem cells are infused back into the patient. No anti-GVHD medication is needed because the infused cells are the patients’ own (Duncombe 1997).

2.6.2 Allogeneic stem cell transplantation

Allogeneic SCT is used to treat haematological malignancies and some other haematological diseases. The bone marrow of the patient is destroyed before transplantation. A classical treatment is total body irradiation (TBI) with 12 Gy (lungs 10 Gy) divided into fractions of 2 Gy and given over five days. TBI is combined with chemotherapy, which in most cases consists of a high dose of cyclophosphamide. There are protocols using other regimens with or without TBI. Thereafter stem cells, which have been harvested either from an HLA-identical sibling or from a matched unrelated donor, are infused into the patient (Duncombe 1997, Bensinger and Spielberger 2004, Shank and Hoppe 2004).
2.6.3 Graft-versus-host disease

Graft-versus-host disease (GVHD) is a situation in which transplanted immune cells attack the recipient’s cells causing clinically variable, and at worst life-threatening, symptoms. All patients receiving allogeneic SCT get immunosuppressive treatment against GVHD. The basic immunosuppressive regimen has traditionally been cyclosporine A (CyA). In most cases a short course of methotrexate (MTX) is used at the beginning of the procedure. Glucocorticoids especially methylprednisolone (MP) also belong to the protocols. In recent years new drugs, which are widely used to prevent rejection in solid organ transplantations, like mycofenylate mofetil (MMF) and tacrolimus, have also been studied in SCT. Antilymphocyte globulin can be used instead of MP especially in patients with an unrelated donor. In spite of prophylaxis, acute or chronic GVHD can complicate the course of SCT. Acute GVHD is treated with a high-dose of MP and chronic GVHD with MP, CyA, MMF or thalidomide (Thomas et al 1975, Duncombe 1997, Sullivan 2004).

2.7 Transplantation associated bone disease

2.7.1 Differences between solid organ and stem cell transplantations

Patients receiving SCT are immobilised and receive immunosuppressive treatment like other transplantation patients (Stern et al 1996). There are, however, some differences between solid organ and stem cell transplantations. Patients receiving SCT are often younger and their illness has lasted for a shorter time than that of patients with solid organ transplantation. If SCT recipients do well, immunosuppressive treatment can be stopped about one year after SCT, which is not the case in patients with solid organ transplants. Another major difference in the treatment is that SCT patients receive high-dose chemotherapy and in many cases total body irradiation (TBI) before transplantation (Bensinger and Spielberger 2004, Shank and Hoppe 2004).

2.7.1.1 Effect of chemotherapy

Glucocorticoids are a part of many haematological treatment protocols. They inhibit bone formation and enhance bone resorption when measured by biochemical markers of bone metabolism (Carlson et al 1994, Arikoski et al 1999b). Their effects on bone are discussed in more detail later (Chapter 2.7.7.4). BMD has shown to be reduced after leukaemia treatment (Arikoski et al 1999a) and after high-dose chemotherapy for non-Hodgkin lymphoma and breast cancer (Banfi et al 2001). Methotrexate (MTX) is a folate antagonist, which has been related to severe osteopenia especially in children, but also in adults (Epstein
1996). MTX is thought to increase bone resorption and urinary and faecal excretion of calcium (Kaste et al 1999). Ifosfamide-associated bone loss has also been described (Kaste et al 1999). One mechanism behind chemotherapy-associated bone loss might be its toxicity to bone marrow osteoprogenitors, which are precursors of bone-forming cells (Bhatia et al 2001). High-dose cyclophosphamide leads to gonadal impairment and to amenorrhea, infertility and lack of oestrogen in females and gonadal impairment and at least in high doses infertility in males (Kelly et al 1990, Sanders et al 1996, Boomsma et al 2002).

2.7.1.2 Total body irradiation

TBI impairs growth hormone excretion (Hovi et al 1990, Talvensaari et al 1994). Lack of GH leads to impairment of skeletal growth in children, but also reduces BMD in adults (Holmes et al 1994). Skeletal irradiation may directly interfere with skeletal growth and cause direct bone loss (Neuhauser et al 1952). The survivors of childhood ALL, who had received cranial irradiation, had lowered BMD (Gilsanz et al 1990). Instead in those survivors of childhood ALL, who had not received TBI, BMD of the lumbar spine (LS) and total body did not differ from healthy controls (Brennan et al 2005). TBI may also damage the gonads and thereby cause hypogonadism (Shubert et al 1990, Kauppila et al 1998).

2.7.1.3 Effect of haematological disease

It is well known that plasmacytomas of bone cause significant morbidity to patients with multiple myeloma (Body et al 1998). Patients with acute lymphoblastic leukaemia (ALL) also have reduced bone turnover at the time of diagnosis either due to cytokines produced by ALL-cells or secondarily to depressed procollagen synthesis caused by occupation of the synthetic capacity by leukaemia cell mass (Sorva et al 1997). Infections associated with malignant diseases enhance the production of cytokines (Mundy 1996). It has been shown that after allogeneic SCT the serum concentrations of osteoclast-activating cytokines IL-6 and TNF-α increase (Lee et al 2002b, Lee et al 2004).

2.7.2 Bone mineral density after solid organ transplantation

It has been known since the 1990s that the recipients of heart, kidney, lung and liver transplants are at increased risk of osteoporosis. The decrease in BMD is fastest during the first 6 months after transplantation, but it continues at least one year after the transplantation (Katz and Epstein 1992, Rodino and Shane 1998). Older patients had significantly higher rates of bone loss (Olivari et al 1988, Muchmore et al 1992).
In cardiac transplant recipients, the reduction in BMD was from 6 to 10% at the lumbar spine during the first 6 months (Sambrook et al 1994, Välimäki et al 1999) and from 6% to 14% at the femoral neck (FN) during the first year (Van Cleemput et al 1995, Välimäki et al 1999). The lumbar BMD stabilises after 6 months, the hip BMD after the first year, but the radial BMD continues to fall during the second and third year (Cohen and Shane 2003, Maalouf and Shane 2005).

Children, who had received a cardiac transplant at least one year before BMD measurement, had lower Z-scores at the lumbar spine (LS), femoral neck (FN) and total body than age-matched healthy controls (Daniels et al 2003).

In kidney transplant recipients, BMD decreases by 4 to 9% at the LS and 5 to 8% at the FN during 6-18 posttransplant months. At the same time 17-49% patients have osteoporosis at the LS, 11-56% at the FN and 22-52% at the radius (Julian et al 1991, Grotz et al 2001, Cohen and Shane 2003, Maalouf and Shane 2005). There is one study of kidney transplant recipients, which showed only 2.4% decrease in lumbar spine BMD and no changes at the femoral neck within six months post transplantation (Miklus et al 2003). A steroid-sparing immunosuppressive protocol was used in this study.

One year after kidney transplantation child patients had lowered z-scores in the total body (Daniels et al 2003).

The spinal BMD of the liver transplant recipients decreased by 2-24% during the first year (Meys et al 1994, Cohen and Shane 2003, Compston 2003). A spontaneous recovery of BMD during the second and third year after liver transplantation has been documented (Cohen and Shane 2003, Maalouf and Shane 2005).

After lung transplantation the lumbar and femoral BMD decreased by 2-5% during the first year and 73% of the patients had osteoporosis (Cohen and Shane 2003, Maalouf and Shane 2005).

### 2.7.3 Bone mineral density after allogeneic stem cell transplantation

Table 1 summarises the major findings of studies of bone metabolism after SCT. Before the present study there were several small studies in patients with SCT: A cross-sectional study (n=23) showed lowered bone mass after SCT (Kelly 1990). In another study 33% of the patients had osteopenia and 18% osteoporosis about three year after receiving SCT (total n=27) (Castañeda et al 1997). The only small (n=9) longitudinal study showed a reduction in BMD 9 months after the beginning of the prophylaxis of GVHD (Stern et al 1996).

In a more recent longitudinal study 29 recipients of allogenic SCT lost 11.7% of their femoral neck BMD, 3.9% of their lumbar spine BMD and 3.5% of their
total body mineral content (TBBMC) during approximately 30 months after SCT (Ebeling et al 1999). In another study (n=81) bone loss was 7.2 % at the lumbar spine BMD, 11.9 % at the femoral neck BMD and 3.8 % at the TBBMC after one year of SCT (Schulte et al 2000). The third study (n=67) showed that 49 % of SCT patients had osteopenia even before the transplantation. During the first six and twelve post transplant months trabecular bone BMD decreased by 13 % and 17 % and cortical BMD by 9.8 % and 9 %, respectively, when measured by QCT (Messenkeil et al 2001). In the fourth study (n=67) the lumbar spine BMD decreased by 3.3 % and the femoral neck BMD by 8.9 % during the first year after SCT (Lee et al 2002a).

A 2-year longitudinal study of 35 patients showed a slight increase in the spinal BMD from one to two years after SCT, and a further loss in the femoral neck BMD at the same time (Schulte et al 2000). A cross-sectional study performed approximately three years (range 1-10 years) after SCT (n=41) showed that BMD in transplantation patients was significantly lower than in controls and 37 % of them had osteopenia and 15 % osteoporosis at the LS (Tauchmanova et al 2002). With phalangeal osteosonogrammetry 60 % of patients had osteopenia and 7 % osteoporosis. The longer after transplantation the better bone status patients had especially in the lumbar spine (Tauchmanova et al 2002). Other studies (n=44-102) have demonstrated that the femoral neck BMD is lowered several years after SCT (Buchs et al 2001, Gandhi et al 2003). The latest study, in which 280 SCT recipients were followed for at least 4 years, demonstrated that the nadir in the reduction of BMD was achieved at 6 months in the lumbar spine and at 24 months in the femoral neck (Schulte and Beelen 2004). Another cross-sectional study (n=22) showed that 28 % of SCT recipients had osteopenia at the LS and 48 % at the FN approximately 5 years after SCT (Kersch-Schindl et al 2004).

Studies of paediatric populations (combined n=95) have shown a slight decrease in Z-scores at the LS, FN and in the whole body at least 1 year after SCT (Nysom et al 2000, Daniels et al 2003). The latest study of paediatric SCT patients (n=48) showed that 26 % of them had osteopenia (defined as a Z-score between −2 and −1) and 21 % osteoporosis (Z-score lower than −2) approximately 5.5 years after SCT (Kaste et al 2004). In one study, which included both paediatric and adult patients (n=28), total body BMD in Z-scores was significantly lower in paediatric patients (Z-score −0,5) than in the adult population (Z-score 0,0) when measured about two years after SCT (Bhatia et al 1998).
2.7.4 Fractures and avascular necrosis after solid organ and stem cell transplantation

2.7.4.1 Fractures

The recipients of solid organ transplants have an increased risk of fractures. Eight per cent of patients with renal transplantation had fractures during the first two post transplant years (Ramsay-Goldman et al 1999). The fractures affected mostly appendicular sites like the hip, long bones and feet (prevalence 10-50 %) than axial sites (prevalence 3-10 %). Females and patients with diabetes had a higher risk of fractures (Ramsay-Goldman et al 1999, Cohen and Shane 2003).

Cardiac transplant recipients have a 22-35 % prevalence of the vertebral fractures during the first post-transplant year (Shane et al 1993), the incidence of new vertebral fractures during the first year being 28-36 % (Shane et al 1996, Välimäki et al 1999, Leidig-Bruckner et al 2001).

After liver transplantation the fracture rate ranges from 24 to 65 % during the first year after the transplantation and the majority of fractures affect the ribs and vertebrae (Leidig-Bruckner et al 2001, Cohen and Shane 2003). There has been a tendency to a decreased incidence of fractures in recent years, which is proposed to be due to steroid-sparing immunosuppressive protocols (Compston 2003).

Eighteen to 37 % of lung transplant recipients get fractures (Shane et al 1999, Cohen and Shane 2003).

In stem cell –transplanted patients the incidence of vertebral or non-vertebral fractures during the first year after SCT has varied from 1 to 16 % (Stern et al 1996, Ebeling et al 1999, Schulte et al 2000).

2.4.7.2 Avascular necrosis

Avascular necrosis of bone is characterised by areas of necrosis of bone and bone marrow of the long bones, especially femur, tibia and humerus (Enright et al 1990). The incidence of the avascular necrosis after allogeneic SCT is 8.1-10.4 %. It is described as affecting most commonly hip, knee, ankle and shoulder joints (Enright et al 1990, Socié et al 1994, Tauchmanova et al 2003). The incidence of avascular necrosis in patients with autologous transplantation was 0-1.9 % (Enright et al 1990, Tauchmanova et al 2003). In all of these studies the incidence of avascular necrosis was correlated to acute or chronic GVHD needing glucocorticosteroids, increasing age and in one study to the male gender (Tauchmanova et al 2003). Patients with avascular necrosis have decreased fibrinolytic potential (Van Veldhuizen et al 1993). In paediatric patients 44 % had had osteonecrosis
approximately 5.5 years after SCT (Kaste et al 2004).

2.7.5 Markers of bone turnover after solid organ transplantation

In a histomorphometric analysis of bone biopsies from liver transplant recipients, bone turnover, both formation and resorption, was increased when compared to the low turnover state before the transplantation (Vedi et al 1999). After kidney transplantation, histomorphometric studies have shown increased bone resorption and osteoblastic dysfunction with a defect in the mineralisation (Julian et al 1991, Cohen and Shane 2003). After cardiac transplantation, the markers of bone formation (serum PINP, PICP, OC) decrease and the markers of resorption (serum ICTP, urinary HOP) increase within the first weeks and months. Bone turnover remains high at least 6-12 months after the procedure (Rich et al 1992, Shane et al 1993, Sambrook et al 1994, Thiebaud et al 1996, Välimäki et al 1999). In lung transplant recipients, bone turnover markers are consistent with both increased resorption and formation (Cohen and Shane 2003).

2.7.6 Markers of bone turnover after allogeneic stem cell transplantation

Table 1 shows major changes in bone markers after SCT. Bone ALP and PINP decreased 3 weeks to 3 months after SCT. Thereafter, these markers returned to the baseline by 6 months and tended to be even higher at 12 months after than before SCT (Carlson et al 1994, Ebeling et al 1999). Similar results have been reported in studies with serum OC as the marker of bone formation (Gandhi et al 2000, Kang et al 2000, Buchs et al 2001). Several years after the transplantation, serum OC was within the reference limits (Castañeda et al 1997).

In one small study (n=27) the markers of bone resorption, ICTP and HOP were elevated several years after SCT (Castañeda et al 1997). Serum ICTP has been shown to be elevated even before transplantation and the level further increased during the first months after SCT and then decreased but remained higher than before SCT (Kang et al 2000, Lee et al 2002a). The acceleration of bone resorption after SCT has been documented also by using assays of urinary HOP (Stern et al 1996), CTX (Banfi et al 2001), NTX (Schulte et al 2000) and pyridinium cross-links (Ebeling et al 1999).
2.7.7 Mechanisms of transplantation associated bone loss

2.7.7.1 Role of underlying disease

There are many reasons why BMD might be worse in chronically ill patients. The impaired hydroxylation of 1,25-(OH)$_2$-D, secondary hyperparathyroidism and consequent osteitis fibrosa, adynamic bone disease and osteomalacia are important metabolic complications of kidney dysfunction and lead to bone morbidity (Gonzáles and Martin 2003). Cholestatic liver disease may impair the formation of 25-OH-D (Katz and Epstein 1992). Heart failure patients use loop diuretics, which lead to a negative calcium balance, and many have smoked (Katz and Epstein 1992). Patients with chronic lung diseases might be smokers and many of them have used glucocorticoids (Cohen and Shane 2003).

2.7.7.2 Immobilisation

Severe chronic disease leads to immobilisation, which leads to rapid bone loss especially in hospitalised patients both in adults (Le Blanc et al 1990) and in children (Tillman et al 2002). Resistant exercise training has been shown to restore BMD in heart transplantation patients (Braith et al 1996).

2.7.7.3 Immunosuppressive treatment

All transplanted allograft organs are at a great risk of undergoing a rapid and progressive immune-mediated injury or rejection, which threatens the prognosis of the transplanted organ and in the worst cases also that of the patient receiving the allograft. Thus, all patients receiving a transplanted organ need immunosuppressive regimens. The traditional strategy consists of CyA, azathioprine and glucocorticoids, most often methylprednisolone (MP). During the past decade new agents have also been developed and in many cases azathioprine is replaced by mycophenolate mofetil (MMF), and also tacrolimus instead of CyA and sirolimus instead of azathioprine or MMF are in clinical use (Denton et al 1999). CyA and MP, in particular, may have an important role in transplantation-associated bone disease (Epstein 1996, Rodino and Shane 1998).

2.7.7.4. Glucocorticoids

Although glucocorticoids inhibit T-cell proliferation and the expression of the genes of such cytokines as IL-1, IL-6, interferon and TNF, which are all implicated in bone resorption, they produce rapid and profound bone loss (Epstein
Approximately 10 mg of prednisolone and prednisone or their equivalents daily is enough to cause bone loss during the first six months after the start of treatment. Even a lower daily dose of 7.5 mg prednisolone may lead to bone loss when used for long periods (Shane and Epstein 1994, Shaker and Lukert 2005). Besides osteoporosis glucocorticoids expose their users to avascular necrosis and even fractures, when BMD is still normal (Canalis et al. 2004, Shaker and Lukert 2005).

Glucocorticoids have many systemic effects, which indirectly affect the bone. They reduce intestinal calcium absorption and increase urinary calcium excretion, which both secondarily increase PTH excretion. Furthermore, glucocorticoids decrease the secretion of growth hormone and gonadal and adrenal androgens and oestrogens (Lukert and Raisz 1990). They also enhance osteoclastic activity possibly through secondary hyperparathyroidism (Rodino and Shane 1998, Canalis 2003).

In the bone they decrease osteoblast recruitment and differentiation, increase osteoblast apoptosis and inhibit synthesis of type I collagen, and then decrease bone formation (Schäcke et al. 2002, Shaker and Lukert 2005). Two mechanisms behind increased resorption are the up-regulation of RANKL and the inhibition of OPG, which have been demonstrated in in vitro studies (Hofbauer et al. 1999a, Hofbauer et al. 1999b) and in patients who have received prednisolone for acute respiratory obstruction (Bornefalk et al. 1998).

2.7.7.5 *Cyclosporine A*

CyA is a small fungal cyclic peptide, which upon administration forms a complex with cytoplasmic receptor protein. This primary complex binds calcineurin A and B, calcium and calmoduline and forms secondary complexes, which prevent the action of new nuclear regulatory protein and T-cell genes (Epstein 1996). In animal models rapid bone loss has been demonstrated after administration of 7.5 to 15 mg/kg of CyA (Movsowitz et al. 1989). CyA enhances both bone resorption and formation but the rate of resorption is higher. Consequently the markers of bone formation and resorption increase after CyA administration (Guañabens et al. 1992, Thiébaud et al. 1996). CyA also inhibits the synthesis of 1,25-(OH)\(_2\)-D in the kidneys (Rodino and Shane 1998) and the production of OPG and stimulates the production of RANKL (Hofbauer et al. 1999a, Hofbauer et al. 1999b). In cardiac transplant recipients, the cumulative dose of CyA is inversely correlated with age-matched BMDs (Rich et al. 1992).
2.7.7.6 Other immunosuppressive agents

Azathioprine is a derivative of mercaptopurin. It may suppress osteoblastic activity and activate osteoclasts but it does not seem to affect bone mass (Bryer et al 1995).

Tacrolimus is a fungal macrolide, which acts in a similar fashion as CyA (Epstein 1996). In animal models it seems to have the same kind of effect on bone as CyA (Rodino and Shane 1998). After cardiac transplantation, patients receiving tacrolimus had a remarkable bone loss (Stemple et al 2002). In rats MMF had no effect on bone accumulation but human data on MMF action in the bone is lacking (Rodino and Shane 1998). In animal models rapamycin or sirolimus increase bone remodelling and inhibit longitudinal bone growth (Epstein 1996).

2.7.7.7. Osteoprotegerin and RANKL

In animal models OPG-deficient mice develop rapid and severe osteoporosis (Bucay et al 1998, Krane 2002, Fohr et al 2003) and at the same time their vessels calcify (Bucay et al 1998). In a cross-sectional study serum OPG levels were lower in cardiac transplant patients than in healthy controls. In a prospective study of the same authors OPG level was normal before cardiac transplantation but decreased after transplantation and the decrease was correlated to bone loss after transplantation (Fahrleitner et al 2003). After renal transplantation the serum OPG level has also been shown to decrease (Sato et al 2001). In another study of this patient group, serum OPG and RANKL did not differ from healthy controls 42 months after transplantation (Malyszko et al 2003). In patients with liver transplantation, serum OPG did not differ from healthy controls when it was measured about 20 months after transplantation (Fahrleitner et al 2002).

In a very recent study serum OPG increased progressively during the first three weeks after SCT and declined thereafter. In this study sRANL was not determined (Baek et al 2004). In another cross-sectional study, SCT recipients had an elevated serum OPG level approximately 5 years after transplantation (Kerschan-Schindl et al 2004).

2.7.8 Prevention of transplantation-associated bone loss

2.7.8.1 Calcium and vitamin D

It is recommended that all transplant recipients should receive calcium (1000 to 1500 mg /day) and vitamin D (400 to 800 IU/ day) supplements although without other measures they are insufficient to prevent bone loss (Van Cleemput et al
1996, Välimäki et al 1999, Pisani et al 2002, Cohen et al 2004). High doses of more potent vitamin D metabolites have in some studies increased BMD of heart-transplanted patients (Van Cleemput et al 1996, Garsia-Delgado et al 1997). In one study 0.25 µg alfacalcidol per day decreased, but did not totally prevent, bone loss at the LS and the FN at one year after kidney transplantation (El-Agroudy et al 2003). In the latest study of cardiac transplant recipients, calcitriol prevented lumbar spine and reduced femoral neck bone loss as effectively as alendronate (Shane et al 2004).

2.7.8.2 Sex steroid replacement therapy

Severe disease can cause amenorrhea in premenopausal women. Oestrogen replacement therapy with progestin has been shown to inhibit bone loss and to prevent fractures in oestrogen-deficient women (Cauley et al 2003). In one study of postmenopausal women with liver transplants, transdermal oestrogen started approximately 4.1 years after the transplantation and increased both femoral neck and lumbar spine BMD during the following two years (Isoniemi et al 2001).

There is only one study of oestrogen replacement therapy in SCT patients. When started about thirteen months after SCT, it increased BMD, which was at the osteopenic level in nine out of thirteen patients before treatment (Castelo-Branco et al 1996).

Chromically ill men have often hypogonadism and immunosuppressive regimens can impair the production of testosterone even more (Rodino and Shane 1998). Serum testosterone decreases after cardiac transplantation but in most cases returns to the baseline level during the first year after transplantation (Shane et al 1997, Välimäki et al 1999). Testosterone replacement therapy increases BMD in hypogonadal men (Snyder et al 2000). Similar evidence on transplantation patients is lacking but treatment with testosterone of truly hypogonadal men is recommended (Rodino and Shane 1998).

2.7.8.3 Calcitonin

Intra-nasally administrated salmon calcitonin has prevented corticosteroid-associated bone loss (Rodino and Shane 1998) and it can be used in postmenopausal osteoporosis (Thamsborg et al 1991). In one non-randomised study of cardiac transplantation patients, calcitonin slightly reduced bone loss (Muchmore et al 1992) but in randomised studies with cardiac transplant recipients it had no effect on BMD either alone (Garcia-Delgado et al 1997) or with calcium (Välimäki et al 1999).
2.7.8.4 Bisphosphonates

Bisphosphonates are drugs that inhibit osteoclastic function and thus prevent bone resorption. They can be administrated either orally (alendronate, clodronate, etidronate, risedronate) or intravenously (ibandronate, pamidronate, zoledronic acid). They all have a long half-life. Their efficacy has been well documented in the prevention of bone loss and fractures in postmenopausal women (Cummings et al. 1998, Pols et al. 1999), and in long-term users of glucocorticoids (Adachi et al. 2000, Reid et al. 2000). In per oral use gastrointestinal side effects are common, because these preparations are acidic. Infused preparations may cause flu-like symptoms. They can worsen kidney insufficiency and cause adynamic bone disease in patients with decreased kidney function (Canalis et al. 2004).

A relatively weak bisphosphonate etidronate used cyclically (400 mg for two weeks every three months) appeared to be unable to prevent bone loss in patients after cardiac (Garcia-Delgado et al. 1996, Van Cleemput et al. 1996) or liver transplantation (Riemens et al. 1996).

In comparison to historical controls a single intravenous dose of 60 mg pamidronate followed by cyclic etidronate decreased bone loss and reduced fracture rate after cardiac transplantation (Shane et al. 1998). In another study of cardiac transplant patients with osteoporosis before transplantation, intravenous pamidronate (60 mg at 3 months intervals for 3 years) resulted in a significant increase in BMD both at the LS and FN whereas patients with normal BMD treated with calcium and vitamin D, lost bone (Krieg et al. 2001). Thirty mg of intravenous pamidronate given at three months’ intervals improved both femoral neck and lumbar spine BMD in osteopenic cardiac-transplant recipients when started about 1.7 years after transplantation (Dodidou et al. 2003). In the latest study oral alendronate prevented lumbar spine and reduced femoral neck bone loss when started immediately after transplantation (Shane et al. 2004).

After kidney transplantation both LS and FN BMD increased significantly in patients who received oral alendronate 10 mg daily started at least 6 months after transplantation. At the same time patients on calcium and vitamin D supplements only had stable BMD (Giannini et al. 2001). In another study patients who received 1 mg of intravenous ibandronate before and 3, 6 and 9 months after kidney transplantation had significantly less bone loss at the LS and even a slight increase in the FN BMD compared to those who got only calcium and vitamin D (Grotz et al. 2001). In one study two infusions of 4 mg of zoledronic acid with an interval of three months prevented bone loss at the FN and even improved lumbar spine BMD six months after kidney transplantation (Haas et al. 2003).
In osteoporotic liver transplant recipients one-year treatment with cyclic etidronate improved BMD when started approximately 17 months after the transplantation (Valero et al 1995). Of patients with reduced bone mass before liver transplantation, those who received 60 mg of intravenous pamidronate every three months before and for 9 months after transplantation had no new vertebral fractures whereas 38 % of those not receiving pamidronate experienced vertebral compression fractures (Reeves et al 1998).

Lung transplantation patients who received two cycles of etidronate after the transplantation had less bone loss during the first six months compared to untreated controls and the protective effect of etidronate remained for up to one year after the transplantation (Henderson et al 2001).

Except for one uncontrolled study with pamidronate (Buchs et al 2001), the efficacy of bisphophonates in the immediate prevention of bone loss after SCT has not been evaluated to date. When started for treatment of osteopenia or rapid bone loss 17 to 24 months after SCT risedronate and zoledronic acid increased the LS BMD by 4.4-9.8 % and the FN BMD by 5.9-6.4 % at one year (Tauchmanová et al 2003, Tauchmanová et al 2005).
3 AIMS OF THE STUDY

The aim of the present study was to evaluate bone loss following allogeneic SCT and to answer the following questions.

I  What is the magnitude and timing of bone loss?

II  What are the mechanisms of bone loss as examined by bone turnover markers and serum osteoprotegerin and RANKL measurements?

III  Can the bone status of SCT patients improve spontaneously?

IV  How and by what mechanism does allogeneic SCT performed in childhood affect peak bone mass in adolescence and young adulthood?

V  Can bone loss be prevented by calcium, vitamin D, calcitonin, sex steroid replacement therapy or bisphosphonates?
4 PATIENTS AND METHODS

4.1 Patients

The characteristics of the patients are shown in Table 1. All the patients received an allogeneic SCT at the Helsinki University Central Hospital. Those, whose disease had begun in childhood, were treated at the Hospital for Children and Adolescents, and adult patients at the Division of Haematology, Department of Medicine. Patients who had renal insufficiency, diseases affecting bone metabolism or multiple myeloma as an indication of SCT were excluded.

4.1.1 Prospective study of bone loss after stem cell transplantation and the effect of calcium with or without calcitonin (Study I)

Sixty-one patients who underwent allogeneic SCT were included in the first study. Twenty-five patients were lost for the follow-up within six months after SCT (9 due to death, 4 due to a relapse, 2 due to a critical and complicated situation and 10 were unwilling to continue due to nausea caused by the study medication). A further 8 patients died before one year after SCT. Because of the dropouts, 8 participants of an earlier pilot study were included in the BMD analyses. Thus 44 patients (21 men and 23 women) completed 6 months follow-up and 36 (16 men and 20 women) 12 months follow up. The mean age was 40 (SD 9) years. The diagnoses were AML (N=17), ALL (n=6), CML (n=15), MDS (n=5) and Burkitt’s lymphoma (n=1). Due to menopausal symptoms, oestrogen replacement therapy was started for 20 women during the study, with a median of 171 (range 40-319) days after SCT.

4.1.2 Follow-up study of adult stem cell transplant recipients (study II)

For the second study the 29 participants of the first study, who were still alive, were invited to a control visit with a median of 75 (range 54-96) months after SCT. Twenty-seven of them (13 men and 14 women) accepted the invitation. Their median age at the time of the study was 44 years and their diseases were ALL (n=2), AML (n=12), Burkitt’s lymphoma (n=1), CML (n=10) and MDS (n=2). All the women were still using oestrogen replacement therapy and none of the men used regular testosterone replacement. One man had received testosterone for 7 months due to impotence. Twelve patients had used treatment for GVHD longer than one year after SCT. At the time of the study one patient was still using CyA and another patient had used it longer than one year after SCT.
Twelve patients had used glucocorticoids longer than one year. Two of the female patients had started bisphosphonate therapy because of prolonged glucocorticoid use.

4.1.3 Stem cell transplantation in childhood (Study III)

For the third study 25 young adults and adolescents who had received an allogeneic SCT in childhood, were invited to the examinations with a median of 7.1 (range 1.5-20.5) years after SCT. Sixteen (6 males and 10 females) were willing to take part in the study. At the time of the examination, the age of the patients varied from 16 to 34 (median 21) years. The underlying diagnoses were AML (n=5), ALL (n=5), CML (n=2), myelodysplasia (n=1) and aplastic anaemia (n=3). Eight patients had received SCT before puberty.

4.1.3 Stem cell transplantation in childhood (Study III)

One hundred and fifteen adult recipients of an allogeneic SCT were screened and 99 of them were randomised for study IV. Seventy-two patients were followed at least 6 months after SCT. Twenty-three patients died, 1 had a relapse of the hematological disease, 1 got another malignancy and 2 were unwilling to continue. Sixty-seven patients completed the study protocol, also 5 were lost before 12 months’ follow-up; 4 due to death and 1 because bisphosphonate therapy had been started outside the trial. Thus 72 patients were followed at least for 6 months and 66 for 12 months. Their median age was 43 (10) years and the diagnoses were ALL (n=10), AML (n=20), lymphoma (n=4), CML (n=27), CLL (n=4), MF (n=4) and MDS (n=3).

4.1.5 Serum osteoprotegerin and RANKL after SCT (Study V)

Thirty (14 men and 16 women) participants of study IV, whose serum samples had been stored at -80°C Celsius comprised one study group in study V. Fourteen patients had received pamidronate (eight women, six men) and 16 patients (eight women, eight men) were treated with calcium, vitamin D, and sex steroid replacement therapy only. The mean age was 40 (10) years and indications for SCT included ALL (n=4), AML (n=9), lymphoma (n=1), CML (n=10), CLL (n=3), MF (n=2) and MDS (n=1). Another patient group consisted of 28 SCT recipients (14 women and 14 men), whose mean age was 41 (12) years and diagnoses ALL (n=5), AML (n=14), amyloidosis (n=1), CML (n=6), CLL (n=1) and hybrid leukaemia (n=1). All were using calcium and vitamin D and all women except 2 women oestrogen.
4.1.6 Healthy controls

In study II an equal number of sex-matched healthy controls from health care staff were recruited for biochemical analyses. They were somewhat younger than the patients [35 years (SD 12) versus 44 (9) years (p=0,005)]. Also for study III 22 (12 females and 10 males) age- and sex-matched controls were recruited for determination of the markers of bone metabolism.

In study V 21 healthy volunteers (11 women and 10 men) served as controls for biochemical analyses.

4.2 Stem cell transplantation procedure

In study I the patients received stem cells from an HLA-identical sibling except three who had a matched, unrelated donor. Those participating in study II all had sibling donors. Of those who had received SCT in childhood 11 had a related and 5 an unrelated donor from the Finnish national registry or international registries. In study IV 41 patients received a transplant from a sibling and 31 patients from an unrelated donor. In the first group of study V 17 patients had a related and 13 an unrelated donor, and in the second group 15 and 13 patients, respectively.

Before SCT adult patients had been conditioned with 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. Instead of irradiation two patients in studies I and II received busulfan 4 mg/kg BW intravenously daily for 4 days before SCT.

All but one patient, who had received SCT in childhood, had been conditioned with total body irradiation (TBI). The irradiation dose was either of 10 (n=8), 12 (n=6) or 14 (n=1) Gys. In addition cytarabin or CY 60 mg/kg BW was administered on 2 consecutive days or CY 50 mg/kg BW on 4 consecutive days if TBI was not given.

4.3 Prevention and treatment of GVHD

CyA, MTX and MP were most commonly used to prevent and treat GVHD. CyA was initiated on day —4 before transplantation and given with a dose of 3 mg/kg BW per day as a continuous intravenous infusion for two weeks. The dose was modified to keep the whole blood cyclosporine A concentration at the level of 400 µg /l. Thereafter, CyA was taken orally and for the next 10 weeks the whole blood cyclosporine A concentration was kept between 100 and 200 µg/l if
the donor was a sibling, and between 200 and 300 µg/l if the donor was unrelated. Thereafter, the concentration was kept near 100 µg/l and 100-200 µg/l, respectively. CyA was administered for nine months and tapered off in approximately six weeks. Nine patients in the study IV were switched from CyA on mycophenolate mofetil due to side effects caused by CyA. Six of those were also participants in study V. In another group of study V five patients were using MMF instead of CYA.

MTX was given intravenously 15 mg/m² one day before SCT and 10 mg/m² on days +3, +6 and +11 after SCT.

MP was started orally 14 days after SCT 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 SCT. Four participants of study I did not receive routine MP. Those with an unrelated donor in study IV (n=31) did not receive routine MP but 6-12 mg/kg BW of antilymphocyte globulin (Thymoglobulin®, Sangstat, Lyon, France). Thirteen of those also participated in study V. However, all except seven in study IV and three in study V received MP for acute or chronic GVHD.

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 dose of MP alone or in combination with CyA, thalidomide or MMF.

4.4 Study design

4.4.1 Prospective study of bone loss after stem cell transplantation and the effect of calcium with or without calcitonin

In study I, patients were randomised to three groups. The first group (n=22) received no additional treatment, the second group (=12) got oral calcium (Mega-Calcium®, Sandoz, Basel, Switzerland); calcium lactate gluconate 5.23 g, calcium carbonate 0.8 g) 1 g twice daily and the third group (n=10) oral calcium 1 g twice daily and intranasal calcitonin (Miacalcic®, Sandoz) 400 IU per day during the first month and then 200 IU per day during the next eleven months. All female patients started oestrogen replacement therapy with a median of 170 (range 40-365) days after SCT. All study groups were similar in age [40 years (SD 10) in the reference group, 40 (8) years in the calcium-group and 41 (12) in the calcium and calcitonin group], but differed with respect of female preponderance in the calcium group (10 women and 12 men in the reference
group, 9 women and 3 men in calcium group, 4 women and 6 men in the calcium and calcitonin group).

BMD was measured before and 6 and 12 months after SCT. Spine x-ray was taken before and 12 months after SCT. Venous blood samples were taken for determination of serum bone ALP, PICP, PINP, ICTP, ionised calcium, phosphorus, creatinine, magnesium and testosterone in men before and 3 and 6 weeks and 3, 6 and 12 months after SCT. In eight people of the reference group (participants of the previous pilot study) only BMD was measured before and 12 months after SCT.

4.4.2 Follow-up study of adult stem cell transplant recipients

In study II BMD was measured and a spine x-ray was taken at the follow-up visit. After an overnight fast, blood was sampled for determination of serum ionised calcium, creatinine, PINP, ICTP, osteocalcin, 25-OH-D and testosterone in men and oestradiol in women. The second 2-hour morning void was collected for determination of urine NTX. Life habits of patients were ascertained using a questionnaire, which was filled in by the patients.

4.4.3 Stem cell transplantation in childhood

In study III blood was sampled for the determination of serum osteocalcin, calcium, albumin, ICTP, PINP, 25-OH-D, TRACP5b, PTH, testosterone in men and oestradiol in women. The second 2-hour morning void was collected for determination of urine NTX. Bone mineral density was measured by DXA.

4.4.4 Prevention of bone loss with calcium vitamin D, and sex steroid replacement therapy with or without pamidronate

Patients were randomized by age and sex to two treatment groups. In one group the patients (19 women and 18 men) received calcium carbonate 1000 mg and vitamin D 800 IU daily (Ideos®, Meda, Solna, Sweden). Two weeks after SCT female patients started percutaneous estrogen replacement therapy using patches, which release 50 mg of estradiol (Estraderm Matrix®, Novartis, Basel, Switzerland) per 24 hours, and oral hydroxyprogesterone acetate 10 mg (Provera®, Pharmacia, Bohrs, Belgium) daily during the first ten days of every month. At the same time male patients started testosterone replacement therapy using patches, which release 2.5 or 5 mg of testosterone per 24 hours (Atmos®, Astra-Zeneca, Gothenburg, Sweden). The dose of testosterone was adjusted on the basis of serum testosterone level at the start of medication. In another group the
patients (17 women and 18 men) received the same treatments mentioned above plus six intravenous infusions of 60 mg of pamidronate (Aredia®, Novartis, Basel, Switzerland); first just before and then 1, 2, 3, 6 and 9 months after SCT. BMD of the lumbar spine (lumbar vertebrae L1-L4) and of the three femoral sites (femoral neck, trochanter, and total hip) was measured before and 6 and 12 months after SCT.

Serum was sampled for the determination of creatinine, ionised calcium, PINP, ICTP, CTX, TRACP5b and estradiol in females and testosterone in males. The second void urine samples were collected for the determination of NTX. Serum and urine samples were collected in the fasting state in the morning by 10 a.m. before and 1, 3, 6 and 12 months after SCT.

4.4.5 Serum osteoprotegerin and RANKL after SCT

In the first part of the study patients were randomized into two groups. One group (eight women, eight men) received calcium, vitamin and sex steroid replacement as mentioned above. In another group patients (eight women, six men) received the same treatments mentioned above plus intravenous pamidronate infusions as in study IV.

Venous blood was sampled for determination of serum OPG and sRANKL before, 1, 3, 6 and 12 months after SCT and BMD before, and 6 and 12 months after SCT in the first part of the study. In the second part of the study OPG and sRANKL were measured with a median of 122 (range 88-163) days after SCT. In this part BMD was not measured.

4.5 Bone mineral density measurements

In studies I, II and IV BMD of the LS (lumbar vertebrae L1-L4) and the femoral sites (FN, trochanter and total hip in study IV and those with Ward’s triangle in studies I and II) was measured by dual energy X-ray absorptiometry (DXA) using Hologic QDR-1000 densitometer (Hologic, Waltham, MA, USA). In study III the measurements were done using Lunar 10068 equipment (GE Lunar, Madison, WI, USA) and total body BMD was measured. The precision of the method (coefficient of variation) was 0.9 % at the lumbar spine and 1.2 % at the femoral neck.

BMD was expressed as g/ cm² and in study III also as standardised Z-scores, which compare individual results with those of healthy people of the same age and gender. BMD values were compared to the manufacturers’ Finnish databases and in study III the American NHANEJ-database for the total body BMD.
For subjects aged less than 20 years the manufacturer’s gender-specific pediatric reference data was used to produce lumbar spine and total body Z-scores; reference data was unavailable for the upper femur. In the adults T-scores, which compare individual bone density measurements to those of the young (20-40 years), normal population of the same gender, were also analysed. According to the criteria defined by WHO, T scores equal to or more than 2.5 SD below the mean of the young normal population of the same gender, represent osteoporosis, whereas T scores equal to or less than –1.0 but more than – 2.5 represent osteopenia (Kanis 1994).

4.6 Biochemical measurements

Intact PICP, PINP and ICTP were determined by RIA kits from Orion Diagnostica (Oulunsalo, Finland). The intra- and inter-assay CVs for these assays ranged from 2 to 9%. The ALP isoenzymes were determined using a kit from Boehringer Mannheim (Mannheim, Germany), in which bone-specific isoenzyme is precipitated by lectin, and bone ALP activity is calculated from total and residual ALP activity. The intra- and inter-assay CVs were 4 and 5 %, respectively. Serum osteocalcin was measured by an IRMA recognising intact osteocalcin and N-Mid-fragment of the peptide (CIS Bio International, Gif-Sur-Yvette, France) with intra- and inter-assay 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 inter-assay CVs ranging from 2 to 10 %; the measured values were proportioned to urinary creatinine excretion. Serum 25-OH-D concentration was measured by a RIA after acetonitrile extraction (DiaSorin, Stillwater, MN, USA). The sensitivity of the method was 5 nmol/l, intra-assay imprecision 5.9 - 8.9 %, and interassay imprecision 6.0 - 9.0 %. Serum testosterone was assayed by an automated luminooimmunoassay (Chiron Diagnostics, Medfield, USA) with intra- and inter-assay CVs ranging from 4% to 7%. Serum oestradiol was measured by a RIA (Orion Diagnostica) with a sensitivity of 0.02 nmol/l, and intra- and inter-assay CVs ranging from 3 to 12 %. For determination of serum ionised calcium blood samples were centrifuged immediately after being drawn, and the serum analysed with an ion selective analyser (Microlyte, Kone Inc, Finland) within a few hours of blood collection (intra-assay CV 1.6%). Serum concentration of TRACP5b was assessed by an immunextraction method with boneTRAPTM reagents from Suomen Bioanalytiikka Oy (Turku, Finland). The analytical sensitivity of this assay was 0.1 U/l, and intra- and inter-assay CVs of TRACP5b were 6 % or less at relevant concentration. Serum CTX was assayed by an ELISA method (Serum CrossLaps ELISA, Nordic Bioscience Diagnostics, Herlev, Denmark). Intra- and inter-assay CVs of the method ranged from 7% to 10%. OPG and sRANKL were determined using EIA kits from Biomedica (Vienna, Austria) with the intra- and inter-assay coef-
ficient of variation being 9.8% and 7.8% for OPG and 11.0% and 7.0% for sRANKL, respectively. Serum phosphorus, magnesium and creatinine and urine creatinine were determined by routine methods.

4.7 Life habits

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

4.8 Statistics

In all the studies, data with normal distributions are expressed as means with SDs, otherwise as medians with ranges or interquartile ranges. The significance level used in overall tests, as well as in linear contrasts, was 0.05.

Analyses were performed as two-sided and using SAS System and SAS/MIXED procedure in study I, NCSS 2000 software (NCSS Statistical Software, Kaysville, UT, USA) in studies II, IV and V and SPSS software (SPSS Inc, Chicago, IL, USA) in studies III, IV and V.

In comparisons between and within the study groups, normally distributed variables were studied using repeated measures ANOVA or two group t-tests. If the assumptions for repeated measures ANOVA (even after log transformation) were not fulfilled, Geisser-Greenhouse adjusted P values were used. Two-sample t-test or one-way ANOVA (percent changes) were used as appropriate. The data not distributed normally after the log-transformation were tested with Mann-Whitney U test or Kruskal-Wallis one-way ANOVA on ranks. BMD changes were also analysed with ANOVA using percent changes from baseline to 6- and 12-month time-points. In the analyses of bone marker data log-transformed percent changes from baseline to 1-, 3-, 6- and 12-month time-points were used. Spearman and Pearson correlations were used when appropriate.

In study III to evaluate the effect of height (as SDs from the normal population), weight and BMI on BMD, two-way analysis of variance for Z-scores was performed by putting each demographic variable one by one together with the group (= normal or reduced BMD) and interaction of the variable and the group into the model. Pearson correlation coefficients between demographic variables and Z-scores were calculated for the whole study population and also separately for the groups, if the above-mentioned interaction existed.
4.9 Ethics

The Ethical Committee of the Department of Medicine, Helsinki University Central Hospital approved study I. The Ethical Committee of the Department of Medicine, Helsinki University Central Hospital approved studies II, IV and V. Study III was approved by the Ethical Committee of the Diseases of Children and Adolescents and Psychiatry, Helsinki University Central Hospital. A written consent was obtained from all the patients.
5 RESULTS

5.1 Magnitude and timing of bone loss

5.1.1 Bone mineral density

In study I no significant difference in bone loss between the treatment groups (no treatment either calcium with or without calcitonin) was observed. Thus, the groups were combined. Figure 3 shows the changes in the BMD in the combined group. Bone loss at the LS, expressed as per cents from baseline, was 5.8 % at 6 months and 3.6 % at 12 months after SCT (p<0.001). At the FN the respective decreases in BMD were 7.0 % and 8.0 (p<0.001). The trochanter BMD decreased by 8.2 % at 6 months and 8.3 % at 12 months after SCT (p<0.001).

The majority of bone loss occurred during the first six months after SCT. At the lumbar spine BMD even slightly recovered from 6 to 12 months after SCT (p<0.01).

5.1.2 Osteopenia and osteoporosis

The number of patients fulfilling the WHO criteria for osteopenia and osteoporosis in study I is shown in Tables 3 and 4. Thirty-nine per cent of the patients had either osteopenia or osteoporosis at the LS before SCT, 50% 6 months and 47 % 12 months after SCT. The respective percentages at the FN were 25 %, 45 %, and 58 %. Tables 2 and 3 also show that in study IV the patients not receiving pamidronate had osteopenia and osteoporosis in quite similar percentages as those in study I.

5.1.3 Fractures

Sixteen per cent out of 25 assessable patients in study I had vertebral fractures. Two experienced a single vertebral compression fracture, and two had multiple fractures one year after SCT. Two of these patients belonged to the reference group and two to the calcium supplement groups.
5.2 Mechanisms of bone loss after stem cell transplantation

5.2.1 Markers of bone formation

Figure 4 shows changes in the markers of bone formation (bone ALP, PINP and PICP) in study I. Because there were no significant differences in biochemical markers between the treatment groups in study I, the groups were combined. The markers of bone formation (bone ALP, PINP, PICP) all decreased 3 weeks to 3 months after SCT. Maximum reductions were 20% in bone ALP at 3 weeks (p=0.027), 40% in PICP at 6 weeks (p<0.0001) and 63% in PINP at 6 weeks (p<0.0001). Thereafter all these markers returned to the baseline by 6 months and tended to be even above the baseline at 12 months after SCT.

Approximately six years after SCT the markers of bone formation (PINP and OC) were similar in patients and controls (study II). Serum PINP had reduced by 30% from the level at 12 months after SCT (p=0.024).

In study III the young patients after SCT performed during childhood did not differ from the healthy controls with respect to the markers of bone formation (serum osteocalcin and PINP), and the marker levels were similar for those with normal vs. reduced BMD.

5.2.2 Markers of bone resorption

Figure 4 shows changes in the marker of bone resorption (ICTP) in study I. Serum ICTP was above the reference range even before SCT and it increased after the procedure reaching the maximum (77% above the baseline) at 6 weeks (p<0.0001). Thereafter it decreased but was still above the baseline at 12 months after SCT (p>0.05).

In study II approximately 6 years after SCT, serum ICTP was still significantly higher in patients than controls [3.93 (1.99) μmol/l vs. 2.78 (0.69) μmol/l, p=0.0001] but it had significantly decreased from the level at 12 months after SCT [6.85 (3.31) μmol/l, p=0.018]. On the other hand, urine NTX was significantly lower in SCT patients than in the controls [31.1 (20.2) nmol/mmol vs. 52.5 (22.0) nmol/mmol, p=0.0002].

In study III SCT patients did not differ from the healthy controls in respect to the markers of bone resorption (urine NTX, serum ICTP, serum TRACP 5b). Furthermore, the marker levels were similar for those with normal and reduced BMD.
5.2.3 Testosterone and oestradiol

In the male patients serum testosterone decreased being at the lowest level (a 57 % reduction from the baseline) at 6 weeks after SCT (p=0.0003) (study I). Thereafter, it returned near the pre-transplantation level by 6 months after SCT. In study II mean serum testosterone level had decreased from 17.4 nmol/l at 12 months to 11.7 nmol/l at the follow-up (approximately 6 years after SCT) visit (p=0.005). Four out of the 13 men had serum testosterone level below the lower limit of the reference range (9.0 nmol/l) and 11 men had values lower than before SCT.

In study II all women were using oestrogen replacement therapy and had a mean serum oestradiol level of 0.23 nmol/l, which falls within the target range (0.1-0.3 nmol/l) in oestrogen-treated women.

5.2.4 Osteoprotegerin

Figure 5 shows changes in serum OPG in the treatment groups of the first part of the study IV. Before SCT they were similar in patients combined [3.66 (1.76) pmol/l] [mean (SD)] and in controls [2.89 (1.44) pmol/l] (p = 0.11). Over time the study groups did not differ from each other (p = 0.38). During the first 6 post transplant months, the mean serum OPG increased by 26 % in the pamidronate group (p = 0.028) and by 27 % (p = 0.002) in the other group. With the study groups combined, serum OPG levels were higher in patients than controls post transplant (p = 0.009 at 1 month, p = 0.003 at 3 months, p = 0.002 at 6 months, and p = 0.09 at 12 months). In the second part of the study serum OPG levels for patients with a median of 122 days after SCT [6.57(1.53) pmol/l] were clearly higher than for controls (p < 0.001).

5.2.5 sRANKL

Figure 5 shows median changes in serum sRANKL after SCT. At the baseline of the first part of study V serum sRANKL levels were similar in patients combined [0.33(0.15) pmol/l] and in controls [0.26(0.13) pmol/l] (p=0.12). Over time the study groups differed from each other (p=0.050). In the pamidronate group the mean sRANKL level decreased by 42 % at 3 months (p=0.0007) and by 38 % at 6 months (p=0.0061). In the other group it did not change significantly. In the second part of study V serum sRANKL concentrations were similar for patients [0.20 (0.07) pmol/l] and controls [0.26(0.13) pmol/l] (p=0.13). Serum OPG and sRANKL concentrations correlated inversely.
5.2.6 Serum 25-OH-D

In study II patients examined several years after SCT had lower serum 25-OH-D concentrations than healthy controls (27.4 [7.4] nmol/l vs. 40.6 [10.6] nmol/l, p= 0.0002). Twenty-four out of 27 patients had a 25-OH-D level below the target of 37.5 nmol/l; the respective numbers for the controls were 11 out of 28.

In study III the median serum level of 25-OH-D of the SCT patients with reduced BMD was not different from the healthy controls (p=0.43). Hypovitaminosis D [25-OH-D ≤ 37.5 nmol/L] (22) was diagnosed in 6 out of 16 SCT patients (38%), but also in 8 out of 23 healthy controls (35%).

5.3 Recovery of bone after stem cell transplantation in adults

5.3.1 Bone mineral density

Study II showed that BMD increased at all measurement sites from one year after SCT to the follow-up visit at approximately 6 years after SCT. The recovery was statistically significant at the LS, where BMD increased by 2.4 % (p= 0.002). The changes at the femoral sites were +4.1 % at the FN (p=0.087), +4.0 % at the trochanter (p=0.095) and +1.4% in the total hip (p=0.23).

To find out the effect of the treatment for GVHD, the participants were divided into those who had the treatment up to one year after SCT, and to those who needed it longer. The amount of MP used, but not that of CyA, was significantly higher in the long-term users’ group. There was no significant difference in the recovery of BMD between long-term versus short-term users of GVHD treatment. Instead, total bone loss at the femoral sites from the pre-transplantation level to the follow-up measurement was significantly higher in long-term versus short-term users; -10. 5 % vs. –1.9 % at the FN (p=0.04), -13.6 % vs. –2.1 % at the Ward’s triangle (p=0.04) and –10.1 % vs. –2.6 % at the total hip (p=0.03).

5.3.2 Osteopenia and osteoporosis and fractures

At the time of the follow-up visit 26 % of the patients had abnormally low BMD at the LS and 41 % at the FN, the respective percentages at 12 months had been 50 % and 48 % (Tables 3 and 4).

No patients had experienced new vertebral fractures between 12 months after SCT and the follow-up visit approximately 75 months after SCT. One man and one woman had had a distal radial fracture; both patients had used GVHD-treatment longer than one year after SCT.
5.4 Effect of stem cell transplantation performed in childhood on peak bone mass

5.4.1. Bone mineral density

BMD data of study III are presented in Table 5. Six patients (38%) had a Z-score less or equal to -1 at least one measurement site; they comprised the group of patients with reduced areal bone mineral density. Z-score $\leq$ -1 was detected in the lumbar spine, the femoral neck, the total hip, and the total body in 3/16 (19%), 2/16 (12%), 1/16 (6%) and 4/16 (25%) patients, respectively.

5.4.2. Risk factors of reduced BMD

All young adult patients with reduced BMD were females (p=0.02) and they were younger (p=0.08) at the time of the SCT than the patients with normal BMD. The SCT patients with normal BMD did not differ from the patients with reduced BMD in respect to a cumulative dose or the duration of use of glucocorticoids, a cumulative dose of CyA, or alcohol or calcium consumption. All 6 patients with reduced BMD exercised regularly at least 1-2 times/week compared to 4/10 of the patients with normal bone mineral density (p=0.07). All patients were non-smokers.

Five of the 6 patients with reduced BMD had been diagnosed with a growth delay $\geq$ –1 SD after SCT as opposed to 3/10 of the patients with normal BMD (p=0.03). Four of the six patients with reduced BMD had had growth hormone insufficiency compared to 3/10 patients with normal BMD (p=0.10). The majority of patients with reduced BMD had been prepubertal at the time of SCT (5/6 vs. 3/10, p=0.03). Five of the six patients with reduced BMD had needed sex hormone substitution to induce pubertal development in contrast to 2 of the 10 patients with normal BMD (p=0.02).

5.4.3. Effect of weight and height

Since growth delay was a risk factor for reduced BMD, the effect of weight and height (and BMI) was studied in a more detailed way. The effect of weight and BMI on BMD was not dependent on the group (normal or reduced BMD) and therefore, Pearson’s correlation coefficients were calculated for the whole study population. Except for the femoral neck, the r-values between BMD and weight or BMI varied from 0.63 to 0.69 (p = 0.003-0.02) for weight and from 0.66 to 0.76 (p = 0.0007-0.02) for BMI at the measurement sites. Instead, the effect of height in SDs was significantly dependent on the group in the lumbar spine (p = 0.002 for the interaction between height and group) and the total body (p = 0.007) and therefore, the correlations between height and BMD were calculated separately for the groups with normal and reduced BMD. In patients with nor-
mal BMD an expected positive correlation between BMD and height was found for the lumbar spine (p = 0.05) and the total body (p = 0.04). However, in patients with reduced BMD the correlations were uniformly negative and significant for the lumbar spine (p=0.008) and of borderline significance for the total body (p = 0.09).

5.5 Prevention of stem cell transplantation- associated bone loss

5.5.1 Calcium and vitamin D

Study I showed that calcium neither alone nor with calcitonin could prevent bone loss after the SCT. In the calcium group the BMD of the LS decreased by 3.9 % in 6 months (p<0.0001) and by 1.2 % (p<0.0001) in 12 months after SCT and at the FN by 6.3 % in 6(p<0.0001) and 6.1 % in 12 months (p<0.0001).

In study IV calcium substitution with vitamin D and sex steroid replacement therapy was not enough to prevent bone loss after SCT. The BMD of the LS decreased by 3.2 % (p= 0.005) at 6 months and by 2.9 % (p=0.031) at 12 months. At the FN the reductions were 4.9 % (p<0.001) and 6.2 % (p<0.001), at the trochanter 8.9 % (p<0.001) and 9.8 % (p<0.001) and in the total hip 7.6 % (p<0.001) and 7.8 % (p<0.001), respectively (Figure 6).

5.5.2 Calcitonin

In study I patients with calcitonin and calcium lost 6.0 % of their lumbar spine BMD in 6 months (p<0.0001) and 3.9 % in 12 months (p<0.0001). The respective changes at the femoral neck were 6.3 % (p<0.0001) and 8.5 % (p<0.0001). The changes did not differ from those in the control group, not receiving any osteoporosis prevention.

5.5.3 Oestrogen and testosterone

Twenty out of 23 female patients in study I started oestrogen replacement with a median of 171 (range 40-319) days after SCT and they were still using it by the time of study II. However, it could not prevent the reduction in bone mass compared to the males of study II, who had lowered testosterone levels but were not using any substitution. In study IV all patients received sex steroid replacement therapy but it together with calcium and vitamin D did not prevent bone loss. (Figure 6) In the group that received only calcium, vitamin D and sex steroid replacement therapy the reductions in BMD were even higher (see 5.5.1) than in the combined study groups of study I (receiving calcium with or without calci-
tonin or no prevention of osteoporosis) [in the trochanter (8.4 % and 8.5 % at 6 and 12 months, respectively) (p=0.07) and in the total hip (6.2 % and 6.4 %, respectively) (p=0.007)].

5.5.4 Bisphosphonates

5.5.4.1 Effect on BMD

In study IV the study groups (calcium, vitamin D and sex steroid replacement therapy with or without pamidronate) differed significantly over time from each other at the LS (p=0.0084), the trochanter (p=0.0040), and the total hip (p=0.0015); at the FN the difference was of borderline significance (p=0.074) (Figure 6). In the pamidronate group lumbar spine BMD remained stable, but decreased in the other group by 3.2 % (p=0.005) at 6 months and by 2.9 % (p=0.031) at 12 months. In the femoral neck BMD of the pamidronate group the decrease was 2.5 % (p=0.001) and 4.2 % (p<0.001) at 6 and 12 months, and in the other group 4.9 % (p<0.001) and 6.2 % (p<0.001), respectively. The patients on pamidronate lost 3.8 % of their trochanter BMD in 6 months (p<0.001) and 4.9 % in 12 months (p=0.001). In the other group, the respective losses were 8.9 % (p<0.001) and 9.8 % (p<0.001). In the total hip bone loss in the pamidronate group was 4.8 % at 6 months (p<0.001) and 5.5 % at 12 months (p<0.001) and in the other group 7.6 % (p<0.001) and 7.8 % (p<0.001), respectively.

5.5.4.2 Effects on bone markers

Changes in the markers of bone metabolism in study IV are shown in Figure 7. Over time the study groups differed from each other with respect to changes in urinary NTX (p=0.035) and S-PINP (p=0.03); the differences in serum CTX (p=0.10) and TRACP5b (p=0.077) were of borderline significance. In the pamidronate group, S-PINP decreased 79 % (p=0.025) during the first three months, and remained lowered by the end of the follow-up (p=0.002). Serum ICTP increased by 61 % (p<0.001) during the first post-transplant month. It was still elevated at 6 months (+48 %, p<0.001). Urinary NTX decreased by 68 % during the first three months (p=0.014). Serum CTX dropped 49 % (p=0.054) below the baseline at 3 months but was back at the baseline at 6 months. Serum TRACP5b increased non-significantly by 40 % (p=0.16) during the first month. By the end of the follow-up it decreased to 70 % of the pre-SCT level in the (p=0.073). In the other group, serum ICTP doubled during the first month (p=0.001) and was significantly elevated at 6 months (+77 %, p =0.027) and non-significantly so at 12 months. Serum TRACP5b increased by 51 % with-
in the first post-transplant month (p=0.022). By the end of the study it decreased to the pre-SCT level. Other bone markers did not change significantly (Figure 7).

5.5.4.3 Osteopenia and osteoporosis and fractures

In study IV 31% of the patients receiving pamidronate had at least osteopenia at the LS before SCT, 26% after six months and 24% after twelve months. In the other group, the number of patients with reduced BMD were 41%, 46% and 34%, respectively. At the FN 34% of the patients in the pamidronate group had at least osteopenia before SCT, 37% six months and 40% 12 months after SCT. In the other group the respective percentages were 43%, 56% and 48%.

In study IV eight patients experienced a new, radiologically demonstrated vertebral fracture; three (8.6%) in the pamidronate group and five (13.5%) in the control group.
6 DISCUSSION

6.1 Patients and methods

6.1.1 Patients

In Finland allogeneic stem cell transplantations of children are concentrated at Helsinki University Central Hospital and for adults they are carried out at Helsinki and Turku University Hospitals. All university hospitals perform autologous stem cell transplantations. All the patients of the present study had their SCT in Helsinki. During the first year after the transplantation patients are closely followed-up by the Division of Haematology, Department of Medicine, Helsinki University Central Hospital. Before SCT most of the patients had been treated in other university and central hospitals in Finland. Therefore, only transplantation-associated treatments are described in this study. The amounts of glucocorticoids, in particular, used before the SCT remained unknown in most cases. However, Finnish haematologists follow the same recommendations in treatment of basic haematological diseases and thereby, it is apparent that the previous treatments did not differ significantly between the patients (Finnish Leukaemia Group).

Patients with multiple myeloma were excluded from the study but otherwise the patients represented a typical SCT population. Also the mortality of our SCT patients, 29 % during the first year, was comparable with that in previous studies (Duncombe 1997). Transplantation procedures and prevention and treatment of GVHD followed international guidelines (Bensinger and Spielberger 2004).

In study I 21 of the original 69 patients stopped the study before six months after transplantation. Because of this high discontinuation rate we decided to include eight participants of an earlier pilot study in this study population. In them the SCT had been performed in a similar way as in the other patients. They did not receive any special prevention for bone loss but had BMD measurements before and 6 and 12 months after SCT.

Currently study IV is the largest published, prospective, randomised study on the prevention of bone loss in SCT patients, and the only one in which preventive therapies were started immediately after SCT.

6.1.2 Measurement of bone mineral density

DXA was chosen as the BMD measurement method because it is easily and rapidly performed, it has a low radiation exposure and the costs are bearable.
(Kanis 1994a). It is also important that in a longitudinal study the repeatability of BMD measurements is good. A disadvantage is that DXA does not take into account the size and the shape of bones and thus gives only an areal, not volumetric bone density (Kanis 1994a). This may bias the results especially when BMD is measured in children. Since growth delay was an important risk factor for reduced areal BMD in patients with SCT in childhood (study III), quantitative computed tomography (QCT) might have been more informative in studying these patients. Also the inability to distinguish between trabecular and cortical bone is a disadvantage in DXA measurements when compared to QCT (Kanis 1994a).

6.1.3 Markers of bone metabolism

Since different markers reflect different phases of the bone remodelling process we used a very broad spectrum of bone turnover markers to study the mechanisms of bone loss (Fohr et al 2003). Serum B-ALP, PINP and PICP were used as the markers of bone formation. Serum OC is mainly a marker of bone formation, but to some degree, also a marker of bone resorption. Serum ICTP was measured as a marker of MMP-related bone resorption, serum CTX and urinary NTX as the markers of cathepsin-K-related bone resorption and serum TRACP5b as a marker of osteoclast function. OC and the degradation products and propeptides of bone-derived collagen are filtered in the kidneys, and thus kidney function may affect their concentrations in serum and urine (Eyre 1996). In all the studies serum creatinine was measured to exclude a clinically significant kidney dysfunction and to estimate an impact of renal function on markers of bone metabolism.

6.2 Magnitude and timing of bone loss

6.2.1 Bone mineral density

In study I the mean bone loss at the LS was 5.8 % at 6 months and 3.6 % at 12 months after SCT. At the FN bone loss was 7.0 % 6 months and 8.0 % 12 months after SCT. In a longitudinal study of 29 recipients of allogenic SCT, patients lost 3.9 % of the lumbar spine BMD, 11.7 % of the femoral neck BMD and 3.5 % of the total body mineral content (TBMC) during approximately 30 post-transplant months after SCT (Ebeling et al 1999). In another study bone loss was 7.2 % at the LS, 11.9 % at the FN and 3.8 % in the TBMC one year after SCT (Schulte et al 2000). A third study showed that 49 % of the SCT patients had osteopenia even before transplantation and that BMD of the trabecular bone, measured by QCT, decreased by 13 % during 6 and 16.9 % during 12 post-transplant months;
the respective cortical bone losses were 9.8 % and 9.0 % (Massenkeil et al 2001).
In a fourth study the lumbar BMD decreased by 3.3 % and the femoral BMD by 8.9 % during the first year after BMD (Lee et al 2002a).

Collectively, our findings are in line with other studies and support the notion that the majority of bone loss occurs during the first six months after SCT and that the upper femur is injured more than the lumbar spine. In the latter site the BMD even slightly recovers from 6 to 12 months after SCT.

These findings differ from those obtained after an autologous SCT. A cross-sectional study of 64 patients, performed with a median of 4.2 years after an autologous SCT, showed that the BMD of the lumbar spine did not differ from healthy controls. Instead, 46 % of patients had osteopenia and 8 % osteoporosis in the FN (Schirmer et al 2001). Another cross-sectional study of 29 patients found no osteopenia five years after an autologous SCT (Kellholz et al 1997). One longitudinal study of 10 patients showed only non-significant changes in the FNBMD (1.1 %) and in the TBBMC (−3.7 %) or even a significant increase (+1.5 %) at the spine one year after an autologous SCT (Ebeling et al 1999). In another longitudinal study of 5 patients LSBMD and FNBMD decreased non-significantly (−0.8 % and −0.5 %, respectively) during the first year after SCT (Schulte et al 2000). Instead, in the same study the total body BMD decreased significantly by 4.1 % (Schulte et al 2000).

Apparently the autologous SCT is, at least at the LS, bone-sparing in comparison to the allogeneic SCT. Instead, at the FN there is discrepancy between the different studies. The most striking difference between these procedures, and a reason for the better bone outcome, is the lack of need of GVHD prevention and treatment in autologous SCT patients.

### 6.2.2 Osteopenia and osteoporosis

In study I 39 % of the patients had either osteopenia (34 %) or osteoporosis (5 %) at the LS before SCT, 50% (43 % osteopenia, 7 % osteoporosis) 6 months and 37 % (34 % osteopenia, 3 % osteoporosis) 12 months after SCT. The respective numbers at the FN were 25 % (all osteopenia), 45 % (42 % osteopenia, 3 % osteoporosis), and 58 % (55 % osteopenia, 3 % osteoporosis). Buchs et al reported in a cross-sectional study that 35 % of SCT patients had osteopenia in the upper femur at the time of transplantation and 43 % osteopenia and 7 % osteoporosis approximately 60 months after SCT (Buchs et al 2001). Another study reported 24% and 4% prevalences of lumbar osteopenia and osteoporosis, respectively, before transplantation (Schulte et al 2000). Thus, taking all these findings together, 25-40 % of SCT patients have decreased BMD even before transplantation, and the number of patients with reduced BMD increases dur-
ing the first six months after SCT both at the LS and the FN. Thereafter, the bone status stabilises at the spine but still worsens at the hip.

### 6.2.3 Fractures

In study I the x-ray of the lumbar spine revealed new vertebral fractures in 16% of the patients. Our findings are in line with the results of other studies of SCT patients, in which the incidence of new vertebral fractures has been up to 14% during the first post-transplant year (Ebeling et al 1999, Gandhi et al 2001). Our study population was small in number from which to draw firm conclusions but in comparison to patients with solid organ transplantations with even a 50% incidence of fractures, the fracture rate seemed to be lower (Shane 1996).

### 6.2.4 Comparison to solid organ transplantations

The amount and timing of bone loss is quite equal after solid organ and stem cell transplantations (Shane 1999, Cohen and Shane 2003). In spite of the similarities in bone loss, patients with solid organ transplantations seem to experience more fractures than patients with haematological transplantations (Cohen and Shane 2003). This might be due to impaired quality of bone in solid organ transplant recipients, which cannot be measured by DXA (Maalouf and Shane 2005).

### 6.3 Mechanisms of bone loss after stem cell transplantation

#### 6.3.1 Markers of bone formation

In study I all the markers of the bone formation (bone ALP, PINP and PICP) decreased 3 weeks to 3 months after SCT. Thereafter they returned to the baseline at 6 months and tended to be even higher at 12 months after SCT. Also other studies have reported similar decreases in bone ALP and PINP during the first months after SCT (Carlson et al 1994, Ebeling et al 1999). Similar results have been reported also in studies that have used OC as a marker of bone formation (Gandhi et al 2000, Buchs et al 2001). Buchs et al (2001) found a positive inverse correlation between decreases in BMD and serum OC during the first months after SCT. As a sign of the normalisation of bone formation, the studies II and III showed PINP and OC levels in the long-term survivors of SCT similar to those in healthy controls.

The finding that the markers of bone formation first rapidly decrease and then increase to an even higher level than before SCT supports the theory that bone loss during the first six post-transplant months is due to glucocorticoids, which
inhibit bone formation (Lukert and Raiz 1990), and thereafter due to CyA, which enhances both formation and resorption (Movsowitz et al 1988). Another explanation for reduced bone formation soon after SCT might be damage to the osteoprogenitor cells. Lee et al reported in an in vitro study that the differentiation of bone marrow stromal cells to osteoblasts was slower in SCT recipients than in healthy controls. Myeloablative therapy was considered a reason for the damage to the osteoprogenitor cells (Lee et al 2002a). It might be concluded that a decrease in bone formation is at least partly responsible for bone loss during the first months after SCT.

6.3.2 Markers of bone resorption

In study I serum ICTP was elevated even before transplantation, increased further during the first months after SCT and then decreased to a level, which was still higher than before SCT. Similar findings have been obtained also in other studies (Kang et al 2000, Lee et al 2002a). The acceleration of bone resorption after SCT has been documented also by using urinary CTX (Banfi et al 2001), NTX (Schulte et al 2000) and pyridinium cross-links (Ebeling et al 1999) as its markers. In one study urinary excretion of deoxypyridoline, which was elevated before SCT, first decreased during the first weeks after SCT and then increased during the next few months to a 20 % higher level than before SCT (Massenkeil et al 2001).

In study II serum ICTP had decreased from the level at 12 months after SCT but it was still higher in long-term survivors of SCT than in controls. At the same time urinary NTX was lower in patients than in controls. In contrast, in study III there were no differences in resorption markers either between patients and controls or between patients with normal and reduced BMD.

In study IV, in patients not receiving pamidronate, who lost bone at all measurement sites, serum ICTP was most consistently elevated; serum TRACP5b was temporarily elevated at 1 month. Serum ICTP was elevated also in patients who received pamidronate and lost bone at the hip; in them other resorption markers (NTX, CTX, TRACP5b) were either consistently or temporarily decreased.

6.3.3 Discrepancy between resorption markers

Even though increased bone resorption after SCT has been demonstrated by other investigators (Ebeling et al 1999, Kang et al 2000, Schulte et al 2000, Banfi et al 2001, Lee et al 2002a) using such resorption markers as NTX, CTX, and pyridinium cross-links, in our studies serum ICTP has been the most consistently elevated resorption marker also in long-term survivors of SCT.
This discrepancy between the bone resorption markers could be explained by two different enzyme pathways, which degrade collagen. It is thought that cathepsin-K mostly release CTX and NTX and MMPs ICTP (Garnero et al. 1998, Atley et al. 2000, Sassi et al. 2000, Garnero et al. 2003). These enzymes are excreted by osteoclasts (Fohr et al. 2003). Collectively, these findings could mean that MMP-mediated resorption of bone is important in immediate bone loss and continues also long after SCT whereas cathepsin-K-mediated resorption is normalised over time either by treatment or spontaneously.

6.3.4 Oestrogen and testosterone

Sex steroids conserve bone mass through action on both osteoblasts and osteoclasts (Riggs et al. 2002). In estrogen deficiency, the activity of osteoclasts exceeds that of the osteoblasts resulting in net bone loss. The delay in menarche until 16 years or older has been associated with an increased risk of vertebral fractures in later life (Rubin et al. 1999). Logically, individuals who achieve a low peak bone mass in young adulthood are likely to develop osteoporosis later in life (Roy et al. 2003). In study III at the highest risk of reduced bone mass were females, which were prepubertal at the time of SCT. They might not have achieved a normal peak bone mass at all due to early gonadal hormone and/or growth hormone insufficiency. TBI evidently has a role in the development of hypogonadism, but the independent effect of TBI on the maturing bone could not be evaluated in our series since nearly all the patients were conditioned with TBI. The fact that testosterone secretion is preserved better in males than estrogen secretion in females after TBI may explain the accumulation of females in the group of patients with reduced BMD in study III. In study I serum testosterone level decreased but in study IV testosterone replacement was unable to prevent bone loss. Thus the lack of sex steroids seems not to be a major mechanism of post-SCT bone loss.

6.3.5 Osteoprotegerin

In study V no decrease in serum OPG was observed after SCT, instead OPG increased significantly in both treatment groups and also in patients not receiving any special prevention of osteoporosis. Thus post-transplant bone loss could not be explained by decreased production of OPG. When compared to healthy controls our patients had similar serum OPG levels before SCT and they even increased after SCT. In a previous study serum OPG levels were normal in patients with leukaemias, low in those with multiple myeloma and high in patients with Hodgkin’s disease or non-Hodgkin’s lymphoma (Lipton et al. 2002).
Perhaps the elevated OPG levels reflect a protective mechanism of the skeleton against increased osteoclastic activity and stimulated bone resorption. Keeping in line with this contention we and others have shown that serum and urine markers of bone resorption rapidly increase after SCT (Välimäki et al 1999, Ebeling et al 1999, Kang et al 2000, Schulte et al 2000, Lee et al 2002a). In one study of liver transplant recipients elevated serum OPG levels correlated positively with elevated serum CTX concentrations (Fahrleitner et al 2002).

6.3.6 sRANKL

In study V before SCT serum sRANKL levels were similar in patients and controls, remained at the control level in patients who received only calcium, vitamin D, and sex hormone replacement therapy, and decreased in those who received additional pamidronate. To our knowledge, serum sRANKL concentrations have not been hitherto studied in haematological malignancies or in conjunction with SCT.

Interestingly and fitting well to the role of OPG as a decoy receptor that is able to neutralise both the cell-bound and soluble forms of RANKL, OPG and sRANKL concentrations inversely correlated in the second part of study V. After all, our findings do not support the view that the excess of sRANKL contributes to bone loss after SCT, but serum concentrations do not necessarily reflect the situation at the tissue level.

6.3.7 Renal dysfunction

In studies I and IV the mean level of serum creatinine increased but none of the patients developed clinically significant renal dysfunction. By the time of study II serum creatinine had returned to the pre-transplantation level. It is unlikely that this slight impairment in renal function affected the levels of the collagen degradation products, which are excreted by the kidneys. On the other hand, the major changes in the makers of bone metabolism took place earlier than the elevation in serum creatinine.

6.3.8 Vitamin D

In study II serum 25-OH-D was significantly lower in patients than in the controls. Twenty-four out of 27 patients and 11 of the 28 controls had hypovitaminosis D, when S-25- OH-D $\geq$ 37.5 nmol/l was used as a cut-off criterion. This is the level at which the serum PTH concentration has begun to rise in cross-sectional studies (Thomas et al 1998). In study III six out of the 16 patients and
eight of 23 healthy controls had low serum 25-OH-D. These findings reflect the poor vitamin D status of the general Finnish population (Kauppinen-Mäkelin et al 2001). In two longitudinal studies both serum 25-OH-D and 1,25-(OH)$_2$-D concentrations were low even before SCT and they further decreased concomitantly with a rise in serum PTH during the first months after transplantation (Massenkeil et al 2001) although patients in the other study received vitamin D 200 IU daily (Schulte et al 2000). After all, the impact of vitamin D status and deranged vitamin D metabolism on SCT-associated bone loss remains open.

### 6.3.9 Role of immunosuppressive agents

As presented before, the changes in BMD are quite small after autologous transplantation. A major difference between autologous and allogeneic SCTs is the use of immunosuppressive regimens in conjunction with the latter. Furthermore, only a minority of patients with autologous SCT receive TBI-based conditionings (Shank and Hoppe 2004). The role of the immunosuppressive medication has been well documented in solid organ transplantation-associated bone loss (Epstein 1996). In study II the long-term users of GVHD prophylaxis [> 1 year, total MP dose 9.7 (5.3) g and CyA dose 81.5 (48.0) g] total bone loss from the pre-transplant level at the femoral sites was significantly higher than short-term users’ [ total MP dose 3.6 (2.6)g and CyA dose 71.3 (17.3)g]. Ebeling et al showed that bone loss after SCT was higher the higher the cumulative dose of glucocorticoids and the longer the duration of exposure to CyA (Ebeling et al 1999). In another study bone loss in patients who did not receive glucocorticoids was significantly lower than in those who were treated with them (Schulte et al 2000).

In the long-term the cortical bone of the upper femur appears to be more vulnerable to SCT than the trabecular bone of the vertebrae. Trabecular bone loss occurs during the first months after SCT, decreases thereafter, and the bone status of the lumbar spine even recovers spontaneously. In the upper femur BMD continues to decrease. During the use of glucocorticoids, the BMD of the trabecular bone decreases faster than that of the cortical bone (Lukert and Raiz 1990). Instead CyA affects both trabecular and cortical bones (Epstein 1996). Consequently, rapid bone loss during the first six months after SCT may be mostly due to glucocorticoids and afterwards CyA is the main drug affecting bone. The remodelling cycles of the trabecular bone are more rapid than those of the cortical bone (Aurbach et al 1992b), also facilitating a quicker recovery of the bone.
6.4 Recovery of bone after stem cell transplantation in adults

Study II demonstrated that BMD increased at all measurement sites from one year after SCT to the follow-up visit approximately 6 years after SCT. The recovery was statistically significant at the LS.

A longitudinal study of 35 patients for two post-transplant years showed a slight increase in the spinal BMD from one to two years after SCT, and a further loss in the femoral neck BMD (Schulte et al 2000). A cross-sectional study performed approximately 3 years (range 1-10 years) after SCT showed that BMD in patients was significantly lower than in controls and 17 % of them had osteopenia and 12 % osteoporosis at the LS and 37 % osteopenia and 15 % osteoporosis at the FN. When phalangeal osteosonogrammetry was used 60 % (25 out of 41) had osteopenia and 7 % (3 out of 41) osteoporosis. The more time had elapsed from transplantation the higher the BMD values, especially in the spine (Tauchmanova et al 2002). It has been demonstrated also in other studies that the BMD of the femoral neck is lowered 60 (5,6) months and 2 and 5 years after SCT (Buchs et al 2001, Gandhi et al 2003). The latest study demonstrated that the lumbar spine BMD reached its nadir at 6 months and the femoral neck BMD at 24 months (Schulte and Beelen 2004).

Taken together all these findings support the view that the lumbar spine BMD recovers but the femoral neck BMD remains lowered for years after SCT. A high rate of osteopenia in the phalanges, which as the upper femur mainly consists of cortical bone, supports the contention that cortical rather than trabecular bone is injured in the SCT.

6.5 Effect on peak bone mass of stem cell transplantation performed in childhood

In study III, six out of 16 patients (38 %) had a significantly reduced areal BMD (Z-score ≤ -1) at, at least, one measurement site, the median Z-scores being – 0.25, -0.4 and –0.2 for the LS, the FN and the total body, respectively. The risk of belonging to the group of reduced bone mass associated with female gender, prepubertal status at the time of SCT, pubertal growth delay, hypogonadism, and low BMI. Also in another study the total body BMD (median Z-score –0,5) 2 (range 1-10) years after SCT was lower (p=0,03) in paediatric patients who had received allogeneic SCT during childhood compared to those who had received SCT as adults (median Z-score 0,0). That study did not find a correlation between reduced bone mass and hypogonadism, intake of glucocorticoids or GVHD (Bhatia et al 1998).

In study III no correlations between the doses of immunosuppressive drugs and BMD were found although they were shown in study II and in adult patients
by others (Ebeling et al 1999). Perhaps the harmful effects of CyA and glucocorticoids in the growing bone at least in part disappear when enough time has elapsed since they stop being taken. Lack of GH and sex steroids may lead to more stable bone loss.

Growth hormone (GH), partly via action of IGF-I, stimulates both osteoblasts and osteoclasts with the net effect being bone accumulation (Olney 2003). GH deficiency impairs the accumulation of bone mass in children (Saggese et al 1992) but also disturbs the maintenance of BMD in adults (Holmes et al 1994). In children with primary pituitary deficiency GH replacement does not totally prevent bone loss (Kaufman et al 1992). GH deficient children achieve lower peak bone mass than healthy controls (Monson et al 2002). It can be thought that a lack of GH is a major threat to bone health of children who undergo a stem cell transplantation. Whether GH therapy should be continued until peak bone mass is achieved, should be examined in further studies.

In study III patients with reduced bone mass had a lower body mass index (BMI) than those with normal BMD. Low BMI predisposes to bone loss (Grisso et al 1997, Bass et al 1999) and increases the risk of fractures in both genders (Roy et al 2003). In our study the patients with low BMI had also a lack of GH, which by decreasing muscle mass affects BMI (Weber 2003).

Height is an important positive determinant of peak bone mass in young adults (Välimäki et al 2004). In the present study, this was true only for those with normal BMD. A new finding was the negative relationship between height in SDs and areal bone mineral density in SCT patients with reduced BMD, especially in the lumbar spine. This implies that the vertebrae were denser the more the patient’s growth was retarded. Dense vertebrae might result from fractures in the lumbar spine. However, none of our patients had been diagnosed with vertebral fractures during the post-transplant follow-up. It could be hypothesised that growth delay might even extend the period of bone mineralisation. Finally, growth retardation in SCT recipients may involve the appendicular more than the axial skeleton.

Taken together, low peak mass in young adults with SCT in childhood associates with female gender, prepubertal status, growth retardation, hypogonadism and low BMI. Unfortunately, due to the small number of subjects we were not capable of performing multivariate analyses to reveal independent determinants of low peak bone mass.
6.6 Prevention of stem cell transplantation associated bone loss

6.6.1 Vitamin D and calcium

In study I calcium either alone or with calcitonin had no significant effect on bone loss. In study IV all patients received calcium and vitamin D but they could not prevent bone loss, the magnitude of which was the same as in study I. Similar results have been obtained in cardiac transplant recipients (Välimäki et al 1999), who in another study benefited from a high dose (32000 IU per week) of calcidiol (25-OH-D) even more than from a low dose of nasal calcitonin or cyclical etidronate with respect to their BMD (Garcia-Delgado et al 1997). Although the evidence to support the role of calcium and vitamin D in the prevention of transplantation-associated bone loss is scarce, their use is generally recommended to all transplant recipients (Cohen and Shane 2003).

6.6.2 Calcitonin

Calcitonin together with calcium had no significant effect on bone loss. The same kind of results have been obtained in cardiac transplant recipients (Garcia-Delgado et al 1997, Välimäki et al 1999). Although calcitonin reduces glucocorticoids-induced bone loss (Rodino and Shane 1998) it does not seem to be effective enough in organ transplantation recipients.

6.6.3 Sex steroids

Oestrogen replacement decreases the risk of fractures in postmenopausal women (Rubin et al 1999, Roy et al 2003). In our studies females who developed amenorrhea as a sign of hypogonadism, received oestrogen replacement therapy. In study I the replacement therapy was started quite late, on average 6 months after SCT, which might have been too late to prevent bone loss at least at the LS. However, in study IV the females who started oestrogen replacement without pamidronate immediately after SCT lost bone at the LS and the FN no less than the women in study I over the first 6 post-transplant months. In line with our findings, Gandhi et al showed that females, who started oestrogen replacement during the first year after SCT, had a bone loss equal to that of other SCT patients (Gandhi et al 2003). In one study oestrogen improved BMD in osteopenic females when started 13 months after SCT (Castelo-Branco et al 1996). Although oestrogen replacement therapy is effective in postmenopausal osteoporosis and possibly in SCT patients when started later on, it does not seem to prevent immediate bone loss after SCT. If oestrogen therapy is not contraindi-
cated, it might be useful for female recipients of SCT to treat hypogonadal symptoms but not to prevent bone loss.

Study I showed a remarkable reduction in the serum testosterone level of male patients with the nadir at six weeks after SCT. In line with the results of another Finnish study (Kauppila et al 1998), four out of 13 males in study II were hypogonadal and all men had lower serum testosterone levels than before transplantation. At the time of the re-examination there was no significant difference in BMD between hypo- and eugonadal men but the hypogonadal men were all short-term users of immunosuppressive drugs. In study IV, in which for all males testosterone replacement therapy was started early after SCT, those who did not receive pamidronate lost bone both at the LS and the FN. Although treatment of male hypogonadism of various causes has improved BMD (Snyder et al 2000), our findings do not support the role of testosterone replacement therapy in the prevention of SCT-associated bone loss. It is, however, recommended for truly hypogonadal men (Rodino and Shane 1998).

6.6.4 Bisphosphonates

In study IV pamidronate prevented bone loss at the LS but only decreased it at the femoral sites in comparison with the combination of calcium, vitamin D, and sex steroid replacement only.

There are no other randomised, prospective studies where the efficacy of bisphosphonate therapy has been tested in SCT recipients. In a non-randomised study (n=33), where 79% of patients either used oestrogen replacement therapy or three-monthly infusions of 30 mg pamidronate, BMD of the LS remained after SCT but decreased at the FN by −4.2% at 6 and by −5.6% at 12 months after SCT (Buchs et al 2001). Instead in randomised studies where treatment was started not earlier than 17 to 24 months after SCT, risedronate or zoledronic acid either prevented further bone loss or even increased BMD at the femoral neck (Tauchmanova et al 2003, Tauchmanova et al 2004).

Our results are comparable to those obtained in a recent study of cardiac transplant recipients where alendronate prevented bone loss at the LS but only decreased it at the FN during the first post-transplant year (Shane et al 2004). A prospective, randomised study in kidney transplantation patients showed that intravenous zoledronic acid prevented femoral bone loss and even increased BMD at the LS during the first six months after the transplantation (Haas et al 2003). In another prospective, randomised study of kidney transplant recipients intravenous ibandronate prevented lumbar bone loss and even improved femoral BMD while patients without treatment lost bone at both sites (Grotz et al 2001). A single dose of pamidronate followed by cyclic etidronate reduced bone
loss both at the LS and the FN compared to historical controls after cardiac transplantation (Shane et al 1998). Liver transplantation patients with reduced bone mass before transplantation had no new vertebral fracture when treated with intravenous pamidronate before and nine months after the procedure. At the same time 38 % of untreated patients had new fractures (Reeves et al 1998). Lung transplantation patients treated with cyclic etidronate had less bone loss both at the LS and the FN than controls (Henderson et al 2001). Bisphosphonates have also either prevented further bone loss or increased BMD when they have been started from six months to several years after cardiac, kidney or liver transplantation (Valero et al 1995, Giannini et al 2001, Dodidue et al 2003).

High serum levels of ICTP might offer one mechanistic explanation for the relative inefficacy of pamidronate in the prevention of bone loss at the hip. ICTP is thought to reflect matrix metalloproteinase (MMP)-mediated bone resorption and CTX and NTX cathepsin-K-mediated resorption (Atley et al 2000, Sassi et al 2000, Garnero et al 2003). Bisphosphonates inhibit cathepsin-K mediated resorption and decrease NTX and CTX (Garnero et al 1994, Christcau et al 2000, Garnero et al 2003), but not serum ICTP. Consequently, a possibility remains that MMP-mediated bone resorption significantly contributes to immediate bone loss in SCT patients and this is not prevented with bisphosphonates.

TRACP5b is a novel and specific marker of the osteoclast function (Halleen et al 2001, Janckila et al 2001) and should reflect both MMP- and cathepsin-K-mediated bone resorptions by osteoclasts. In the pamidronate-treated patients TRACP 5b was lowered below the pre-transplantation level but ICTP remained elevated 6 to 12 months after the procedure. Consequently, a question arises where the possibly increased MMP activity resides - in osteoclasts or possibly in osteoblasts (Chanbers et al 1985, Blavier and Delaisse 1995).

The mechanisms by which bisphosphonates inhibit osteoclasts are not totally clear. Data concerning the effect of bisphosphonates on the OPG/RANKL system is controversial. In a rat osteosarcoma cell line, pamidronate and clodronate down-regulated the expression of mRNA of RANKL (Pan et al 2004). In one study different concentrations of alendronate or pamidronate did not change the messengerRNA expression of RANKL or OPG in bone cells of mice (Kim et al 2002). In another study zoledronic acid and pamidronate stimulated OPG production in human osteoblast cell culture (Viereck et al 2002). In the only human study of patients with Paget’s disease elevated serum OPG decreased after the start of tiludronate (Alvarez et al 2003). In our study the V serum OPG concentration increased similarly in patients receiving and not receiving pamidronate but serum sRANKL decreased in patients treated with pamidronate. Thus, at least part of the bone-sparing effects of bisphosphonate might be mediated through the inhibition of RANKL production.
7 CONCLUSIONS

1) BMD both at the lumbar spine and at the upper femur reduced significantly by 5.8 % and 7.0 % during the first six months after allogeneic stem cell transplantation. BMD of the lumbar spine slightly recovered during the next six months, whereas BMD of the upper femur further decreased at least up to one year after SCT.

2) The changes in bone turnover markers indicated that the reduction in BMD after allogeneic SCT was a net effect of decreased bone formation and increased bone resorption. The markers of bone formation decreased during the first 3 months after SCT but returned to the pre transplantation level by six months after SCT. The marker of bone resorption serum ICTP increased rapidly and remained elevated even several years after SCT. Increased serum ICTP levels support the contention that metalloproteinase-mediated bone resorption is an important mechanism in SCT-associated bone loss. Deficiency of OPG or excess of sRANKL did not seem to contribute to bone loss after SCT.

3) In adult SCT-recipients BMD of the lumbar spine spontaneously returned near to the pre-transplantation level during the follow-up of a mean duration of six years. Also the femoral BMD slightly recovered over the years after SCT but remained lower than before transplantation.

4) On average one third of the patients, who had received a SCT during childhood, had a reduced bone mass in adolescence and young adulthood. Female gender, hypogonadism, prepubertal status at the time of SCT, growth retardation and low BMI were risk factors of reduced peak bone mass.

5) Bone loss after SCT could not be prevented by calcium with or without calcitonin or sex hormone replacement therapy. Instead, intravenous pamidronate prevented bone loss in the lumbar spine and decreased but did not totally abolish it in the upper femur. The relative inefficacy of pamidronate in the prevention of bone loss at the hip might be related to MMP-mediated bone resorption, which is not inhibited by bisphosphonates.
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Table 1 Summary of studies of bone metabolism after allogeneic stem cell transplantation

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Type of study</th>
<th>LBMD</th>
<th>FNBMB</th>
<th>Prevalence of osteopenia / -porosis</th>
<th>S-OPG</th>
<th>S-OC</th>
<th>S-ICTP</th>
<th>U-NTX</th>
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<td>Baek et al 2004</td>
<td>36</td>
<td>Prospective</td>
<td>-5.2 % in 1 year</td>
<td>-11.6% in 1 year</td>
<td>+23 % in 3 months</td>
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<tr>
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<td>-4.2 % in 1 year</td>
<td>-5.6% in 1 year</td>
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<td></td>
<td></td>
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<tr>
<td>Castañeda et al 1997</td>
<td>27</td>
<td>Cross-sectional</td>
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<td></td>
<td>33 %/18% 13 months after SCT</td>
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<tr>
<td>Ebeling et al 1999</td>
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<td>Gandhi et al 2003</td>
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<td>Prospective</td>
<td>-2.4 % in 6, 0.001 % in 24 months</td>
<td>-5.2 % in 6, -4.2 % in 24 months</td>
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<td>all had lowered BMD 1 year after SCT</td>
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<td>+ 30 %</td>
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*= Includes 30 patients with autologous transplantation

Abbreviation: FBMD= bone mineral density of femoral neck, ICTP= type I collagen carboxyterminal telopeptide, LBMD= bone mineral density of lumbar spine, NTX= type I collagen aminoterminal telopeptide, OC= osteocalcin; OPG= osteoprotegerin, SCT= stem cell transplantation
Table 2: Characteristics of the patients

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<td>41 (12)</td>
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<td>Cumulative dose of MP (g)</td>
<td>at 6 months</td>
<td>3.6 (0.5)</td>
<td>5.5 (0.7)</td>
<td>5.1 (0.8)</td>
<td>3.8 (2.2)</td>
<td>2.7 (2.2)</td>
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<td>at 12 months</td>
<td>4.4 (2.6)</td>
<td>5.8 (0.8)</td>
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<td></td>
<td>total</td>
<td>6.3 (5.0)</td>
<td>4.0 (0.8)</td>
<td>2.1 (1.2)</td>
<td>4.3 (2.7)</td>
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<tr>
<th>Study</th>
<th>Analysis</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative dose of CyA (g)</td>
<td>at 6 months</td>
<td>38.2 (4.0)</td>
<td>40.4 (5.3)</td>
<td>46.2 (5.8)</td>
<td>43.8 (17.0)</td>
<td>43.3 (14.4)</td>
</tr>
<tr>
<td></td>
<td>at 12 months</td>
<td>62.1 (4.3)</td>
<td>68.1 (5.7)</td>
<td>77.2 (7.0)</td>
<td>67.1 (20.8)</td>
<td>67.6 (31.6)</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>75.8 (34.1)</td>
<td>25.6 (9.1)</td>
<td>28.0 (9.1)</td>
<td>43.5 (22.8)</td>
<td>51.9 (25.6)</td>
</tr>
</tbody>
</table>

* = the same patients as in study I, # = the same patients as in study IV

Abbreviations: pami = pamidronate, AML = acute myeloblastic leukaemia, ALL = acute lymphoblastic leukaemia, CML = chronic myeloid leukaemia, CLL = chronic lymphocytic leukaemia, MDS = myelodysplastic syndrome, MF = myelofibrosis, AA = amyloidosis, MP = methylprednisolone, CyA = cyclosporine A
Table 3 The number of patients having osteopenia or osteoporosis at the lumbar spine

<table>
<thead>
<tr>
<th>Time</th>
<th>Study I</th>
<th>Study II</th>
<th>Study IV</th>
<th>Pami +</th>
<th>Pami -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>6 mt</td>
<td>12 mt</td>
<td>Follow-up</td>
<td>before</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>15 (10)</td>
<td>17 (12)</td>
<td>16 (12)</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>(also in study II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>2 (1)</td>
<td>3 (1)</td>
<td>1 (1)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(also in study II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>17 (11)</td>
<td>20 (13)</td>
<td>17 (13)</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>(% of whole group (also in study II))</td>
<td>39 (41)</td>
<td>50 (57)</td>
<td>47 (50)</td>
<td>26</td>
<td>31</td>
</tr>
</tbody>
</table>

Table 4 The number of patients having osteopenia or osteoporosis at the femoral neck

<table>
<thead>
<tr>
<th>Time</th>
<th>Study I</th>
<th>Study II</th>
<th>Study IV</th>
<th>Pami +</th>
<th>Pami -</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>6 mt</td>
<td>12 mt</td>
<td>Follow-up</td>
<td>before</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>11 (7)</td>
<td>17 (9)</td>
<td>20 (11)</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>(also in study II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>(also in study II)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>11 (7)</td>
<td>18 (10)</td>
<td>21 (12)</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>(% of whole group (also in study II))</td>
<td>25 (27)</td>
<td>45 (43)</td>
<td>58 (48)</td>
<td>41</td>
<td>34</td>
</tr>
</tbody>
</table>

Table 5 Bone mineral density in young adults medially 7.1 (range 1.5-20.5) years after stem cell transplantation. Bone mineral density is presented as standardized for age and gender (Z-score). Percentages indicate the proportion of the whole study group (n=16).

<table>
<thead>
<tr>
<th></th>
<th>Z-score (SD)</th>
<th>Z-score less than equal to –1 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1-L4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>-0.2 (1.0)</td>
<td>3 (19) 0 (0) 3 (19) 0 (0)</td>
</tr>
<tr>
<td>Men</td>
<td>0.6 (0.7)</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Women</td>
<td>-0.5 (1.1)</td>
<td>3 (30) 0 (0) 3 (30)</td>
</tr>
<tr>
<td>Femoral neck*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>-0.4 (1.3)</td>
<td>2 (13) 0 (0) 2 (13) 0 (0)</td>
</tr>
<tr>
<td>Men</td>
<td>-0.3 (0.4)</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Women</td>
<td>-0.7 (1.8)</td>
<td>2 (33) 2 (33) 2 (33)</td>
</tr>
<tr>
<td>Total hip*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>-0.1 (1.2)</td>
<td>1 (6) 0 (0) 1 (6) 0 (0)</td>
</tr>
<tr>
<td>Men</td>
<td>0.2 (0.4)</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Women</td>
<td>-0.9 (1.6)</td>
<td>1 (17) 1 (17) 1 (17)</td>
</tr>
<tr>
<td>Total body</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>-0.2 (1.0)</td>
<td>4 (25) 0 (0) 4 (25) 0 (0)</td>
</tr>
<tr>
<td>Men</td>
<td>0.3 (0.5)</td>
<td>0 (0) 0 (0) 0 (0)</td>
</tr>
<tr>
<td>Women</td>
<td>-0.4 (1.1)</td>
<td>4 (40) 4 (40) 4 (40)</td>
</tr>
</tbody>
</table>

* not available in five subjects less than 20 years
**Figure 1** Schematic view of bone remodelling

**Figure 2** Local regulation of bone remodelling. IL-1, 6, 11= interleukins 1,6,11, TNF = tumour necrosis factor, GM-CSF=granulocyte-macrophage colony stimulating factor M-CSF= macrophage-colony stimulating factor, RANKL = receptor activator of NF-κB ligand, RANK = receptor activator of NF-κB, OPG = osteoprotegerin, TGFβ= transforming growth factor β
Figure 3 Bone loss in the combined population of the study I. Changes in BMD (mean and SE) as per cent from baseline between 0 and 6 months, 6 and 12 months and 0 and 12 months after SCT. * p<0.0001 for changes from baseline.

Figure 4 Markers of bone turnover (mean and SE) in the combined population of study I. (a) serum bone-specific alkaline phosphatase; (b) serum procollagen I carboxyterminal propeptide; (c) serum procollagen I aminoterminal propeptide; (d) serum collagen I carboxyterminal telopeptide. *
Figure 5 Serum osteoprotegerin (A) and sRANKL (B) concentrations [mean(SE)] in SCT patients receiving and not receiving pamidronate. RM ANOVA for a difference between the groups over time. * $P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for changes from baseline.
Figure 6 Per cent changes in bone mineral density [mean (SE)] in the study groups. A. Lumbar spine. B. Femoral neck. C. Trochanter. D. Total hip. P values in the figures denote significancies between the groups over time (RM ANOVA). * P<0.05, ** P<0.01 for differences between the groups at different time points. †P<0.05, †† P<0.01, ††† P<0.001 for within-group changes from baseline.
Figure 7 Per cent changes in bone turnover markers (median with interquartile ranges) in the study groups. A. PINP = serum type I procollagen aminoterminal propeptide; B. NTX = urinary type I collagen aminoterminal telopeptide related to creatinine; C. CTX = serum type I collagen carboxyterminal telopeptide b; D. ICTP = serum type I collagen carboxyterminal telopeptide; E. TRACP5b = serum tartrate resistant acid phosphatase 5b. * P<0.05, ** P<0.01, *** P<0.001 for differences between the groups at different time points. † P<0.05, †† P<0.01, ††† P<0.001 for within-group changes from baseline.