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ORIGINAL ARTICLE

Unexpected low incidence of vertebral fractures in heart transplant recipients: analysis of bone turnover

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Summary

Heart transplantation (HTX) is associated with a reduction in bone mineral density (BMD). Different markers of bone metabolism have been used, and the applied immunosuppressive regimens have also changed over time. This study was performed to re-investigate bone metabolism in HTX recipients. Twenty-five HTX recipients were compared with 25 HTX candidates in respect of biochemical parameters of bone metabolism, BMD, and the frequency of fractures for 1 year. Osteopenia or osteoporosis was observed in approximately two-thirds of the HTX recipients. Nevertheless, only three (12%) HTX recipients developed a vertebral fracture within 1 year after transplantation; no peripheral fractures occurred. Compared with HTX candidates, HTX recipients had lower serum levels of osteocalcin, and higher serum levels of cross-linked-N-telopeptide of type I collagen (NTX). In HTX recipients, osteocalcin initially reached a nadir, increased during the first 3 months, and decreased thereafter. Bone-specific alkaline phosphatase initially increased and then decreased. Serum levels of NTX and parathyroid hormone remained high throughout the year. Despite a high bone turnover, an unexpectedly low rate of vertebral fractures was registered. Nevertheless, each fragility fracture is a serious complication and we need to take steps to prevent this complication.

Introduction

Osteoporosis is a systemic skeletal disease characterized by low bone mass and deterioration in the microarchitecture of bone tissue, resulting in fragility of bone and susceptibility to fractures [1]. Bone disease is a clinically significant complication, frequently observed in patients suffering from terminal heart failure as well as in heart transplant recipients. The reported bone loss during the first year after heart transplantation (HTX) is 10% [2], which is higher compared with the mean bone loss of 0.53% per year in healthy women and 0.3% in healthy men [3,4].

Changes in bone-specific laboratory parameters during the first year after HTX have been reported in some studies. Sambrook *et al.* [5] registered a transient decrease in bone formation (osteocalcin) and an increase in bone resorption (urinary hydroxyproline) during the first few months after transplantation. In men, a transient but significant decline in mean serum testosterone was seen immediately after HTX. Shane *et al.* [6] observed similar

changes in bone formation and resorption during the first year after transplantation. Serum levels of 1, 25-dihydroxyvitamin D fell by 3 months and remained significantly below baseline levels throughout the first year. Serum testosterone was reduced only at 1 month post-HTX. However, Thiebaud *et al.* [7] registered increased levels of osteocalcin and urinary hydroxyproline excretion throughout the first year after transplantation. Parathyroid hormone levels showed a biphasic pattern with an initial decrease at 2 months after transplantation, but increased thereafter. A further study focusing on the initial post-transplant period showed increases in bone formation markers shortly after transplantation, a return to baseline levels at 3 months, and a further increase thereafter. Bone resorption was increased throughout the observation period of 12 months [8].

The causal role of immunosuppressive therapy in respect of post-transplant bone loss is beyond question. Particularly, glucocorticoids and calcineurin inhibitors are believed to be harmful to bone metabolism.

The pathogenesis of bone loss in heart transplant candidates and recipients has been a subject of considerable investigation [9,10]. New serum markers of bone metabolism have been introduced over time. The bone resorption marker cross-linked-N-telopeptide of type I collagen (NTX), which is a more specific marker of bone resorption than hydroxyproline excretion [11], was not used in the above mentioned studies focusing on the initial post-transplant phase. Additionally, the immunosuppressive regimen has changed in the last few years. We investigated HTX candidates and recipients (1 year after transplantation) with regard to bone metabolism.

Patients and methods

Study population

Twenty-five HTX recipients were investigated a few days after transplantation and followed up for 1 year. In addition, 25 HTX candidates were investigated once. The subjects had to be at least 20 years of age. Exclusion criteria were diseases other than chronic heart failure leading to secondary osteoporosis, nonosteoporotic metabolic bone diseases, and the use of an assist device before transplantation. Calcium (1000 mg) and vitamin D (800 IE) supplementation were prescribed immediately after HTX. The protocol was approved by the ethics committee of the Medical University of Vienna and written informed consent was obtained from all participants.

Immunosuppression and surveillance protocol

All HTX patients were given methylprednisolone intravenously at a dose of 500 mg during surgery, and 125 mg at 8, 16, and 24 h post-transplantation. Thereafter, oral methylprednisolone was started at 10 mg daily in most patients; four patients took less or no oral methylprednisolone. The oral dose of methylprednisolone was reduced within the first few months after transplantation to an alternating dose of 10 mg/5 mg or 5 mg daily.

Starting on the first postoperative day, all patients received antibody induction therapy with polyclonal antithymocyte globulin (Thymoglobuline, Cambridge, MA, USA) at a dose of 2 mg/kg for 3–7 days (depending on platelet count). Cyclosporine A (Sandimmun Neoral, Novartis, Basel, Switzerland) was started 3–7 days after transplantation with target serum levels of 200 ng/ml in all but three patients who received tacrolimus (Prograf, Astellas Pharma, Deerfield, IL, USA; target serum level: 10 ng/ml).

All patients received mycophenolate mofetil (Cell Cept, Hofmann-La Roche, Grenzach-Wyhlen, Germany) 1.5–3 g/day (depending on leukocyte count) intravenously on the first postoperative day and orally thereafter.

Diagnosis and prevention of infection

Episodes of infection were identified clinically and confirmed by microbiological, serological, or histological tests. These included bacterial and fungal cultures in biopsies or blood. Cytomegalovirus (CMV) screening was performed using serologic tests (complementary binding reaction) and measurement of CMV early antigen as well as PCR for CMV in blood [12]. Patients received prophylaxis for CMV by administration of 100 ml anti-CMV IgG hyperimmunoglobulin (Cytotect, Biotest, Dreieich, Germany) intravenously immediately after transplantation and on postoperative days 7, 14, and 21. Patients with CMV mismatch and those with a positive/positive CMV serological status were given antiviral preemptive therapy with intravenous ganciclovir (Cymevene, Roche, Vienna, Austria) at 10 mg/kg/day for 21 days after transplantation, followed by oral treatment with 3×500–1000 mg/day for additional 70 days. Patients also received antibacterial prophylaxis with cefazolin (3×2 g/day) and vancomycin i.v. for 5 days, and antifungal prophylaxis with nystatin (100 ml/day) until the time of discharge from the hospital [12].

Bone mineral density measurement, X-ray

The bone mineral density (BMD) of the lumbar spine and that of the femoral neck was measured by dual energy X-ray absorptionmetry (DXA) using QDR 4500 (Hologic Inc, Waltham, MA, USA) in HTX recipients 4 weeks, 6 and 12 months after transplantation. In HTX candidates, BMD of the lumbar spine and the femoral

neck was measured once. All measurements were performed using the standard procedures recommended by the manufacturer. Biplanar roentgenograms of the thoracic and lumbar spine taken 4 weeks and 12 months after transplantation were used to identify vertebral deformities caused by fractures. A semi-quantitative method of fracture determination was used. Vertebrae were graded as normal, mildly deformed (20–25% reduction in height), moderately deformed (25–40% reduction in height), and severely deformed (at least 40% reduction in height) [13]. The X-ray results were also taken into account for the analysis of BMD.

Biochemistry

Venous blood samples were taken in the morning after overnight fasting 1, 2, and 4 weeks as well as 3, 6 and 12 months after heart transplantation and, additionally, in HTX candidates at the time of inclusion. The following parameters were studied: calcium, phosphate, alkaline phosphatase, creatinine, blood urea nitrogen (AU 5400 analyzer Olympus, Japan), osteocalcin, sex hormone binding globulin, estradiol, testosterone, and parathyroid hormone (electrochemoluminescence immunoassays, Modulat <EEE> analyzer, Roche Diagnostics, Mannheim, Germany), bone-specific alkaline phosphatase (BAP; Metra, Mountainview, CA, USA), and 25-hydroxyvitamin D (25-OH-vitamin D; RIA, DiaSorin, Stillwater, MN, USA). Bioavailable testosterone was calculated from molar concentrations of testosterone, sex hormone binding globulin, and albumin, according to the law of mass action [14] using previously published association constants [15]. The second morning urine was tested for NTX (ELISA, BRAHMS Diagnostica GmbH, Berlin, Germany).

Statistical analysis

Continuous parameters are described by their medians and quartiles while categorical parameters are mentioned in frequencies and percentages (with exact confidence intervals). According to the WHO criteria for BMD [16], patients were classified as normal (T-score > -1), presenting with osteopenia ($-2.5 \leq$ T-score ≤ -1) or presenting with osteoporosis (T-score < -2.5). The Mann–Whitney *U*-test was used to compare BMDs (Z score) of the lumbar spine and the femoral neck as well as biochemical data between HTX candidates and the first measurement of HTX recipients. McNemar's test was used to compare percentages within patients at different time points. In addition, BMD values are shown graphically as mean and SD of the changes from baseline values.

To register the essential features of the development of the various parameters over time, a linear mixed model was fitted. However, according to previous research experiences, the time trend is not modeled to be linear over the whole time period, but instead to be linear between weeks 1 and 2, between weeks 2 and 12, and between weeks 12 and 52, thus allowing for changes in slopes at weeks 2 and 12 [17]. The random portion of this piecewise linear mixed model was used to account for correlations between a patient's measurements over time. Piecewise linear time courses and their confidence intervals as estimated by the model are shown. Estimated slopes between weeks 1 and 2, between weeks 2 and 12, and from week 12 onward are given, with *P*-values corresponding to the null hypothesis of a slope equal to zero.

P-values <0.05 were considered statistically significant. Adjustment for multiple testing was not performed because of the exploratory nature of this study. The statistical software packages SPSS 10.0 (SPSS Inc., Chicago, IL, USA, 2000) and SAS 9.2 (SAS Institute Inc., Cary, NC, USA, 2000) were used for statistical analysis.

Results

Study population

The groups of HTX recipients and HTX candidates consisted of 25 patients each. With regard to age, no relevant differences were registered between the two groups. The underlying heart disease was ischemic cardiomyopathy in 14 patients, dilated cardiomyopathy in eight, and inflammatory heart disease in three.

Bone mineral density

Inter-group comparison of BMD values expressed as Z scores between HTX candidates and HTX recipients at their first measurement did not reveal any differences.

The changes in BMD over time are presented as mean percent change to baseline and the percentage of subjects with T scores in the normal, osteopenic, and osteoporotic ranges are given in Fig. 1. None of the changes was statistically significant. The T score values 4 weeks after transplantation were -1.6 [-2.4; -0.4] and -1.3 [-2.5; -0.4] for the lumbar spine and the femoral neck, respectively. One year after transplantation, they were -1.7 [-2.7; -0.7] and -1.0 [-2.1; -0.5] for the lumbar spine and the femoral neck, respectively.

Fractures

Two male HTX recipients (8%) developed a new vertebral fracture. One male HTX recipient (4%) who had prevalent osteoporosis according to the BMD measurement at baseline experienced a deterioration of a previous vertebral fracture. No peripheral fragility fractures occurred.

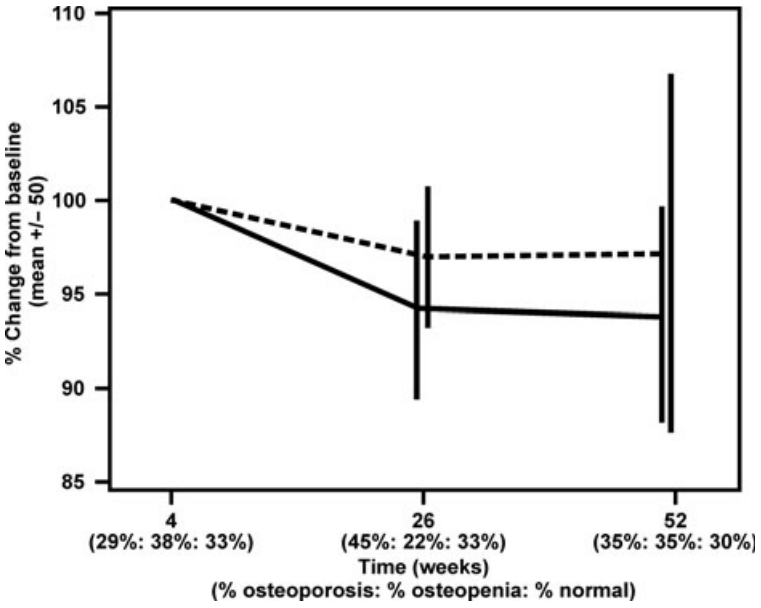

Figure 1 The percent change of bone mineral density (BMD) compared with baseline in heart transplantation (HTX) recipients (lumbar spine: solid line; femoral neck: dashed line) and the percentage of subjects with T scores in the normal, osteopenic, and osteoporotic ranges.

Table 1. Demographic and biochemical data of HTX transplantation candidates and HTX recipients 1 week after transplantation.

	HTX candidates $n = 25$	HTX recipients $n = 25$
Age (a)	56 [54; 60]	55 [46; 62]
Male:female†	22:3	24:1
Osteocalcin (ng/ml)	21.2 [11.1; 31.3]	9.5 [6.1; 12.5]*
Bone-specific alkaline phosphatase (U/l)	17.7 [13.9; 26.1]	18.1 [15.3; 22.6]
Cross-linked-N-telopeptide of type I collagen (nmol/l)	3.7 [2.6; 5.4]	7.3 [4.7; 9.8]**
Estradiol (pg/ml)‡	34.0 [26.0; 46.0]	30.5 [26.3; 39.5]
Bioavailable testosterone (ng/ml)‡	1.12 [0.72; 1.76]	0.60 [0.27; 1.00]*
25-hydroxyvitamin D (nmol/l)	45.1 [26.6; 62.3]	35.6 [21.9; 48.0]
Serum calcium (mmol/l)	2.5 [2.4; 2.6]	2.1 [2.0; 2.2]**
Serum phosphate (mmol/l)	1.1 [1.0; 1.4]	1.0 [0.8; 1.2]
Parathyroid hormone (pg/ml)	57.5 [40.9; 105.0]	62.1 [51.0; 77.1]
Creatinine (mg/100 ml)	1.27 [1.08; 1.62]	0.98 [0.86; 1.11]**
Blood urea nitrogen (mg/100 ml)	27.8 [20.3; 54.3]	18.3 [13.2; 25.1]*

HTX, heart transplantation.
Values are expressed as median [quartiles].
*$P < 0.05$, **$P \leq 0.001$.
†All female participants were postmenopausal.
‡Testosterone and estrogen values in males only.

Biochemistry

Compared with HTX candidates, recipients had significantly lower serum levels of calcium, osteocalcin, creatinine, blood urea nitrogen, and bioavailable testosterone but higher NTX at their first measurement (Table 1).

Serial biochemistries of HTX recipients are shown in Fig. 2. The bone formation marker osteocalcin was reduced at the first measurement, increased by 7.6 ng/ml ($P < 0.001$) per week until week 2, increased subsequently by 2.8 ng/ml per week ($P < 0.001$), and decreased by 0.2 ng/ml per week ($P < 0.001$) from 3 months post-transplant onward. Bone-specific alkaline phosphatase initially increased, showing a significant change of 3.8 U/l ($P = 0.023$) per week. The subsequent reduction was statistically significant from the third to the twelfth month (−0.1 U/l; $P = 0.0173$). Bone resorption (NTX) was already increased at the first measurement. It increased further by 1.5 nmol/l until week 2 ($P < 0.001$). Changes until 3 months post-transplant were not significantly different from zero. From 6 to 12 months, the weekly reduction in serum levels of NTX was 0.05 nmol/l ($P < 0.001$). With regard to sex hormones, bioavailable testosterone levels in men increased

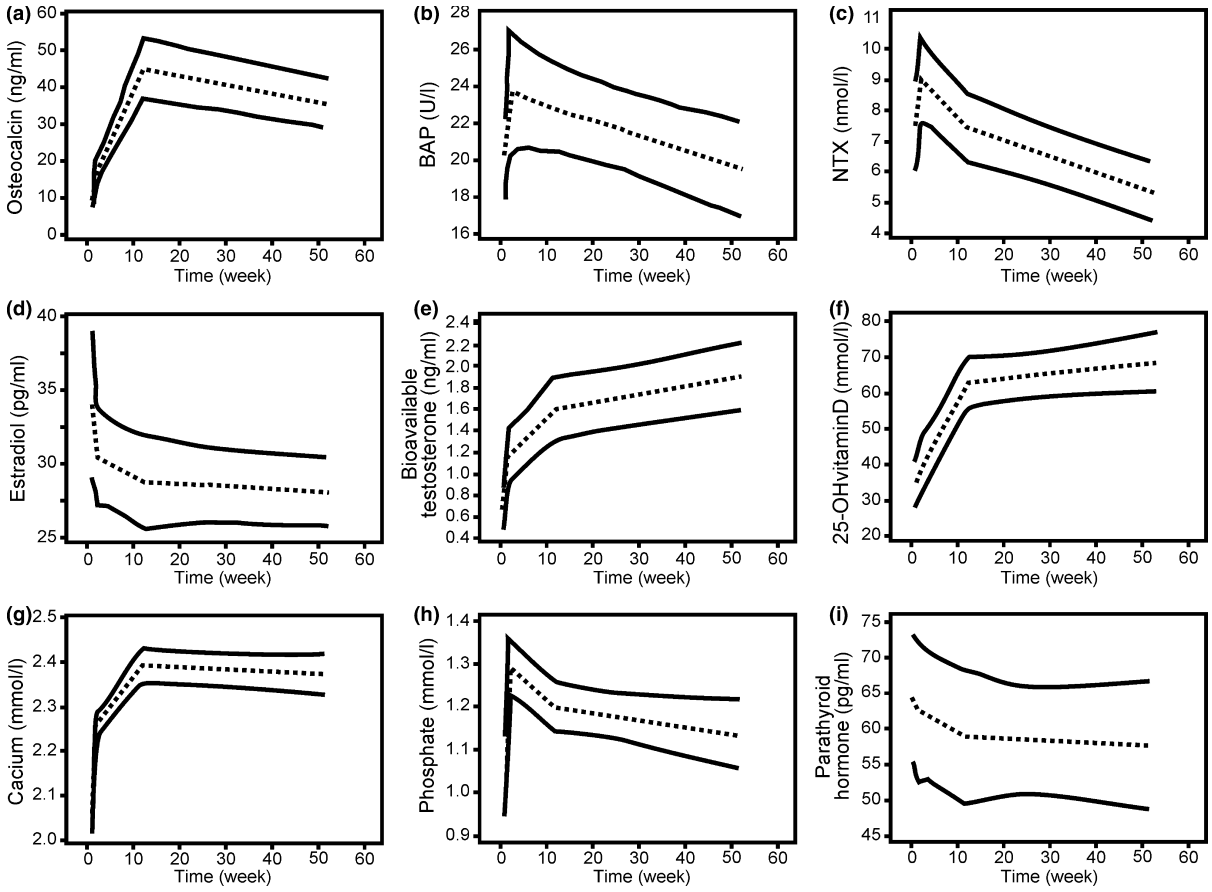

Figure 2 Estimated piecewise linear time courses and their confidence intervals for osteocalcin, (a) bone-specific alkaline phosphatase (BAP), (b) cross-linked-N-telopeptide of type I collagen (NTX), (c) estradiol, (d) bioavailable testosterone, (e) 25-OH-vitamin D, (f) calcium, (g) phosphate, (h) and parathyroid hormone, (i) (estimated slopes between weeks 1 and 2, between weeks 2 and 12 and from week 12 onward are given in the text).

by 0.5 ng/ml within the first week ($P < 0.001$), 0.04 ng/ml per week ($P < 0.001$) thereafter, and by 0.01 ng/ml per week ($P = 0.030$) from 3 months post-transplant onward. In men, only the decrease in serum levels of estradiol was significant within the first week (−3.5 pg/ml; $P = 0.028$). Serum levels of 25-OH-vitamin D were at the lower level of the normal range at the beginning, but increased by 4.8 nmol/l within the first week ($P = 0.023$), and by 2.4 nmol/l per week until week 12 ($P < 0.001$). Serum calcium levels increased by 0.2 mmol/l ($P < 0.001$) within the first week, and by 0.01 mmol/l per week ($P < 0.001$) from week 2 to 12. Serum phosphate increased by 0.26 mmol/l ($P < 0.001$) during the first week. Serum levels of parathyroid hormone were in the upper range of normal values at all six measurements. Serum creatinine and blood urea nitrogen fluctuated in the upper third of the normal range; a significant change was only registered for serum creatinine levels within the first week (0.2 mg/100 ml, $P = 0.002$).

Discussion

The biochemical profile in the early post-transplant period is consistent with the uncoupling of bone formation and bone resorption. Although bone turnover increased after transplantation, changes in BMD within the first year after transplantation were not significant. Our patients had an unexpectedly low rate of vertebral fractures.

The nonsignificant change in BMD after transplantation is in contrast to previous studies. In most studies, BMD decreased significantly during the first year after transplantation [2,5,6,18,19]. Vertebral fractures are reported to occur at a rate of 21–36% within the first year post-transplant [6,18,20–23]. One study even identified a fracture rate of 44% [24]; only two studies described lower fracture rates in untreated HTX recipients [2,5]. Thus, the fracture rate of 12% in our patient group who received no bone-specific treatment is very low. So far, a vertebral fracture rate in HTX recipients as low as 14% was observed only once [25].

Immunosuppressive drugs, especially glucocorticoids, are toxic to bone. As glucocorticoids are known to inhibit bone formation and enhance bone resorption [26], they were very likely responsible for changes in markers of bone turnover during the early post-transplant period when glucocorticoids are given at high doses. Studies investigating the effects of glucocorticoid withdrawal on bone metabolism in organ transplantation recipients support this thesis [27,28].

Various immunosuppressive regimens have been used in studies. Compared with a very significant study by Elisabeth Shane [6], our patients received less methylprednisone within the first 6 months after transplantation. In the former study, patients were started on 100 mg prednisone daily compared with a starting dose of 10 mg prednisone daily in our patients. The fact that serum levels of osteocalcin peaked prior to the former study shows that bone formation was less suppressed by corticoids in our patients. We were able to administer a lower prednisone dose because our patients started to receive an anti-thymocyte globulin on the first postoperative day. This immunosuppressive medication appears to be less deleterious to bone metabolism than corticoids. Clinical studies on the effect of anti-thymocyte globulin on bone metabolism have not been published thus far. However, according to experimental studies, anti-thymocyte globulin may even have a positive effect on bone metabolism [29–32].

The calcineurin inhibitor cyclosporine A (CsA) is also a very important part of the immunosuppressive regimen used to protect the allograft. CsA stimulates both osteoclast and osteoblast activity *in vivo*, but resorption rates exceed formation rates, resulting in a net bone loss [33,34]. Only three patients in our collective received tacrolimus instead of CsA. The two medications apparently do not differ in respect of bone loss [35–37].

Compared to the immunosuppressive regimen used by Shane *et al.*, our patients received mycophenolate mofetil – a fungal macrolide – instead of azathioprine. Both immunosuppressive medications were shown to exert no effect on bone volume in the rat model [38,39]. Thus, this part of the immunosuppressive regimen appears to be identical in respect of bone loss.

The reason why serum levels of 25-OH-vitamin D doubled within the first year after transplantation may have been the regular intake of 800 IU of vitamin D and the fact that HTX recipients improve rapidly and even surpass their pretransplant mobility. They probably spend more time outdoors and are exposed to sunlight, which is important for the vitamin D metabolism.

The increase in 25-OH-vitamin D is responsible for the normalization of serum calcium levels. Serum calcium levels increased to the middle range of normal values and serum phosphate levels also were normal. Nevertheless, serum levels of parathyroid hormone were in the upper range of normal at all six measurements after transplantation. This persistent hyperparathyroidism concurs with the data published in previous reports on heart [6,9] as well as renal transplant recipients [40]. Secondary hyperparathyroidism was observed in patients with untreated and treated congestive heart failure [41]. Immediately after HTX, serum levels of calcium may be rather low because of secondary hyperparathyroidism. Persistent secondary hyperparathyroidism may then lead to autonomous overproduction of parathyroid hormone.

Hypogonadism has been held partially responsible for bone loss after transplantation. The question of hypogonadism in male HTX recipients was investigated by analyzing serum levels of whole testosterone and was registered in the majority of [6,8,42,43] but not all [20] studies addressing this subject. Percentages of male HTX recipients with hypogonadism vary between 20% [44] and 52% [19]. We measured bioavailable testosterone, which was low in HTX candidates. This is in line with a previously reported testosterone deficiency in men with chronic heart failure [45]. It was even less in HTX recipients, but increased during the first year post-transplant, mainly during the early post-transplant period. The absence of a further initial decrease in bioavailable testosterone during the first few weeks after transplantation may be because of the very short administration of glucocorticoids and their well known negative effect on the production of gonadal sex hormones. As previous studies with higher doses of glucocorticoids showed a return to baseline values after 6 to 12 months [5,6] the rise of serum testosterone levels may also be caused by the proximity in time to surgery.

Serum levels of creatinine and blood urea nitrogen were significantly lower at the first measurement in HTX recipients compared with HTX candidates. Renal function is frequently impaired in patients with chronic heart failure. The renal insufficiency associated with the basic disease usually ameliorates after transplantation, leading to a reduction in serum creatinine and blood urea nitrogen. However, some renal impairment may persist and, additionally, the immunosuppressant cyclosporine A is nephrotoxic [46]. These two facts may be responsible for fluctuations in serum creatinine and blood urea nitrogen in the upper third of the normal range throughout the first year after transplantation.

The preponderance of males in our study group is in agreement with previous studies, which also reported a majority of males among HTX candidates and recipients. However, the fact that only few women were included may make the study less generalizable.

We were unable to investigate our HTX recipients before transplantation. To compensate for the absence of appropriate baseline values we investigated 25 HTX candidates, and registered exactly the same changes between pre- and post-transplant values as reported by Shane *et al.* [6].

Similar to previous investigations using a different immunosuppressive regimen, this study also showed a high incidence of defective bone metabolism with an uncoupling of bone formation and bone resorption in the early post-transplant period. Osteopenia or osteoporosis was observed in approximately two-thirds of HTX patients. Nevertheless, only 12% of the HTX recipients developed a vertebral fracture within 1 year after transplantation. Serum levels of 25-OH-vitamin D doubled within the first year. Serum calcium levels normalized but hyperparathyroidism persisted. Bioavailable testosterone levels in men were low, but increased markedly within the first 3 months after transplantation. The unexpectedly low rate of vertebral fractures in our patients may have been because of the different immunosuppressive regimen we used compared with those used in previous studies. But, each fragility fracture is a serious complication and we need to take steps to prevent this complication.

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Authorship

KK-S: designed study, performed study, analyzed data, wrote paper. MR, SM, MP, CK, CB, VF-M, RP, and MG performed study, collected data. AG: performed statistical analysis. PP: designed study, interpreted data.

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