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Lower serum β-CrossLaps in male cardiac transplant recipients treated without prednisolone

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Abstract Post-transplantation bone disease is a frequent problem after successful cardiac transplantation. We performed a cross-sectional analysis of male heart transplant recipients in the late post-transplantation period. Nine patients (group A) had received immunosuppressive therapy with cyclosporin A and mycophenolate mofetil (steroid-free treatment), and 12 patients (group B) remained on triple-drug therapy, which included glucocorticosteroids. Bone mineral density status was analyzed by osteodensitometry and by markers of bone turnover. Osteopenia was common in both groups (44.4% in group A and 50% in group B) as was osteoporosis (30% and 33.3% in groups A and B, respectively). β-CrossLaps were significantly lower in sera of cardiac transplant recipients on double immunosuppressive (i.e., glucocorticosteroid-free) regimen than in sera of patients on triple-drug therapy (428.3 ± 109.4 vs 661.7 ± 337.0 pg/ml, $P < 0.05$). Lower serum β-CrossLaps levels in patients undergoing glucocorticosteroid-free treatment may indicate a lower risk of bone deterioration in the long term.

Keywords Corticosteroids · Bone metabolism · Osteoporosis · Vertebral fracture · Immunosuppression

Introduction

Osteoporosis is a frequent complication after cardiac transplantation (CTX) [26]. The patient's quality of life may be reduced substantially by osteoporotic fracture or pain. There is an increased incidence of fracture particularly within the first 6 months after CTX, when the highest bone loss occurs [28] and vertebral fractures are most predominant [18, 21, 27]. The pathogenesis of post-transplantation bone disease is multifactorial. Glucocorticosteroids and cyclosporin A (CyA), which are both associated with loss of bone density, are used in most cardiac transplant recipients. For glucocorticosteroids it is well documented that reduction in bone density results in increased fracture incidence in a dose-dependent manner [31]. CyA also exhibits a negative influence on bone [4, 22, 30]. Partially, the changes in bone turnover are secondary to CyA-induced moderate renal impairment, and secondary hyperparathyroidism due to decreased renal function or 25-OH-vitamin D3 deficiency is a significant problem after cardiac transplantation too [13]. In addition, glucocorticosteroids may induce (partial) hypogonadism in men and women, which enhances bone loss, especially if high doses of glucocorticosteroids are necessary. Many cardiac transplant recipients already have a low bone mass at the time of CTX. Relative immobilization and the use of loop diuretics in patients with heart failure at NYHA III and IV are important factors contributing to bone loss before CTX [2, 18].

The bone-formation marker osteocalcin is suppressed during the first 6 months after CTX [29]. Bone resorption markers such as urinary deoxypyridinoline are increased in the early post-transplantation period and return to normal by 12–24 months. At the present time, data on serum β-CrossLaps in cardiac transplant recipients are scarce. The aim of our study was to analyze bone metabolism, particularly the bone-turnover marker serum β-CrossLaps, as well as bone mineral density status and fracture prevalence, in cardiac transplant recipients before osteo-protective therapy was instituted. Furthermore, we retrospectively compared the effects of two immunosuppressive regimens on bone metabolism and osteoporosis, as well as on fracture prevalence.

Materials and methods

Setting

In 1994 a CTX outpatient clinic was founded at our institution in an attempt for the institution to provide heart recipients with adequate treatment within a short distance from their homes. The regular monitoring of clinical and biochemical parameters, including plasma levels of immunosuppressive drugs and endomyocardial biopsy, are readily available here. In 1996 we assessed the follow-up outcome of the patients and showed that treatment strategies resulted in a very good outcome that was comparable to published results of large transplantation units [15].

Study design and patients

We performed a cross-sectional study of all male heart transplant recipients at our institution in the late post-transplantation period (4.2 years ±2.6 SD after CTX, $n=21$). The patients did not differ with regard to reasons for transplantation. The main diagnosis leading to CTX was idiopathic dilated cardiomyopathy followed by ischemic heart disease.

Therapy

In the first year after CTX all patients received triple-drug therapy, which consisted of prednisolone, CyA, and azathioprine. Thereafter, due to a clinical decision, we switched nine of our patients with the best and stable myocardial biopsy results (≤ 1B International Society for Heart Transplantation (ISHT) Standardized Grading System) [1] to mycophenolate mofetil and CyA, stopped azathioprine administration, and gradually decreased the prednisolone dose until it was stopped. We allocated these patients on immunosuppressive therapy with CyA and mycophenolate mofetil to group A ($n=9$) and those remaining on triple-drug therapy, which included glucocorticosteroids (azathioprine, CyA, and prednisolone), to group B ($n=12$). The mean time (±SD) without glucocorticosteroids in group A was 9.8±8.1 months, and the prednisolone dose averaged 6.9±3.4 mg/day in group B at the time of the study analysis.

There were no acute severe late rejections (≥1B ISHT) in either group, and endomyocardial biopsies demonstrated the absence of clinically relevant chronic rejection requiring high-dose methylprednisolone. The patients were given no bone protective therapy until the study started. After collecting the data of the present study we instituted adequate therapy with calcium, vitamin D3 (or the activated 1,25 formulation if renal function was impaired), and bisphosphonates if indicated. Because hypogonadism was ruled out in our patients, sex hormone replacement therapy was not necessary.

Bone mineral density measurement and radiographic assessment

We analyzed bone mineral density (BMD) status by osteodensitometry (DXA Lunar Prodigy, Lunar, Madison, Wis., USA). Lumbar spine measurements represented the average of four vertebrae (L1–L4). Readings from fractured vertebrae were excluded from the osteodensitometric results. BMD was expressed as grams per square centimeter (g/cm^2) and as standardized T-score value. T scores of 2.5 or more SDs below the mean were classified as osteoporosis, and T scores between −1.0 and −2.5 SDs as osteopenia according to WHO criteria [16]. Anterior–posterior and lateral radiographs of the chest and the thoracic and lumbar spine were performed so that we could assess the presence of fractures. We classified fractures by combining a semi-quantitative and quantitative morphometric approach as described previously [10, 23]. For the latter, a vertebral fracture was defined as a 20% or greater reduction, of at least 4 mm, in any vertebral height in any vertebra from T4 to L4.

Biochemical analysis

As bone resorption markers, serum β-CrossLaps (Elecsys 2010 Systems, Roche Diagnostics, Mannheim, Germany) and urinary *N*-telopeptide NTx (Osteomark NTx, Ostex International, Seattle, Wash., USA) were determined. To assess bone formation activity we measured Bone-specific alkaline phosphatase (Access Ostase, Beckmann Coulter, Fullerton, Calif., USA). Serum creatinine, alkaline phosphatase, calcium, and phosphate were analyzed by means of the Hitachi 717 System (distributed by Boehringer Mannheim, Mannheim, Germany). Total testosterone (Bayer Vital, Fernwald, Germany), free testosterone (Diagnostic Products, Los Angeles, Calif., USA), estradiol (Bayer Vital), sex hormone-binding globulin (SHBG; Diagnostic Products), calcitriol (DiaSorin, Stillwater, Minn., USA), 25-OH-vitamin D3 (Immunodiagnostic Systems, Tyne and Wear, UK), and intact parathyroid hormone (iPTH; Nichols Institute Diagnostics, San Juan Capistrano, Calif., USA) were determined with routine techniques.

Statistical analysis

Statistics were computed via the Mann–Whitney *U* test, and all data are expressed as mean ± SD. Additionally, the main results are described by means of box plots. For correlation analysis, bivariate Spearman's rho correlation coefficients were calculated. Furthermore, we performed multiple regression analysis for serum β-CrossLaps and the other biochemical parameters. For all tests, a two-tailed *P* value of less than 0.05 was considered to be statistically significant. Statistical analyses were performed with SPSS 10.1 for Windows.

Results

There was no significant difference between the patient groups with regard to age, height, weight, and body mass index (BMI), as shown in Table 1. In addition,

adequate control of systolic and diastolic blood pressure was achieved in both groups (Table 1).

Also, creatinine clearance was similar in both groups (Table 2). Calcium was within the reference range in both groups, and iPTH levels were borderline or moderately elevated in only a few patients of both groups, without significant difference between the groups. 25-OH-vitamin D and calcitriol (1,25-OH-vitamin D) were comparable in groups A and B (all data shown in Table 2). Hypogonadism was ruled out in our patients by normal values for free testosterone, total testosterone, SHBG, and estradiol (Table 2).

Serum β-CrossLaps were significantly lower ($P < 0.05$) in male cardiac transplant recipients on a double immunosuppressive (i.e., glucocorticosteroid-free) regimen than in patients on triple-drug therapy (Fig. 1, Table 3). There was no significant correlation between creatinine clearance and serum β-CrossLaps (Fig. 2). In addition, serum β-CrossLaps were not correlated with any other serum or urinary biochemical parameter as determined by multiple regression analysis. The bone-turnover markers urinary *N*-telopeptide NTx and bone-specific alkaline phosphatase did not differ significantly between the two groups (Table 3), although there was a non-significant trend towards lower urinary *N*-telopeptide NTx levels in group A.

Osteopenia (lumbar and/or femoral neck dual X-ray absorptiometry (DXA) T score < -1 SD) was present in four of nine patients (44.4%) in group A and in six of 12 patients (50%) in group B (*P* n.s.). Osteoporosis, as defined by lumbar and/or femoral neck DXA T score ≤ -2.5 SDs, was seen in three of nine patients (30%) in group A and in four of 12 patients (33.3%) in group B (*P* n.s.). Therefore, only 22.2% of the patients in group A and 16.7% of group B (*P* n.s.) had BMD results within the reference range of young male adults (Table 4).

Vertebral fractures were present in four of nine patients (44.4%, 12 vertebral fractures) in group A and five of 12 patients (41.7%, 14 vertebral fractures) in group B (*P* n.s.). No fractures of the hip or the radius were recorded.

Table 1 Baseline clinical characteristics. Data are shown as mean ± SD

Characteristic	Group A	Group B	*P*
Age (years)	53.2 ± 8.3	55.1 ± 10.2	0.702
Height (m)	1.721 ± 0.066	1.72 ± 0.059	0.345
Weight (kg)	80.5 ± 13.7	81.7 ± 14.5	0.754
BMI (kg/m^2)	27.1 ± 3.8	27.5 ± 3.8	0.651
RR systolic	130.5 ± 14.6	138.5 ± 18.2	0.508
RR diastolic	81.4 ± 10.9	85.9 ± 10.1	0.277

Discussion

The main finding of our study is the reduction of bone resorption—as measured by serum β-CrossLaps—in patients on a double immunosuppressive regimen that used mycophenolate mofetil and CyA, compared with those on a triple-drug therapy (CyA, azathioprine, prednisolone). Bone-specific alkaline phosphatase and urinary *N*-telopeptide NTx did not exhibit significant differences between the groups, which suggested a specific discriminatory property of serum β-CrossLaps with regard to bone metabolism in post-transplantation bone disease.

BMD measured by DXA was correlated with the risk of osteoporotic fracture. In a large study, low hip BMD in elderly women predicted an increased risk of hip fracture [3]. The correlation of BMD with osteoporotic fracture risk was demonstrated in men too [19]. Each reduction of BMD by 1 SD increases the risk of vertebral fracture (odds ratio: 1.8) and hip fracture (odds ratio: 2.3). In our patients, vertebral fractures occurred even before DXA showed substantial reduction of BMD. The short period of glucocorticosteroid-free immunosuppressive therapy did not allow us to establish a difference in osteoporosis and fracture prevalence between the groups.

The lower serum β-CrossLaps level of group A, however, may indicate a lower risk of further bone deterioration in a glucocorticosteroid-free immunosuppressive regimen in the long term. This is supported by

Table 2 Biochemical parameters of renal function, calcium/phosphate metabolism, and sex-hormone levels. Data are shown as mean ± SD

Variable	Group A	Group B	*P*	Reference range
Calcium	2.44 ± 0.15	2.42 ± 0.06	0.193	2.02–2.60 mmol/l
Phosphate	1.16 ± 0.17	1.20 ± 0.16	0.382	0.87–1.45 mmol/l
Creatinine clearance	89.6 ± 33.1	78.0 ± 35.9	0.345	75–125 ml/min
iPTH	50.3 ± 20.3	53.2 ± 20.2	0.824	10–65 ng/l
Calcitriol	79.4 ± 19.7	77.5 ± 24.2	0.904	38–134 pmol/l
25-OH-vitamin D3	65.3 ± 34.3	46.8 ± 37.5	0.177	20–100 nmol/l
Free testosterone	60.3 ± 12.8	57.2 ± 17.0	0.754	31.2–163.0 pmol/l
Total testosterone	17.7 ± 5.2	15.3 ± 5.2	0.219	6.0–29.2 nmol/l
Estradiol	131.8 ± 45.2	105.7 ± 44.8	0.473	36.7–128.5 pmol/l
SHBG	28.0 ± 11.0	24.0 ± 7.8	0.464	16–120 nmol/l

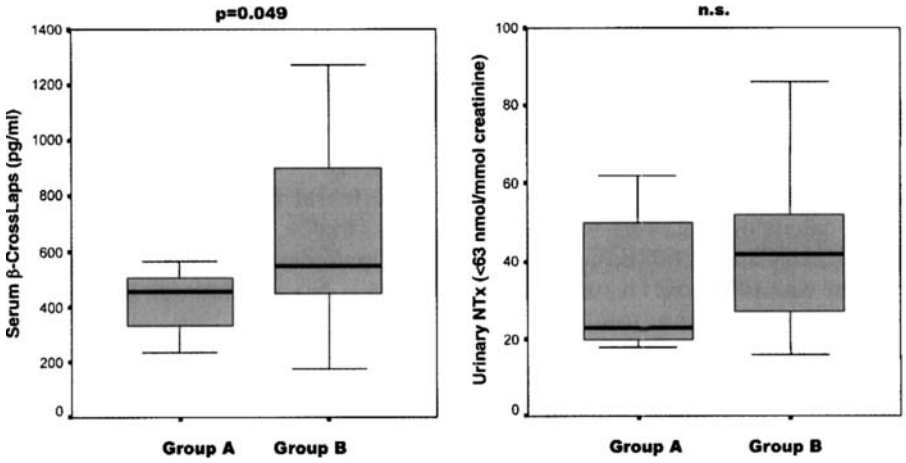

Fig. 1 Box plots of serum CrossLaps and urinary *N*-telopeptide NTx. *The line within the box plot* represents the median. *The ends of the box* are at the upper and lower quartiles so that the middle 50% of the cases fall within the range of scores defined by the box. *The long lines coming out of the sides* are whiskers (=1.5-times the interquartile range) enclosing approximately 95% of the data points. Group A consisted of nine patients on CyA and mycophenolate mofetil (steroid-free), group B of 12 patients on CyA, azathioprine, and prednisolone (triple-drug therapy)

data by Garnero et al. [9], who demonstrated an increased risk of osteoporotic fractures in postmenopausal women with high serum β-CrossLaps. The analysis for urinary *N*-telopeptide NTx in that study detected a trend towards an increased risk of osteoporotic fracture in patients within the highest quartile of this marker; however, in contrast to the serum β-CrossLaps this result was not statistically significant. The reasons for the

Table 3 Markers of bone turnover. Data are shown as mean ± SD. *BCE* bone collagen equivalents

Marker	Group A	Group B	*P*	Reference range
Serum β-CrossLaps	428.3 ± 109.4	661.7 ± 337.0	0.049	100–580 pg/ml[a]
Urinary *N*-telopeptide NTx	33.3 ± 17.9	42.9 ± 20.0	0.792	< 63 nmol BCE/mmol creatinine
Bone-specific alkaline phosphatase	13.5 ± 6.3	12.9 ± 4.4	0.758	< 20.2 µg/l
Alkaline phosphatase	115.0 ± 31.4	118.5 ± 67.2	0.862	70–175 U/l

[a]30 to 50-year-old male healthy probands (reference range for 50 to 70-year-old male healthy probands: 70–710 pg/ml)

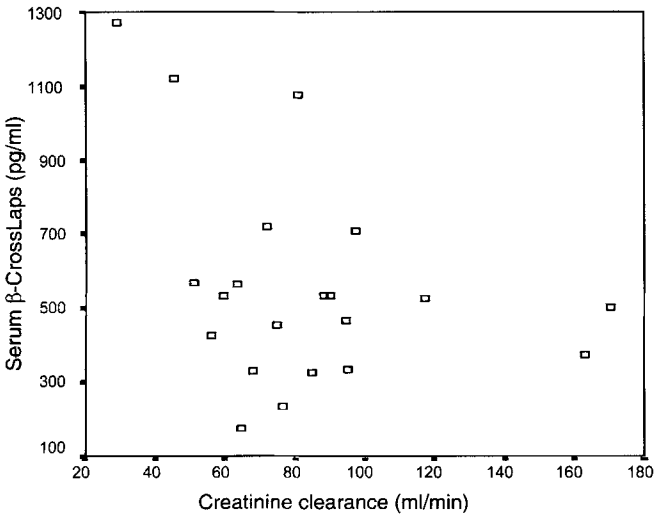

Fig. 2 Bivariate Spearman's rho correlation analysis of serum CrossLaps vs creatinine clearance of all patients ($n=21$)

discrepancy between resorption markers in our study and in the OFELY study mentioned above [9] are unclear. Urinary *N*-telopeptide NTx is likely to reflect the degradation of both isomerized and non-isomerized type-I collagen [8] in contrast to serum CrossLaps, which specifically recognize the β-isomerized form [6]. β-isomerization, a spontaneous post-translational modification, is believed to reflect aging of type-I collagen [6, 8] and was shown to be more prominent in bone than in soft tissue [11]. That pattern might explain the lower sensitivity of urinary *N*-telopeptide NTx, which was also not a significant predictor of hip fracture in the EPIDOS study [7]. Thus, the amount of degradation of β-isomerization type-I collagen measured by serum CrossLaps may reflect more specifically the resorption of mature bone matrix than urinary *N*-telopeptide NTx. Our observation of lower serum β-CrossLaps in cardiac transplant recipients undergoing steroid-free treatment is the first report to show the ability of serum β-CrossLaps to characterize the influence of low prednisolone doses in a

Table 4 BMD data. Data are shown as mean ± SD

Parameter	Group A	Group B	*P*
Lumbar spine (BMD, g/cm^2)	1.070 ± 0.207	1.047 ± 0.175	0.917
Lumbar spine (T score)	−1.344 ± 1.846	−1.609 ± 1.434	0.972
Lumbar spine (Z score)	−0.989 ± 1.864	−1.236 ± 1.515	0.972
Femoral neck right (BMD, g/cm^2)	0.858 ± 0.093	0.858 ± 0.099	0.910
Femoral neck right (T score)	−1.41 ± 0.956	−1.59 ± 0.71	0.910
Femoral neck right (Z score)	−0.888 ± 0.879	−0.933 ± 0.636	0.910

small group of patients at high risk of osteoporotic fracture.

In addition, a recent study showed lumbar spine BMD recovery 3 years after CTX if glucocorticosteroids were stopped at least within the 3rd year after CTX [17]. In contrast, earlier data from CTX patients with a higher mean daily prednisone dose (12.5 mg) did not demonstrate lumbar spine BMD recovery [14].

Patients with severe renal impairment, and especially with terminal renal insufficiency on dialysis, are known to have elevated serum β-CrossLaps. Most of these patients have hyperparathyroidism with consecutive activation of bone turnover; but also the reduced renal clearance rate of serum β-CrossLaps is assumed to contribute to the elevated levels in advanced renal insufficiency [25]. In the present study, we analyzed patients with normal renal function, and only few had moderate renal impairment, while parathyroid hormone levels were normal or borderline–elevated in both groups (Table 2). In order to rule out a predominant effect of different renal function or parathyroid hormone metabolism on the serum β-CrossLaps result, we calculated the correlation coefficient, which did not give a significant correlation between both. Thus, the difference in serum β-CrossLaps regulation in CTX patients was most likely not due to different renal elimination of the telopeptide, but due to the influence of low-dose glucocorticosteroids on serum β-CrossLaps release from bone in CTX patients.

Osteoporosis and increased prevalence of vertebral fracture was frequent in our male cardiac transplant recipients and constitute a major problem in the late post-transplantation period. Similar results were published for a mixed male and female population earlier [12, 20, 27]. A significant proportion of patients can be treated with a double immunosuppressive regimen (CyA and mycophenolate mofetil, no prednisolone) without rejection events being increased [5, 24]. However, the mean time without glucocorticosteroids was relatively short, and our study was not planned to analyze the safety of the glucocorticosteroid-free therapeutic regimen with regard to rejection episodes.

Our data show excellent preservation of renal function and very good clinical status of the patients in the late post-transplantation period. Arterial hypertension was treated by means of various combinations of antihypertensive agents, which resulted in satisfactory blood pressure reduction to or near the target range in most CTX patients. The glucocorticosteroid-free treatment in group A and the low glucocorticosteroid dose in group B, as well as good renal function, explained the absence of hypogonadism. A similar situation was described by Shane et al. [29].

Our study clearly demonstrates the differential effects of the various immunosuppressive strategies (triple-drug therapy vs glucocorticosteroid-free double-drug therapy) on bone metabolism in men. The male cohort powers our results by enabling us to eliminate confounding variables such as pre-menopausal and post-menopausal status (with/without HRT or use of oral anti-conceptives). Further studies that include more patients are necessary to clarify the role of serum β-CrossLaps as a sensitive marker of glucocorticosteroid-induced bone-turnover changes and its possible superiority over other markers of bone turnover.

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