Bone mineral density and associated changes after kidney transplantation

Thesis

Szilveszter Dolgos

Under the Cotutelle agreement between the Faculty of Medicine, University of Oslo and the Faculty of Medicine, Semmelweis University

Oslo 2009

Supervisors: Professor Anders Hartmann (University of Oslo)
Professor Jens Bollerslev (University of Oslo)
Professor László Rosivall (Semmelweis University)

Adjudication committee:
Professor Bjarne Magnus Iversen (University of Bergen)
Professor Leif Mosekilde (University of Aarhus)
Associate Professor Judit Kapocsi (Semmelweis University)

Introduction

Chronic kidney disease (CKD) is an irreversible condition associated with progressive loss of renal function. The rising number of patients with CKD is largely due to the increasing prevalence of diabetes mellitus and nephrosclerosis, furthermore the growing numbers of older patients are also a contributing factor. In end-stage renal disease (glomerular filtration rate [GFR] below 15ml/min/1.73m^2) the severe loss of kidney function is incompatible with life therefore initiation of renal replacement therapy (RRT) is needed. There are two options for RRT: chronic dialysis therapy or renal transplantation (RTx). Overall, renal transplantation provides the best quality of life for these patients. The present work relates primarily to kidney transplant patients.

As CKD progresses, disturbances in mineral homeostasis and various metabolic bone disorders may develop. The classical term "renal osteodystrophy" has recently been replaced by "CKD-Mineral and bone disorder" and is characterized by three components: laboratory abnormalities, bone disease, and vascular calcification. Almost all patients already have various metabolic bone disorders at the time of renal transplantation. Following RTx, there is an improvement in the balance of mineral homeostasis due to the functioning kidney allograft (most cases has a GFR of 30-60 ml/min), however, further substantial bone loss can be observed. The main clinical implication of low bone mineral density and osteoporosis is the increased risk of osteoporotic bone fracture. The majority of post-transplant bone loss occurs within the first 6 months after RTx, mainly due to high dose glucocorticoid exposure. Nonetheless, it seems there are other factors which may contribute to the pathogenesis of bone loss early after transplantation.

According to the current guidelines, the most accurate diagnostic test for determining the type of renal osteodystrophy is bone biopsy. Since bone biopsy is an invasive and painful procedure, it is only recommended in specific cases. In the clinical practice dual-energy X-ray absorptiometry (DXA) is a preferred, widely used, non-invasive method to measure bone mineral content (g/cm²) and to identify osteopenia or osteoporosis. Furthermore, beyond the routine blood tests (calcium, phosphate, PTH etc.), a number of biochemical markers are available clinically to assess bone metabolism in patients with chronic renal failure.

Moreover, altered metabolic function together with changes in body composition are important and common complications in this population. Malnutrition in chronic renal failure is a frequent finding in haemodialysed patients, while obesity often develops after renal transplantation.

Aims

Overall, our aims were to assess bone mineral density and body composition in solid organ transplant recipients, particularly in kidney transplant candidates.

Aims of the different papers included in this thesis:

Paper I

- To measure bone mineral density in kidney allograft patients and to identify predictors of bone loss and cumulative fracture rate at the time of renal transplantation.

Paper II

- To quantify the early changes in body composition after renal transplantation and identify predictors of these changes in a large number of generally well-nourished patients.

Paper III

- To describe the magnitude and distribution of early bone loss following renal transplantation and to evaluate the association between biochemical bone markers and alterations in bone mass.

Paper IV

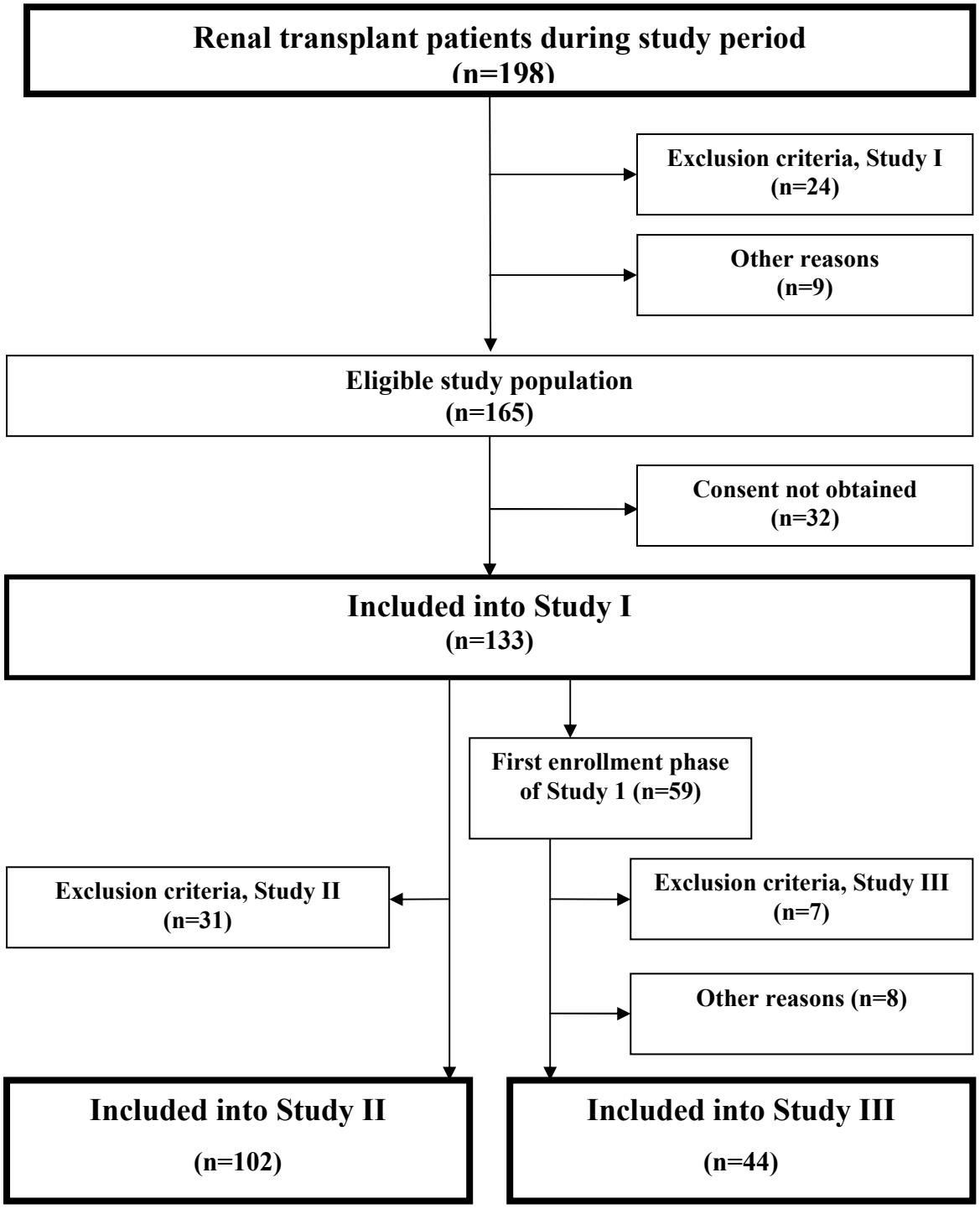
- To compare Z-scores among patients with different types of end-stage organ failure awaiting transplantation.

Methods

Study population (Paper I, II and III)

In Norway, all transplantations are performed at a single centre, Rikshospitalet University Hospital, Oslo. A total of 198 patients received a kidney allograft between February and early December 2006 at the author's centre. This patient cohort was considered for enrollment in the studies. The disposition of patients for the three different studies is shown in Figure 1.

Figure 1: Patients' disposition in Papers I, II and III



Exclusion criteria:
Study I – less than 18 years of age, severe medical complication;
Study II – previous transplantation, conditions which may influence body composition and hydration status;
Study III – previous transplantation, conditions or treatment which may influence bone resorption

Immunosuppressive treatment

For every patient, immunosuppressive therapy consisted of initial high-dose glucocorticoids and an interleukin-2 blocker (basiliximab) for induction. Maintenance therapy consisted of prednisolone, MMF and either a calcineurin inhibitor (cyclosporine A or tacrolimus) or in a few cases (n=33) an mTOR inhibitor (sirolimus or everolimus).

Measurement of bone density and body composition

Bone mineral density of the lumbar spine, total femur and total body was measured between 5 to 10 days after transplantation using DXA (LUNAR Corp, Madison, WI, USA) in Study I. The participants were positioned according to standard techniques. The values were evaluated by the enCORE 2006 software (General Electrics Healthcare, V10.10, Madison, WI, USA) and were expressed as absolute BMD values in g/cm², and as T-score and Z-score. The same DXA machine was used to analyze of total and segmental body composition at baseline and 10 weeks after renal transplantation in Study II.

Biochemistry

Fasting blood samples were collected as a part of the routine control procedure and were measured using standard automatic analyzer techniques. Beyond the routine blood sample analyses, serum samples were taken for biochemical assessment of bone turnover in Study III. Intact parathyroid hormone (iPTH), human osteocalcin, C-terminal cross-linked telopeptide (CTX-1), intact fibroblast growth factor 23 (FGF-23), 25-hydroxyvitamin D (25-OHD) were measured at baseline and 10 weeks after renal transplantation. Ten weeks after RTx, on the day when the blood sample was collected, each patient's renal function was assessed via plasma clearance of ^{51}Cr-labeled EDTA and the result was adjusted to 1.73 m^2 body surface.

Statistics

Differences between and within groups were analysed by paired and unpaired samples T-test. Crude associations between BMD and potential bone loss predictors were tested using bivariate correlations. Variables with p values less than 0.2 in this analysis were entered into a stepwise multiple linear regression model. The same statistical methods were used to examine

the relationship between changes in body composition from baseline until follow-up and potential predictors as well as between changes in BMD and baseline biochemical markers. In all studies the statistical program package SPSS was applied, and a two-sided p value <0.05 was considered statistically significant.

Study population (Paper IV)

This single centre study assessed BMD in a large cohort of patients with the four most common types of end-stage organ failure (lung, liver, heart and kidney). Patients included in this study were registered on waiting list and all received subsequently a transplant at our centre. In total, 291 adult first-time transplant patients with end-stage organ failure were included in the study between August 2003 and December 2006. Of these, 210 were waiting for either lung (n=60), liver (n=84) or heart (n=66) transplantation, and were transplanted within one year after being measured for BMD. Furthermore, a random selection of a comparable number of consecutive kidney allograft recipients (n=81) were included in the study. They were all from the main cohort described in Paper I. In our daily practice kidney patients are measured for BMD at the time of transplantation.

The study population represented 82% of lung-, 72% of liver-, and 86% of heart transplant candidates who were enrolled on waiting list and were transplanted in Norway in this time period. Similarly, the kidney patients studied comprised 88% of kidney transplants in the same time period.

Results

Paper I

At the time of renal transplantation, Z-scores were significantly lower ($p<0.05$) in the study population compared with the reference population. Osteopenia was present in more than third of the total group and osteoporosis in 11-15% in varying parts of the skeleton. The cumulative fracture rate was 29%. The multivariate linear regression analysis revealed a significant relationship between total body BMD and age, former transplantation, female sex, iPTH, time on haemodialysis, BMI, and physical activity. Cumulative fracture rate was associated with physical inactivity (RR: 4.6 $p = 0.003$), osteopenia (RR: 2.7 $p = 0.019$) and BMI (RR: 2.5 $p = 0.040$)

Paper II

There was a numerical though statistically non-significant weight loss of 0.9 kg [95% CI 0.3 to 2.2, $p=0.106$] (78.1 kg to 77.2 kg) in the study population at follow-up. However, significant changes in body composition were found during the same period. There was a significant increase in total body fat mass (1.3 kg) accompanied by a significant reduction in fat-free mass (2.5 kg). In the multivariate model increasing age, low-tertile body fat mass, prednisolone dose, longer time on dialysis, and lower C-reactive protein (CRP) level were independent predictors of increasing fat mass.

Paper III

Loss of bone mass occurred rapidly following renal transplantation. A significant association between serum osteocalcin and CTX-1 was seen at baseline ($r=0.25$, $p=0.001$) and at follow-up ($r=0.40$, $p<0.001$), indicating synchronisation of bone remodelling.

In the multivariate model, baseline osteocalcin was independently associated with bone loss in the total body ($p=0.049$), and CTX-1 in total femur ($p<0.001$). Baseline iPTH was significantly, though not consistently, associated with changes in the different bone compartments.

Paper IV

Although low bone mass was found in all four groups of patients with different end-stage organ failure, lung failure patients consistently had the lowest Z-scores, followed by advanced liver, kidney and heart disease patients.

Discussion

Transplant related bone disease (Papers I, III and IV)

Our studies (Paper I and III) convincingly demonstrate that low bone mass is already present at all measured skeletal sites at the time of RTx, and significant bone loss can be observed as early as 10-12 weeks after renal transplantation. A large number of consecutive, and more than 80% of the eligible renal transplant recipients in the given time period, were included in the first study. Our findings, therefore, are likely to be representative of the ESRD patients who are eligible for renal transplantation in Norway.

In Study I we found that one of the major general risk factors for low BMD was ageing. This finding can be explained by changes in hormonal status, nutrition and physical activity that parallel ageing. Female gender was also an independent risk factor, suggesting that the female skeleton is more vulnerable to transplant-related hormonal changes than that of the male. High body mass index and also regular weight-bearing, physical activity had a beneficial effect on bone mass. One of the uremia-related factors associated with low BMD was former transplantation, most likely due to the long-term immunosuppressive treatment principally exposure to glucocorticoids. Another risk factor for low bone mass was secondary hyperparathyroidism with a high level of circulating PTH which may induce significant bone loss within the first few months post-transplantation. The length of dialysis treatment was another risk factor, the longer patients had received chronic dialysis the greater the likelihood for bone loss.

It is generally considered that bone remodelling is desynchronized in patients with CKD and that this leads to bone abnormalities before and shortly after transplantation. Interestingly in Study III, we found a significant positive correlation between osteocalcin (marker of bone formation) and CTX-1 (marker of bone resorption), suggesting ongoing coupled bone turnover during the early post-transplant period. Our findings can be explained by the relatively large number of patients with pre-emptive RTx, and the overall short length on dialysis (on average only one year). Since the serum levels of these biochemical markers of

bone turnover were elevated in the majority of patients, we hypothesized that increased rates of bone turnover contribute to early post-transplant bone loss. We did not find an association between FGF-23 and other bone markers or BMD measurements, suggesting that this hormone does not directly affect bone metabolism early after RTx**.**

Non-renal (lung, liver and heart) transplantation and osteoporosis

Interestingly, in Study IV, lung and liver transplant candidates had significantly lower Z-scores compared to kidney transplant patients. We found it somewhat surprising that kidney failure patients had higher BMD than both lung and liver patients since CKD per se has a deleterious effect on bone. This may at least in part be explained by a high percentage of pre-emptive kidney transplantation and patients with a short time on dialysis.

Alteration of body composition in kidney transplant patients (Paper II)

There was a marked increase in body fat mass with a significant decrease in fat-free mass, without any significant changes in total body weight at 10 weeks after RTx. Patients had an increase in total body fat with a central accumulation of fat mass early after RTx. Advancing age was an independent predictor of weight gain due to age-associated decreases in basal metabolic rate and physical activity. Cumulative prednisolone dose was an independent predictor of fat mass distribution, mainly abdominal fat accumulation resembling Cushingoid features. The duration of dialysis before transplantation was another significant predictor of body fat changes. Furthermore, we found that patients with poorer initial nutritional status, such as low-tertile fat mass, were more likely to have increased body fat mass. C-reactive protein, which is a marker of chronic systemic inflammation, was associated with body wasting.

Lean body mass is the key marker of nutritional enhancement. Cumulative prednisolone dose was the only independent predictor of decreased fat-free mass which can be explained by that high doses of glucocorticoids introduced early after RTx induce protein catabolism leading to negative nitrogen balance and muscle wasting.

Conclusions

Paper I

Significantly reduced bone mass and high cumulative fracture rate were found in this nationwide study involving a representative sample of ESRD patients who were eligible for renal transplantation in Norway. Beyond the well-known, generally accepted risk factors for reduced BMD, uremia-related independent factors were identified. For cumulative fracture rate, inactivity was the strongest predictor, followed by presence of osteopenia. Therefore, after normalization of the transplanted patients' physical performance, regular exercise should be strongly advised.

Paper II

Recovery from uremia with nutritional decline shortly after RTx can lead to significant changes in body composition despite no change in total body weight. Significant increase in fat mass along with reduction of fat-free mass was observed. The clinical consequence of these early changes remains to be explored in ongoing prospective studies. However, early nutrition intervention guided by qualified dietitians could be useful to avoid excessive weight gain early after transplantation.

Paper III

A significant reduction in BMD was observed due to increased bone turnover with inappropriate bone formation shortly after RTx. Serum osteocalcin and telopeptid in combination with iPTH seems to be a reasonable choice for routine assessment of bone metabolism. FGF-23 was not associated with either bone loss or any of the traditional bone markers. The exact role of FGF-23 on transplant-related bone loss needs further investigation.

Paper IV

Patients with end-stage lung-, heart-, liver- or kidney disease who are candidates for organ transplantation are all at risk for osteoporosis, most pronounced in lung patients. The finding suggests that increased awareness of bone disease before transplantation is warranted in all solid organ transplant groups.

Future perspectives

Reviews and meta-analysis of literature relating to this topic does not allow evidence-based pharmacological interventions to reduce bone loss and bone fractures in potential solid organ transplant recipients beyond what is known in the general population. Randomized controlled trials assessing effects of interventional drugs on fractures in this patient group are needed.

By today bisphosphonates may be the most promising agent.

Accordingly we have initiated an ongoing prospective, randomized, double bind, placebo controlled clinical study (Ibandronate Versus Placebo in the Prevention of Bone Loss After Renal Transplantation. Protocol no.: SMR-1471) including 130 patients to investigate whether bone loss and bone fracture may be better prevented with the addition of bisphosphonates to active vitamin D_3 compared to active vitamin D_3 alone.

Publications

The candidate's publications related to the Thesis:

I. Szilveszter Dolgos, Anders Hartmann, Stine Bønsnes, Thor Ueland, Gunhild Aker Isaksen, Kristin Godang, Per Pfeffer and Jens Bollerslev. Determinants of bone mass in end-stage renal failure patients at the time of kidney transplantation.
Clinical Transplant 2008; 22: 462-468.

II. Szilveszter Dolgos, Anders Hartmann, Trond Jenssen, Gunhild Aker Isaksen, Per Pfeffer and Jens Bollerslev. Determinants of short-term changes in body composition following renal transplantation.
Scandinavian Journal of Urology and Nephrology 2009; 43: 76-83.

III. Szilveszter Dolgos, Anders Hartmann, Stine Bønsnes, Gunhild Aker Isaksen, Kristin Godang, Thor Ueland, Per Pfeffer and Jens Bollerslev. Early changes in bone mass, biochemical bone markers and fibroblast growth factor 23 after renal transplantation.
The Scandinavian Journal of Clinical & Laboratory Investigation 2009; 69: 161-167.

IV. Szilveszter Dolgos, Anders Hartmann, Gunnhild Aker Isaksen, Svein Simonsen, Øystein Bjørtuft, Kirsten M Boberg and Jens Bollerslev. Osteoporosis is a prevalent finding in patients with solid organ failure awaiting transplantation – a population based study.
Submitted in Clinical Transplantation 2009

The candidate's other publications

Dolgos Sz, Hartmann A, Bollerslev J, Pfeffer P, Jenssen T, Vörös P, Rosivall L. *Comparison of different modalities for measuring body composition in kidney transplant recipients.* In Hungarian. (A testösszetétel meghatározására alkalmas módszerek összehasonlítása vesetranszplantált betegeken.)
Hypertonia és Nephrológia 2008; 12 (2): 57-64.

The candidate's poster presentations, lectures

- Oral presentation at the 18th Congress of the Hungarian Diabetes Society, Tihany, Hungary, April 20-23, 2006. *Diabetes among the homeless.* In Hungarian. (Hajléktalan cukorbetegek.)
- Poster presentation at the 29th Congress of the Nordic Society of Nephrology, Gothenburg, Sweden, May 23-26, 2007. *Determinants of bone mass in end-stage renal failure patients at the time of kidney transplantation.*
- Oral presentation at the 24th Congress of the Hungarian Nephrology Society, Pécs, Hungary, Sept 6-8, 2007. *Determinants of short-term changes in body composition following renal transplantation: A longitudinal study comparing different methods.* In Hungarian. (A testösszetétel változásaiban szerepet játszó tényezők vesetranszplantáció után. Longitudinális vizsgálat különböző módszerek összehasonlítására.)
- Poster presentation at the 13th Congress of the European Society for Organ Transplantation (ESOT/ETCO), Prague, Check Republic, Sept 28-Oct 03,. 2007. *Determinants of bone mass in end-stage renal failure patients at the time of kidney transplantation.*
- Poster presentation at the American Transplant Congress 2008, Toronto, Canada, May 31-June 4, 2008. *Biochemical markers as predictors of early bone loss after renal transplantation.*
- Poster presentation at the 24th Congress of the Scandinavian Transplantation Society, Oslo, Norway, May 14-16, 2008. *Determinants of short-term changes in body composition following renal transplantation.*

- Poster presentation at the 25th Congress of the Hungarian Nephrology Society XXV. Annual Meeting in Szeged, Hungary, Sept 25-28, 2008. *The effect of biochemical bone markers on vascular calcification in diabetic and non-diabetic haemodialysed patients.* In Hungarian. (Csontanyagcsere-markerek és az érfali meszesedés diabéteszes és nem diabéteszes hemodializált betegekben.)
- Poster presentation at the World Congress of Nephrology, Milano, Italy, May 22-26, 2009. *Osteoporois is a prevalent finding in patients with solid organ failure awaiting transplantation – a population based study.*
- Poster presentation at the 26th Congress of the Hungarian Nephrology Society, Siófok, Hungary, Sept 17-19, 2009. *Association between post-transplant bone metabolism and glucose intolerance after kidney transplantation.* In Hungarian. (A poszt-transzplantációs csontanyagcsere és a glükóz intolerancia kapcsolatának vizsgálata vese transzplantáció után.)