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Post-Transplantation Bone Disease

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1. Introduction

Solid organ or stem cell transplantation is a well established procedure in the treatment of end-stage diseases (renal disease, chronic liver failure, end-stage pulmonary disease, heart failure). Improved outcome for these patients has allowed us to study some of the complications. One of these is metabolic bone disease, which can hinder their long-term survival and quality of life. In this chapter we have reviewed our current understanding of the pathophysiology of bone loss before and after solid organ transplantation, and review recommendations for the prevention and treatment of osteoporosis in patients accepted into organ transplantation programs. There are a number of risk factors contributing to bone loss in these patients: hypogonadism, vitamin D deficiency, malabsorption, low body weight, physical inactivity, excessive use of tobacco or alcohol and immunosuppressive therapy. Management of pretransplant risk factors has improved, resulting in better bone mineral density (BMD) levels before transplantation (Guichelaar et al., 2006). After transplantation, rapid and marked bone loss is observed in the first 3-6 months. The speed of the bone loss suggests that corticosteroids are heavily involved. Greater bone loss at vertebral and hip sites and high rates of incident fragility fractures have been reported (Leidig-Bruckner et al., 2001).

2. Pathogenetic factors

Many factors contribute to the pathogenesis of osteoporosis after organ transplantation. These include bone disease preceding transplantation, immunosuppressive medications, nutritional and lifestyle factors, and derangements of the parathyroid-calcium-vitamin D and the pituitary gonadal axes (Table 1). However, specific pathophysiological features can also be found in different forms of end-stage diseases.

2.1 Pre-existing bone disease

2.1.1 Kidney disease

End-stage renal disease (ESRD) is associated with a form of bone disease that is generically referred to as “renal osteodystrophy”. Many mechanisms are involved in its pathophysiology including calcitriol deficiency, hypocalcemia, hyperphosphatemia, secondary hyperparathyroidism, metabolic acidosis, and aluminum overload. Kidney transplantation will improve many aspects of renal osteodystrophy, but parathyroid hyperplasia may not regress even when normal kidney function returns.

General Risk factors
Vitamin D Deficiency and secondary Hyperparathyroidism
Hypogonadism
Inactivity / Immobilization
Poor Nutrition
Low body weight
End Stage Renal Disease
Secondary hyperparathyroidism
Adinamic bone disease
Osteomalacia
Mixed Uremic disease
Metabolic Acidosis
Long term hemodialysis
Medications: loop diuretics, heparin and warfarin.
Diabetic nephropathy
β-microglobulin amyloidosis
End-Stage Lung disease
Smoking
Chronic use of glucocorticoids
Hypercapnia
Hypoxia
Pancreatic insufficiency (cystic fibrosis).
Failure to attain peak bone mass (in patients who have cystic fibrosis).
Heart failure
Mild renal insufficiency
Medication: loop diuretics, heparin and warfarin.
Failure to attain peak bone mass (in patients with congenital heart disease).
End-Stage Liver Disease
Alcohol abuse
Cholestatic liver disease
Bone marrow transplant recipients
Chronic use of glucocorticoids
Chemotherapy
Growth hormone deficiency (in children)

Table 1. Risk Factors contributing to bone fragility before transplantation

There are several histological subtypes of renal osteodystrophy. The most common is osteitis fibrosa, characterized by increased bone turnover and typically associated with high serum PTH levels (secondary hyperparathyroidism). Osteomalacia is the least common type, with low bone formation and accumulation of unmineralized osteoid. A mixed disease of both, combining increased resorption and increased osteoid, can also exist. Adynamic bone disease is characterized by low bone formation without evidence of fibrosis.

Compared to the general population ESRD patients are 4.4 fold more likely to have a hip fracture and the prevalence of vertebral fracture is 21% higher (Alem et al., 2000). Some of the risk factors for fractures in general population are also seen in patients with renal osteodystrophy: older age, female gender, or low body weight. Specific risk factors in end-stage renal patients are duration of dialysis and peripheral vascular disease (Sethman-Breen et al., 2000). Although BMD in patients with renal osteodystrophy tends to be lower in cortical sites (forearm and hip) than cancellous sites (spine), there is not a clear relationship between the different histological types of renal osteodystrophy and bone density (Gerakis et al., 2000). Furthermore, BMD does not consistently predict fractures after kidney transplantation (Grotz et al., 1994). It is important to note that measurement of bone mineral density (BMD) and WHO criteria cannot be used to diagnose osteoporosis in patients with ESRD. This is because any of the several possible histological forms of renal bone disease may all be associated with low, normal or even elevated BMD.

2.1.2 Lung disease

Patients who are candidates for lung transplantation are highly likely to have osteoporosis before surgery. A retrospective study in patients with diffuse parenchymal lung disease referred for lung transplantation revealed that 30% and 49% of patients had lumbar or femoral osteoporosis respectively (Shane et al., 1996). Other authors found osteoporosis in 50% of the patients at lumbar spine and 61% at femoral neck (Tschopp et al., 2002). Several risk factors such as hypoxemia, malnutrition, vitamin D deficiency, smoking, decreased immobility and low body weight are involved. Cystic fibrosis is associated with additional risk factors such as hypogonadism, inflammatory bone-resorbing cytokines and pancreatic insufficiency that may impair the absorption of calcium and Vitamin D. In addition, most of the patients who undergo lung transplantation have experienced prior glucocorticoid therapy (Tschopp et al., 2002).

2.1.3 Cardiac disease

Osteoporosis and osteopenia are common in patients with severe congestive heart failure (CHF). Lumbar spine osteopenia has been found in 43% of patients, and spine osteoporosis (T-score ≤ -2.5 or Z-score ≤ -2.0) in 12-40% of patients. Biochemical markers of bone turnover suggest the presence of increased bone resorption. Involved factors that contribute to bone loss include low serum 25-OH vitamin D, hypogonadism, immobilization and loop diuretic use. Long-term therapy with heparin has been associated with bone loss and vertebral fractures. However, in CHF oral anticoagulants (warfarin) usually are used chronically instead of heparin. Warfarin blocks vitamin K-dependent gamma-carboxylation of osteocalcin and impairs its binding with calcium. Secondary hyperparathyroidism may occur due to impaired renal function and abnormal vitamin D metabolism. Hypogonadotropic hypogonadism appears to be very common in males with CHF. Up to

30% of males with CHF evaluated before transplantation have low levels of testosterone, and this proportion could further increase after cardiac transplantation (Cohen et al., 2003). We have found that trabecular bone loss was related to pretransplantation time of waiting. Also bone resorption markers were increased at this stage reflecting a high bone turnover (Garcia Delgado et al., 2000).

2.1.4 Liver disease

Osteoporosis and osteopenia are frequent complications of chronic liver disease. Its prevalence is high in patients waiting for liver transplant, especially in cholestatic liver disease. In the most important series, densitometric osteoporosis has been described between 31% to 44% (Lopez et al., 1993, Newton et al., 2001, Solerio et al., 2003;Ninkovic et al., 2001; Guichelaar et al., 2006).

A low bone turnover state has been found in biochemical measurements and histomorphometric analysis (Diamond et al., 1989). Osteocalcin levels are low and correlate with bone formation rate on bone biopsies, and show increases after successful transplantation. However, some reports describe increases in parameters reflecting bone resorption (osteoclast number and bone resorption surface) (Cuthbert et al., 1984).

Reduced bone formation has been related to several toxic factors that could inhibit osteoblast function as excessive alcohol intake or hyperbilirrubinemia. Also a lower IGF-1 synthesis, that has a direct trophic action upon the osteoblast could be involved. Glucocorticoid used simultaneously reduces bone formation and increases bone resorption. Vitamin D metabolism plays a pivotal role. Patients with end-stage liver disease frequently have low serum levels of 25(OH) vitamin D, since 25-hydroxylation of colecalciferol occurs at the hepatocyte. Fat malabsorption also decreases 25 (OH) vitamin D. In addition, increased vitamin D catabolism, reduced levels of vitamin D-binding protein (DBP), or reduced sunlight exposure can further decrease vitamin D serum levels. However, true osteomalacia is rare in cirrhotic patients (Table 2). Typically, successful liver transplantation reverses most of these factors. Hypogonadism frequently associated in these patients, could be partially responsible for the increased bone resorption.

Reducing bone formation:
-Ethanol excess
-Iron overload (hemochromatosis)
-Hyperbilirrubinemia
-Glucocorticoid use
-Decreased IGF-1
-Decreased 25 (OH) vitamin D ₃
Increasing bone formation:
-Hypogonadism
-Glucocorticoid use

Table 2. Involved factors in hepatic osteodystrophy.

In the past two decades significant changes have occurred in the management of chronic liver disease, the waiting time for transplantation and immunosuppressive therapy. Recently an improvement in lumbar spine BMD T-scores pretransplant from -2.5 before 1990 to -1.7 after 1996 has been described (Guichelaar et al., 2006). This data can help to clarify the etiology of bone loss: the severity of liver disease has not changed, the duration of disease before transplantation has been extended and patients can reach older ages. However, nutritional status has improved and bilirubin values decreased. These factors may have contributed to increase BMD before transplantation.

2.1.5 Bone marrow

Bone marrow transplant (BMT) recipients have many known risk factors for developing bone loss: Failure to attain peak bone mass in children and adolescents, hypogonadism, inactivity and induction and consolidation regimens with high dose of chemotherapy, glucocorticoids and irradiation that may damage bone marrow stromal cells and colony-forming unit fibroblast, reducing osteoblastic differentiation. A study in patients before BMT (after chemotherapy) show osteopenia in 24% and osteoporosis in only 4% (Schulte et al., 2000).

2.2 Related to transplantation: Immunossuppressor drugs and other factors

2.2.1 Glucocorticoids

Early bone loss has been observed in all solid organ transplants in the first 3 to 6 months, increasing the incidence of osteoporosis and osteopenia (Rodino et al., 1998; Leidig-Bruckner et al., 2001; Eastell et al., 1991). Bone loss primarily affects the spine and proximal femur. Some authors found greater impairment at this level (Keogh et al., 1999; Ninkovic et al., 2002). In patients who already have osteopenia or osteoporosis, this subsequent bone loss can result in a higher number of fractures (Eastell et al., 1991; Leidig-Bruckner et al., 2001). Traditionally, it has been assumed that high doses of glucocorticoids required for immunosuppression play a major role in this loss. High doses (≥ 1 mg/kg/day) are commonly prescribed immediately after transplantation, with gradual dose reduction over several weeks or months. Total GCs exposure depends on the transplanted organ, number of rejection episodes, and different immunosuppressive regimens.

The natural history of post-transplantation osteoporosis suggests that there are two main phases: the early one and the late one. The factors affecting the skeleton differ between this two phases.

The mechanisms associated with bone loss due to glucocorticoid treatment in the first phase are (Table 3):

1) An increase in bone resorption as a result of increased urinary calcium, decrease in intestinal calcium absorption, secondary hyperparathyroidism and hypogonadotropic hypogonadism; 2) Activation of osteoclastogenesis caused by increase of RANKL and decrease of osteoprotegerin (OPG). 3) Corticosteroid treatment decrease the proliferation and function of osteoblasts (by inhibiting the gene expression of osteocalcin, collagen type 1 and IGF-I) and induces its apoptosis (Canalis et al., 2002). **(Fig 1)**

In addition to their direct effects on bone tissue, glucocorticoids can induce severe myopathy, impairing balance and mobility, decreasing weight-bearing activity and increasing fall risk and fractures.

Increase in bone resorption as a result of increased urinary calcium.
Decrease in intestinal calcium absorption.
Secondary hyperparathyroidism.
Hypogonadotropic hypogonadism.
Activation of osteoclastogenesis caused by increase of RANKL and decrease of osteoprotegerine (OPG) levels.
Decrease in proliferation and function of osteoblasts (by inhibiting the gene expression of osteocalcin, collagen type 1 and IGF-I).
Decrease anabolic effects of TGF-beta.
Induces Osteoblast apoptosis.

Table 3. Effects of high doses of glucocorticoids in bone loss.

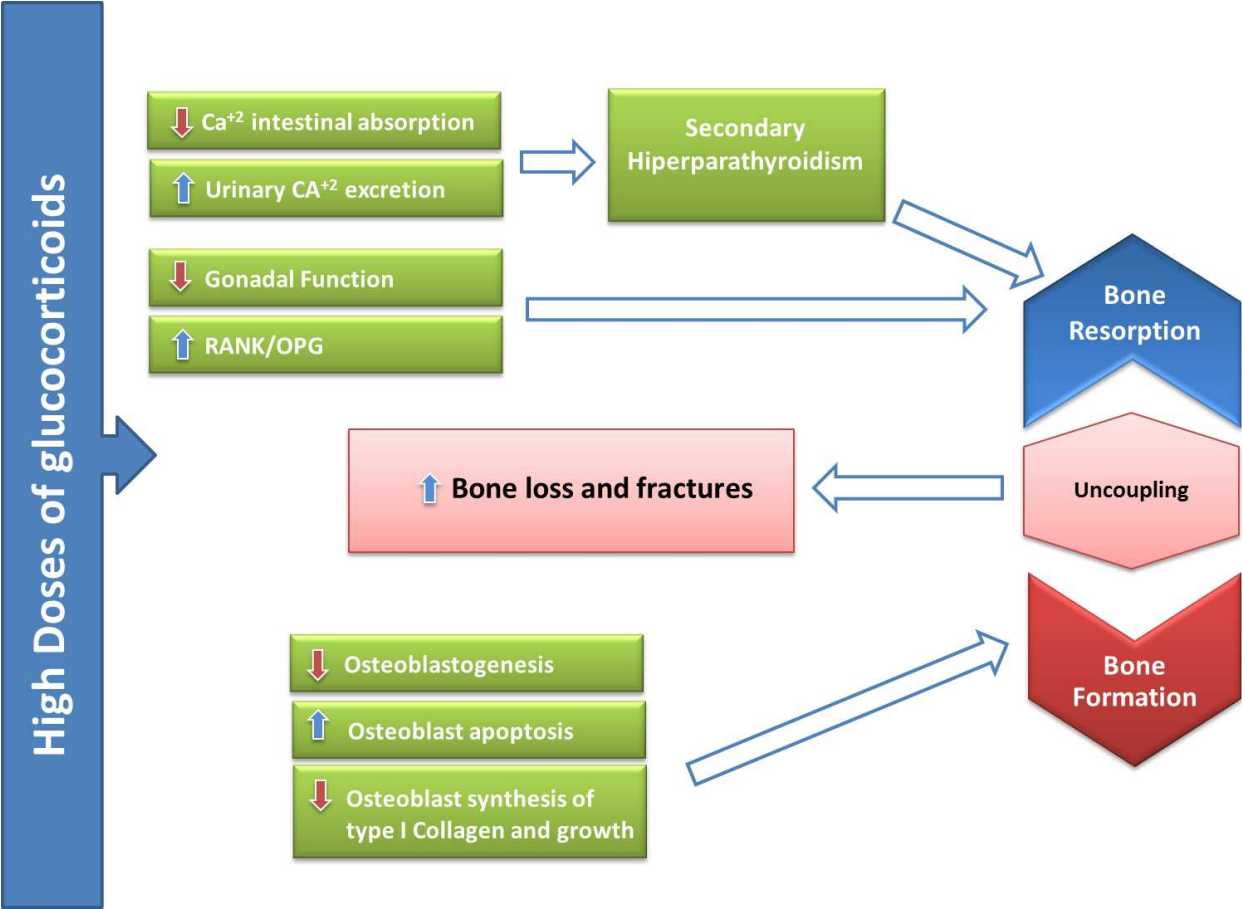


Fig. 1. Effect of high doses of glucocorticoids in bone loss

Effect of glucocorticoids in the WNT pathway: The Wnt pathway and the Bone Morphogenetic Protein (BMP) seem to be involved in the pathogenesis of glucocorticoid-induced osteoporosis, suppressing osteoblast differentiation and activity. BMP and Wnt pathway are regulated by several mechanisms: one of them are proteins that act as extracellular antagonists of BMP (Noggin, Chordin, Twisted gastrulation, Grelin, Sclerostin,

Follistatin and Dan), while others act as Wnt antagonist: Frizzled-related protein (sFRP), Dickkopf (Dkk) and Cerberus. Glucocorticoids can affect these signaling pathways, but the exact mechanisms are not been clarified. Recent studies suggest that glucocorticoids induce an alteration in osteoblast function by increasing Wnt antagonists with the subsequent suppression of this pathway. Recent reseach has shown that dexametasone, increases follistatin and DAN (BMP antagonists), sFRP-1(Wnt antagonist) and axin-2 (inhibitor of Wnt signal) . Simultaneously, alendronate and PTH (1-34), which have demonstrated to be effective in the treatment of steroid osteoporosis, were able to antagonize the increase in this proteins induced by dexametasone (Hayashi et al., 2008).

The potential impact of glucocorticoid dose as a determinant of bone loss is supported by the absence of bone loss at the lumbar spine and proximal femur in renal transplant patients treated with low doses of steroids and tacrolimus (Goffin et al., 2001). We have previously reported that steroid withdrawal in patients who have undergone a successful liver transplant accelerates the recovery of lumbar spine bone density without adverse effects on graft tolerance (Martínez Díaz-Guerra et al., 2002).

Moreover, higher rates of fracture occurring after cardiac (Shane et al., 1996) and lung (Shane et al., 1999) transplantation, in which higher doses of steroids are use, would be consistent with their role in the pathogenesis of post transplant osteoporosis.

The late phase observed in the postrasplant period takes place when the glucocorticoid doses are usually tapered below 5 mg per day. Then, osteoblast function recovers and consequently, an increase in bone formation and recoupling of bone remodeling activity is observed. During this later phase, rates of bone loss slow and there may even be some recovery, particularly in cancellous bone (Kulak et al., 2006). It is also found that despite an initial decrease in post transplant BMD, biopsies in the iliac crest showed improvement in histomorphometric parameters 4 months later (Guichelaar et al., 2003).

In conclusion, current evidence suggests that bone loss after transplantation is caused by an initial increase in bone turnover and resorption, plus a decrease in bone formation. Later, increases in bone formation could overcome resorption. These changes would be consistent with the rapid decrease in BMD observed in the first months after transplantation and recovery to baseline values, as most of studies show.

2.2.2 Other immunosuppressive drugs

The effect of these drugs in humans is difficult to study because they are used in conditions that by themselves affect bone remodeling, and they are rarely used in monotherapy so, the potential deleterious effect of one single agent could not be ascertained.

The role of calcineurin inhibitors in post-transplant bone loss is unknown. Tacrolimus is a calcineurin inhibitor that suppresses T cell activation and the production and release of IL-2 and other cytokines. It induces severe trabecular bone loss in rats, but this effect appears to be less severe in humans (Epstein, 1996). Cyclosporin A (CyA), another calcineurin inhibitor, also appears to have adverse effects in mouse models, inducing high turnover and reducing bone mass. Some studies in humans suggest a similar effect in patients with liver (Giannini et al., 2000), and cardiac (Thiebaud et al., 1996) transplantation. In a research of 360 patients with liver transplantation due to chronic cholestatic liver disease, the post transplant bone gain was lower, and the number of fractures was higher in patients treated with CyA than in those receiving tacrolimus (Guichelaar et al., 2007). Other authors have found greater

fracture incidence in patients receiving CyA treatment than in those treated with tacrolimus (Monegal et al., 2001).

In other liver transplanted recipients study, although bone mass losses were similar in patients on CyA regimen than in those on tacrolimus, histomorphometric changes after transplantation were different between groups. Patients treated with tacrolimus had an improvement in trabecular bone architecture compared with patients receiving CyA (Guichelaar et al., 2004). These findings suggest that patients treated with tacrolimus may have faster recovery of bone metabolism after the initial phase of bone loss compared with those treated with CyA.

A study comparing CsA monotherapy versus prednisone and azathioprine regimen in renal transplant recipients did not find any differences in bone loss or bone histomorphometric parameters (Cueto-Manzano et al., 2003). Furthermore, a major side-effect of CsA therapy is dose-related nephrotoxicity, often leading to secondary hyperparathyroidism, which may also adversely affect bone health.

Other immunosuppressive agents such as Mycophenolate mofetil (104), rapamycin or azathioprine have shown no effects on bone in murine models (Maalouf et al., 2005).

3. Bone loss and fractures after transplantation

The majority of longitudinal studies show a decrease in bone mineral density at the lumbar spine and hip that occurs in the first year after solid organ transplantation. The amount of bone loss ranges between 3% and 10%, particularly in the first 3-6 months. Rapid bone loss and major involvement of lumbar spine (trabecular bone tissue) are findings probably related to the large doses of glucocorticoids used immediately after transplantation. Rates of lumbar spine bone loss slow thereafter, with stabilization by 6-12 months and even some recovery after liver, lung, and heart transplantation. However, most studies do not document recovery of bone mass at the hip. BMD changes after renal transplantation are different since continued bone loss after the rapid initial bone loss may be observed. Prevalence of densitometric osteoporosis is quite variable depending on type of organ transplantation and time elapsed since transplantation (Hawkins et al., 1994).

With regard to fractures, a high incidence of them (between 20% and 40% in most studies) has been documented. In heart and liver transplant recipients, the incidence of new fractures parallels the timing of the most rapid bone loss, with most fractures occurring within the first year after transplantation (Eastell R, 1991; Henderson et al., 1995; Shane et al., 1996; Ramsey-Goldman et al., 1999).

Fractures usually affect the spine and ribs in liver, cardiac, or lung recipients, whereas long bones are more easily fractured in renal transplant recipients. However, fracture incidence may have decreased in the last years. This is probably related to the wide implementation of immunosuppressive regimens that use lower doses of glucocorticoids and for a shorter period of time. Indeed, bone loss and fractures remain unacceptably high in several recent studies.

Risk factors for fractures after transplantation include older age, prevalent fractures before transplantation, postmenopausal status, and lower body mass index. Additional risk factors in renal transplant recipients include the presence of diabetes mellitus and prolonged dialysis. The predictive roles of pretransplantation BMD and cumulative glucocorticoid dose are controversial. Associations between these risk factors and bone loss or fractures are

not consistent across studies. Even patients with normal pre-transplant BMD may suffer fracture after transplantation. Therefore, it is usually not possible with current clinical tools to predict whether individual transplant recipients will fracture after transplantation.

In a nested case-control study of transplant recipients (kidney, liver, lung, heart), multivariate analysis showed that post-transplant fracture rate was highest among those with a history of hyperthyroidism, pretransplant diabetes, fracture, or corticosteroid use, and among those currently exposed to antidepressants, narcotics, sirolimus, and loop diuretics (Shane et al., 2009 update). Use of bisphosphonates or calcitonin was also a predictor of fracture, likely indicating the presence of pre-transplant osteoporosis.

In some studies, the rate of post-transplant fracture is decreasing, in part due to increased recognition of the problem, which has resulted in changes in immunosuppressive regimens (reduction in dose and duration of glucocorticoids) and earlier identification and treatment for osteoporosis (Shane et al., 2004; Compston et al., 2003)

3.1 Kidney transplantation

Rates of bone loss are greatest in the first 3-18 months and range from 4-9% at the spine and 5-8% at the hip.

After renal transplantation fractures affect appendicular sites (hips, long bones, ankles, feet) more commonly than axial sites (spine and ribs). The majority of fractures occur within the first 3 years. Fracture prevalence varies from 7-11% in nondiabetic renal transplant recipients, but is considerably higher in patient transplanted because of diabetic nephropathy and in those who receive kidney-pancreas transplants. (Nowacka-Cieciura et al., 2006)

With regard to the difference in the prevalence of fracture in end stage renal disease patients referred to kidney transplant or those who continued dialysis, a large study realized with 101,039 patients with end stage renal disease demonstrated that kidney transplantation was associated with a 34% greater risk of hip fracture than continued dialysis (Nisbeth et al., 1994).

3.2 Lung transplantation

During the first year after lung transplantation, rates of bone loss at the lumbar spine and femoral neck range from 2 to 5%. In another study conducted with 70 patients awaiting lung transplantation the prevalence of vertebral fractures was 29% in patients with chronic obstructive pulmonary disease and 25% in patients with cystic fibrosis (Shane et al., 1996). Fracture rates are also high during the first year after lung transplantation, ranging from 18 to 37%.

3.3 Cardiac transplantation

The most rapid rate of bone loss occurs in the first year. Spinal BMD declines by 6-10% during the first 6 months, whereas femoral neck BMD falls by 6-11% in the first year. The rate of bone loss slows after the first year and spine BMD may increase after the third year (Cohen et al., 2003). Densitometric osteoporosis at the lumbar spine and femoral neck has been reported in approximately 28% and 20% respectively of long term transplant patients (Chou et al., 2006).

Vertebral fracture incidence ranges from 20-36% during the first 1 to 3 years after cardiac transplantation. One prospective study shows that 36 percent of all patients and 54 percent

of women sustained a fracture after this procedure, 85 percent of which occurred within the first six months (Shane et al., 1996). Women with the lowest pretransplant hip BMD were at highest risk of fracture. In men, pretransplant BMD did not differ between those who went on to fracture and those who did not.

3.4 Liver transplantation

Spine BMD declines by 2-24% during the first year in earlier studies, particularly during the first 3-6 months. Some authors report higher rates of bone loss and fracture in patients who have alcoholic cirrhosis, primary biliary cirrhosis, and primary sclerosing cholangitis (Lopez 1992). Rates of bone loss have been lower in more recent studies. In the second year after transplantation, lumbar BMD recovered or even exceeded baseline levels (Guichelaar et al., 2006). Although early studies showed a predominance of post-transplant bone loss and fractures in the lumbar spine (Compston et al., 2003), more recent studies reported higher bone loss at the hip (Keogh et al., 1999; Ninkovic et al., 2002; Crawford et al., 2006). It seems that there are differences in the natural evolution of lumbar and femoral BMD, with greater loss of femoral bone that persists after the first year after transplantation. As an example, after 3 years, BMD at the femoral neck improved, but still remained below baseline levels (Monegal A, 2001). Other studies found a decrease in BMD at the femoral neck at 6 and 12 months, even despite treatment with bisphosphonates, suggesting a lesser effect of these drugs at cortical bone (Keogh et al., 1999), (Ninkovic et al., 2002), (Monegal et al., 2009).

Fracture rates after liver transplantation are highest in the first 6-12 months. Range from 24 to 65% and the ribs and spine are the most common sites. Women with primary biliary cirrhosis and the most severe preexisting bone disease appear to be at greatest risk. In a study of 37 patients receiving liver transplantation between 1993 and 1995, an incidence of 27% of vertebral fractures in the first three months after transplantation was found (Ninkovic et al., 2000). A subsequent study of the same group, done between 1995 and 1998, showed that this incidence was only 5%. Between both studies there was a considerable reduction in the dose and duration of glucocorticoid treatment, although the use of cyclosporine and tacrolimus barely changed (Ninkovic et al., 2002).

3.5 Bone marrow transplantation

The pattern of bone loss after bone marrow transplantation (BMT) is different from other forms of osteoporosis, being more persistent and severe in cortical bones, such as femoral neck than in trabecular bones, such as lumbar spine (Hatutman et al., 2011)). Bone marrow transplant (BMT) recipients have many known risk factors for developing decreased bone mineral density after transplantation. The pathogenesis of bone disease following BMT differs from others form of post-transplantation osteoporosis; recipients are usually younger and the time from the diagnosis to the BMT does not exceed 2 years; history of prolonged bed rest is uncommon. Immunosuppressive drugs are used in relatively low doses and for short periods of time (Ebeling et al., 1999). Glucocorticoid use is restricted to the treatment of graft-versus- host disease (GVHD).

Lumbar spine BMD declines by 2-9% and femoral neck BMD falls 6-11% during the first year following transplantation. Lumbar spine BMD begins to recover after 12 months, returning to baseline levels at 48 months. The extent of recovery at the femoral neck is less (Schulte et al., 2004). The presence of chronic GVHD is another factor associated with higher risk of osteoporosis in these patients. Avascular necrosis develops in 10-20% of allogenic

BMT patients; its development may be facilitated by a deficit in bone marrow stromal stem cell regeneration and low osteoblast number.

4. PTH. 25-OH-D. Bone remodeling in postransplantation bone disease

4.1 PTH

Elevated PTH levels have an adverse effect on bone health increasing turnover and decreasing bone mass, especially in cortical bone.

Some studies reflect a slight increase in PTH levels in the first month after transplantation. It could be related to vitamin D deficiency, calcium malabsorption or decreased tubular reabsorption of calcium, consequence of steroid treatment (Compston et al., 1996). PTH levels may remain elevated in the long-term due to chronic renal failure induced by cyclosporin (Floreani A, 2001; Crosbie et al., 1999).

4.2 25-OH vitamin D

Inadequate levels of vitamin D have been described in patients with end-stage liver diseases prior to liver transplantation, and this may play a role in the aetiology of lower mineralization after transplantation. Our group found that 91% of liver transplanted patients had insufficient serum levels of 25(OH)D at transplantation time. After adequate supplementation, serum 25(OH)D levels increased from 3 months onwards (Guadalix et al., 2011). A positive correlation between serum 25(OH)D levels at 3 months and BMD increase at 6 months was found suggesting that this vitamin has a positive effect on mineralization (Crosbie et al., 1999). In our study serum 25(OH)D levels showed positive correlation with the percentage change in total hip and femoral neck BMD at 12 months of treatment (Guadalix et al., 2011). These results suggest that vitamin D could have a main role in bone loss prevention after liver transplantation.

4.3 Bone remodeling in postransplantation bone disease

Bone turnover markers can provide information about the mechanisms of bone loss in post-transplant period. Our group has previously reported an increase in bone turnover markers such as osteocalcin after liver transplantation (Valero et al., 1995; Hawkins et al., 1994). No significant changes in urinary hydroxyproline, one and two years after transplant were found; however urinary excretion of NTX (amino-terminal telopeptide of collagen type I) showed a significant decrease after two years compared with baseline values (Giannini et al., 2000), while other found that values of deoxypyridinoline doubled compared to baseline, two months after transplantation (Crosbie et al., 1999). We also found that β -CTX decreased as from 3 months both in patients on bisphosphonate treatment as in patients receiving only calcium plus vitamin D, reflecting a reduction of bone resorption after liver transplantation (Guadalix et al., 2011). Greater reductions in β -CTX may be obtained with intravenous bisphosphonates. A significant decrease in urinary deoxypyridinoline in heart transplant recipients after intravenous pamidronate treatment (Shane et al., 1998) and in β -CTX levels 6 months after liver transplanted in 13 patients treated with intravenous zoledronic acid (Misof et al., 2008) was found. Other investigators found an early increase (one and 3 months after heart transplantation) in resorption markers hydroxyproline, pyridonoline and deoxypyridinoline and a decreased in osteocalcin, recovering all baseline values at 6 months (Shane et al., 1997). Several studies have investigated the OPG / RANKL in the post-transplant period. Results are

not homogeneous. High levels of both, OPG and RANKL in the first 14 days after liver transplantation compared to the control group were found (Fabrega et al., 2006). In the other hand, serum OPG in 57 patients at 3 and 6 months after cardiac transplantation was correlated with bone loss at the lumbar spine and femoral neck sites, after 6 months. Serum OPG alone accounted for 67% of the variance of lumbar spine bone density changes over the first 6 months post transplantation leading to the conclusion that serum OPG levels decline consistently in all patients following initiation of immunosuppressive therapy and are independently correlated with changes in bone density (Fahrleitner et al., 2003). In another study in patients with kidney transplant, levels of OPG and RANKL did not differ between healthy volunteers and transplant patients (Malyszko et al., 2003).

5. Gonadal status and postransplantation bone disease

Hypogonadotropic hypogonadism is frequently found both before and after transplantation and may play a role in the multifactorial pathogenesis of immunosuppression-induced bone loss. Sex-steroid deficiency in either sex results in an increase in bone turnover with an imbalance in bone formation and bone resorption. Few studies have assessed the status of gonadal function after transplantation and its relationship with bone mass. Many premenopausal women and men undergoing solid organ transplantation have temporary hypogonadism, most often related to the effects of glucocorticoids and chronic illnesses (Fleischer et al., 2008; Tauchmanová et al., 2005). In some cases (i.e. chemotherapy and/or radiation therapy for hematopoietic stem cell transplantation), hypogonadism is permanent (Tauchmanová et al., 2003). In men and women undergoing transplantation, testosterone and estrogen-progestin replacement, respectively, have been shown to slow bone loss (Isoniemi et al., 2001; Kananen et al., 2005). Hypogonadism is a common finding among patients with terminal liver disease, especially in males. Incidence was estimated up to 70% (Guichelaar et al., 2004). There are few studies about change in sex hormones after liver transplantation, some authors have reported an increase in free testosterone, although the recovery of normal levels has not been achieved in all patients (Floreani et al., 2001; Monegal et al., 2001). In a study of 10 liver transplant recipients followed for 12 months after transplantation, before transplantation, 90% of patients had a decrease in testosterone levels and reported decreased libido and erectile dysfunction. After transplant, total testosterone levels had doubled, and free testosterone increased tenfold. Patients reported early improvement in sexual function (6 to 8 weeks after transplantation). It was suggested that pretransplant abnormalities in gonadal function are mainly due to liver failure and are reversible in most patients (Madersbacher et al., 1996). In premenopausal women, normal menses usually resumes after liver transplantation (Mass et al., 1996).

Low levels of testosterone are quite common early after cardiac transplantation, and may be found in up to 50% of men (Guo et al., 1998). In addition, a significant relationship between low levels of serum testosterone and rates of femoral neck bone loss during the first 6 months after transplantation have been found by some authors (Shane et al., 1997). Fleischer et al., (2008) studied 108 male heart transplant patients. One month after transplantation, total testosterone levels were below normal in 63% of them while 33% had decreased levels of free testosterone. 15% of patients had elevated gonadotropin a month after transplantation, increasing to 29% at 6 months. These data suggest a suppression of the hypothalamic-pituitary-gonadal axis immediately after transplantation, with subsequent recovery. Authors attributed this to steroid therapy. Prednisone dose was found to be the main

determinant of the values of total and free testosterone). No relationship was found between post-transplant bone loss and testosterone levels, probably because patients were treated with calcitriol or alendronate.

In most studies, testosterone levels return to normal by 6 to 12 months after transplantation (Sambrook et al., 1994) but up to 20% of patients receiving prednisone and cyclosporine A may persist with low serum total testosterone levels 3 years after cardiac transplantation (Stempfle et al., 1999).

Regarding the role of other immunosuppressive agents on gonadal function cyclosporine A decreases testosterone by affecting both the hypothalamic-pituitary- gonadal axis (Sikka et al., 1988) and by direct inhibition of testicular synthesis of testosterone in murine models (Seethalakshmi et al., 1990). However, cyclosporine did not seem to affect testosterone levels in humans (Fleisher et al., 2008; Samojlik et al., 1992).

Some authors recommend hormonal treatment in post transplant osteoporosis in men and premenopausal women with hypogonadism, if there are no contraindications (Shane et al., 2009 up-to-date). Androgen replacement therapy has shown skeletal benefit (increase in BMD) only in men with hypogonadism. It has been demonstrated in an uncontrolled study of postmenopausal liver transplantation recipients, that the use of transdermal estradiol was effective in increasing BMD of lumbar spine and femoral neck over a period of two years (Isoniemi et al., 2001).

6. Prevention and treatment of osteoporosis

6.1 General measures before transplantation

The same measures used to prevent osteoporosis in the general population apply to transplant recipients, regardless of the pretransplant BMD measurement. It is recommended that all candidates for organ transplantation follow a thoroughfuly evaluation in order to identificate and correct risk factors and to implement measures to improve bone health (Table 4).

Before Transplantation	After transplantation
<ul style="list-style-type: none">- Measurement lumbar spine and hip BMD. If BMD is low, it should be evaluated secondary causes of osteoporosis.- Patients with kidney failure should be avaluated and treated for renal osteodystrophy.- Perform spine radiograph to detect prevalent fractures.- Recommend appropriate intake of calcium (1000-mg/ day) and vitamin D (800 IU).- Patients with osteoporosis should be evaluated and treated according to general guidelines.	<ul style="list-style-type: none">- Consider initiating preventive therapy in most patient (even those with normal BMD): calcium, vitamin D and antiresorptive agents.- Perform annual BMD measurement.- Perform annual Spine Radiograph.- Annual measurement of bone turnover markers.

Table 4. General recommendations for prevention and treatment of osteoporosis

Lifestyle factors, such as immobilization, smoking, and alcohol abuse, should be avoided. Concomitant use of medications that can negatively impact skeletal health should be minimized whenever possible. Hypogonadism should be sought and corrected, particularly in males, where symptoms are easily confounded with those of preexisting chronic disease or adverse effects of concomitant medication. All patients should receive the recommended daily allowance for calcium (1000-1500 mg/day) and vitamin D (800 IU/day). Higher vitamin D doses should be given if the patient is vitamin D deficient (serum 25-hydroxyvitamin D level >20 ng/ml [50 nmol/L]). Although calcium and vitamin D do not prevent transplantation-related bone loss, randomized trials assessing antiresorptive therapy, such as bisphosphonates, have been carried out in the setting of concomitant calcium and vitamin D repletion.

Prevention of early bone loss after transplantation have been reported with specific resistance training programs (Mitchell et al., 2003). Regular weight-bearing exercise (30 minutes, three times per week) has proved also to be beneficial for the prevention and treatment of osteoporosis. Because of the high prevalence of osteopenia and osteoporosis in patients awaiting transplantation and the morbidity caused by osteoporosis after transplantation, it is recommended that candidates for organ transplantation undergo measurements of BMD of the hip and spine, preferably at the time of acceptance to the waiting list. Low BMD before transplantation has been pointed out as a risk factor for fractures after transplantation. In addition, spine radiographs should be performed to detect prevalent fractures. Patients who have a history of fracture or have osteoporosis on DXA (T-score \leq -2.5) before transplantation should be evaluated for secondary causes. When a secondary cause (i.e. hypogonadism) is identified, appropriate treatment is recommended prior to transplant. In addition to the treatment (when possible) of secondary causes, some patients may benefit from additional osteoporosis therapy, such as bisphosphonates, while awaiting transplant. Patients with osteopenia should be considered for prevention (calcium, vitamin D and/or antiresorptives) evaluating risk factors. Alternatively, patients with normal BMD can defer medical therapy until immediately after transplantation. For patients with end-stage renal disease, an evaluation and treatment for renal osteodystrophy according to accepted guidelines is highly recommended.

6.2 Therapeutic measures of post-transplantation bone loss

Several drugs have been studied for the treatment of osteoporosis after transplantation. Many of these studies were done with small number of patients, were not randomized or not compared with control group. Also, patients were not selected based on T-score or risk factor (beyond the transplant). There is no consensus on candidates for the treatment or the drug of choice. Given the accelerated bone loss that occurs immediately after transplantation many experts recommend preventive treatment for all patients receiving solid organ transplantation, regardless of pretransplant BMD (Maalouf NM, 2005; Cohen et al., 2006). This approach is based on observational data that show an overlap in BMD values between the pre-transplant patients with posttransplant fracture and those without fractures (Leidig-Bruckner et al., 2001; Shane et al., 1996). The lack of reliable clinical predictors to identify individual patients who will experience osteoporotic fractures renders all transplant recipients candidates for preventive therapy. Treatment should be started immediately after transplantation.

Since lumbar BMD starts to recover in many patients at 12 months after transplantation, long-term treatment may be unnecessary (Cohen et al., 2006). Duration of treatment

depends on patient characteristics, such as time of steroid therapy withdrawal, presence of other risk factors for low bone mineral density and fractures as well as information provided by the measurement of BMD.

Another approach to the management of transplanted patients is to apply similar guidelines as those used for the prevention of glucocorticoid-induced osteoporosis. There are several guidelines for the prevention of glucocorticoid-induced osteoporosis. Collectively, they suggest preventive therapy for patients with clinical risk factors for osteoporosis and fracture (age ≥ 65 years, previous fragility fracture) or in patients without other risk factors if BMD T-score is below -1.0 or -1.5 (Shane E, 2009).

6.2.1 Bisphosphonates

These potent antiresorptive agents are an obvious option in preventing the rapid bone loss, that occurs mainly in the early phase after transplantation. Bisphosphonates are considered the medical therapy of choice for the prevention of transplantation-related bone loss. Although there are conflicting data both oral and intravenous bisphosphonates appear to be effective in these patients.

Below shows some of the results obtained with bisphosphonates treatment in different types of transplants.

In a study of 99 subjects receiving stem cell transplantation, patients were randomly assigned to receive calcium and vitamin D or calcium and vitamin D plus pamidronate (60 mg intravenously six times over the first post-transplant year). Treatment with pamidronate prevented spine bone loss (0 percent in pamidronate group versus -2.9 percent in calcium group), and reduce hip bone loss (-5.5 percent and -7.8 percent in the pamidronate and calcium-vitamin D groups, respectively) (Kananen et al., 2005).

In a trial of 62 adults undergoing liver transplantation, patients were randomly assigned to receive infusions of zoledronic acid (4 mg) or placebo within seven days of transplantation. BMD was measured 3, 6, 9, and 12 months later. Zoledronic acid group lost significantly less bone at the hip at all time points (Crawford BA, 2006). In the spine, the zoledronic acid group lost less bone at three months, but the difference between the two groups was no longer significant at 12 months because of improvements between 3 and 12 months in the placebo group. Zoledronic acid sometimes caused postinfusion hypocalcemia and temporary secondary hyperparathyroidism.

Oral bisphosphonates are also effective in preventing bone loss after transplantation (Shane et al., 2004; Atamaz et al., 2006). As an example, in a trial of 98 subjects receiving a liver transplant, subjects randomly assigned to alendronate (70 mg weekly) versus no alendronate had significant increases in lumbar spine (5.1 and 8.9 percent) and femoral neck (4.3 and 8.7 percent) BMD at 12 and 24 months, respectively, compared with the control group (Atamaz et al., 2006). All subjects received calcium (1000 mg daily) and calcitriol (0.5 mcg daily).

Our group studied the effect of risedronate in liver transplant patient. The main findings of our study are that liver transplanted patients with low bone mineral density who receive either Risedronate combined with calcium and vitamin D3 or vitamin D3 and calcium alone showed a significant increase in spine BMD at 12 months compared to baseline values. In addition, risedronate patients showed a significant increase in intertrochanteric BMD, but we were not able to find any significant differences between groups. Hence, these results suggest that weekly risedronate after liver transplantation combined with 1000 mg/day of calcium and 800 IU/day of vitamin D are not superior to the administration of calcium and vitamin D alone (Guadalix S, 2011). Significant

improvement in BMD at the lumbar spine was also observed 12 months after liver transplant in the control group. This response may be related to the administration of calcium and vitamin D3 itself, but also to improvement in general health, mobility, muscle mass, and nutrition as a consequence of better liver function.

A recent meta-analysis in 364 liver transplant patients from 6 randomized controlled trials have found that bisphosphonate therapy improved lumbar spine BMD by 0.03 g/cm² (95% CI 0.01-0.05 g/cm², $p=0.02$) at 12 months post-liver transplantation compared to the control group. However, a statistically significant change in femoral neck BMD could not be demonstrated in this meta-analysis. Data on fractures could not be analyzed (Kasturi et al., 2010). In a study of 34 lung transplant recipients (with cystic fibrosis antecedents), pamidronate therapy versus calcium and vitamin D showed a significant increase in bone mass at 2 years in lumbar spine and total femur. There was no difference in fracture incidence (Aris et al., 2000).

In patients after allogeneic stem cell transplantation pamidronate reduced bone loss at the spine, femoral neck and hip by 5.6, 7.7 and 4.9% respectively after 12 month of treatment. However, at 24 month, only differences at BMD of total hip remained statistically significant between study groups (Grigg et al., 2006). Other study in 12 patients treated with zoledronic acid show that 12 month after infusion, total hip BMD increased in 75% of the patients and femoral neck BMD increased in 11 of 12 patients. Spinal BMD only increase in four (D'Souza et al., 2006).

Based upon the above trials, we suggest bisphosphonates as first choice for prevention of transplantation-related bone loss. There are few data to support the use of one bisphosphonate over another. Many of the trials used intravenous bisphosphonates due to ease of administration, especially in post-transplant patients who are required to take many oral medications. There are no trials comparing oral to intravenous bisphosphonates in the immediate post-transplant setting. The decision should be based upon individual patient preferences and abilities. The safety and efficacy of bisphosphonates for prevention of transplantation-related bone loss in patients with chronic kidney disease has not been carefully evaluated, and in general, there is limited data on the level of renal impairment at which bisphosphonate use should be avoided and whether this level is the same for iv bisphosphonates. In the majority of trials, individuals with serum creatinine concentrations above the upper limits of normal were excluded from participation. Despite these concerns, however, it is usually recommend their use after renal transplantation, at least during the first year when rates of bone loss are most rapid.

6.3 Other therapies

Replacement doses of calcium and vitamin D (400-1000 IU/day) do not prevent clinically significant bone loss after transplantation, but active metabolites of vitamin D could reduce post-transplantation bone loss, probably by reversing glucocorticoid-induced decreases in intestinal calcium absorption.

6.3.1 Calcidiol (25-hydroxivitamin D) and alfacalcidol (1 α -hydroxivitamin D)

In renal transplant recipients calcidiol (40 μ gr/day) prevents spine and femoral bone loss and is associated with a significant decrease in vertebral deformities (Talalaj et al., 1996). Consistently with these findings, our group have found that in patients randomized immediately after cardiac transplantation to 32000 IU/week of oral calcidiol for 18 months,

lumbar spine BMD increased ~5%, whereas those who received cyclical etidronate or nasal calcitonin, showed decreases in spine BMD (Garcia-Delgado et al., 1997). Also, alfacalcidol therapy has been associated with an increase in BMD or prevention of additional bone loss in renal (El-Agroudy et al., 2003) and cardiac recipients (Van Cleemput et al., 1996).

6.3.2 Calcitriol

Calcitriol may suppress bone resorption indirectly by facilitating intestinal calcium absorption and suppressing PTH secretion. Studies of calcitriol alone and those that compare calcitriol and bisphosphonates suggest that calcitriol may also prevent early post-transplant bone loss, particularly at the proximal femur. Positive effects on BMD have been found with higher doses of calcitriol ($> 0.50 \mu\text{gr/day}$) in heart, lung or renal transplantation, whereas lower doses ($0.25 \mu\text{gr/day}$) are relatively ineffective. Use of calcitriol requires close monitoring of serum and 24 hour urine calcium, because it is associated with hypercalcemia and hypercalciuria in $>50\%$ patients. In a study of 65 patients undergoing cardiac or single lung transplantation, patients were randomly allocated to receive placebo or calcitriol ($0.5\text{--}0.75 \mu\text{g/day}$), the latter for either 12 months or 24 months. Bone loss at the proximal femur was significantly reduced or prevented by treatment with calcitriol for 2 years compared with treatment with calcium alone (Sambrook et al., 2000).

Other randomized study compared the efficacy of 6 months treatment with either calcitriol ($0.5 \mu\text{g/day}$) or two cycles of etidronate plus calcium in preventing bone loss in 41 patients undergoing cardiac or lung transplantation. Compared with an untreated reference group, both therapies offered significant protection at 6 months in lumbar spine and etidronate provided significant protective carryover after therapy had been discontinued (Henderson et al., 2001). Although calcitriol appears to be effective in preventing bone loss after transplantation (Sambrook et al., 2000; Shane E, 2004) it should not be selected as first-line treatment because of their limited effectiveness and narrow therapeutic window.

6.3.3 Calcitonin

Although calcitonin is effective in preventing bone loss in postmenopausal women, it has not been shown to be superior to calcium in transplant recipients (Välimäki et al., 1999). In one trial, the combination of continuous oral calcitriol ($0.5 \mu\text{g/day}$) and nasal salmon calcitonin (200 U/day) for the first 3 months was inferior to intravenous pamidronate (0.5 mg/kg body weight) every third month in attenuating bone loss three months after cardiac transplant but similar at 18 months in 26 cardiac transplant recipients (Bianda et al., 2000).

6.3.4 Recombinant parathyroid hormone (PTH)

PTH 1-34 (teriparatide) has been shown to improve BMD in patients with glucocorticoid-induced osteoporosis, but there are few studies evaluating PTH for the prevention of post-transplant osteoporosis. In a small trial 24 kidney recipients patients were treated with $20 \mu\text{g}$ of teriparatide/daily/6 months, it was shown that femoral neck BMD was stable compared to the placebo group. Lumbar spine BMD and radial BMD, histomorphometric bone volume and bone matrix mineralization status remained unchanged in both groups. (Cejka et al., 2008). Recombinant parathyroid hormone (PTH) has not been well studied in this population. Patients who have received total body irradiation during hematopoietic stem cell transplantation or who have primary or secondary elevations in PTH are not candidates for recombinant PTH therapy.

6.3.5 New therapies

Promising new agents for transplantation osteoporosis include new potent anticatabolic drugs such as human antibodies to receptor activator of nuclear factor κ -ligand (RANKL) (denosumab), and cathepsin K inhibitors.

6.4 Monitoring

There is no consensus on the optimal strategy for monitoring patients on therapy. Patients on antiresorptive therapy are measured BMD every year after transplantation. Patients with normal BMD can be followed up with DXA every two years, depending also of other risk factors. In patients who require continuous treatment with glucocorticoids (prednisone ≥ 5 mg / day), BMD measurement is recommended annually. An effort should be made to find the lowest prednisone dose compatible with graft survival.

7. Summary and conclusions

Although bone loss and fractures after transplantation seem to be lower than those reported years ago, they remain being a main long term posttransplant complication. An effective approach should incorporate pre-transplant measures to detect and to treat preexisting bone diseases. Oral or intravenous bisphosphonates, in conjunction with calcium and vitamin D, are effective in preventing post-transplantation bone loss when started shortly after grafting. The optimal dose, timing, and frequency, particularly of intravenous bisphosphonate administration, remain to be determined. At present, most controlled trials lack sufficient statistical power to demonstrate efficacy for fracture prevention, so treatment regimens are based on results of effects on the surrogate end-points, bone densitometry, and bone turnover markers. More studies are required to determine the best agent and route of administration to prevent this common complication of organ transplantation. The future challenge is to achieve adequate immunosuppression without corticosteroids, with drugs not damaging bone. This approach, together with improved bone health before the transplant would be an effective strategy to reduce post-transplant osteoporosis.

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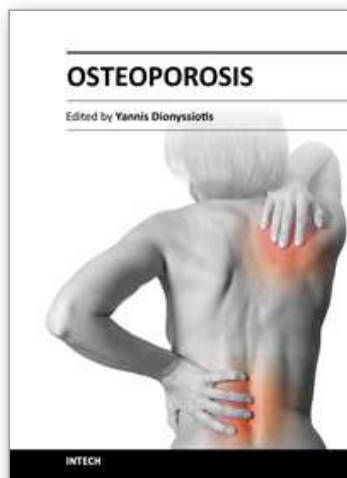
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Osteoporosis is a public health issue worldwide. During the last few years, progress has been made concerning the knowledge of the pathophysiological mechanism of the disease. Sophisticated technologies have added important information in bone mineral density measurements and, additionally, geometrical and mechanical properties of bone. New bone indices have been developed from biochemical and hormonal measurements in order to investigate bone metabolism. Although it is clear that drugs are an essential element of the therapy, beyond medication there are other interventions in the management of the disease. Prevention of osteoporosis starts in young ages and continues during aging in order to prevent fractures associated with impaired quality of life, physical decline, mortality, and high cost for the health system. A number of different specialties are holding the scientific knowledge in osteoporosis. For this reason, we have collected papers from scientific departments all over the world for this book. The book includes up-to-date information about basics of bones, epidemiological data, diagnosis and assessment of osteoporosis, secondary osteoporosis, pediatric issues, prevention and treatment strategies, and research papers from osteoporotic fields.

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