Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 62; Numer/Number 5/2011
ISSN 0423–104X

Transplantation osteoporosis

Osteoporoza u osób po przeszczepach

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Abstract

Transplantation provides a valuable, often life-saving, treatment for end-stage failure of many organs, including the heart, kidneys, liver, pancreas and lungs. It is also an important therapeutic option in diseases of the bone marrow and the immune system. Despite the undoubted benefits for transplant patients, it is associated with an increased risk of many complications. The potential causes include: poor general health of the patient, heavy burden of the surgery itself and the need for the long-term use of immunosuppression. In addition, the patients are also on numerous other medications, e.g. anti-coagulants, diuretics. Osteoporosis and high risk of fractures have emerged as frequent and devastating complications of the transplantation process. This article provides a review of the current literature on osteoporosis after transplantation, and the treatment options for this serious illness. **(Pol J Endocrinol 2011; 62 (5): 472–485)**

Key words: *transplantation, osteoporosis, treatment*

Streszczenie

Transplantacja stanowi cenną i często jedyną metodę leczenia schyłkowej fazy niewydolności wielu narządów, między innymi serca, nerek, wątroby, trzustki oraz płuc. Z powodzeniem stosuje się ją również w chorobach układu odpornościowego i szpiku. Pomimo niewątpliwych korzyści dla chorego transplantacja wiąże się ze zwiększonym ryzykiem wielu powikłań z uwagi na duże obciążenie samym zabiegiem, często ciężki stan ogólny chorego oraz konieczność długotrwałego stosowania immunosupresji. Jednym z powikłań po przeszczepie może być również osteoporoza oraz związane z nią wysokie ryzyko złamań. Powyższa praca stanowi aktualny przegląd piśmiennictwa dotyczącego osteoporozy po przeszczepach oraz możliwości leczenia tej groźnej choroby. **(Endokrynol Pol 2011; 62 (5): 472–485)**

Słowa kluczowe: *przeszczep, osteoporoza, leczenie*

Introduction

In 1988, there were 12,619 organs transplanted in the USA. That number increased to 25,468 by 2003 and, with the use of spouse donors, this will likely increase further [1].

The recognition and identification of the role of T lymphocytes and subsets, as well as of B lymphocytes in mediating immune reaction, involved in virtually every disease process, including, among others, infection, cancer, cardiovascular disease and organ transplantation, have allowed the development of a class of drugs known as immune modulators [2, 3]. These drugs either enhance or suppress immune reaction, depending upon what type of modulation is required to affect disease outcome.

In organ transplantation, the main requirement is to prevent or inhibit donor-organ rejection by the recipient's immune system. These immune-modulating drugs have an enormous impact on prolonging the lifespan of patients. This impact has not, however, been possible without certain costs, one of them being the effect of some of these agents on bone status.

This review will focus on the effects of drugs, other than glucocorticoids, on bone. These drugs include calcineurin inhibitors (CIs), cyclosporine and tacrolimus and non-CIs: rapamycin, mycophenolate mofetil, methotrexate, and azathioprine. A number of other immunosuppressive drugs are at the disposal of physicians, and the list is steadily growing, but data on the effects of these other drugs on bone is either missing or has yet to be studied.

Immunosuppressive agents

Glucocorticoids

Glucocorticoids constitute an integral component of most post-transplantation regimens, and a brief description of their effects on bone and mineral metabolism in the setting of organ transplantation is given here.

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The immunosuppressive properties of glucocorticoids are related to their inhibition of the expression of a variety of cytokines, including IL-1, IL-2, IL-6, interferon, and tumour necrosis factor (TNF) [3, 4]. This inhibition presumably occurs via binding of the glucocorticoid–glucocorticoid receptor protein complex to a glucocorticoid-responsive element in the regulatory region of the target genes. Glucocorticoids also inhibit the IGF regulatory system, including IGF-I expression in osteoblasts, an effect that probably contributes to their inhibitory effect on bone formation. In addition, glucocorticoids inhibit T-cell proliferation [3]. It is of great interest that cytokines IL-1, IL-6, TNF, and interferon, which are suppressed by glucocorticoids, have been found to stimulate bone resorption [3]. These observations suggest that glucocorticoids, which inhibit these cytokines, must cause bone loss and fractures via other mechanisms. These mechanisms are thought to include:

— inhibition of gastrointestinal calcium absorption and stimulation of renal calcium loss, both of which predispose to negative calcium balance and secondary hyperparathyroidism [4];
— suppression of the hypothalamic–pituitary–gonadal axis which decreases gonadal steroidogenesis;
— direct suppression of osteoblast recruitment and osteoblast function, including inhibition of osteocalcin synthesis;
— decreased transcription and synthesis of skeletal growth factors, such as IGF-I, TGF-, and fibronectin;
— enhanced synthesis of collagenase in osteoblasts;
— induction of apoptosis of osteoblasts and osteocytes, both *in vitro* and *in vivo* [4].

Recently, disruption of vascular channels in the bone micro-environment, as well as an increased production of receptor activator of NFkappa B ligand (RANKL) and a decreased synthesis of osteoprotegerin, both stimulating osteoclast activation, have added to the multifactorial aetiologies of glucocorticoid-induced bone loss [5–8].

Calcineurin inhibitors

Cyclosporine (CsA) and tacrolimus (FK506) are inhibitors of calcineurin. They both require binding to intracellular proteins: CsA to cyclophilins [9–11] and FK506 to FK binding proteins [12, 13]. These proteins are called immunophilins and are peptidyl-prolyl cis-trans isomerase enzymes [9, 13]. This binding is essential but not sufficient for immunosuppression. The complexes, in turn, inhibit the intracellular phosphatase enzyme calcineurin [9–13], which prevents transcription of T lymphocyte cytokine genes and the genes that control membrane molecules, such as CD 40 ligand [14, 15]. Calcineurin is a serine–threonine phosphatase that is uniquely regulated by Ca^{2+} and calmodulin. Calcineurin enzyme interacts with NF-AT (Nuclear Factor of Activated T cells) [16], which is a family of transcription factors necessary for activation of genes, involved in the inflammatory and the immune systems. Thus, by inhibiting calcineurin [17–22], CsA and FK 506 (see below) prevent the activation of NF-AT, with a consequent inhibition of growth and differentiation factors critical to the immune response. CsA and FK506 have made a huge impact on preventing organ rejection and preserving life. One of the drawbacks to their use, however, is their propensity to cause rapid and profound bone loss [23, 24], a propensity best illustrated in experimental models. This bone loss was first observed in the rat model, where administration of immunosuppressive doses to normal, young or old, male or female oophorectomised rats produced a significant loss of trabecular and cortical bone after four weeks. The loss was reversible after stopping the drug and was dose-dependent [25]. Histomorphometry showed this to be an extremely high-turnover bone loss with increased markers of resorption and formation. This action in causing high turnover bone loss is dependent on the presence of T lymphocytes.

Tacrolimus (fk506)

This immunosuppressant, which acts in a similar fashion to CsA, except that it binds to specific FK binding proteins, is frequently used as a first-line immunosuppressant in place of CsA or to prevent rejection, when CsA has failed. It is reputed to have less nephrotoxicity than CsA. *In vivo* effects of FK506 produce the same histomorphometric picture of high-turnover bone loss as seen with CsA [26].

Clinical studies supporting the role of calcineurin inhibitors in bone loss after transplantation

Clinically, there are studies directly implicating the role of calcineurin inhibitors in bone loss after organ transplantation [27, 28], which confirms the experimental evidence. The first study, linking CsA to bone abnormality, was published in 1988 [29]. In that study, histology in renal transplant patients revealed unexpectedly high bone turnover, unlike that seen with glucocorticoid administration or in secondary hyperparathyroidism [30]. In fact, the turnover resembled that observed in experimental studies in the rat. A clinical study in heart transplant recipients also attributed the bone loss, seen after cardiac transplantation, to CsA, and again, the biochemical findings revealed a high-turnover osteoporosis [27]. Those studies were all confounded by triple immunosuppression, and the role of one drug as the culprit cannot be ascertained. Studies with CsA monotherapy in transplanted patients, compared to other CsA-containing regimens, showed that, after

12 months, lumbar BMD did not decrease, and in fact increased after 18 months [31–34].

The most convincing study was a comparison of CsA monotherapy with a non-CsA (prednisone and azathioprine) regimen to isolate the effects of CsA alone in renal transplant patients [30]. This study utilised BMD, as well as bone histomorphometry as effected targets of immunosuppression. The results showed that both regimens decreased bone density at the distal radius, and less significantly at the lumbar spine, with no differences in the degree of bone loss. A histopathological analysis showed an increased number of osteoclasts and, surprisingly, a decrease in the number of osteoblasts and mineral apposition and bone formation rates, again with no differences between the groups. The findings did not support a role for PTH, either biochemically or histomorphometrically, despite the fact that PTH was considered by some investigators to be pivotal for bone loss after transplantation. The surprising decrease in bone formation in that study may be related to the time post-transplant, as the subjects were 140 ± 75 months after transplantation. Similarly, the role of tacrolimus (FK506) was also hard to separate from the other immunosuppressants used to prevent the rejection, despite the animal studies, showing either equal or more severe loss of bone with FK506 than with CsA, with a high turnover remodelling state similar to that after CsA [35]. Studies to resolve the role of FK506 were done with patients receiving low-dose prednisone and FK506, compared to normal-dose glucocorticoids and CsA [36]. Those studies showed that, when a cumulative dose of steroids was adjusted for both groups, the FK506 patients after one year did not lose bone, compared to the CsA and low-dose prednisone groups. There was no relationship to PTH levels. A prospective, longitudinal, randomised, double blind study was undertaken to assess the effects of FK 506, glucocorticoids, azathioprine, or mycophenalate mofetil (instead of azathioprine) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) against the same immunosuppressive regimen without $1,25(OH)_2D_3$ (placebo group) [37]. The study duration was two years. The objective was to determine whether tacrolimus produced bone loss and whether 1,25-dihydroxyvitamin D_3 could modify or prevent the bone loss. The obtained results showed that BMD decreased in both groups after transplantation, compared to normal age-matched subjects. However, BMD increased significantly in the lumbar spine in the group receiving a vitamin D analogue, although no significant differences between the groups could be shown. In the femoral neck region, BMD was maintained, but the placebo group lost bone significantly. Again, no between-group significance could be shown. The conclusion of the study could be such that FK506 was associated with rapid bone loss, comparable to CsA, which could then be modified by the low-dose calcitriol therapy over two years. A more convincing study would compare FK506 against CsA in comparable, post-transplant patients, not receiving glucocorticoids to determine whether there is any difference between the two CIs in their effect on bone mass status.

The question of the calcineurin inhibitors and bone loss is a very complex issue, due to the underlying renal osteodystrophy disease, the difference in patient profiles, multiple drug administration etc. A direct head-to-head comparison of the calcineurin inhibitors vs. glucocorticoids alone may never occur, due to patient numbers and, perhaps, for ethical reasons.

Other immune-modifying drugs

Rapamycin

Rapamycin (sirolimus) is also used to prevent organ rejection after transplantation. Rapamycin is a macrolide and a product of *Streptomyces hygroscopicus*. It does not inhibit the production of interleukins resulting from antigen T-cell activation, but it does inhibit the cellular proliferation stimulated by growth factor signal transduction in response to alloantigens [38, 39]. Rapamycin binds to the same intracellular protein, immunophilin FKBP12, to form a complex, which does not target calcineurin, but rather targets mTor (mammalian target of rapamycin). mTor inhibits the translation of mRNAs, that encode for cell cycle regulators and T-cell proliferation [39]. *In vitro* studies have shown that, in bone marrow stromal cells, rapamycin, like CsA and FK506, decreases osteoprotegerin (OPG) mRNA and protein levels and increases RANKL, which can potentially induce bone loss [40]. Rapamycin may, in addition, act via TGF-β to enhance osteoclastogenesis by inducing monocyte–macrophage cell differentiation into osteoclasts [41]. Conversely, *in vivo* studies in rats demonstrate that rapamycin does not cause bone loss but may interfere with longitudinal bone growth and, at high doses, decrease cortical bone in young rats, which are still rapidly growing [38]. An effect on gonadal function has also been described with rapamycin [38]. In human subjects, studies of rapamycin therapy without glucocorticoids and calcineurin inhibitors, investigating bone density to ascertain an effect has not been reported, and may not be feasible in a transplant population. There have been no studies on human populations evaluating the effect of rapamycin on bone density alone (only in combination with glucocorticoids and calcineurin inhibitors) so far. Conducting such a study in patients after transplantation could be difficult.

There is a possibility, however, that bone loss with calcineurin inhibitors may be mitigated by combining

rapamycin with a low-dose CsA, which has been shown in rats to prevent bone loss and not compromise immune suppression [42]. Very recently, two open labelled, randomised phase 2 studies compared the effects of sirolimus (rapamycin) vs. cyclosporine on bone turnover markers in 115 patients post-transplantation over one year. The patients were all receiving glucocorticoids and/or mycophenolate mofetil or azathioprine. Urinary excretion of N-telopeptides and serum osteocalcin were consistently higher in the patients on CsA, compared to sirolimus [43]. Everolimus, which is a derivative of rapamycin, has a similar mechanism of action to that of rapamycin, but its effect on bone has not yet been studied [44].

Mycophenolate mofetil

Mycophenolate mofetil, another additive to the immune-modifying drug armamentarium, is now becoming the choice to replace azathioprine as part of triple therapy, together with CsA or FK506 and glucocorticoids, to prevent organ rejection. Mycophenolate mofetil is converted *in vivo* to mycophenolic acid. This natural product of penicillium fungi selectively inhibits the proliferation of T and B cells, as well as arterial wall smooth muscle cells. Most other tissues are resistant to the action of mycophenolate mofetil because of an alternate nucleotide synthesis. It also causes less bone marrow suppression than azathioprine. Experimentally, in *in vivo* conditions, there is no evidence for bone metabolism alteration, nor for bone volume loss [45]. Recently, it has been reported that prednisone and mycophenolate mofetil, in the absence of CsA, may also be associated with high turnover bone loss on bone histomorphometry [46]. This requires further study.

Azathioprine

Azathioprine has long been an essential part of therapy in combination with CsA and glucocorticoids to prevent organ rejection. It is also a purine antagonist and, therefore, inhibits rapidly proliferating cells, which include T and B lymphocytes and bone marrow haematopoietic cells [47]. However, in rats, given azathioprine, albeit for a short period, no effect on bone except on bone formation markers was seen [47]. How this would translate with long-term use into clinically relevant outcomes is at present unknown.

Summary

The nonsteroidal immunosuppressants belonging to the calcineurin inhibiting family, have been shown experimentally and clinically to produce severe and rapid high-turnover bone loss. In the clinical setting, however, these drugs are often used with glucocorticoids, which are known to produce severe and rapid bone loss. Despite the production of a low turnover bone loss by glucocorticoids, the bone biopsy histomorphometry in the combination treatment reveals a high turnover state. The end result of this combination of drugs is rapid and severe bone loss with a very high rate of fractures. Other immunosuppressants, such as sirolimus, azathioprine, and mycophenolate mofetil, have clearly demonstrated adverse effects on the skeleton in experimental conditions. The more recent immunomodulators have not been well studied in regard to bone loss and fracture occurrence in clinical trials. The ability to separate the contribution of an individual drug as the culprit for producing adverse skeletal effects is extremely difficult, given the clinical situation and the other confounding variables, found in patients awaiting and post-transplantation. The development of immunosuppressant drugs that can prevent organ rejection and other adverse side effects, including bone loss, would be a major advance in the field of organ transplantation.

Clinical impact of transplantation on bones

The majority of candidates for organ transplantation reveal risk factors that predispose toward osteopenia, osteoporosis and subsequent fractures. These factors include general debilitation, loss of mobility, poor nutrition, cachexia and exposure to certain drugs, including glucocorticoids. In addition, many women are postmenopausal, and both premenopausal women and men with chronic illness may have gonadal dysfunction. When the disease is present during childhood or adolescence, as is the case with cystic fibrosis, there may be interference with the attainment of peak bone mass. After transplantation, episodes of rejection, usually reversed by large doses of glucocorticoids and cyclosporine, compound the bone loss. Consideration of particular issues related to transplantation of specific organs follows.

Kidney transplantation

Patients who undergo renal transplantation have severe chronic renal insufficiency or end-stage renal disease (ESRD) and most of them have been dialysed for varying intervals before transplantation. Pre-existing bone disease is almost universal in this population. Renal osteodystrophy is a general term that encompasses all the bone histological alterations that may occur in uraemic patients [48]. In a given individual, there may be evidence of hyperparathyroidism with or without osteitis fibrosa, osteomalacia, low turnover, or a dynamic bone disease due to aluminum accumulation (or other as yet poorly understood factors), osteoscle-

rosis, particularly of the vertebrae, and macroglobulin amyloidosis. Many patients will have 'mixed' renal osteodystrophy, i.e. a combination of one or more of the aforementioned lesions. Other factors that may affect the skeletal integrity of patients with ESRD include both type 1 and type 2 diabetes, hypogonadism secondary to uraemia and diseases such as systemic lupus erythematosus. Several drugs used routinely in the management of patients with renal disease, such as loop diuretics and aluminum-containing phosphate binders, can also affect bone and mineral metabolism. In addition, some patients, who are candidates for transplantation, may have been previously exposed to glucocorticoids or cyclosporine, applied as a therapy for immune complex nephritis or other diseases, and thus may already have sustained significant bone loss prior to transplantation.

After renal transplantation, several investigations have documented a decline in bone mass [49, 50] and increased fracture rate [51, 52]. While the greatest insult to the skeleton is related to glucocorticoid and cyclosporine exposure, persistent hyperparathyroidism probably contributes to the declining bone mass. Recently, a factor relating to the development of secondary hyperparathyroidism has been the identification of very low levels of 25-hydroxyvitamin D_3 (25(OH) D_3) (geometric mean =10.9 ng/mL) in renal transplant patients when measured at the end of winter [53]. The rate of bone loss is greatest during the first six months after transplantation and at sites where cancellous bone predominates, such as the lumbar spine [49, 50]. Indeed, some reports suggest an increase in bone mass at the radius, a site that consists predominantly of cortical bone [50]. The rate of lumbar spine bone loss varies between 6% and 18% per year, but tends to be somewhat lower than that observed for other transplanted organs, such as the liver. This lower rate of bone loss is possibly because lower doses of glucocorticoids and cyclosporine are used for immunosuppression or newer bone sparing immunosuppressants are administered after kidney transplantation than after transplantation of other solid organs [49, 50].

Moreover, rejection is more easily diagnosed, and therefore detected earlier, than with other organs, and lower doses of immunosuppressive drugs are more effective in reversing rejection when it is diagnosed earlier. It is accepted that the adverse skeletal effects of glucocorticoids and cyclosporine are experimentally and clinically dependent upon both dose and duration of exposure [25, 54]. Julian et al. reported a decrease in lumbar spine BMD of 5.6% at six months, and 7.0% at 18 months, after transplantation [49]. Moreover, by 18 months, bone density was below the 'fracture threshold' in ten out of 17 patients. There appears to be a gender-determined difference in the site at which bone loss occurs [50, 55]. Men have been shown to lose more bone at the proximal femur than women in the first few months after transplantation. In contrast, radial bone density increased in men at six months post-transplantation, but not in women. Bone biopsies, performed prior to transplantation, revealed changes typical for hyperparathyroidism. However, by six months after transplantation, the histomorphometric picture was more typical for a glucocorticoid effect, demonstrating osteoblast dysfunction and decreased mineral apposition [55]. Unfortunately, bone biopsies were not performed at 18 months when glucocorticoid doses were lower. Thus, there was no data either to exclude or to incriminate cyclosporine as a factor contributing to bone loss. However, there is some evidence in the literature to support a role for cyclosporine in the pathogenesis of high turnover state, often apparent in renal transplant recipients one year after transplantation [29]. The increased frequency of cyclosporine use as monotherapy in renal transplant patients should clarify the relative roles of glucocorticoids and cyclosporine in the pathogenesis of post-transplantation bone loss. Recently, 45 renal transplant recipients have been evaluated with quantitative histomorphometry 120 months after transplantation. Those treated with cyclosporine monotherapy demonstrated significantly lower mineral apposition rates than those treated with azathioprine and prednisone [30].

In general, vertebral fractures are less common after kidney transplantation than after transplantation of other organs. However, appendicular fractures are extremely common, particularly in patients after transplantation for diabetic nephropathy, in whom the incidence of fracture has been reported to be as high as 45% [51]. While the reasons for this are not clear and are multifactorial (including the accumulation of glycosalated end-products), microvascular disease and neuropathy (the lack of proprioception and pain sensation) may affect fracture incidence by increasing the risk of falls. In addition, bone mass may be below normal in patients with type 1 diabetes mellitus, even before renal transplantation, thus placing such patients at higher risk for fracture after transplantation.

Avascular necrosis occurs commonly after renal transplantation [56–61]. The incidence in children is 6% [56–59] and in adults is 8%. The hip is the most commonly affected site. While the association of avascular necrosis with glucocorticoids is well established, cyclosporine has also been incriminated in producing avascular necrosis and bone pain of the hip and other weight-bearing bones, such as the knees [62]. The known vasospastic or vasoconstrictive properties of cyclosporine may contribute to the development of avascular necrosis.

Cardiac transplantation

Osteoporosis and fractures constitute a major cause of morbidity after cardiac transplantation [63–67]. In early cross-sectional studies, the prevalence rate of vertebral fractures in cardiac transplant recipients ranged between 18% and 50%, and moderate to severe bone loss was present in a substantial proportion of subjects at both lumbar spine and the femoral neck [63, 67]. Risk factors that may predispose patients with end-stage cardiac failure to bone loss even before transplantation include exposure to tobacco, alcohol and loop diuretics, physical inactivity, hypogonadism, and anorexia which may contribute to dietary calcium deficiency. Hepatic congestion and prerenal azotemia may also affect mineral metabolism. Although the average bone mineral density of patients awaiting cardiac transplantation may not differ significantly from normal, it has been observed that approximately 8% to 10% of the patients fulfill the World Health Organisation's criteria for osteoporosis and 40% to 50% have osteopenia or low bone mass [64, 68]. Prospective longitudinal studies have documented rates of bone loss ranging from 2.5% to 20%, predominantly during the first year after transplantation [68, 69]. Biochemical changes after cardiac transplantation include sustained increases in serum creatinine and decreases in $1,25(OH)_2D_3$ concentrations [68]. On average, serum testosterone concentrations decrease in men, with recovery by the sixth post-transplant month [68]. Serum osteocalcin falls precipitously and there is a sharp increase in markers of bone resorption (hydroxyproline and pyridinium crosslink excretion) during the first three months, with return to baseline levels by the sixth month [68, 69].

This biochemical pattern coincides with the period of most rapid bone loss and highest fracture incidence and suggests that the early post-transplant period is associated with uncoupling of formation from resorption. It is of interest that at least two studies of subjects treated with high doses of glucocorticoids alone, confirmed the decrease in serum osteocalcin but demonstrated no increase in the markers of bone resorption [70,71]. This suggests that the pathogenesis of early bone loss after transplantation may be related both to the well-known inhibitory effects of glucocorticoids on bone formation, and to the effects of cyclosporine A or of another agent increasing bone resorption. There is also evidence for a high bone turnover state later in the post-transplant course, perhaps due to cyclosporine, characterised by elevations of both serum osteocalcin and urinary excretion of resorption markers [27, 72]. In a recent cross-sectional study, Eastell et al. [72] evaluated 50 men, ranging from 0.5 to 47 months after cardiac transplantation. They concluded that bone turnover was increased after cardiac transplantation and that the increase was due in part to secondary hyperparathyroidism related to renal impairment. Thus, biochemical changes later in the post-transplant course may be mediated, at least in part, by cyclosporine A-induced renal insufficiency. The pattern of bone loss after cardiac transplantation is similar to that observed after renal [29] or liver transplantation [73–75]. Despite the predilection for glucocorticoids to affect the cancellous bone of the vertebrae to a greater extent than other sites, Shane et al. [64, 65] have reported that there is as much, or even more, bone loss at the hip. Moreover, while bone loss at the lumbar spine slows or stops after the first six months, femoral neck bone loss continues during the second half of the first year after transplantation [64, 65]. Moreover, these investigators in a recent longitudinal study have demonstrated that 36% of patients (54% of the women and 29% of the men) suffered from one or more fractures in the first year, despite daily supplementation with calcium (1,000 mg) and vitamin D (400 IU) [65]. Although the majority of fractures affected the vertebral bodies, two patients suffered multiple rib fractures and two had fractures of the femoral neck. In women, low pretransplant femoral neck bone density predicted the risk of vertebral fracture after transplantation. In men, however, it was the rate of bone loss after fracture rather than the pretransplant bone density that was associated with fracture risk. Also of note was the observation that patients with normal bone mass also fracture frequently [65]. A European study of 159 cardiac transplant recipients reported similar findings [66]. This study underscores the need for a complete bone evaluation and bone mass measurements prior to, or immediately after, transplantation, as well as aggressive intervention to prevent bone loss and fractures in all patients, regardless of age, sex, or pretransplant bone density. There is very little longitudinal data available on the pattern of bone loss during the second year after transplantation. However, data from Shane et al. suggests that the rate of bone loss slows or stops in the majority of patients, with some recovery at the lumbar spine noted during the third year of observation [65]. Bone loss also slows at the hip after the first year; however, in contrast to the spine, there has been no significant recovery by the fourth post-transplant year [65].

Liver transplantation

Patients with liver failure also have risk factors that may predispose to fracture after transplantation. In addition, the doses of immunosuppressive drugs used are much larger than those commonly employed after renal transplantation. Moreover, since the liver plays a major role in cyclosporine metabolism, hepatic dysfunction

may also influence its serum concentrations, possibly predisposing to cyclosporine toxicity. These factors may account for the observation that liver transplant recipients have higher rates of bone loss than cardiac and renal transplant recipients during the first year after transplantation [73, 75, 76]. However, the type of liver disease may also be an important risk factor for osteoporosis. In one study, 13 out of 20 women with primary biliary cirrhosis, a disease characteristically associated with low turnover osteoporosis, suffered atraumatic fractures of vertebrae, ribs, hips, and long bones during the first year after transplantation [76]. Patients with alcoholic cirrhosis may also have very low bone mass prior to transplantation, perhaps due to hypogonadotrophic hypogonadism and excess iron deposits in the skeleton. Eastell et al. [76] reported that despite the high incidence of fractures in liver transplant recipients, bone mass recovered and bone histology normalised with increasing survival time after transplantation. This, however, has not been a uniform finding, and other studies have revealed continued losses rather than recovery [75]. Depending upon the bone density at the time of transplantation, these patients may always be at risk for fractures as survival rates and duration increase. As is the case with renal and cardiac transplantation, the independent role of glucocorticoids and calcineurin phosphatase inhibitors in the pathogenesis of bone disease in liver transplant patients is difficult to assess since single drug therapy is uncommon. The mechanism of bone loss after liver transplantation has been studied by bone biopsy in 21 patients, who underwent tetracycline labelling and transiliac crest bone biopsy prior to and three months after transplantation.

Before transplantation, a low turnover state was observed, with decreased wall width and erosion depth. Postoperative biopsies showed high turnover with increased formation rates and activation frequency and a trend toward increased indices of resorption [77]. In an earlier study, these investigators documented a significant increase in PTH concentrations after liver transplantation [78]. While increased PTH could account for these histomorphometric findings, similar effects are observed in animals treated with calcineurin inhibitors without a rise in PTH concentrations.

Lung transplantation

There have been several reports of skeletal complications in lung transplantation candidates and recipients. As with any chronic illness severe enough to require transplantation for survival, patients who undergo lung transplantation have predisposing factors for low bone mass prior to transplantation. In particular, tobacco exposure, chronic hypoxemia, immobility, glucocorticoid use and certain underlying diseases, such as cystic fibrosis, are common in candidates for lung transplantation. The prevalence of osteoporosis (bone mineral density two standard deviations below aged-matched controls) varies between 55% and 66% at the spine and up to 78% for the hip [79, 80]. The prevalence of vertebral fracture in candidates before transplantation is also high, varying between 25% and 29% [79, 80]. Adults with cystic fibrosis are probably at even greater risk for bone disease than other patients who undergo lung transplantation. A recent study of 70 adults with cystic fibrosis observed virtually all to have either osteoporosis or low bone mass. Reported fracture rates were double those in the general population, while rib and vertebral fractures were 10-fold and 100-fold greater, respectively. The strongest predictors of BMD were body mass index, cumulative prednisone dose and pubertal age [81].

The incidence of new fractures after lung transplantation is also very high [82], even in patients who receive antiresorptive therapy. It has recently been reported that 11 (10 women) of 30 lung transplant recipients (37%), all of whom had received daily calcium (1,000 mg) and vitamin D (800 IU) and antiresorptive therapy (injectable calcitonin, cyclic etidronate, pamidronate, or alendronate) sustained a total of 54 new fractures during the first year after transplantation [82]. The average time to first fracture was 4.5 months. The commonest fracture sites were ribs, vertebrae, pelvis, and sacrum [82, 83]. In addition, the fracture incidence may be underestimated, as patients with existing rib or vertebral fractures may not be candidates for lung transplantation because of the effect on lung function post operatively. Moreover, despite antiresorptive therapy, 50% had a significant decrease in BMD. Biochemical markers of bone turnover were significantly higher in those who lost bone and in those who fractured. Paediatric lung transplantation is becoming more frequent and both osteoporosis and reduced growth velocity can be expected in these children [84]. Similar to other transplanted organs, a high bone turnover state with elevated osteocalcin concentrations has been reported after lung transplantation [85].

Bone marrow transplantation

Bone marrow transplantation is performed with increasing frequency and for expanding indications. Low BMD has also been reported in patients after bone marrow transplantation [86], probably related to both pre- and post-transplant factors. In preparation for transplantation, patients receive myeloablative therapy (alkylating agents and/or total body irradia-

tion) and commonly develop profound and frequently permanent hypogonadism, which almost certainly contributes to bone loss. Two studies have documented low BMD in hypogonadal women after bone marrow transplantation [87] and shown that hormone replacement therapy is associated with significant increases in BMD [88]. A study of nine adults, undergoing high-dose glucocorticoid and CSA therapy for graft-versus-host disease (GVHD), observed significant bone loss in most patients [89]. Other factors include low vitamin D levels, physical inactivity and the malignancy itself [90]. A more recent study has documented that low bone mass antedates bone marrow transplantation, particularly in subjects with prior glucocorticoid exposure, and that post-transplant bone loss is particularly severe in patients who undergo allogeneic bone marrow transplantation, probably because of their increased propensity for GVHD [91]. In these patients, the rate of bone loss was 11.7% at the femoral neck and 3.9% at the spine [91]. In the first prospective study in children and adolescents, there was a 15% increase in osteopenia and a 3% increase in osteoporosis in the first year post-transplant. The reduction in lumbar spine BMD at six months correlated with the cumulative dose of glucocorticoids [90]. Biochemical data suggests that bone loss after marrow transplantation is a high turnover state with increases in resorption markers and alkaline phosphatase activity [91–93], although some studies show an initial decrease, followed by a recovery towards baseline at six months [90]. In adult patients, non traumatic fractures occurred in 10.6% of patients three years after marrow transplantation [94].

Evaluation of candidates for transplantation

Abundant data is now available documenting the high prevalence of bone disease in candidates for all types of transplantation. Therefore, a complete skeletal evaluation is indicated. This evaluation should occur prior to transplantation, so that potentially treatable abnormalities of bone and mineral metabolism may be addressed and the skeletal condition of the patient optimised before transplantation. This evaluation should include a full history with particular emphasis upon risk factors for osteoporosis such as family history, prevalent low-energetic fractures, medical conditions (thyrotoxicosis, diabetes mellitus, renal disease, rheumatoid, and intestinal or liver disorders), poor lifestyle choices (physical inactivity, dietary calcium and vitamin D deficiency, excessive caffeine and alcohol intake, tobacco use), and exposure to drugs (diphenylhydantoin, antiepileptics, lithium, loop diuretics, glucocorticoids, prolonged and large doses of heparin, excessive doses of thyroid hormone etc.). Additional risk factors important in women include premature menopause, postmenopausal status, a history of anorexia nervosa or prolonged episodes of amenorrhea. In men, it is important to exclude hypogonadism. A physical examination should focus upon diseases that predispose to osteoporosis, such as hypogonadism, thyrotoxicosis and Cushing's syndrome. Risk factors for falling (sight, hearing, balance, and muscle strength) should also be assessed. Bone density of the spine and hip and plain radiographs of the thoracic and lumbar spine are the most important tests to obtain prior to transplantation. The biochemical evaluation should include a chemistry panel, thyroid function tests, intact PTH and vitamin D metabolites, and total and free testosterone, follicle-stimulating hormone (FSH), and luteinising hormone (LH) concentrations in men. Markers of bone formation (serum osteocalcin and bone-specific alkaline phosphatase) and resorption (urinary deoxypyridinoline or serum/urinary C and N-telopeptide) can also provide valuable information.

Summary of the evaluation of candidates for organ transplantation

— history and physical examination, with attention to risk factors for osteoporosis;
— bone densitometry by dual-energy X-ray absorptiometry (DXA);
— thoracic and lumbar spine radiographs or vertebral fracture assessment (VFA) by DXA technique;
— serum calcium, parathyroid hormone, 25-hydroxyvitamin D, thyroid function tests and bone turnover markers;
— in men, serum total and/or free testosterone, FSH, and LH;
— urine for calcium and markers of bone resorption (optional).

In the interest of cost control, it could be argued that the battery of biochemical tests may be unnecessary if the bone density measurement is normal and calcium and vitamin D supplementation are planned. However, if the pre-transplant bone density is low, the biochemical evaluation can alert the physician to the aetiology of low bone mass and guide appropriate therapy.

After transplantation, serum and urine indices of mineral metabolism are less crucial. Measurement of bone density remains important and should be performed at 6–12 monthly intervals for the first two years and annually thereafter. Bone biopsy may be necessary after renal transplantation, since many experts remain reluctant to use bisphosphonates in patients with adynamic bone disease, despite the lack of any data either supporting or excluding the use of these drugs

in this setting. Although transiliac crest bone biopsy remains a research tool, more histomorphometric studies would be very helpful in confirming the theories of the pathogenesis of transplantation osteoporosis. With the advent of non invasive techniques to measure bone quality reflecting microarchitectural changes and bone strength, new information on the pathogenesis and risk of fracture, as well as monitoring the benefits of therapy, may become more logical.

Management of transplantation osteoporosis

The general principles for the treatment of transplantation osteoporosis are similar to those for any type of osteoporosis. Therapy may be initiated during the waiting period before transplantation or in the initial 6- 12 months after transplantation. In addition, no long-term transplant recipient with established osteoporosis and/or fractures should be neglected. It must be emphasised that prevention of bone loss accompanying transplantation in a transplant recipient is probably more effective in reducing morbidity than the treatment of already established osteoporosis. During the waiting period before transplantation, rehabilitation therapy should be prescribed, as tolerated, to maximise the patient's condition and physical fitness. In general, calcium supplementation should be prescribed at doses of 1,000–1,500 mg per day, depending upon age, gender, menopausal status, and dietary intake. Either calcium citrate or calcium carbonate is acceptable; however, the carbonate form should be taken with food to enhance absorption and it can cause constipation. All patients should receive the Recommended Daily Allowance of vitamin D (800 IU daily). However, given the frequency of vitamin D insufficiency in these patients, the amount needs to be increased if measurement of 25-hydroxy-vitamin D is low. Hormone replacement therapy should be considered in all postmenopausal women, as well as in premenopausal amenorrheic women, where there are no contraindications to such therapy. Hypogonadal men should also be offered testosterone replacement. Generally accepted guidelines for gonadal hormone replacement should apply to these patients.

After transplantation, pharmacologic strategies should be instituted immediately to prevent bone loss and fractures. It must be emphasised that very few controlled prospective studies have been reported that support the use of specific therapies. The recommendations described in this chapter are based upon such data as currently exists, together with experience, acquired in similar clinical situations, and with supportive experimental evidence.

Summary of recommendations for the management of organ transplant recipients

— encourage transplant physicians to use the lowest possible dose of glucocorticoids and to consider alternative therapies for rejection (e.g. OKT3, rapamycin and mycophenolate mofotil);
— obtain bone mineral density routinely in patients accepted for transplantation and refer for evaluation/therapy all patients with low bone mass (T score between –1.0 and –2.5) or osteoporosis (T score –2.5);
— ensure calcium intake of 1,000-1,500 mg daily, both before and after transplantation;
— ensure vitamin D intake of 800–1,000 IU, or as needed to maintain serum 25-OHD concentrations above 30 ng/mL;
— encourage participation in a physical rehabilitation programme, both before and after transplantation;
— replace gonadal steroids in hypogonadal women and men;
— begin antiresorptive therapy, preferably a bisphosphonate, before transplantation in patients with antecedent osteoporosis/fractures or low bone mass;
— begin antiresorptive therapy, preferably a bisphosphonate, immediately or as soon as possible after transplantation in patients with normal or low bone mass and continue for at least the first post-transplant year;
— measure BMD at 6-12 monthly intervals for the first two years after transplantation.

Potential therapies for transplantation osteoporosis

Potential therapies for transplantation osteoporosis include:
— vitamin D and analogues;
— oestrogen;
— testosterone;
— calcitonin;
— bisphosphonates;
— newer drugs such as Denosumab.

Vitamin D and analogues

Administration of vitamin D or its analogues is usually recommended after transplantation. Sambrook et al. [95] found calcitriol to be efficacious in patients with glucocorticoid-induced osteoporosis. Although the role of vitamin D and its analogues in transplantation osteoporosis remains unclear, these investigators recently reported, in an abstract form, that calcitriol (0.5–0.75 mcg/day) prevented spine and hip bone loss during the first six months after heart or lung transplantation and was as effective as cyclic etidronate [96]. Hypercalcemia and hypercalciuria are the major side

effects of the therapy with the active analogue and may develop either suddenly or at any time during the course of treatment. Thus, frequent urinary and serum monitoring may be required. If hypercalcemia occurs, it must be recognised and reversed promptly because of the adverse effects on renal function and the life-threatening potential of a severely elevated serum calcium concentration. Supplemental calcium and any vitamin D preparations must be discontinued until calcium and 25(OH)D_3 values normalise. Although one may be tempted to permanently discontinue pharmacologic doses of vitamin D or its metabolites in view of the necessary serial monitoring and potential dangers, it seems reasonable to recommence therapy at a lower dose. The exact mechanism by which vitamin D and its analogues may influence post-transplantation bone loss is uncertain. They may overcome glucocorticoid-induced decreases in intestinal calcium absorption, reduce the potential for secondary hyperparathyroidism, promote differentiation of osteoblast precursors into mature cells, or influence the immune system and potentiate the immunosuppressive action of cyclosporine [97, 98].

In summary, given the requirement for serial monitoring and the narrow therapeutic window with regard to hypercalcemia and hypercalciuria, we regard pharmacologic doses of vitamin D and its analogues as adjunctive rather than primary therapy for the prevention and treatment of transplantation osteoporosis.

Oestrogen

In postmenopausal women, or premenopausal women with amenorrhea or irregular menses, oestrogen replacement should be recommended, provided that there are no contraindications. The dose is the same as that used for prevention or therapy of postmenopausal osteoporosis and may be given either orally or transdermally. In women with an intact uterus, progesterone must be prescribed in addition to prevent endometrial cancer. Continuous rather than cyclic therapy is preferred after transplantation, as oestrogen enhances hepatic metabolism of cyclosporine (and presumably FK506) and may theoretically compromise immunosuppression, although it is not known if such an effect occurs in these patients. For patients who cannot take oestrogen, tamoxifen [99] or newer selective oestrogen receptor modulators (SERMs) such as raloxifene [100], may well be a suitable alternative. Although no trials of these drugs in organ transplant recipients have been published, raloxifene has been shown to reduce cyclosporine-induced bone loss in the rat model [101]. Hyperlipidaemia, produced by glucocorticoids, cyclosporine, and its analogues, may also be ameliorated by oestrogen, tamoxifen, and raloxifene, although no systematic studies have been conducted that address this particular issue.

Few trials have evaluated the efficacy of oestrogen in preventing bone loss and fractures after transplantation. Therefore, the recommendation to prescribe oestrogen for this purpose is based in part upon the observation that 17-estradiol prevents cyclosporine-induced bone loss in the oophorectomised rat [102]. In addition, a wealth of clinical data supports the protective effect of oestrogen on the skeleton and one cross-sectional study suggests that oestrogen and progesterone therapy are associated with higher bone density in women on glucocorticoids [103]. The exact mechanism by which oestrogen protects the skeleton is unknown. However, oestrogen is associated with the inhibition of bone resorbing cytokines, such as IL-1, IL-6, and IL-11. Recent work implicates IL-1 [104] as a potential candidate in the pathogenesis of high turnover bone loss in the cyclosporine-treated rat, making it conceivable that oestrogen may modify bone loss in this model by inhibiting this cytokine. However, potential side effects on cardiovascular and breast cancer have to be recognised.

In bone marrow transplantation, in which hypogonadism is a predominant feature, 12 months of hormone replacement therapy (HRT) was associated with significantly increased bone density in a small number of women, without adversely affecting liver enzymes [88]. There may also be an advantage to combine HRT with a bisphosphonate, particularly in the light of a recent study in which an addition of alendronate in a group of postmenopausal women on stable doses of HRT caused a significant increase in BMD, but no fracture data for combined therapy is available.

Testosterone

Men with serious chronic illnesses, such as hepatic, renal or cardiac failure, are commonly hypogonadal. In addition, high doses of both glucocorticoids [103] and cyclosporine (greater than 15 mg/kg body weight) suppress the hypothalamic–pituitary–gonadal axis and produce hypogonadism [105, 106]. Testosterone deficiency, both before and after transplantation, may contribute to the risk of bone loss and fractures [67, 69, 95]. However, given the complexity of the clinical situation after transplantation, it is not possible to ascertain whether testosterone deficiency is an independent risk factor for fracture or bone loss. In any event, since androgen deficiency is known to cause low bone mass in men, it is not unreasonable to prescribe testosterone in men who are truly hypogonadal. Testosterone therapy is not without risks. Of special concern is the potential for induction or exacerbation of hyperlipidaemia in

patients already prone to accelerated atherosclerosis from hypertension, diabetes, glucocorticoid, and cyclosporine drug therapy [107]. In addition, prostatic hypertrophy and liver abnormalities are side effects of androgen therapy.

However, after successful transplantation, serum testosterone levels frequently normalise, so that androgen therapy may only be required as a temporary measure [67, 69, 95]. Thus, it can be argued that temporary administration of testosterone after transplantation constitutes physiologic hormone replacement rather than pharmacological therapy. The risks of prostatic hyperplasia and liver abnormalities can be minimised by administering testosterone transdermally, rather than by injection. If testosterone therapy is recommended, patients must be cautioned about potential risks and benefits. Monitoring should include monthly measurement of serum lipids and hepatic enzymes and regular prostate examinations. Whether testosterone replacement with calcium supplements and vitamin D is sufficient to prevent transplantation osteoporosis is not known. However, it would be more prudent to include additional antiresorptive therapy to protect the skeleton.

Calcitonin

In the therapy of osteoporosis, calcitonin has been shown to increase bone density and reduce vertebral fractures in patients with osteoporosis, although it is not as effective as other current therapies. Despite many years of experience, the optimum dose, the route of administration and efficacy of continuous versus intermittent dosing remain unclear. Both injectable and inhaled calcitonin has been used successfully to treat glucocorticoid-induced bone loss in humans [108]. While its use in transplant recipients has not been established by controlled trials, an experimental work in a rat model has demonstrated that cyclosporine-induced bone loss can be prevented by calcitonin [109]. However, literature reports are rather far from being consistent regarding the use of calcitonin to prevent bone loss and fractures after transplantation. The usual practice is to prescribe synthetic salmon calcitonin, 100 units daily by subcutaneous injection, or intranasal calcitonin (100–200 IU), as soon as immunosuppressive therapy is begun. This intranasal dose varies according to the transplanted organ [110, 111]. Valero et al. administered either injectable calcitonin or etidronate to liver transplant recipients and found that lumbar spine bone density increased by 6–8% with no difference in the efficacy between the drugs [112]. By contrast, other investigators have not found calcitonin to be particularly effective [111, 113].

Bisphosphonates

Bisphosphonates, which act by inhibiting osteoclastic bone resorption, have been used successfully to prevent and treat glucocorticoid-induced bone loss. Published studies include first-, second-, and third-generation bisphosphonates, such as etidronate, pamidronate, tiludronate, alendronate, risendronate, ibandronate and, most recently, zolendronate [114–123]. Because transplantation osteoporosis can be considered as one form of glucocorticoid-induced osteoporosis, and as cyclosporine- and tacrolimus-induced bone loss are characterised experimentally by simultaneously increased bone formation and bone resorption, bisphosphonates may prove to be highly successful in the prevention of transplantation osteoporosis. Some [121–123], although not all [124], studies suggest that bisphosphonates can prevent bone loss and fractures after transplantation. A single intravenous dose of pamidronate (60 mg), given during the first two weeks after transplantation and followed by cyclical editronate for the remainder of the first year, prevented lumbar spine and femoral neck bone loss and significantly reduced the fracture incidence, compared to patients who received only calcium and 400 IU of vitamin D [122]. A similar experience has been reported with intravenous pamidronate in kidney [121] and, even more dramatically, in liver [123] transplant recipients, where fractures were prevented in the group treated with pamidronate. Alendronate has been used in the prevention of osteoporosis after cardiac, liver, and renal transplantation and the Shane group's own clinical experience suggests that this drug is effective in this setting [125]. At present, these drugs constitute the most promising approach to the prevention of this often crippling form of osteoporosis. As with other forms of therapy, many issues remain to be resolved, such as whether continuous or intermittent (cyclical) therapy should be used and at what level of renal impairment these drugs should be avoided. However, a very recent study, intravenously administering a long-acting potent N-containing bisphosphonate, zoledronic acid, in a prospective, double blind, placebo controlled trial to 62 post-liver transplant patients at seven days, and then at months 1, 3, 6, and 9 has shown that, after adjusting for weight and PTH levels, BMD increased at the lumbar spine, femoral neck and total hip by 1.1%, 2.7%, and 2.4%, respectively after one year [120]. Before the administration of N-containing bisphosphonates, the patient should be made aware of the very rare but important side effects, such as osteonecrosis of the jaw, subtrochanteric fractures and joint and muscle pains besides the upper GIT disturbances, which can be overcome with intravenous preparations. However, as no direct cause and effect for these side effects has

been proven, they should not preclude physicians from prescribing these drugs as the first line of therapy.

Other therapeutic options

At the present time, the use of the lowest possible doses of glucocorticoid and calcineurin phosphatase inhibitors offers the best option. The regimens which do not use either glucocorticoids or calcineurin inhibitors may prove the most advantageous to the skeleton. A human monoclonal antibody to RANKL (denosumab) has recently been approved to treat osteoporosis [126] and trials are underway to examine its effect in glucocorticoid induced bone disease. This may have a place in post-transplant disease, as it potently inhibits bone resorption and can be given twice per annum as a s.c. injection. This drug inhibits the osteoclast cell cycle but has little or minimal side effects on renal function and may exacerbate hypocalcemia seems promising but no data exists in transplant patients. Currently, the most exciting areas of investigation involve the agents that stimulate bone formation (growth hormone, growth hormone releasing peptide, PTH or its analogues, the IGF family, including the IGF binding proteins, prostaglandins, particularly of the E series [127, 128], and the TGF-superfamily, including bone morphogenic protein). PTH (1-34) and other agonists of PTH1 receptor may enhance cell differentiation into the osteoblastic line and reduce their adipogensis [129, 130], but further studies are necessary to prove this hypothesis. Antibodies, which inhibit the action of sclerostin, a major factor in the WNT-beta catenin pathway promoting bone resorption, are also undergoing clinical trials and this may have impressive anabolic effects. Such drugs are of theoretical value, particularly in the setting of glucocorticoid therapy, where the inhibition of bone formation is a major contributor to bone loss. Newer analogues of vitamin D, that promote calcium absorption and stimulate bone formation without hypercalcemia [131], may also be valuable additions to the therapeutic armamentarium. Prospective, controlled clinical trials are sorely needed, not only to evaluate the existing regimens, but also to study these newer therapies. This may be unrealistic, given the trial design, the required number of patients, different immunosuppressive regimens, comorbid conditions etc.

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