Chapter 10
Metabolic Bone Disease Following Organ Transplantation

Se-Min Kim, Sol Epstein, Tony Yuen, Michael Pazianas, Li Sun, Barbara Murphy, and Mone Zaidi

Introduction

Solid organ transplantation offers a valuable therapeutic option to patients with terminal organ failure. Over the years, technical and therapeutic progress, especially the advent of new immunosuppressive agents, has significantly improved outcomes. The survival rate, for example, of a kidney transplant recipient at 1-year today exceeds 95 % [1]. Graft half-life has also increased dramatically almost to 10 years [2]. As transplant recipients live longer, patients and health care providers alike have become increasingly aware of complications related to transplantation.

Metabolic bone disease, such as osteoporosis and avascular necrosis (AVN), in post-transplant patients are ones that are most debilitating. They take a significant toll on wellbeing, with pain and discomfort. Another major issue is the high incidence of hip and vertebral fractures that increases both morbidity and mortality. Many epidemiologic studies have shown a strong association of the risk of fracture and solid organ transplantation. Organ transplant recipients were reported to have almost a fivefold increase in the risk of any fracture compared to general population [3]. Even when compared to the patients on the transplantation waiting list, the risk of fracture still remains significant. Of note is that the relative risk of hip fracture increased ~34 % following transplant, with the highest incidence in the

S.-M. Kim, MD • S. Epstein, MD • T. Yuen, PhD • L. Sun, MD, PhD
B. Murphy, MD • M. Zaidi, MD, PhD (✉)
Department of Medicine, Mount Sinai School of Medicine,
1 Gustave L. Levy Place, New York 10029, NY, USA
e-mail: ksm8099@gmail.com; bonedocsol@aol.com; tony.yuen@mssm.edu; li.sun@mssm.edu; barbara.murphy@mssm.edu; mone.zaidi@mssm.edu

M. Pazianas, MD
Department of Orthopedics, Oxford University, Oxford, UK
e-mail: michael.pazianas@ndorms.ox.ac.uk

R.K. Aaron (ed.), *Diagnosis and Management of Hip Disease*,
DOI 10.1007/978-3-319-19905-4_10

early post-transplant period (3.3 fractures per 1000 person-year) [4]. Likewise, a longitudinal study (1997–2010) also showed a high hip fracture rate (3.8 fractures per 1000 person-years) [5].

It is important to note that in a post-transplant situation, fractures can, and do occur at relatively conserved, and often at near-normal bone mineral density (BMD) values. Fragility fractures, in themselves, in such cases initiate a formal diagnosis of severe osteoporosis. However, the diagnosis of osteoporosis or low bone mass (formerly termed osteopenia) based on BMD is equally common. A population-based study from Taiwanese National Transplant Registry reported significantly higher incidence of osteoporosis (and related fractures) in transplant patients compared with general population. The overall hazard ratio (HR) for osteoporosis and osteoporosis-related fractures was 5.14 (95 % CI, 3.13–8.43) and 5.76 (95 % CI, 3.80–8.74), respectively [6].

The risk of fracture in this population is clearly multifactorial. Decreased BMD from metabolic bone disease, a previous fracture, old age, a first-degree relative with fracture, low body weight, smoking, rheumatoid arthritis or celiac disease, glucocorticoid use, and excessive alcohol consumption are all considered risk factors for fracture. A meta-analysis of studies on renal transplant recipients suggested that advanced age, female gender, and a history of diabetes were compounded to increase fracture risk [7]. The higher rate of fracture in diabetes among renal transplant recipients was also noted in a separate study [8].

Metabolic Bone Disease After Transplantation

Pre-existing Bone Disease

Most transplant patients also have pre-existing bone disease, most prominently those with chronic kidney disease (CKD). Therefore, *albeit* challenging, it is imperative to assess, prevent, and treat metabolic bone disease in pre- and post-transplant period.

Renal Osteodystrophy

Renal osteodystrophy arises fundamentally from the disruption in calcium and phosphate homeostasis. The kidney is a principal organ that regulates blood calcium and phosphate levels. Parathyroid hormone (PTH) increases phosphate excretion by the inhibition of Type I/IIa sodium-phosphate co-transporter in renal proximal tubule. It also stimulates the activity of 1α-hydroxylase, which, in turn, hydroxylates 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol. In a negative feedback loop, 1,25-dihydroxycholecalciferol produced in kidney inhibits PTH production. It is important to note that in addition to increasing calcium absorption from the gut, vitamin D also stimulates phosphate absorption, and in turn inhibits PTH secretion indirectly. The recent advance in the understanding of fibroblast growth factor FGF-23, FGFR and the klotho complex also helps to shed light on the

bone-kidney-parathyroid gland axis in calcium and phosphate homeostasis. Bone-derived FGF-23 suppresses Na/P co-transporter and excretes phosphate. Klotho, which is expressed in kidney and parathyroid gland, works as a cofactor promoting FGF-23 activity. FGF-23 level is high in CKD patients, and has been studied as a marker for CKD. Hyperphosphatemia is a hallmark of CKD despite elevated FGF-23 level, and it is postulated that FGF-23 does not exert its phosphaturic effect in the absence of klotho. As a matter of fact, marked reduction of urinary klotho was observed in early phase of CKD preceding FGF-23 elevation and electrolyte imbalance. To maintain the CaxP product within a normal range, elevated serum phosphate pushes serum calcium down; this causes the earliest elevations in serum PTH levels (secondary hyperparathyroidism). There is some evidence that hyperphosphatemia in CKD can directly stimulate PTH synthesis and contribute to parathyroid hyperplasia [9, 10].

As renal function deteriorates further, activity of 1α-hydroxylase in proximal tubules also decreases. With decreased enzyme activity and insufficient reserves of vitamin D, 1,25-dihydroxyvitamin D levels fall, which also stimulate the parathyroid gland contributing further to secondary hyperparathyroidism. Other cytokines such as IL-1, -6, and -11 also play a part in increased PTH expression [9]. Inasmuch as the mechanism of calcium and phosphate regulation is intricate, the characteristics of renal osteodystrophy vary among individuals. Renal osteodystrophy is, traditionally, classified into *four* different categories: osteitis fibrosa cystica, adynamic bone disease, osteomalacia, and mixed renal osteodystrophy [11].

Osteitis fibrosa cystica, or high turnover disease, is due to secondary hyperparathyroidism. Continuous exposure to PTH is key to the pathophysiology of high bone turnover [12]. At the same time, mesenchymal precursor cells differentiate into fibroblast-like cells, resulting in marrow fibrosis. However, and paradoxically, elevated PTH levels seem to be required to maintain normal rates of bone formation in patients with CKD. Uremia can itself cause PTH resistance, with the down-regulation of PTH receptors, increased levels osteoprotegerin and decreased bone morphogenetic proteins (BMPs) [13].

One of the tenants of CKD therapy is to reduce the effects of secondary hyperparathyroidism on the skeleton. This means that serum PTH must be suppressed therapeutically, using either phosphate-binding agents or calcium sensing receptor antagonists. Excessive suppression of PTH leads to *adynamic bone disease* (also termed low-turnover bone disease). Thus, *per* the Kidney Disease: Improving Global Outcomes (KDIGO) position statement, it is recommended that in patients with stage 5 CKD, serum PTH should be maintained at 2–9 times the upper limit if normal in order to prevent low-turnover disease [14]. Adynamic bone disease is more common in diabetic patients [11].

In addition to adynamic bone disease, CKD patients also display varying degrees of *osteomalacia.* In osteomalacia, mineralized bone volume is low because of an increase in mineralization lag time with relative osteoid excess and thick osteoid seams. This mineralization defect arises from vitamin D deficiency and resistance.

These subtypes of renal osteodystrophy tend to co-exist as *mixed renal osteodystrophy* and this ambiguous classification complicates management. In response, KDIGO released the bone turnover, mineralization, and bone volume (TMV) classification [15].

Malluche et al. [16] reported the histomorphometric analysis of 630 cases using TMV in CKD patients. They reported that 58 % of patients exhibited low bone turnover, with 18 % and 24 % patients exhibiting normal and high turnover respectively. This finding is consistent with Moe et al., who demonstrated that low-turnover bone disease was more prevalent in CKD patients [15]. Interestingly, Malluche et al. also found a racial difference, with whites exhibiting predominantly low-turnover disease (62 %), whereas blacks displayed mostly normal or high-turnover (68 %). Osteomalacia was observed in only 3 % of the study participants [16].

As expected, the risk of fractures in CKD patients on dialysis is many times higher than general population. Likewise, given their co-morbidities, mortality from hip fracture is also significantly higher in dialysis patients [17–19]. This has led to efforts to screen patients at risk of fracture. Notably, Coco et al. showed that patients with lower PTH levels were more likely to sustain hip fractures than patients with higher PTH levels [17]. However, this inverse relationship with PTH was not confirmed with other studies. Danese et al. [19] reported U-shaped relationship with risk of fracture and PTH level with the lowest risk observed at ~300 pg/mL, suggesting that the risk of fracture is high at both ends. So far the optimal PTH level in terms of skeletal health in CKD patients is still unclear.

Finally, renal osteodystrophy also causes heterotopic calcification, mainly arterial calcification, which is a major predictor of cardiovascular mortality. It is thought to be triggered by dyslipidemia, oxidative stress, advanced glycation end-products (AGEs), and hyperphosphatemia, which cause transformation of vascular smooth muscle cells to osteogenic cells. In vitro studies show that high phosphate levels directly stimulate vascular smooth muscle cell transformation to “osteoblast-like” cell [20, 21].

End-Stage Liver Disease

End-stage liver disease is the cause of pre-existing hepatic osteodystrophy in >80 % of patients undergoing liver transplantation evaluation [22]. It is more prevalent in patients with cholestatic liver disease, such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) [23]. Histomorphometric analysis of bone biopsies from patients with PBC and PSC have shown decreases in bone volume and reduced bone formation. Osteoblast numbers, mean wall thickness, and mineralization rate are all reduced. Elevated bone resorption has also been documented with increased areas of eroded surface and osteoclast numbers [24].

The pathophysiology of hepatic osteodystrophy is not clearly understood. Unconjugated bilirubin reduces osteoblast proliferation in vitro [25]. Hyperbilirubinemia not only down-regulates *Runx2* expression, but also increases the RANKL/OPG ratio favoring bone resorption [26]. However, an association between serum bilirubin levels and BMD has not yet been established [27]. Finally, there are other risk factors in patients with chronic liver disease that contribute to bone disease; these include alcoholism, hypogonadism, vitamin D deficiency, and genetic factors [28].

End-Stage Heart Failure

About one third of heart transplantation candidates showed osteopenia and osteoporosis [29]. Intrinsic risk factors with heart failure, such as advanced age, vitamin D deficiency, CKD, and medication use (loop diuretics) can all contribute to the increased risk of osteoporosis in heart failure patients [30]. Furthermore, although unproven, immobilization and limited physical activity due to exercise intolerance are also considered to have a negative effect on skeletal health.

The pathophysiologic link between heart failure and osteoporosis is not well understood. A few studies have suggested that hyperparathyroidism in heart failure may be a contributor [31, 32]. Activation of the renin-angiotensin-aldosterone (RAA) system in congestive heart failure and hypertension has also been considered as a culprit. Osteoblasts and osteoclasts both express angiotensin II receptors. Angiotensin II induces the differentiation and activation of osteoclasts directly, and also increases *Rankl* expression in osteoblasts, which, in turn, stimulates osteoclast differentiation [33–35]. Leistner et al. [36] provided direct evidence for increased RANKL/OPG ratio in patients with systolic heart failure; this was repeated in the mouse following the induction of ischemic cardiomyopathy.

End-Stage Lung Disease

About 70 % of patients of lung failure have osteoporosis based on reports regardless of type of underlying lung disease [37, 38]. Hypoxia, hypercapnia, smoking history, and glucocorticoid exposure all contribute to bone disease [39]. Interestingly, Kneidinger et al. demonstrated that patients with COPD have decreased Wnt/β--catenin signaling; the latter pathway plays a key role in osteoblast differentiation [40].

Hematopoietic Disorders

About 70 % of 81 patients display normal BMD values prior to bone marrow transplantation. Although patients with high-dose chemotherapy tend to show lower BMD, only 4 % have documented osteoporosis [41]. However, a population-based cohort study of myeloproliferative disorders showed an increased risk of fracture [42]. Chemotherapy, steroid use, hypogonadism, and other co-morbidities likely contribute. In contrast, in pediatric patients with acute leukemia, 75 % of patients showed radiographic abnormalities, among which 40 % had osteoporosis, 20 % had pathologic fractures, and 1.2 % had AVN [43]. As expected, the incidence of fracture in children with acute lymphoblastic leukemia was very high [44]. Lean mass, age at diagnosis, systemic and/or intrathecal chemotherapy were found to predict bone loss [45].

Bone Disease in Diabetes Patients

Diabetes has an unequivocal and strong association with fracture risk, besides being a prevalent co-morbidity in patients with end-stage organ failure. Pre-transplant diabetes was found to be an independent risk factor for fracture (OR: 1.94, 95 % CI: 1.5–2.6) when adjusted for age, sex, previous fracture, and immunosuppressant (including glucocorticoid) use [46]. Furthermore, one of the most common etiologies of end-stage renal disease is diabetes, and patients with ischemic cardiomyopathy often have diabetes. One survey reported about 30 % of patients with liver cirrhosis had diabetes [47]. About 20 % of renal transplantation patients have type 1 diabetes mellitus, and a high rate of fracture (40 %) was noted in diabetes group compared to non-diabetes group. Of note, diabetic patients had fractures early in the post-transplant period, commonly in their appendicular skeleton, such as ankle and foot fractures [8]. The impact of diabetes on skeletal health after transplantation is therefore highly significant.

Transplantation-Related Risks

Immunosuppressant Use

Glucocorticoid-Induced Bone Loss and Osteonecrosis

Glucocorticoid use is strongly associated with the risk of vertebral and non-vertebral fracture. The risk of fracture is dose- and time-dependent, and is seen at doses as small as 2.5 mg prednisone per day when utilized for prolonged periods [48, 49]. Of note is that longer duration and continuous use shows an approximately fivefold increase in the risk of hip fracture [48].

The mechanism of glucocorticoid-induced osteoporosis is relatively well studied. The high fracture risk arises mainly from decreased bone formation. Histomorphometry shows decreased trabecular bone area and trabecular width. Bone formation rate (BFR) and mineral apposition rate (MAR) are consistently reduced in glucocorticoid users in a dose-dependent manner [50]. Glucocorticoids inhibit osteoblasts, but can also stimulate osteoclasts. Osteoblast differentiation is reduced with evidence for increased apoptosis [50]. Glucocorticoids also negatively affect osteocytes, resulting in the accumulation of micro-damage, which leads directly to impaired bone quality and a high fracture risk [50]. It has recently been shown that osteocytes can also undergo autophagy with low dose glucocorticoids [51]. Increased osteoclast perimeters, but with decreased osteoclast progenitor number has also been noted with glucocorticoid use [50]. An effect of glucocorticoids on osteoclasts was directly demonstrated in transgenic mice that over-expressed 11β-HSD2, an enzyme that converts cortisol to cortisone [52].

AVN is another debilitating skeletal complication in transplant recipients. Its prevalence has, however, decreased dramatically with introduction of newer immunosuppressants and reduced steroid doses. A recent epidemiologic study over

9 years reported the prevalence of AVN at 4.6 % in the transplant recipients. Male patients were more affected than females, and the femoral head was most commonly involved. Glucocorticoid usage is strongly associated with AVN [53]. A meta-analysis of 22 studies showed a strong correlation between daily total dose of glucocorticoid and AVN rate ($r = 0.61 - 0.80$) [54]. The cumulative steroid dose was statistically significantly higher in AVN group compared to control group [55].

The histopathology of AVN is characterized by osteocyte necrosis with or without loss of structural integrity [53]. On light microscopy, osteocyte necrosis is reflected by condensed nuclei and empty osteocyte lacunae. Hematopoietic marrow necrosis and surrounding interstitial edema are also commonly seen. With glucocorticoid use, fat emboli and lipid deposits increase intraosseous extravascular pressure, which compromise blood flow and cause ischemia [53]. The repair process with capillary angiogenesis and revascularization begins in the area of necrosis. Bone resorption occurs followed by bone formation; however, reduced bone formation due to glucocorticoid use leads to net bone loss. This net bone loss, *albeit* locally, leads to the loss of integrity and structural collapse [56].

Skeletal Effects of Calcineurin Inhibitors

Calcineurin inhibitors, a commonly used class of immunosuppressant drugs, notably cyclosporine (CsA) and tacrolimus (FK506), inhibit the activity of the enzyme calcium/calmodulin-sensitive phosphatase, calcineurin. Our group has shown that calcineurin plays a major important role in bone remodeling. The first evidence came from Epstein's group: cyclosporine injections in vivo were found to result in significantly elevated levels of bone resorption and trabecular bone loss [57]. This was shown to be T cell, dose, as well as duration dependent. Subsequent, histomorphometric studies in patients with cyclosporine monotherapy showed not only increases in osteoclast activity, but also decrements in osteoblastic bone formation [58]. CsA has been found to be more detrimental to bone than FK506 [59].

We found that calcineurin Aα, the target for both CsA and FK506, was expressed both in osteoblasts and osteoclasts [60]. We thereafter went on to characterize the skeletal phenotype of a mouse in which calcineurin Aα was deleted genetically [61]. We found that 6-week-old calcineurin $A\alpha^{-/-}$ mice were osteoporotic both at cancellous (lumbar spine) and cortical sites (femur and tibia). A marked reduction in cortical bone thickness and a modest reduction in trabecular bone were obvious upon histological examination. Labeling with tetracycline showed a (~60 %) reduction in MAR, which indicated attenuated bone formation [62]. Surprisingly, however, while there was little difference in resorbed surfaces in calcineurin $A\alpha^{-/-}$ mice, osteoclast formation from $A\alpha^{-/-}$ hematopoetic stem cells was markedly impaired ex vivo (~40 %). Overall, the studies confirmed that either the inhibition of calcineurin activity by chemical inhibitors, such as CsA or FK506, or genetic deletion of a predominant isoform, caused osteoporosis reflecting the clinical situation.

To substantiate this concept, we also performed gain-of-function studies using osteoblasts and osteoclasts. We created a fusion protein between calcineurin Aα and TAT, a 12 amino acid-long, HIV derived, Arg-rich sequence that was able to

traverse cell membranes [63]. We transduced mature osteoclasts, osteoclast precursor (RAW264.7) cells, and pre-osteoblastic (MC3T3.E1) cells in separate experiments, essentially with 100 % efficiency. The transduced protein stimulated the expression of the osteoblast differentiation markers *alkaline phosphatase*, *bone sialoprotein* and *osteocalcin* [62]. Likewise, it significantly enhanced osteoclast formation from both RAW-C3 cells and bone marrow precursors [64].

High turnover bone disease with elevated bone turnover markers have been noted clinically after the initial effect of glucocorticoids causing a low turnover, but when stopped the CsA effect becomes evident. This is accompanied by continuous bone loss at cortical bone sites the femur while the spine tends to recover. Histomorphometry performed years later in patients post-transplant only on CsA showed elements of high turnover [58]. Renal patients on CsA monotherapy post-transplant continued to lose BMD [65].

Finally, it is notable that calcineurin inhibitor-free immunosuppression protocols have worse outcomes with regard to long-term treatment outcome compared to steroid-free regimens.

Other Immunosuppressants

Sirolimus (rapamycin), an mTOR inhibitor, has not been studied in detail regarding its bone effects, but is considered safer. Rapamycin in vivo does not show significant loss of trabecular bone compared to CsA [66]. Bone turnover markers, including urine N-telopeptides and serum osteocalcin, were also consistently lower in sirolimus—compared with CsA-treated patients [67]. Consistent with this, patients on a sirolimus-based regimen showed reduced serum levels TRAP-5b and RANKL compared with calcineurin inhibitor-based regimens. In vitro studies have, however, shown reduced osteoclast differentiation and osteoclast precursor proliferation [68]. This anti-resorptive property of sirolimus might actually be beneficial in reducing the accelerated bone loss in the early transplant period. It allows the use of lower dose of glucocorticoids and calcineurin inhibitors without compromising organ survival. Everolimus, also an mTOR inhibitor, might have beneficial effects on bone, as mTOR inhibition is associated with decreased osteoclast survival and activity [69].

Another immunosuppressant, mycophenolate mofetil (MMF) did not show significant effects on histomorphometric parameters, although osteocalcin levels were suppressed in vivo [70]. The effects on bone of newer immunosuppressants, including monoclonal antibodies, such as alemtuzumab or basiliximab, have not been investigated.

Other Considerations in Renal Transplant Patients

Effect of Hypophosphatemia in Renal Transplant

Hypophosphatemia, a frequent accompaniment in the early phase after renal transplant, is multifactorial [71]. Persistently elevated PTH and/or FGF-23 will increase phosphate excretion from healthy transplanted kidneys. That said, there continues

to be relative 1,25-vitamin D deficiency as renal function may not be not fully restored—this will result in a persistent lowering of intestinal phosphate absorption. Immunosuppressant, such as glucocorticoids and CsA, can by themselves inhibit renal phosphate reabsorption [72, 73]. Persistent hypophosphatemia will invariably negatively affect bone mineralization, and result in skeletal complications that we have learned from diverse pathologies, such as X-linked hypophosphatemic rickets and oncogenic osteomalacia.

Persistent Secondary Hyperparathyroidism in Renal Transplant

PTH levels normalize during the first 3–6 months of transplant as renal function normalizes. Functional parathyroid gland mass is thereby reduced in most cases, except for those with monoclonal glandular hyperplasia [74, 75]. This category of patients normally has a highly elevated PTH level at the time of transplant [76]. Persistently increased PTH can result in hypercalcemia (and hypophosphatemia). However, bone turnover does not correlate well with PTH levels in transplant recipients. Histomorphometric parameters do not correlate with hypercalcemia in patients with post-transplant hyperparathyroidism [77], to the extent that PTH may not be the main determinant of bone turnover following transplant [78]. Recently, persistent hyperparathyroidism has been shown to be a major determinant of fractures 5 years post-transplantation [79].

Vitamin D Levels in Renal Transplant Patients

Vitamin D insufficiency and deficiency continues to be prevalent in patients with transplant [80–84]. 1,25-dihydroxyvitamin D levels often remain low, even after renal function improves. This could be caused, in part, by the normally insufficient vitamin D reserves in patients with renal transplant. Vitamin D deficiency could indeed be prolonged even after successful transplantation [85], particularly since other factors related to transplantation, such as immunosuppressant use, can also directly affect vitamin D metabolism.

Acute Rapid and Severe Bone Loss Post-transplantation

Rapid and acute bone loss may be promoted by secondary causes of osteoporosis, such as glucocorticoid-induced bone disease, immobilization, organ transplantation, and acute estrogen withdrawal. We have termed this as acute, rapid and severe bone loss (ARSBL) [86]. The etiology is multifactorial, arising from glucocorticoid and calcineurin inhibitor use, pre-existing osteodystrophy, hyperparathyroidism, poor nutrition, immobilization, and vitamin D deficiency.

BMD by dual energy X-ray absorptiometry (DXA) scan is, as of now, the most cost-effective and widely utilized non-invasive measurement of skeletal health. It is most generally accepted as a predictor of the risk of fracture in all population. The decrease of 1 standard deviation (SD) in BMD increases the relative risk of fracture about twofold [87]. DXA measures vertebral spine, radial shaft, and hip, respectively, representing mainly trabecular bone, cortical bone, or both types of bone. However, it is well known that BMD declines may not explain the high fracture risk noted, for example, with high dose glucocorticoid therapy. Patients can and do fracture with near-normal BMDs.

Longitudinal studies have shown a correlation between time-elapsed after transplantation and change in BMD. Regardless of type of organ, a significant decline occurs in early phase, mostly within 3–12 months; this is generally followed by BMD stabilization or even an increase at the spine [88–93]. Notably, Julian et al. showed that BMD at the lumbar spine decreased by 6.8 % from the time of transplantation, with more than half of their patients falling below the "fracture threshold." The bone loss during the first 6 months post-transplant contributed significantly to the overall bone loss of 8.8 % over 18 months. In this context, postmenopausal women may lose bone at the rate of 2 % per year in the early years of menopause, when such declines are most rapid. Thus, the bone loss in transplant patients is, by comparison, much more rapid and acute. Interestingly, however, BMD in radial shaft was near normal to begin with (*Z* score: −0.67) and there was no significant bone loss noted within 6 months [91]. This suggested that trabecular bone was more affected than cortical bone during post-transplantation period.

Yet another study looking at renal transplant recipients within first 5 months calculated an absolute mineral loss of 40 g (total skeleton has ~1 kg calcium). The bone was lost mainly in the trabecular bone compartment, with the rate of vertebral BMD loss at 1.6 ± 0.2 % per month [94]. This rate was significantly higher rate than that reported (1.7 % per year) in renal transplant recipients based on 8-year longitudinal study [95].

This pattern of ARSBL was also noted in other organ transplantation. Among patients with orthotopic liver transplantation, a high rate of bone loss at the lumbar spine (15.9 % per year) was observed in the first 4 months. Pre-transplant BMD at lumbar spine was in the osteopenic range (*Z*-score: −1.39), which further decreased (1.77) at the 4-month post-transplant time point. Almost 36 % of patients in this cohort developed fractures within 1 year following transplantation [96]. Interestingly, BMD slowly stabilized and even increased after 8 years follow-up [97]. Consistent with these dramatic declines, a cross-sectional study reported decreases in BMD by 8.6 ± 1.0 % at lumbar spine and by 11.3 ± 2.2 % at the femoral neck within 1 year [92]. Likewise, while patients undergoing bone marrow transplantation displayed normal pre-transplant BMDs at both lumbar spine and femoral neck, their femoral neck BMD declined whereas their lumbar spine remained conserved 3 months following transplantation [98, 99].

The noted decrements in BMD in transplant patients pose a significant risk of fracture [3, 5, 6, 100]. This risk is especially high in early period, due to the rapidity of bone loss over a short time [4]. Pre-transplant BMD and length of use of glucocorticoids are key determinants of the high risk of fracture [93].

Some studies have, however, questioned the association between BMD and fracture risk in transplant patients. Although BMD is most generally used as a surrogate for fracture risk, the association is relatively weaker in transplant recipients compared with general population [100, 101]. The insensitivity of areal BMD (aBMD) has been noted in patients treated with glucocorticoids and in patients with diabetes. Patients with type 2 diabetes tend to have higher BMDs, despite the increased risk of fracture [102].

It is well known that areal BMD (measured by DXA) is not able to assess bone quality, including its microarchitecture, which is as crucial as BMC in determining bone strength. Newly developed technologies like quantitative computed tomography (QCT), micro-MR imaging, finite element modeling (FEM), and microindentation allow us to examine cortical and trabecular bone compartments separately, as well as to assess mechanical properties of bone directly. Rehman et al. [103] demonstrated that volumetric BMD (vBMD) at the lumbar spine measured by QCT is a better predictor of vertebral fracture than areal BMD (by DXA) in postmenopausal women receiving long-term glucocorticoids. In renal transplant recipients, one study looked at bone stiffness and failure strength using micro-MR and FEM. Stiffness and failure strength declined in both cortical and trabecular compartments over the initial 6 months after transplantation. Importantly, these changes did not correlate with a change in areal BMD [104].

Serum bone turnover markers have also been studied as predictors of skeletal health in transplant patients, being non-invasive, readily available, and repeatable measures. Bone-specific alkaline phosphatase and osteocalcin reflects BFRs, whereas collagen degradation products, such as procollagen type 1, N-terminal pro-peptide, C-terminal telo-peptide, among others, are used as bone resorption surrogates. However, these markers are significantly affected by renal function [105], and studies to use these in transplant recipients have not been particularly useful [106–110].

Histologic features of post-transplant bone loss in renal transplant recipients vary. A cross-sectional study reported persistent high-turnover bone disease in renal transplant recipients, with ~50 % or more patients having osteitis fibrosa cystica [111]. A considerably smaller proportion displayed adynamic bone disease (5.3 %) or osteomalacia (3.5 %). Other studies, however, have demonstrated predominantly decreased bone formation, with high-to-normal bone resorption. Julian et al. reported decreased mean wall thickness and reduced MAR. Characteristics of secondary hyperparathyroidism, such as woven bone and marrow fibrosis, were shown to disappear as PTH levels normalized with renal allograft [91]. Consistent histomorphometric changes were noted using paired bone biopsies (pre- and post-transplant) at the 1- to 3-month time point. These showed evidence of reduced BFR, prolonged mineralization period, and importantly, an increased number of apoptotic osteoblasts [112]. Bone biopsies at 5.6 ± 0.8 years after transplantation similarly showed decreased BFRs in more than 50 % of patients, and prolonged mineralization in most patients [78]. Fortunately, these histologic changes do not seem to be persistent. After a period of 10 years, osteoid volume and surface became greater than normal, and BFRs and mineralization surfaces remained low, but almost approached normal values [113].

In liver transplantation, bone resorption compared to pre-transplant biopsies persisted. However, interestingly bone formation parameters increased, although mean wall thickness remained low at 4 months after transplant [24, 96]. Histomorphometric data on lung and cardiac transplantation are limited. A study of postmortem vertebral bone biopsy from post-transplanted, and non-transplanted patients with cystic fibrosis did not show any significant differences in terms of osteoblast and osteoclast activity, although cortical and trabecular bone mass was found to be somewhat lower in the transplantation group [114].

Management of Bone Disease After Transplant

Monitoring Bone Disease After Transplantation

The National Kidney Foundation recommends serial BMD measurements at time of transplant, 1 year, and 2 years post-transplant, and treat protocols according to *T*-score [115]. As BMD and other biomarkers cannot identify patients at a high risk of fracture, and the accelerated rate of bone loss occurs in early period, it is considered generally prudent to initiate preventive measures immediately following transplantation.

Preventive and Therapeutic Interventions

Exercise

There is evidence that structured exercise programs could potentially be helpful for maintaining skeletal health and increasing BMD in lung transplant patients [116]. Heart transplant recipients also regained BMD in axial and peripheral bones towards pre-transplantation levels with specific resistance exercise training [117]. Along the same lines, resistance exercise plus alendronate was more efficacious than alendronate alone in restoring BMD in heart transplant recipients [118]. These observations support the importance of physical activity and mechanical loading after organ transplantation.

Early Steroid Withdrawal or Avoidance

Because of the detrimental multisystem complications of glucocorticoid use, including skeletal fragility, attempts have been made to minimize their use in transplant patients. The skeletal benefit of early steroid withdrawal has recently been observed. Early steroid taper showed a significantly reduction in fracture risk, but that this was noted at the expense of a higher risk of graft rejection [119, 120]. Therefore, current

guidelines do not recommend early steroid withdrawal [115]. With the advent of newer immunosuppressant agents, such as MMF, sirolimus, and selective subsets of T cell inhibitors, non-glucocorticoid immunosuppressant regimes are being used more frequently and successfully to prevent organ rejection.

Anti-resorptive Agents

Bisphosphonates are currently the most effective therapies for post-transplant bone disease. These agents have been shown to prevent bone loss and increase BMD in transplant patients. The early generation intravenous bisphosphonate, pamidronate was studied in renal transplant recipients, being given at 1, 2, 3, and 6 months post-transplantation. Whereas spine BMD in the treatment group was preserved, the control group showed declines at 6 and 12 months (4.8 and 6.1 %, respectively) [121]. This preventive effect was also observed in different settings, where pamidronate was administered at the time of and 1 month following transplantation. At 12 months, the treatment group showed preserved BMD compared to a significant decrease at both lumbar spine and femoral neck in the control group [122]. Another bisphosphonate, ibandronate, was shown to be similarly effective in renal transplant patients [123–125]. Likewise, zoledronic acid, given two times within 3 month after transplantation, was shown stabilize or increase BMD at both sites [126]. Looking at fracture risk, a meta-analysis including nine studies showed that bisphosphonate use reduced number of subjects with fractures (OR: 0.53, 95 % CI: 0.31–0.91) [127].

Similar preventive effects were noted with transplant of other solid organs. Pamidronate increased BMD in lung transplant recipients [128]. Significantly less bone loss at the lumbar spine and femoral neck at 12 months was seen in cardiac transplant patients with ibandronate. The incidence of vertebral fracture also appeared to be lower in the ibandronate group, *albeit* not statistically significantly. However, in liver transplant recipients, a single dose of pamidronate before liver transplantation did not show skeletal preservation [129].

Although the preventive effect of bisphosphonate has been consistently observed in several clinical trials, there is still the concern that bisphosphonates might in fact exacerbate low bone turnover disease, occasionally prevalent in transplant recipients. Indeed, pamidronate use was actually associated with development of adynamic bone disease [121]. Another unanswered question is the duration and frequency of bisphosphonate use. Bisphosphonate might not continue to remain effective in the long-term, as the patients' BMD can stabilize (or declines very slowly) on its own after a significant early bone loss phase [90, 95].

For patients with AVN, anti-resorptive medications are possibly beneficial to prevent the loss of structural integrity, particularly as subchondral resorption can trigger femoral head collapse [56]. We have provided proof-of-concept that the pituitary hormone ACTH can, in a rabbit model, prevent steroid-induced AVN through its ability to enhance VEGF production and in turn stimulate angiogenesis [130, 131].

Anabolic Agents

Teriparatide, recombinant PTH (rPTH), is a compelling agent given its anabolic effect on bone remodeling. It is expected to counteract the early bone loss, which is characterized by decreased osteoblast differentiation and increased osteoblast apoptosis. Teriparatide has shown efficacy in the therapy of glucocorticoid-induced osteoporosis. Saag et al. [132] showed that treatment with teriparatide increased BMD and lowered vertebral fracture rate. Since glucocorticoid use plays a key role in early post-transplant bone loss, teriparatide mechanistically appears to be a logical choice, particularly if low bone turnover is the predominant feature. However, the protective effect of teriparatide has not been demonstrated yet. A small-sized randomized controlled trial comparing rPTH with placebo did not show any beneficial effect on BMD at lumbar spine or distal radius, although BMD at femoral neck was stable in teriparatide group and decreased in control group [133]. In the future the use of a sclerostin inhibitor potentially has the advantage of increasing bone formation and reducing fracture risk.

Vitamin D Supplements

Active vitamin D supplementation, not parent vitamin D, such as calcidiol, alfacalcidiol, and calcitriol showed an overall beneficial effect [134]. Calcidiol was compared to the non-nitrogen containing bisphosphonate etidronate in heart transplant recipients; an improvement in BMD was noted [135]. Two randomized trials have shown a protective effect of alfacalcidiol in renal transplant recipients [136, 137]. Calcitriol may be particularly beneficial in renal transplant since calcitriol production can remain persistently low even after renal function normalizes with allograft. A randomized, double blind study demonstrated that patients with renal transplant treated with calcitriol and calcium displayed increased or preserved BMDs at lumbar spine and femoral neck [59, 138]. In addition, there is possible additive benefit from the pleiotropic effects of vitamin D, since there is evidence that vitamin D can act as an immunomodulator. A retrospective cohort study reported less acute rejection in patients with renal transplant when treated with calcitriol [139].

Surgical Intervention for Skeletal Complication

Patients with AVN, osteoarthritis, and fractures oftentimes require and benefit from surgical intervention. In the case of AVN, hip preservation can be attempted depending on the presence of structural failure or collapse. There are several methods for surgical treatment for AVN of femoral head; core decompression with or without biologic augmentation, non-vascularized bone grafts, vascularized fibular grafts, intertrochanteric osteotomy, and cemented or un-cemented total hip replacement depending on the severity of bone loss [56]. Overall, total hip and knee arthroplasty can be safely performed and provides excellent functional outcomes in lung and

liver transplant recipients [140–142]. The patients with renal transplant also show good outcomes after total hip replacement, but a high rate of early failure was noted [142]. Indeed, there is ongoing concern for increased post-surgical complications, such as graft failure and infection because of co-morbidities, immunosuppressant use, and co-existing metabolic bone disease [141–143].

Conclusions

Skeletal complications after solid organ transplantation can compromise a patient's quality of life and increase mortality and morbidity. Pre-existing bone disease from underlying end-organ damage needs to be screened, and any reversible cause needs to be addressed before transplantation. Persistent pre-existing metabolic bone disease in addition to other factors specifically related to transplantation is the rule rather than an exception. Immunosuppressant use, especially high-dose glucocorticoids and calcineurin inhibitors, causes accelerated bone loss in the early post-transplant phase. The ensuring skeletal fragility is characterized predominantly by suppressed bone formation with mildly increased bone resorption. Decreased osteoblast differentiation and osteoblast apoptosis are key to the pathophysiology. Screening and diagnosing patients at high risk of fracture during pre- and post-transplantation thus becomes critical. It is recommended that BMD is measured at periodic intervals before and after transplant, notwithstanding the limitations of DXA. Newly developed technologies such as QCT, micro-MR, FEM and microindentation should provide more valuable information on bone quality. The most well-established preventive and therapeutic option, as of now, is a bisphosphonate. Although there is limited data in terms of fracture risk reduction, bisphosphonates have shown promise in increasing or stabilizing BMD at both trabecular and cortical sites. However, the optimal frequency and duration of treatment is unknown, and there is lingering concern that the drugs may exacerbate low turnover disease. Active vitamin D with calcium supplementation has shown beneficial effects on BMD. The only available anabolic agent, rPTH, as of yet has not shown a beneficial effect (in a preliminary study). Other newly developed therapeutics like RANK inhibitor and sclerostin inhibitor has not been fully studied.

Acknowledgements M.Z. acknowledges support of the National Institutes of Health [DK80459 (to M.Z. and L.S.), AG40132 (to M.Z.), AR06592 (to M.Z.), and AR06066 (to M.Z)].

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