CKJ REVIEW

Bone mineral density and mortality in end-stage renal disease patients

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ABSTRACT

Osteoporosis characterized by low bone mineral density (BMD) as assessed by dual-energy X-ray absorptiometry (DXA) is common among end-stage renal disease (ESRD) patients and associates with high fracture incidence and high all-cause mortality. This is because chronic kidney disease-mineral bone disorders (CKD-MBDs) promote not only bone disease (osteoporosis and renal dystrophy) but also vascular calcification and cardiovascular disease. The disturbed bone metabolism in ESRD leads to 'loss of cortical bone' with increased cortical porosity and thinning of cortical bone rather than to loss of trabecular bone. Low BMD, especially at cortical-rich bone sites, is closely linked to CKD-MBD, vascular calcification and poor cardiovascular outcomes. These effects appear to be largely mediated by shared mechanistic pathways via the 'bone–vascular axis' through which impaired bone status associates with changes in the vascular wall. Thus, bone is more than just the scaffolding that holds the body together and protects organs from external forces but is—in addition to its physical supportive function—also an active endocrine organ that interacts with the vasculature by paracrine and endocrine factors through pathways including Wnt signalling, osteoprotegerin (OPG)/receptor activator of nuclear factor-κB (RANK)/RANK ligand system and the Galectin-3/receptor of advanced glycation end products axis. The insight that osteogenesis and vascular calcification share many similarities—and the knowledge that vascular calcification is a cell-mediated active rather than a passive mineralization process—suggest that low BMD and vascular calcification ('vascular ossification') to a large extent represent two sides of the same coin. Here, we briefly review changes of BMD in ESRD as observed using different DXA methods (central and whole-body DXA) at different bone sites for BMD measurements, and summarize recent knowledge regarding the

relationships between 'low BMD' and 'fracture incidence, vascular calcification and increased mortality' in ESRD patients, as well as potential 'molecular mechanisms' underlying these associations.

INTRODUCTION

The kidneys play an important role in the systemic regulation of mineral metabolism. The decline in renal function in patients with chronic kidney disease (CKD) leads to the systemic syndrome of CKD-mineral bone disorders (CKD-MBDs) that feature, on one hand, impaired bone health caused by renal osteodystrophy and osteoporosis, and, on the other hand, cardiovascular disease (CVD) with arteriosclerosis and generalized vascular calcification including coronary artery calcification (CAC). These common interlinked features of CKD-MBD contribute to premature ageing [1] with severe and seldom fatal complications leading to markedly increased morbidity and high mortality, especially in patients with end-stage renal disease (ESRD).

In addition to 'age-related osteoporosis', bone status in CKD patients is affected by 'renal osteodystrophy', a collective term for a heterogeneous group of metabolic bone diseases associated with CKD-MBDs that are characterized by alterations of bone morphology due to abnormal bone turnover rate (high and low bone turnover diseases), defective mineralization and volume [2]. Bone disease in ESRD is a mixture of reduced bone density and impaired bone quality due to microdamage and disorders of microarchitecture and collagen. It associates not only with increased risk of fractures but also with poor nutritional status with reduced muscle strength and low lean body mass, and increased vascular calcification involving both intimal calcification linked to atherosclerotic plaque formation and medial calcification linked to arteriosclerosis, vascular stiffening and vascular senescence [3]. Altogether these alterations increase the risk for CVD events and mortality [4–10].

In the general population, according to meta-analysis of prospective cohort studies, low bone mineral density (BMD) levels at 'all investigated sites' are associated with increased CVD-related and all-cause mortality [11]. In patients with ESRD, low BMD is even more strongly associated with poor outcomes due to alterations in the bone–vascular axis and metabolic and hormonal abnormalities linked to CKD-MBD such as disturbances in mineral metabolism, vitamin D deficiency, secondary hyperparathyroidism, and excess or deficiencies of molecules influencing bone formation [12–16]. Bone status in ESRD is therefore more closely linked to accelerated vascular calcification and premature cardiovascular events than in the general population. Accordingly, in ESRD patients, low BMD determined by dual-energy X-ray absorptiometry (DXA) associates with markedly increased CVD-related and all-cause mortality [17–20].

There are still many unexplored research territories in CKD-MBD including factors that may explain the links between low BMD and mortality in ESRD patients. For instance, few scientific reports have so far explored whether the association between low BMD and mortality depends on the sites of BMD measurement. The molecular mechanism of bone–vascular axis is another area that remains to be explored. In this review, we (i) present available data on associations of BMD measured by DXA at various bone sites with vascular calcification and mortality in ESRD and (ii) discuss possible mechanisms behind bone–vascular axis alterations that may explain these associations.

Bone physiology and pathophysiology

The association of impaired bone status with poor clinical outcomes may reflect one or more of the many functions of bone:

(i) physical: body support, facilitation of movement and protection of internal organs against external forces;
(ii) haematopoiesis: harbours bone marrow, producing blood cells;
(iii) nutritional: storage of minerals and fat, and indirectly of muscle protein through harbouring skeletal muscles;
(iv) metabolic: mineral metabolism and acid–base balance; and
(v) endocrine and paracrine: bone–vascular axis pathways including Wnt signalling, the OPG/receptor activator of nuclear factor-κB (RANK)/RANK ligand (RANKL) system and the Galectin-3/receptor of advanced glycation end products (RAGE) axis.

In humans, 80% of the skeleton is composed of cortical bone and 20% of trabecular bone [21]. Cortical bone is much harder and denser than trabecular bone to protect soft tissues, while trabecular bone is a honeycomb-like network consisting of myriads of highly interconnected bony trabeculae [21]. The proportions of cortical to trabecular bone vary between different skeletal sites: vertebrae are trabecular rich sites, and contain >40% of trabecular tissue [22, 23]. In long bones, the diaphysis consists of mainly cortical bone, while the metaphysis and epiphysis are a mixture of cortical bone and trabecular bone, and the proximal femur and mid-radius are cortical rich sites [22]. Due to the differences in the ratio of cortical to trabecular content, the bone turnover rate differs between different bones and between different parts of the long bones, and the proportion of cortical to trabecular bone and bone turnover, are largely affected by age, sex, metabolic diseases and drugs. For example, bone turnover rate is generally lower in the peripheral skeleton than in the central skeleton, and the mean iliac trabecular bone turnover in post-menopausal women is twice that of cortical bone (18% versus 8%/year) [24]. In post-menopausal as compared with pre-menopausal women, bone remodelling rates assessed by iliac biopsy are 2-fold increased [25], the cortical bone becomes thin and porous and there is loss of trabecular bone and trabecular connectivity [26]. These changes in bone microarchitecture, which lead to fragile bones and increased risk for fractures following external forces such as falls, are magnified by hyperparathyroidism, which increases bone turnover rate [27–29].

> **Key points:**
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> Cortical and trabecular bone differ as regards their functions, metabolic activity and implications for clinical outcomes.
> The location of cortical and trabecular bone should be considered in bone status assessments.

Changes of cortical and trabecular bone in CKD

While this review focuses on BMD measures by DXA, there are several other options to assess changes in bone status in CKD, such as bone biopsy, quantitative ultrasound (QUS), digital X-ray radiogrammetry and peripheral quantitative computed tomography (pQCT) including high-resolution pQCT (HRpQCT). However, these techniques have drawbacks, such as invasiveness (biopsy), lower reproducibility (QUS and CT) and less documentation (all), and they are still not generally used for diagnosis and for monitoring effects of treatment.

Bone biopsy is considered as a gold standard tool for the classification of turnover, mineralization and volume defects in CKD patients; however, findings may vary due to differences in age, race, ethnicity, cause of ESRD, dialysis vintage and sample size between studies [30–33]. Nevertheless, histomorphometric measurements show that bone morphology might improve following therapeutic interventions. For example, after 12 months of treatment with cinacalcet, a calcimimetic agent, trans-iliac bone biopsies showed normal bone histology in 20 out of 77 haemodialysis (HD) patients, in parallel with normalizing serum parathyroid hormone (PTH) levels [29].

Changes of trabecular and cortical bone appear to be different in CKD as compared with the general population. Trabecular bone, which makes up two-thirds of total bone surfaces, shows greater metabolic activity than cortical bone [24]. Trabecular bone receives blood supply through the surrounding bone marrow and the metabolic activity is regulated by hormones and growth factors through this blood supply [34]. Trabecular bone is more rapidly lost than cortical bone in postmenopausal women in early phase [35, 36], and, based on results by QCT in a community-based study, this may explain, in large part, why fragility fractures are more common in elderly women than in elderly men [37]. However, in contrast, while CKD patients had rapid cortical bone loss during 1.5 years of follow-up, there was no trabecular bone loss as assessed by HRpQCT; there was even an increase of trabecular area [38]. This was supported by another study in CKD patients showing better preservation of trabecular bone than cortical bone as assessed by bone biopsy [28]. A possible explanation for this difference in bone loss pattern between the general population and CKD patients could be that trabecular bone loss is attenuated in CKD patients because of increased levels of PTH, a hormone that has anabolic effects on trabecular bone and catabolic effects on cortical bone [39]. In CKD patients, cortical bone loss associates with increased cortical porosity and decreased cortical thickness [38]. These changes, which may be partially explained by secondary hyperparathyroidism, are in general more severe in patients undergoing dialysis compared with patients without dialysis therapy [38, 40].

Key points:

In contrast to the general population, loss of cortical bone is more severe than loss of trabecular bone in patients with CKD.

DXA

Bone densitometry by DXA was developed and eventually approved by US Food and Drug Administration in 1980s, and DXA of the hip is the dominating technique to diagnose osteoporosis. DXA is a machine that generates two X-ray beams with different levels of energy, 45 and 100 keV, or ~39 and 71 keV depending on the type of equipment. Because the tissues absorb (attenuate) the X-rays differently depending on their energy and the electron density of the tissue, the software of the DXA machine can calculate the tissue composition and the bone density. Though DXA is an X-ray-based imaging method, the radiation dose is very low and the risk from the exposure can be considered almost negligible. The effective radiation dose from a single whole-body DXA (<10 μSv) [41] is equivalent to <1 week of background radiation.

When BMD is assessed by 'central DXA', the DXA machine quantifies BMD of the central skeleton including lumbar spine and hip. According to the International Society for Clinical Densitometry, central DXA, and not whole-body DXA (see below), is recommended for diagnosis of osteoporosis in clinical practice [42], but for historical reasons DXA of the forearm is also accepted for making the diagnosis. Among central skeleton sites, BMD of the hip has the best prediction of fracture risk, but when monitoring response to treatment BMD of the spine is preferred [43, 44].

Another application of DXA, 'whole-body DXA', is increasingly used to assess skeletal mineral status of the whole body as well as for evaluating body composition (i.e. muscle and fat content). These applications have further promoted the use of whole-body DXA, not only in clinical practice, but also in clinical research. While the lower resolution of whole-body measurements makes the diagnostic performance not equivalent to that obtained by central DXA, BMD from central DXA of the hip, spine and distal forearm correlate closely with BMD at sub-regions obtained from whole-body DXA, especially in spine [45–47]. The validity of BMD using whole-body DXA was also confirmed by another study using pQCT [2]. However, as regards diagnosis of osteoporosis, prevalence estimations by sub-regions analysis of whole-body DXA were generally lower (i.e. underestimated) than corresponding central site-specific measurements of BMD [46].

Thus, as whole-body DXA and central DXA are not equivalent, the interpretation of BMD values yielded by these two methods may vary. The diagnostic criteria for osteoporosis by World Health Organization are based on femoral neck BMD by central DXA [44], and not by whole-body DXA, using data from the National Health and Nutrition Examination Survey III database [42]. Nevertheless, abundant data and clinical evidence support the usefulness of whole-body DXA in the day-to-day clinical practice in various disciplines of medical science.

Key points:

BMD data by central DXA and whole-body DXA differ. Central DXA is the recommended method for diagnosis of osteoporosis.

Diagnostic methods in CKD-MBD

The pathogenesis and characteristics of bone disease linked to CKD-MBD in ESRD differ from primary osteoporosis. Bone biopsy has been recommended as the gold standard for the diagnosis and classification of renal osteodystrophy by Kidney Disease: Improving Global Outcomes [48] and ERA-EDTA [49]. Histological findings by bone biopsy are often different from estimations based on serum biomarkers, such as high levels of serum intact PTH (iPTH). However, in clinical practice, bone

biopsy is rarely performed due to its invasive nature. In addition, it is expensive and time consuming, often requiring weeks to get a final histopathological report. Recently, Carvalho *et al.* [40] reported a significant association between BMD by DXA and bone histomorphometry data by bone biopsy, supporting the usefulness of DXA in CKD patients.

While DXA cannot provide details regarding the relative proportions of the cortical and trabecular bone or distinguish between different types of renal osteodystrophy, the potential of DXA to predict fracture risk not only in the general population but also in specific subgroups such as in CKD has gradually become clearer. Iimori *et al.* [50] reported that BMD in hip and femoral neck, but not in spine, was associated with any type of fracture in a prospective study of 485 dialysis patients. Similar results were replicated in another study of 384 incident fractures in 2754 individuals including 587 (21%) CKD patients [51].

> **Key points:**
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> Bone biopsy is the gold standard but is not performed routinely in clinical practice.
> DXA is increasingly used to analyse fracture risk in CKD patients.

Associations of low BMD with vascular calcification

As discussed below, mechanisms of vascular calcification and bone mineralization share several common pathways, which may explain why low BMD is strongly related to vascular calcification in the general population [52, 53] and in patients with ESRD [9, 54–58]. Not only low bone mass but also bone loss is an independent predictor for progression of vascular calcification [59, 60]. In a population-based cohort study with 25 years follow-up of older men and women, Kiel *et al.* [60] demonstrated that bone loss at the second metacarpal of the hand as assessed by simple plain radiographs is associated with increased aortic calcification index in women, but not in men. This suggests that endocrine factors such as oestrogen might play a role in the interplay between bone and vasculature. Low trabecular bone volume, as assessed by iliac bone biopsy, has been shown to correlate with the progression of calcification as assessed by CAC, and lower bone turnover (i.e. decreased bone resorption) was associated with less rapid CAC progression in HD patients [58]. These observations may imply that normalizing bone metabolism could represent an effective option to prevent vascular calcification; however, so far there is no strong clinical evidence confirming that such interventions will reduce established vascular calcification. Also, the crucial mechanism(s) of the bone–vascular axis are not fully understood.

> **Key points:**
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> Low bone mass and bone loss are linked with vascular calcification.

Association of BMD with vascular calcification depends on bone site for BMD measurement

There is no consensus on which specific bone location should be used for measurement of BMD when studying relations between BMD and signs of vascular calcification. There are discrepancies between studies regarding the relation of vascular calcification, including CAC, with BMD measured at different anatomical bone sites. It is possible that such variations might be explained by the heterogeneity of study populations and the bone sites selected for BMD measurements. In the general population, an age-stratified random sample of 200 women showed that higher BMD of the spine but not of the hip was independently associated with less aortic calcification [53]. In postmenopausal women, carotid plaque score showed an inverse association with total body BMD, but not with lumbar spine BMD [61]. In another study in postmenopausal women, reduced BMD at the proximal femur is associated with aortic calcification [62]. In ESRD, the site of BMD measurements also appears to play a role. Thus, in HD patients, CAC score was negatively associated with BMD at hip site but not with lumbar spine BMD [20], while in another study greater bone loss at the ultra-distal (UD) radius is associated with significantly higher all-cause mortality [63].

The degree of atherosclerosis and hence the degree of intimal deposition of calcium, which may influence BMD measurements of the spine, varied between these studies. We have reported that low BMD of arms and legs—rather than BMD at other and more central locations—was associated with high CAC score in ESRD patients [64] and that BMD correlated inversely with CAC and histologically biopsy-verified epigastric arterial vascular calcification in extremities (arms and legs) but not in the pelvis, spine and femoral bone sites in ESRD patients undergoing living-donor kidney transplantation [65]. It is possible that these differences between different sites may be partially explained by absence of extensive arterial calcifications in the extremities, whereas BMD in spine could be overestimated due to aortic calcifications. Compared with central DXA, BMD measured at peripheral sites could represent a more appropriate method for analyses of relations between BMD and vascular calcification in individuals with substantial aortic calcification, such as ESRD patients.

> **Key points:**
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> Spine BMD by DXA does not necessarily associate with vascular calcification due to interference by aortic calcifications.

Low BMD predicts increased all-cause and CVD mortality

Table 1 summarizes reported associations between BMD and all-cause and CVD mortality in ESRD. Compared with the general population where there is an abundance of data, the relationship between BMD and CVD mortality in ESRD is less clear due to the relatively small number of patients and publications in this field. Most previous reports regarding ESRD patients analysed the association between BMD and all-cause mortality, while only a few studies investigated the relationship between BMD and CVD mortality in ESRD patients [6, 7, 17–20, 66, 67] (Table 1).

In the study by Taal *et al.* [18], reduced total hip BMD measured by central DXA was significantly associated with poor outcomes after adjusting for age, calcium × phosphorus (Ca × P) product and previous allograft, whereas there was no

Table 1. Previous reports on association between BMD and mortality in ESRD patients

References	Location	No. of patients	Sex	Age (years) (mean/median)	Dialysis modality	Design	Follow-up (years)	BMD sites	Measurement method	Outcomes	Factors controlled for in multivariate analysis	Outcomes associated with
Taal et al. [18]	UK	88	F/M	51–70	HD	Prospective observational	3.5	Spine, hip	Central-DXA	All-cause mortality	Age, Ca × P and previous allograft	Hip
Matsubara et al. [7]	Sweden	277	F/M	55	HD/PD	Prospective observational	5	Total	Whole-DXA	All-cause and CVD mortality	Age, sex, DM, CVD, hsCRP, phosphate, Ca × P and SGA	Total
Park et al. [6]	Sweden	361	F/M	53	HD/PD	Prospective observational	5	Total	Whole-DXA	All-cause mortality	Age, fat body mass and SGA	Total
Disthabanchong et al. [20]	Thailand	83	F/M	57	HD	Prospective observational	5	Spine, hip	Central-DXA	All-cause mortality	Age, Davies score, dyslipidaemia, albumin and CAC score	Hip
Chen et al. [66]	Sweden	231	F/M	56	HD/PD	Prospective observational	5	Spine	Whole-DXA/CT	All-cause mortality	Age, sex, diabetes, CVD, hsCRP and SGA	Spine (VBD by CT)
Yap et al. [67]	Australia	58	F/M	58–65	HD/PD	Prospective observational	6	Spine/FN	Central-DXA/pQCT	All-cause mortality	Age, sex, transplant status, albumin, PTH, phosphate, dialysis duration, diabetes and smoking	FN
Orlic et al. [19]	Croatia	134	F/M	59	HD	Prospective observational	4	Spine, hip, forearm	Central-DXA	All-cause mortality	Age and sex[a] (used as a Z-score)	Forearm
Iseri et al. [17]	Sweden	426	F/M	56	HD/PD	Prospective observational	5	Total, head, arms, legs, trunk, hip, pelvis, spine	Whole-DXA	All-cause and CVD mortality	Framingham CVD risk score, SGA, %HGS, albumin, hsCRP, LBMI, recruitment year	Total, head, pelvis (and leg)

DM, diabetes mellitus; FN, femoral neck; %HGS, percent handgrip strength; hsCRP, high-sensitivity C-reactive protein; LBMI, lean body mass index; SGA, subjective global assessment.

[a]Outcomes significantly associated with.

significant association between reduced spine BMD and clinical outcome. In line with this, another study reported similar results after adjusting for age, Davies score, dyslipidaemia, albumin and CAC score [20]. Furthermore, another study reported that forearm sites in addition to femoral neck were associated with high all-cause mortality in univariate analysis [19]. Yap *et al.* [67] used pQCT in addition to DXA in a prospective study of 58 dialysis patients with 6 years follow-up and found that tibia cortical density by pQCT, in addition to BMD at femoral neck measured by DXA, was significantly associated with increased all-cause mortality.

Furthermore, also changes in bone mass were associated with increased mortality [63]. In a study of 269 men on chronic HD, 1-year bone reduction at UD radius was an independent predictor of all-cause mortality [63]. The authors speculated that high iPTH contributed to bone loss as well as to increased mortality.

While most studies in ESRD patients included HD patients, we investigated both peritoneal dialysis (PD) and HD patients and found that low total BMD measured by whole-body DXA was associated with increased all-cause mortality as well as increased CVD mortality [6, 7, 17]. As regards the impact of dialysis therapy on fracture events, some studies showed that compared with HD, PD was associated with lower risk of fracture events [68, 69], which in turn potentially could lead to lower subsequent mortality risk. A report from Taiwan National Database comprising 51 473 dialysis patients showed that HD patients had a 31% higher incidence of hip fractures than PD patients [Hazard ratio (HR) = 1.31, 95% CI 1.01–1.70] [68]. Similar results were reported in patients from the US Renal Data System, where HD patients had 1.6 times greater odds ratio of hip fracture risk than PD patients [69]. While the mechanism(s) for a potential beneficial effect of PD versus HD on fractures is not fully understood, better preservation of residual renal function in PD as compared with HD [69] may conceivably contribute to reduced fracture risk.

Key points:

Bone mass in ESRD patients is associated with all-cause mortality and CVD mortality.
The strength of this association may depend on treatment modality such as HD versus PD.

Association of BMD with mortality depends on bone site for BMD measurement and risk of fractures

Even though methods were somewhat different between the reports discussed above, they all show that low BMD is related to poor survival in CKD. However, it is still unclear which BMD sites are more appropriate and suitable for predicting clinical outcomes. As compared with the general population, vascular calcification may be more important as a factor masking the 'true' value of BMD in ESRD. As mentioned above, BMD at spine was often similar, whereas BMD at hip and radius sites were generally lower in ESRD patients than in the general population. This suggests that existence of aortic vascular calcification could influence BMD measurements in ESRD [48]. To address this issue, we assessed the association between mortality and regional BMD, measured by whole-body DXA (head, arms, legs,
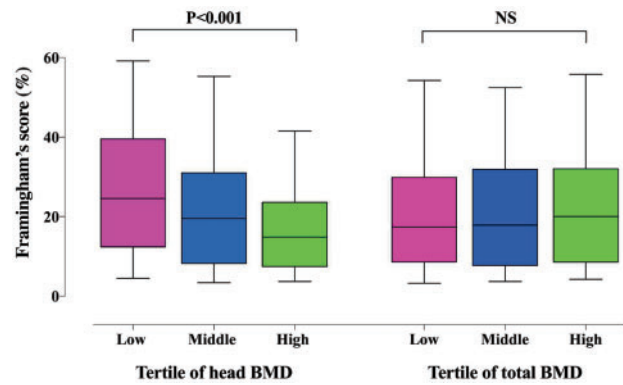

FIGURE 1: Low BMD as assessed by whole-body DXA at cortical-rich sites such as head and pelvis associates with increased mortality. The figure shows distribution of head BMD and restricted cubic spline curve with multivariable-adjusted sub-distribution hazard ratio (sHR) (95% CI) for all-cause mortality across values of head BMD (as continuous variable) in ESRD patients at initiation of dialysis therapy. Median value of head BMD was taken as reference. The results are adapted from a previous study [17] which concluded that cortical BMD appeared to have stronger association to survival in ESRD than trabecular BMD.

trunk, hip, pelvis and spine), in a prospective study of 426 incident dialysis patients. Using multivariate competing risk analysis with renal transplantation as a competing risk, and adjusting for several confounders (including Framingham CVD risk score, nutritional status and inflammation), lower values of total BMD, head BMD and pelvis BMD were associated with increased all-cause and CVD mortality [17] (Figure 1). Leg BMD was associated with CVD mortality, while BMD at other sites (arms, trunk, hip and spine) was associated with mortality. To the best of our knowledge, our study [17] is the first to show the relationship between BMD at specific bone sites measured by whole-body DXA and clinical outcomes in ESRD patients.

Vascular calcification including abdominal aortic calcification is highly prevalent in CKD [70, 71] and may affect measurements of the 'true' BMD, especially of spine BMD, as artefacts and local structural changes influence DXA measurements [72]. Besides, extensive osteophyte formation and degenerative changes are often seen in elderly patients. This might explain why several studies did not observe the association between BMD at spine by DXA and all-cause mortality [17–20, 65].

In a prospective study of 231 ESRD patients comprising incident dialysis patients (mostly PD patients and recipients of living-donor kidney transplant), we measured vertebral bone density (VBD) by cardiac computed tomography (CT) (64-channel detector scanner), which is not influenced by the presence of aortic calcification, and analysed the association between VBD and all-cause mortality [66]. Low VBD [receiver operating characteristics curve (ROC) = 0.72] was more strongly associated with increased all-cause mortality than total BMD by whole-body DXA (ROC = 0.56). VBD (ROC = 0.75) was more strongly associated with higher CAC score (CAC >100 AUs) compared with total BMD by whole-body DXA (ROC = 0.57). This study suggested that bone condition was strongly linked to cardiac calcification as assessed by CAC score in ESRD, a finding supported by the inverse relationship between CAC and BMD by DXA in dialysis patients [8]. Considering the significant association between increased CAC and increased CVD-related and all-cause mortality in CKD and dialysis patients [71, 73, 74], we assume that low BMD by DXA is linked to increased cardiac calcification, which in turn is associated with increased mortality. Although spine BMD by DXA may not reflect the 'true' BMD (as measured

by CT), it appears to be a valid predictor of the mortality risk. However, our study based on VBD [66] suggests that one may measure the 'true' BMD value at the spine site—avoiding the influence of calcification, osteosclerosis and bone spur areas.

One possible mechanism for the link between BMD by DXA and mortality is that reduced BMD may increase incidence of fractures and thereby lead to fatal outcomes associated with fractures. Fractures are common, and increased mortality following fractures were reported in the general population [75, 76], CKD patients [77] and ESRD patients on HD [78]. Specifically, reduced cortical thickness and thin cortical envelope—which is overrepresented in CKD patients—had great impact on the risk of fracture incidence [79, 80]. In non-dialysed CKD patients, the risk of fracture was increased especially in patients with estimated glomerular filtration rate <30 mL/min [81]. Among fracture types, there was a larger effect of GFR decline on hip fracture risk than for other types of fractures, and the incidence of fractures was significantly associated with mortality and major adverse cardiac events. Tentori *et al.* [78] found higher fracture incidence in HD patients compared with general population, with hip fracture (44%) being an especially common fracture location in data from the Dialysis Outcomes and Practice Patterns Study. Substantial increase of subsequent morbidity and mortality following hip fracture was reported in the elderly [82], as well as in HD patients [66]. Long hospitalization and excessive mortality rate following fracture events in HD patients, especially in the first month following the fracture incident, may be related to the high frequency of a frail status in dialysis patients [78].

Another possible explanation, and most likely a much more important factor for the association between low BMD and increased mortality, involves the bone–vascular axis, whereby an impaired bone status may reflect progression of vascular calcification. This will be discussed below, first in relation to different bone sites and then as regards possible molecular mechanisms for the altered bone–vascular axis in ESRD.

> **Key points:**
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> Associations of low BMD with mortality depend on bone site for BMD measurement and risk of fractures.

Importance of cortical bone in ESRD patients

Among several sites measured by whole-body DXA, the head site stands out due to its different characteristics as compared with other bone sites. In a recent study of 704 amateur sportsmen, physical activity was associated with total body BMD and regional BMD by whole-body DXA but not with head BMD [83], conceivably because the head is not a weight-bearing site and not usually involved in physical exercises. Furthermore, head BMD was not influenced by activity or disease condition in children and adolescents [84], unlike the total body mass. As regards assessment of bone fragility in children, a clinical guidance paper suggested that 'cranium should be excluded from the total body scan analysis' because of head BMD having a large proportion of the total body in spite of not reflecting physical activity or disease stage [84]. The head seems to be affected by age and body mass index to a different extent than other parts of the body [85]. Thus, in studies on adult ESRD patients, BMD of the head might be of interest as an indicator of bone health that is not necessarily influenced by physical activity. In

a previous study in ESRD patients, there was a strong negative correlation between head BMD and iPTH and dialysis vintage [86]. We observed that high age, low hand grip strength, low lean body mass index and high serum iPTH were significantly associated with reduced head BMD, even after adjusting for several confounders [17]. Furthermore, in terms of disease conditions, those of our patients with low head BMD had high Framingham's score (FRS) predicting 10-year risk of CVD, whereas there was no significant association between other sub-regional BMDs, or total BMD, and FRS [analysis of variance (ANOVA), P < 0.001] (Figure 2). Considering the fact that cortical bone is largely aggravated by progression of CKD and increase of serum PTH [87], cortical rich sites, such as the skull, may be preferred sites to capture the changes of the patient's bone condition. However, skull BMD may be affected by hyperostosis cranii, an asymptomatic condition that has been reported to affect ∼22% of postmenopausal women [88].

The femoral neck is another cortical-rich site; femoral neck BMD by central DXA had highly significant inverse association with cortical porosity assessed by iliac bone biopsy [89] (Figure 3). Recently, with the use of advanced hip analysis software allowing for the assessment of cortical parameters at femoral neck in DXA, it was shown that bone cortical thickness was reduced in ESRD patients as compared with healthy controls, and that this was associated with increased prevalence of fractures [90]. Thus, DXA may partially represent changes in bone histomorphology. Given the importance of cortical bone in CKD, impaired bone status measured by DXA may reflect not only bone mass but also, partially, bone quality. Cortical-rich sites may be more valuable locations for assessing metabolic alterations linked to CKD-MBD than trabecular-rich sites in CKD patients. These sites may differ depending on different measurement methods. For example, whole-body DXA is not able to divide long bone into portions [i.e. in forearm measurement of UD, mid-diaphysis , one-third distal radius of distal radius], whereas this can be accomplished by using central DXA.

> **Key points:**
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> Bone mass at cortical-rich sites such as the head and femoral neck is more affected than trabecular bone and appears to be useful sites for predicting clinical outcomes in ESRD patients.

Mechanistic insights into the bone–vascular axis in CKD

The observation that low BMD, i.e. less mineralization of bone, associated with increased risk of ectopic calcification in blood vessels and its clinical sequelae [4, 55, 60, 91–94], referred to as the 'calcification paradox' [95], is supported by novel mechanistic insights into the bone–vascular axis. Vascular calcification with 'ossification' of vessels may be a consequence of impaired bone remodelling [96] and osteogenic processes driven by osteogenic transcriptional factors, such as runt-related transcription factor 2 (RUNX2) and Msh homoeobox 2 in cells surrounding calcified arteries [97–99]. Other bone regulatory factors including osteonectin, osteopontin, bone sialoprotein, type I collagen, alkaline phosphatase and matrix-Gla protein (MGP) are also present in extra-skeletal calcification sites [100, 101]. In mouse models, deletion of bone-related genes including OPG, MGP and the fibroblast growth factor-23/
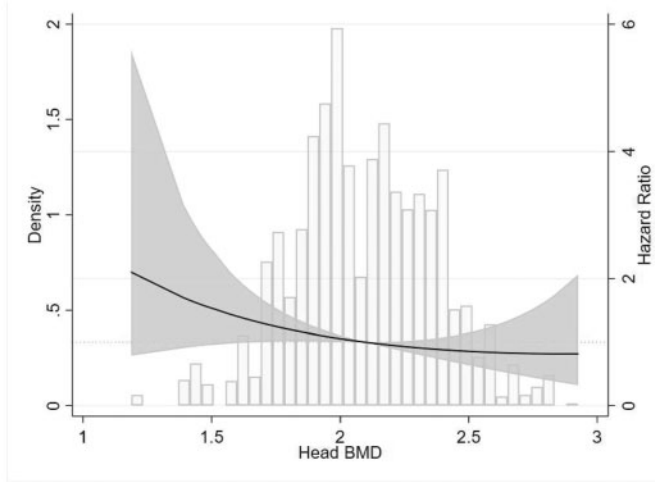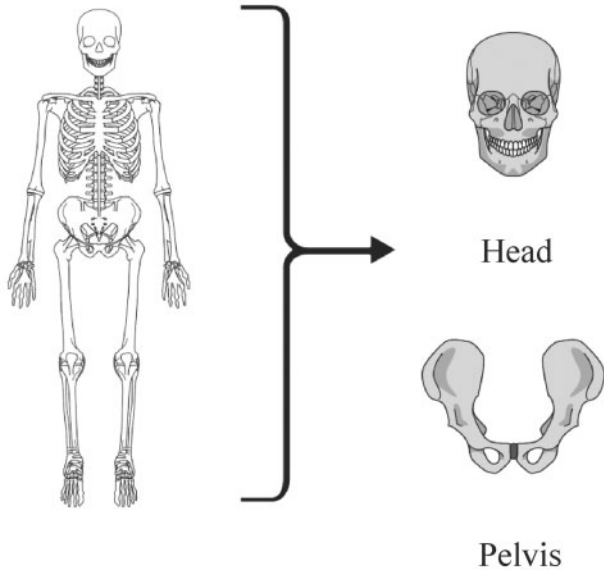
FIGURE 2: Association between tertiles of BMD head and total BMD (assessed by whole-body DXA; median and 10–90th percentiles) and Framingham's cardiovascular risk score (adapted from a previous study [17]). Comparisons between tertiles were assessed with non-parametric ANOVA Kruskal–Wallis test followed by Dunn's test for continuous variables. The figure illustrates that a possible reason why a cortical-rich bone site like the head appears to be suitable for analysing relations with survival in ESRD is that it associates with cardiovascular risk factors.
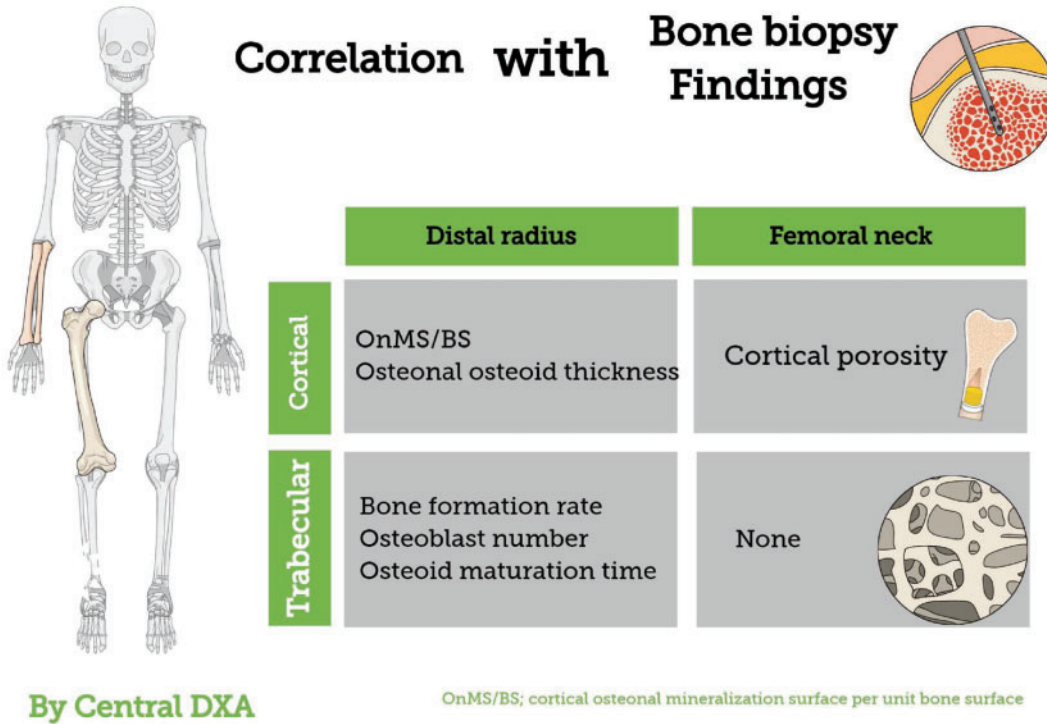

FIGURE 3: Associations between BMD by central DXA and histological findings by bone biopsy as reported in previous studies [40, 89].

klotho axis result in extensive vascular calcification illustrating this 'calcification paradox' [96, 102, 103]. Likewise, *in vitro* studies demonstrated that vascular smooth muscle cells (VSMCs) and vascular pericytes can undergo osteogenic differentiation and produce bone forming transcription factors and proteins in response to high concentrations of phosphate, calcium, glucose, oxidized lipids, inflammatory cytokines and various toxins in uraemic serum [104]. The similarity of cellular and molecular signalling in osteogenesis and vascular calcification has led to the insight that vascular calcification is a cell-mediated active process rather than a passive mineralization routine.

In CKD, the synergism of oxidative stress, hyperphosphataemia, hypercalcaemia, hyperparathyroidism, medication with calcitriol, calcium carbonate and warfarin, and deficient anti-ageing processes provides a 'perfect storm' for bone loss and vascular calcification [105, 106]. Though this paradox—the coexistence of bone loss/osteoporosis and vascular calcification—is well-established among patients with CKD, the underlying mechanism(s) behind such a scenario has not yet been fully elucidated. Below we describe some signalling pathways that potentially could provide mechanistic links between bone loss and vascular calcification.

Wnt signalling

Wnt glycoproteins act as extracellular signalling ligands [107] in processes influencing bone [108] as well as the vasculature [109, 110]. Activation of the predominant signalling pathway, Wnt/β-catenin-dependent signalling, results in increased osteoblastogenesis and downregulated osteoclastogenesis, leading to enhanced bone formation and reduced bone resorption [111]. Genetic alterations in Wnt signalling components can result in either increased BMD or bone loss [112].

Sclerostin, encoded by the *sclerostin (SOST)* gene, is expressed by osteocytes and acts as a soluble inhibitor of wingless and Int-1 (Wnt) signalling to reduce bone formation; however, high serum sclerostin levels are associated with increased vascular calcification [113]. While mutations in SOST may increase bone mass [114, 115], overexpression of Dickkopf-related protein 1 (DKK1), another inhibitor of Wnt signalling, causes osteopaenia, whereas DKK1 insufficiency increases bone formation [116]. Blockade of sclerostin and DKK1 using neutralizing antibodies may increase BMD, trabecular bone volume, osteoblast number and bone formation, suggesting that downregulation of these two proteins potentially could be of value in future treatment strategies to ameliorate osteoporosis and bone loss [117, 118].

The expression of sclerostin and DKK1 is not restricted to bone. DKK1 is expressed in skin, placenta, prostate, kidney and platelets, and, to some extent, in endothelium [119]. Sclerostin is expressed in calcified VSMCs and aortic valves, as well as in the kidney and in chondrocytes [120]. Aside from affecting bone health, the Wnt/β-catenin signalling pathway may promote atherogenesis and vascular calcification [110]. *In vitro* studies indicate that DKK1 could attenuate transcriptional factor RUNX2 expression and thereby inhibit osteogenic differentiation of VSMC [121–123]. The observed expression of Wnt antagonists (sclerostin, DKK1 and Frizzled-related proteins) in calcified VSMCs [120, 124, 125] may represent a counteractive response to prevent progressive vascular calcification, potentially explaining the paradoxical inverse correlations of circulating levels of DKK1 and sclerostin with the extent of vascular calcification. In addition, increased expression of DKK1 in macrophages and endothelial cells may promote matrix metalloproteinase (MMP) activity in calcifying vascular cells, contributing to the instability of the atherosclerotic plaque [126, 127]. Inversely, a low expression of DKK1 hampers the adhesion of macrophages to endothelial cells and thus attenuates inflammation as well as the risk of plaque rupture [128]. It is thus tempting to speculate that Wnt/β-catenin signalling, involving particularly the antagonists DKK1 and sclerostin, affects the interplay between bone and vasculature.

OPG/RANK/RANKL axis

The triad of OPG, RANK and the RANKL are key proteins involved in bone metabolism [129]. RANKL, which is expressed by osteoblasts, stimulates its receptor RANK on cell surfaces including osteoclast cells. OPG, a secreted glycoprotein and a member of the tumour necrosis factor (TNF) receptor superfamily, is expressed in most tissues, including bone and vasculature, and acts as a soluble decoy receptor for RANKL and thus blocks the molecular effects of RANKL [130, 131]. Of note, since OPG can directly counter RANKL-mediated actions through RANK, the OPG/RANK/RANKL axis may influence bone remodelling processes and thus bone mass.

Dysfunction of OPG/RANK/RANKL system is of importance for skeletal disorders manifested either as excessive bone loss or excessive bone formation. Aside from RANKL, OPG also binds the pro-apoptotic factor—TNF-related apoptosis-inducing ligand (TRAIL) (produced by immune cells), and initiates cell death through TRAIL receptors expressed primarily on tumour or transdifferentiated cells [132]. OPG$^{-/-}$ mice exhibit osteoporosis due to excessive RANKL activation with enhanced bone resorption and diminished bone formation [133].

Compared with the relatively well-established role of OPG/RANK/RANKL in bone metabolism, the role of this triad in vasculature, particularly the effector OPG, is less clear. However, while OPG may counteract vascular calcification by suppressing bone resorption and by inhibiting VSMCs osteogenesis thus serving as a local vascular calcification inhibitor, OPG$^{-/-}$ mice develop severe vascular calcification accompanied by formation of bone matrix in the vasculature [96]. Moreover, OPG treatment rescues warfarin-induced vascular calcification in rodent models when administrated simultaneously with warfarin [134]. On the other hand, in CKD, increased circulating OPG levels are associated with increased severity of media vascular calcification [135, 136]. Furthermore, OPG is expressed along the margins of calcified areas [131, 137], and OPG expression is 2- to 4-fold higher in carotid endarterectomy specimens from symptomatic patients as compared with asymptomatic patients [138].

Several potential insights might explain such contradictive findings. First, RANKL stimulates the secretion of inflammatory cytokines including monocyte chemotactic protein-1, TNF-α and interleukin-1, as well as MMPs, which could induce OPG expression in VSMCs [139]. Since OPG is a soluble decoy receptor for RANKL, an elevated OPG level could thus inhibit such pro-inflammatory process as a compensatory and atheroprotective effect. Secondly, the cellular effect of OPG can be independent of RANK/RANKL system as it can also directly bind TRAIL and inhibit TRAIL receptors. This is especially crucial in the development of atherosclerotic plaques as apoptosis is tightly associated with plaque destabilization [140]. Hence, increased OPG expression in plaques could indicate risk of rupture-prone phenotype. The theoretic role of OPG, acting as a compensatory mechanism in response to various harmful stimuli, could partially contribute to the complexity of vascular calcification; yet, it is worth noting that under certain conditions, OPG may also amplify unfavourable capacities, e.g. inducing apoptosis of VSMCs, stimulating secretion of inflammatory cytokines and MMPs [131, 141]. Whether increased OPG has a counteractive effect aiming at preventing the vascular calcification process (either via localized inhibition of inflammatory and atherogenic effects induced by RANKL and TRAIL or via systemic inhibition of bone absorption)—or merely represents progressive plaque development with osteogenesis of VSMCs in the vasculature—remains to be determined.

Galectin-3/RAGE axis

Another novel insight into the 'calcification paradox' is the role of galectin-3 and the RAGEs in bone remodelling and vascular

osteogenesis. Galectin-3 is a protein of the lectin family, encoded by the *Galactin-3 (LGALS3)* gene that influences growth, differentiation and apoptosis of cells in tissues such as bone and vasculature [142, 143], and is another key regulator of the Wnt/β-catenin signalling pathway [144]. Galectin-3 also acts as a receptor of advanced glycation end products (AGEs). Similar to other RAGEs it removes advanced glycation end products (AGEs), molecules that exert detrimental cellular effects and cause tissue injury [145], by binding of AGEs to RAGE [146]. With regards to bone metabolism, galectin-3 is a marker of chondrogenic and osteogenic cell lineages [147] and a critical factor in different stages of bone biology and function, and its expression is mediated by the bone growth regulator RUNX2 [148, 149]. On the other hand, galectin-3 has a role in atherogenesis [150] and thus participates in the bone-vascular interplay.

Both galectin-3 and RAGE are expressed in osteoblastic cell lines [151, 152]; while galectin-3 is critical for osteoblast differentiation by promoting osteoblastogenesis and suppressing osteoclastogenesis, RAGE is essential for osteoclast maturation and function [152]. Galectin-3-deficient (Lgals3$^{-/-}$) mice develop a bone phenotype characterized by reduced trabecular bone compared with wild-type mice [153] and with VSMCs showing decreased activation of Wnt/β-catenin signalling, suggesting that galectin-3 is capable of inducing osteogenesis [149]. The impaired osteogenesis of Lgals3$^{-/-}$ VSMCs is accompanied by RAGE up-regulation, corroborating the counteractive roles of galectin-3 and RAGE in osteoblast-like phenotype differentiation [149]. Galectin-3 and RAGE are also involved in vascular calcification. Galectin-3 may transform VSMCs into osteoblast-like cells via Wnt/β-catenin signalling, contributing to sheet-like calcifications, a process potentially enhanced by its anti-inflammatory effects [154]. In contrast, RAGE favours deposition of spotty or granular calcification by promoting and aggravating inflammation and by counteracting the osteoblastogenic effect of galectin-3, potentially via inhibition of β-catenin activation [155]. In Apo-E null mice, VSMC-targeted expression of RAGE ligand S100A12 results in plaque instability with increased numbers of spotty calcified nodules [156]. The galectin-3/RAGE axis

represents a novel link between bone and vasculature; however, its role in bone loss and vascular calcification in the context of CKD needs to be further studied.

The mechanistic molecular framework of the bone–vascular axis—through which impaired bone status associated with changes in the vascular wall via pathways such as Wnt signalling, OPG/RANK/RANKL system and the Galectin-3/RAGE axis—illustrates that osteogenesis and vascular calcification in fact share many similarities and that low BMD and vascular calcification therefore may represent two sides of the same coin. These insights may be of potential value for future preventive and therapeutic strategies targeting low BMD and its links with increased mortality in ESRD patients.

Key points:

The bone–vascular axis including pathways such as Wnt signalling, OPG/RANK/RANKL system and the galectin-3/RAGE axis may influence concomitantly both osteogenesis and vascular calcification.

SUMMARY AND CONCLUSION

ESRD is associated with profound metabolic and nutritional alterations that affect essentially all organ and tissues, and bone is no exception. Osteoporosis and renal osteodystrophy with reduced density and impaired quality of bone are common manifestations of CKD-MBD that associate not only with increased risk of fractures but also with vascular calcification, arteriosclerosis and increased mortality. Among various measurement methods to assess bone condition, DXA is the one with the strongest documentation of predicting risk of fracture and recent studies show that low BMD as assessed by DXA is also associated with the presence and severity of vascular calcification and increased mortality risk in ESRD patients. BMD measurements by DXA provide important prognostic
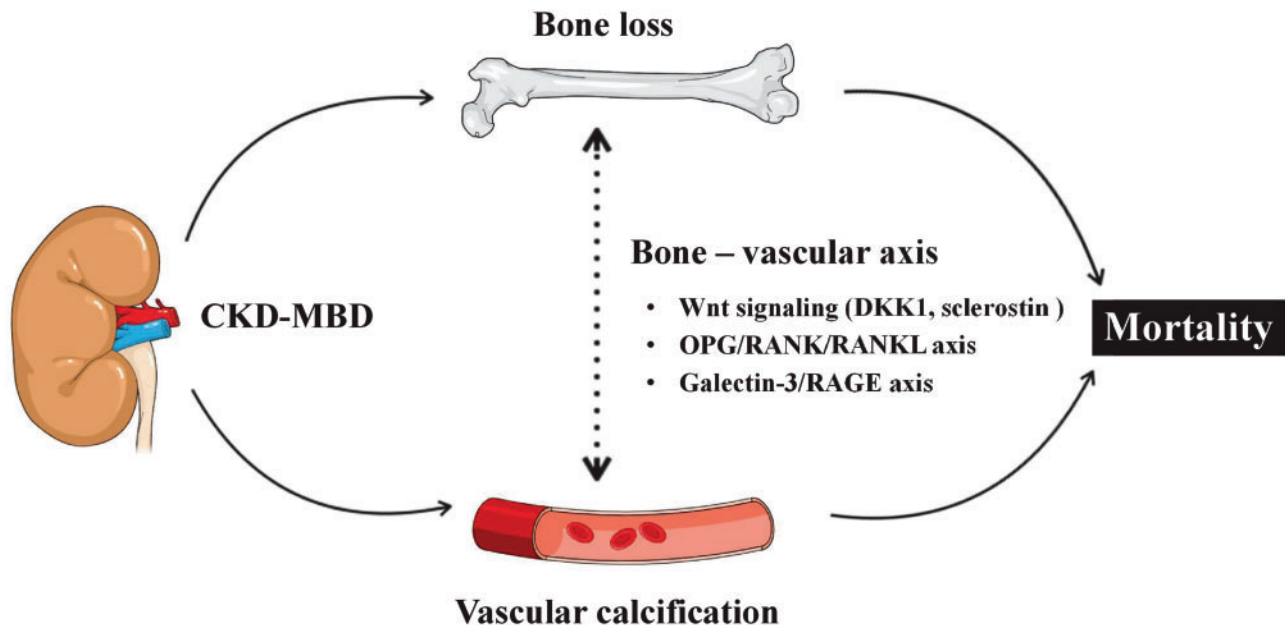
FIGURE 4: Possible mechanisms of bone–vascular interplay in ESRD. CKD-MBDs involve activation of shared molecular signalling pathways that concomitantly may promote impaired bone status with loss of bone mass and vascular calcification.

information of bone status and degree of osteoporosis but have different meaning depending on which bone sites are investigated. Recent evidence regarding bone changes caused by CKD-MBD suggests that cortical-rich bone sites might be more important than trabecular bone sites when assessing prognosis; however, further studies are needed. More importantly, changes of bone status, and in particular cortical-rich sites, reflect pathological conditions linked to vascular calcification, and are strong predictors of worse outcome in ESRD. The association between low BMD and poor clinical outcomes is to a large extent explained by the underlying alterations of the bone–vascular axis as processes involved in bone loss are also involved in ectopic calcification. Thus, low BMD and vascular calcification in ESRD may represent two sides of the same coin (Figure 4). Emerging evidence has indicated the important role of bone–vascular cross-talk involving pathways such as Wnt signalling, OPG/RANK/RANKL axis and galectin-3/RAGE axis, both in the aetiology of vascular calcification and in pathological changes of bone. The translation of these novel findings into new methods for preventive and therapeutic interventions would represent an important step towards improving the poor outcomes in the ESRD population that to a large extent appear to be linked to processes leading to bone disease and vascular calcification. Meanwhile, clinical and epidemiological studies are needed to link observed changes in bone status to biomarkers reflecting pathophysiological pathways including Wnt signalling, OPG/RANK/RANKL axis and galectin-3/RAGE axis. Further investigations of BMD and its relation to fractures, vascular calcification and mortality in ESRD patients receiving different nutritional, pharmacological and renal replacement therapies are warranted to establish optimal methods for DXA assessments, including selection of the 'best' sites for BMD measurements.

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CONFLICT OF INTEREST STATEMENT

B.L. is employed by Baxter Healthcare Corporation. P.S. is on scientific advisory boards of REATA and Baxter. None of the other authors declare any conflict of interest.

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