The connections between vascular calcification and bone health

Jorge B. Cannata-Andia[1], Pablo Roman-Garcia[1], Keith Hruska[2]

[1]Bone and Mineral Research Unit, Hospital Universitario Central de Asturias, RedinRen del ISCIII, Instituto Reina Sofía de Investigación Nefrológica, Universidad de Oviedo, Spain,

[2]Division of Pediatric Nephrology, Washington University in St Louis, St Louis, MO, USA

Corresponding author:

Jorge B. Cannata-Andía
Servicio de Metabolismo Óseo y Mineral
Instituto Reina Sofía de Investigación
Hospital Universitario Central de Asturias
C/ Julián Clavería s/n
33006 Oviedo, Asturias, Spain
Phone: +34 985106137
Fax: +34 985106142
E-mail: metoseo@hca.es

Abstract

Vascular calcification, bone loss and increased fracture risk are age-associated disorders. Several epidemiological studies have suggested a relationship between vascular calcification, impaired bone metabolism and increased mortality. So far, this relationship had been underestimated as osteoporosis and vascular calcification have been considered non-modifiable disorders of aging. Recent data suggest that this association is not simply an artefact of age, stressing that the coincidence of vascular calcification with low bone activity and osteoporosis could be biologically linked.

During the development of vascular calcification, the transition of vascular smooth muscle cells towards an osteoblast-like phenotype promotes the release of the vesicular structures, and mineralization within these structures is promoted by several players, including those related to mineral metabolism, like phosphorus, calcium or PTH, which influence either the supersaturation within the structure or the expression of osteogenic factors.

However, an intriguing question is whether the presence of vascular calcification impacts bone metabolism, thus demonstrating true cross-talk between these tissues. Evidence is now emerging, suggesting that some inhibitors of the Wnt pathway, such as secreted frizzled proteins 2 and 4 and DKK-1, may play a role linking vascular calcification and bone loss.

An additional important question to answer, from the patient's perspective, is whether or not progression of vascular calcification can be prevented or restricted and whether altering this progression we can efficiently impact patients' outcomes. Several evidences suggest the control of the CKD-MBD components, particularly serum phosphorus are the main targets to maintain normal bone turnover and protect against vascular calcification.

Introduction

Vascular calcification, bone loss and increased fracture risk are disorders associated with ageing, both in patients with chronic kidney disease (CKD) [1-7] and in the general population [8-11]. Furthermore, several epidemiological studies suggest a relationship between vascular calcification, impaired bone metabolism and increased mortality [10,12-15]. The relationship between bone metabolism and vascular calcification was a component of the decision made by the Kidney Disease: Improving Global Outcomes (KDIGO) foundation to adopt a new nomenclature, the CKD–Mineral and Bone Disorder (CKD–MBD), for a syndrome of CKD incorporating disturbances in mineral metabolism, vascular calcification, renal osteodystophy and excessive mortality [16]. This review is focused on the system biology between the skeleton, mineral metabolism and vascular calcification that is disordered in the CKD-MBD.

Bone turnover and vascular calcification

The association between bone fragility and vascular calcification has been made repeatedly since a significant inverse correlation between bone mineral density and aortic calcification was reported 20 years ago [12]. However, this association was probably underestimated because osteoporosis and vascular calcification were considered non-modifiable disorders of aging. Recent data suggest that this association is not simply an artefact of age. The role of ageing cannot be completely dismissed, but the clinical coincidence of vascular calcification with low bone activity and osteoporosis suggests that there are direct biological links between arteriosclerosis and osteoporosis, and the coincidence is supported by pathological science.

In support of this concept, a study published in 2004 demonstrated that patients with the highest degree of aortic calcification had the lowest bone density [17]. In the same cohort followed for 2 years, bone loss was greater in patients with progressive vascular calcification

[17]. In agreement with these results, a recent study from one of our groups, carried out in randomly selected general population, has shown that after 4 years of follow up, subjects with the most severe aortic calcification had not only a lower bone mass, but also a higher incidence of new osteoporotic fractures [11].

In addition, another recent study from the same group, involving patients on haemodialysis, demonstrated that vascular calcification of the large and medium calibre arteries was associated with an increased risk of vertebral fractures [4]. Both vascular calcification and vertebral fractures were associated with increased mortality in women participating in this study.

A relationship between vascular calcification and low bone turnover assessed by histomorphometric markers has also been demonstrated in haemodialysis patients [7] (Table 1). Preliminary data have demonstrated a negative relationship between low bone turnover and the degree of coronary artery calcification [18]. Data demonstrating an inverse relationship between mineralized bone volume and both coronary calcification and vascular stiffness have also been recently published [19].

Despite all of this evidence, the relationship between low bone turnover and vascular calcification remains under debate. A recent publication found that vascular calcification was not influenced by bone turnover when multivariate analysis was performed [20]. This is likely due to the fact that both high and low turnover were assessed. In fact, it is not bone turnover itself that is related to vascular calcification, but rather that bone resorption is in excess of bone formation, which can occur at any rate of turnover. This concept has been proven in the several phase three osteoporosis trials, especially with denosumab, wherein, inhibition of bone resorption and equalization with formation results in a major reduction of the serum calcium and phosphorus. These results demonstrate that the serum calcium and phosphorus, although normal in concentration, are being controlled through excess bone resorption. In

agreement with this concept, it has been reported that the correction of the balance in bone turnover, either high or low, protects against the progression of vascular calcification [21]. This is in agreement with translational studies demonstrating that stimulation of bone formation in CKD stage 3-4 corrected hyperphosphatemia [22]. Overall, most of the epidemiological, clinical and translational evidence strongly suggest that the incidence and progression of vascular calcification is inversely related to bone mass and positively related with the degree of mineralized bone loss, and thus with the incidence and prevalence of osteoporotic fragility fractures.

The low turnover osteoporosis of aging is not the only disorder of mineral metabolism that has been linked to vascular calcification. In patients with different stages of CKD, the serum phosphorus is strongly associated with increased vascular calcification and decreased bone strength. Indeed, abnormally high serum phosphorus concentrations have been described as one of the main pathogenetic factors inducing vascular calcification [23,24]. In contrast PTH levels have and have not been associated with vascular calcification. A recent metanalysis demonstrates that of the serum phosphorus, calcium and PTH, only phosphorus is associated with cardiovascular events and mortality associated with vascular calcification [25,26].

Pathophysiology of vascular calcification

Vascular calcification in patients with CKD occurs through precipitation of calcium phosphate as a consequence of unstable supersaturation of the exchangeable calcium and phosphate pools. However, the process is not solely a passive one related to precipitation from the extracellular fluid surrounding vascular smooth muscle cells (VSMCs) of the vascular walls. Rather VSMCs undergo a transition away from their contractile functional state, expressing markers of their osteoblast cousins and develop an exchangeable calcium/phosphorus pool analogous to the site of bone formation wherein calcification of the skeleton occurs. The analogy to bone formation is especially strong in atherosclerotic calcification of the neointima

stimulated by CKD [27]. In the other form of vascular calcification stimulated by CKD, medial arterial calcification, the process represents a complex set of steps in which the normal inhibitors of calcification are diminished and concentrations of calcium and phosphorus produce unstable supersaturation leading to crystal formation and vascular calcification. Calcification appears to be initiated by the release of vesicular structures from VSMCs that contain hydroxyapatite [26]. The transition of VSMCs towards the osteoblastic phenotype promotes the release of the vesicular structures, and mineralization within these structures is promoted by expression of osteoblastic proteins. Osteoblastic morphogens, the bone morphogenetic proteins (BMP)-2 and BMP-4, transcription factors, core-binding factor 1 (Cbfa-1, also know as Runx2), a key transcription factor in osteoblast differentiation, and bone proteins such as alkaline phosphatase and osteocalcin are all components of the osteoblastic transition of VSMCs [28].

The factors that are involved in this change in VSMC phenotype have been the focus of much research in recent years, with evidence suggesting that it is driven both by an increase in factors that promote this change and a decrease in factors that inhibit it. In recent times, a host of these calcification promoters and inhibitors have been identified, some of which may be systemic and others localized (Figure 1). The relative importance of these factors is unclear, and it is likely that some play more of a role in the progression of soft tissue calcification rather than in its initiation.

Several factors related to mineral metabolism have been shown to promote calcification. For example, it has been demonstrated *in vitro* that high calcium and phosphorus levels induced VSMC calcification [29]. As stated above, serum phosphorus seems to be particularly important in the development of vascular calcification. Indeed, serum phosphorus may well be the link between bone turnover and vascular calcification. When bone turnover is low, as in the adynamic bone disorder, the size of the exchangeable calcium and phosphorus pool is reduced leading to larger excursions in the concentrations associated with intake. In addition,

bone resorption is in excess of formation serving to block the reservoir function of the skeleton for excess phosphorus. In the case of high bone turnover, as in secondary hyperparathyroidism, phosphorus is released from bone and again the reservoir function of the skeleton is compromised. Stimulation of skeletal anabolism and increasing bone formation rates above rates of resorption reduce hyperphosphatemia, demonstrating restoration of the reservoir function of the skeleton. In these studies with BMP-7 as the anabolic principle vascular calcification was reduced in part by movement of phosphorus into the skeleton [22]. As renal excretion of phosphorus was not increased in these studies, the decrease in phosphorus levels must have been due to the increase in bone formation. Treatment with phosphate binders, which may serve to decrease the supersaturation of the exchangeable Ca-Pi pool, has also been shown to prevent vascular calcification in mouse models [30].

Phosphorus has also been shown to directly stimulate the osteoblastic transition of VSMC in CKD [31]. The mechanism of phosphorus in inducing calcification has been explored. Knockdown of the putative phosphate sensor, the sodium-dependent phosphate co-transporter, Pit-1, by siRNA inhibited phosphorus-stimulated mineralization of VSMCs [32]. This indicated that vascular calcification might be regulated by cellular uptake of phosphorus. Extracellular Pi signaling or increased intracellular phosphorus stimulate VSMCs to undergo transition to an osteoblastic phenotype, expressing Runx2, Msx2 and osterix the critical osteoblastic transcription factors promoting the expression of the osteoblast transcriptome [33] and stimulating matrix vesicles (Figure 2). An additional role for serum phosphorus and calcium may be to promote VSMC apoptosis, contributing to the initiation of calcification, since apoptotic bodies may function similarly to matrix vesicles in heterotopic mineralization.

The concept of the vasculature under the influence of osteoblastic transition acting as a new reservoir for phosphorus since deposition is in excess or resorption may explain why vascular calcification is present before hyperphosphataemia is detected [34,35]. Vascular calcification has

been detected in 47% of non-diabetic patients with Stage 4 CKD [36] and up to 94% of predialysis patients with diabetes [37], but hyperphosphataemia usually only manifests in Stage 4 and 5 CKD [38]. Data suggest that fibroblast growth factor (FGF)-23 levels increase early in CKD and may be a marker of increased phosphorus load ahead of the development of hyperphosphataemia [35]. Current evidence on the association of FGF-23 with vascular calcification is mixed, but recent studies demonstrate a positive and independent association with aortic calcification especially in early CKD in translational models and in patients [39]. Elevations in FGF23, a hormone secreted mainly by the osteocyte in early CKD, indicate that the skeleton has been affected by renal damage. Then becomes what is the signal for the osteocyte to secrete FGF23? While this remains to be proven, changes in the Ca-Pi exchangeable pool are leading contenders. Thus in CKD prior to hyperphosphatemia, the changes in the systemic environment produced by a high phosphorus load and a blocked skeletal reservoir, leads to vascular calcification which acts together with increased renal excretion to maintain normal serum phosphorus concentrations.

Other mineral metabolism parameters that may contribute to the development and progression of vascular calcification include calcium, vitamin D and PTH.

An excess of calcium load or efflux from bone, both in high or low bone turnover states may favour cardiovascular calcification and or cardiovascular events [40]

VSMCs express vitamin D receptors [41] and pharmacological calcitriol doses induce matrix mineralization of VSMCs *in vitro* [42]. However, the physiological function of vitamin D receptor activation in VSMCs is inhibitory to matrix mineralization through stimulation of smooth muscle differentiation and repression of osteoblastic transition [43]. Patients with CKD generally have low levels of vitamin D, the use of low-dose of vitamin D has been associated with a lower mortality [44] Both vitamin D/calcitriol deficiency and pharmacological doses of active vitamin D analogues stimulate vascular calcification, suggesting a biphasic dose response and underscoring the protective inhibitory physiological actions of calcitriol [45,46].

The role of PTH is also unclear. PTH fragment 1–34 has been shown to inhibit calcification in a murine model of atherosclerotic vascular calcification [47], but PTH 7–84 may act to increase vascular calcification [48] and high PTH levels are often associated with high calcification scores [20]. Some studies demonstrated that PTH itself is not able to induce vascular calcification, but has a synergistic effect with the phosphorus, probably due to an indirect and deleterious effect associated with bone remodelling and osteoclastic activity [49]. A recent meta-analysis of factors related to vascular calcification and mortality has reinforced the role of Pi as a cardiovascular risk factor but failed to indentify the role of PTH [25].

Soft tissue calcification occurs in some patients with CKD well before mineral metabolism is impaired, and recent studies demonstrate onset of vascular calcification in Stage 2 CKD before stimulation of osteoblastic transition is demonstrable. Uraemic serum has been shown to induce osteoblast-like changes in VSMCs, even when blocking Pit-1 restricts the effect of phosphorus. Inflammation and reactive oxygen species are two factors that have been also associated with vascular calcification. Inflammation has been widely described as one component of atherosclerosis and medial vascular calcification. It has been shown that when the inflammatory molecule “tumour necrosis factor alpha” (TNF-alpha) is overexpressed in the vessels, the mice show vascular calcification, with a higher activation of Wnt in their VSMCs, a fact which can promote osteogenesis of aortic smooth muscle cells *in vitro* [50-53]. The latter strongly suggests that inflammation may promote vascular calcification, probably, via the Wnt signalling pathway. All these experiments support the idea that vascular calcification can be mediated by players that could act upstream in the cascade of events that promotes vascular calcification. As osteoporosis has an important inflammatory component, the latter might be part of the pathway linking vascular calcification and bone loss. In addition, several other factors related with inflammation and oxidative stress have been implicated, among them hydrogen peroxide has been reported to stimulate Cbfa-1 directly [54] and BMP-2, which is high in uraemic serum ; increases osteoblastic differentiation

of calcifying cells and may also reduce expression of matrix Gla protein, a calcification inhibitor [55]. Also, leptin, a fat derived circulating factor stimulated by inflammation, has been shown to induce calcification [56]. In summary, there are many inflammation-related molecules that may be present in the uraemic serum that promote vascular calcification and it is unlikely that there is only one definitive initiating factor.

Vascular calcification inducing bone loss

It is clear that impaired bone metabolism and its consequences have an important role in the development of vascular calcification. However, an intriguing question is whether the presence of well established and severe vascular calcification can have an impact on bone metabolism, thus demonstrating true cross-talk between these tissues. Some evidence is now emerging.

In recent CKD translational studies, in rats fed a high phosphorus diet and LDLR-/- mice fed a high fat diet the increase in aortic calcification were associated with decreases in bone mass [29]. In addition, the microarray analysis of areas with severe vascular calcification showed over-expression of the family of secreted frizzled-related proteins (SFRPs) [57]. The SFRPs are circulating wingless/int (Wnt) protein inhibitors. Induction of interstitial nephritis is associated with up-regulation of SFRP4, SFRP2, and DDK1 in the vascular adventitia [58]. SFRPs and DKK1 are inhibitors of the canonical signalling Wnt pathway, which is actively involved in bone formation and vascular calcification [51,52,59]. This increase in SFRPs in areas of severe vascular calcification may be indicative of a vascular wall artery defense mechanism triggered in order to block the activation of the Wnt pathway, with the aim of attenuating mineralization in the calcified aortic wall. As the SFRPs are secreted circulating proteins, they may act not only locally in the artery wall to reduce mineralization, but also in bone impairing mineralization, resulting in reduced bone mass.

This new and challenging feedback hypothesis may help to explain some of the results observed in epidemiological studies in the general and CKD population, in which the more severe cases of progressive vascular calcification were associated to greater bone loss, and more bone fractures [4,11].

The need for further research

The association between impaired bone health and vascular calcification has sparked tremendous research effort in recent years. However, it is clear that for some questions, definitive answers are still being sought.

Can we definitely say that vascular calcification is a consequence of low bone turnover? In our view, it is clear that low bone turnover, a finding which can be observed in osteoporosis and adynamic states represents an environment that favours vascular calcification,. It can be speculated that patients with aging or CKD and adynamic bone disorder are particularly at risk of the damaging effects of high calcium and phosphorus. The strong emerging consensus from observational studies suggests that Pi is a cardiovascular risk, and this risk is heightened in the aged osteoporotic or CKD patient. Low bone turnover is a powerful trigger for the development of abnormalities in the exchangeable calcium and phosphate pool that stimulates vascular calcification. However, increased bone turnover is also present in the CKD population, and this is also likely to increase the risk of vascular calcification, again via the resulting impaired calcium and phosphorus metabolism. No doubt other factors also trigger calcification, and inflammation may be particularly important in patients with diabetes.

One of the most important questions to answer from the patient's perspective is whether or not progression of vascular calcification can be prevented or restricted. In considering this, it is helpful that more is now known about the pathogenesis of this potentially fatal complication of CKD and clear modifiable targets are being identified. However, it is important to state that despite it has been clearly established that cardiovascular calcification is associated with

adverse clinical outcomes, there is no yet strong data proving that altering the progression of cardiovascular calcification impact patients outcomes.

Efforts to maintain normal mineral metabolism, and thus bone health, are at the heart of strategies to prevent soft tissue calcification. A clear target is the control of serum phosphorus, several phosphate binders are available, some of which contain calcium. Whether calcium-based binders contribute to the progression of vascular calcification has been a matter of much debate. Some studies have shown that non-calcium-based binders may attenuate vascular calcification in comparison with calcium-based agents [60], whereas others have not [61]. It has been suggested that the use of calcium-based agents may be of particular concern in patients with adynamic bone disease [3]. Given the clearer evidence for the role of phosphorus, physicians should perhaps give greater consideration to the ability of phosphate binders to reduce serum phosphorus levels and maintain good bone health. Treatment with non-calcium-based-binders has been shown to lead to beneficial changes in bone histomorphometry in patients with either high or low turnover bone disease [62,63]. As phosphorus load appears to increase ahead of the development of hyperphosphataemia this could conceivably contribute to calcification; phosphorus restriction before hyperphosphataemia occurs is therefore an intriguing prospect. Studies assessing the effect of phosphate binders in patients with normal serum phosphorus levels are ongoing and the results will be of interest.

The effect of vitamin D treatments on vascular calcification in patients with CKD is still unclear, but several studies have shown a survival benefit associated with vitamin D [2,64]. This benefit seems more evident with low–dose treatment, in a range of physiological replacement [2]. Some evidence suggests that the calcimimetic cinacalcet may protect against vascular calcification in patients on dialysis [65,66], but the clinical evidence is as yet limited. A recent study investigated the use of cinacalcet plus low-dose vitamin D therapy, compared with vitamin D therapy alone, on coronary artery calcification [67]. Results showed a trend towards attenuation of the progression of coronary artery calcification, although the difference in

calcification scores between groups did not reach statistical significance [66]. Any beneficial effects of cinacalcet may be confined to patients on dialysis; as in the early stages of CKD the actions of cinacalcet on PTH lead to an unwanted increase in serum phosphorus levels [68,69].Agents that act directly on bone may also be effective in attenuating calcification. Preclinical studies have suggested the potential for inhibition of the receptor activator of NF-kappaβ ligand [70], and a potential role for the skeletal anabolic BMP-7 [22].

Summary

There is good evidence to suggest that impaired bone turnover, particularly low bone turnover, promotes the progression of vascular calcification. Several factors have been identified as possible links between bone and calcifying soft tissues, but a greater understanding of the key determinants of vascular calcification is still required. Maintenance of good bone health appears to be critical to maintaining good cardiovascular health in patients with CKD. Intriguingly, the original rationale for controlling serum phosphorus levels was to maintain bone health and it would appear that we have to focus again on this aspect of treatment to reduce cardiovascular mortality. Phosphate binders offer an effective approach to maintaining normal bone turnover and are likely to help to protect against vascular calcification.

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Author's contributions

JCA designed and wrote the revision, PRG wrote the revision and the figure 1 and KH designed and wrote the revision and made figure 2 and Table1

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TABLE LEGEND

Table 1. Correlation of bone histomorphometric parameters and parathyroid hormone levels with calcification score. Adapted from London *et al*. 2004 [7] with permission.

Range 0–4, where 0 = no calcification detected and 4 = generalized calcification in all arterial segments examined. PTH, parathyroid hormone.

FIGURE LEGENDS

Fig. 1. Promoters and inhibitors of vascular calcification Modified from a picture produced by Professor G. London with permission.

ALP, alkaline phosphatase; BMP, bone morphogenetic protein; Ca, calcium; CBfa1, core-binding factor 1 (also known as Runx2); LDLox, oxidized low-density lipoprotein; MGP, matrix GLA protein; P, phosphorus; PTH, parathyroid hormone; PTHrP, parathyroid-hormone-related protein; TNF-α, tumour necrosis factor alpha; Vit D_3, calcitriol; Wnt, wingless/int proteins.

Fig. 2. The active role of phosphorus in vascular calcification. Adapted from Yang *et al.* 2004 [71] with permission; Ca, calcium; Na, sodium; P, phosphorus; Pit-1, sodium-dependent phosphate co-transporter; SMC, smooth muscle cell; VSMC, vascular smooth muscle cell.