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Review Article

Review article: Getting the balance right: Assessing causes and extent of vascular calcification in chronic kidney disease

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SUMMARY: Vascular calcification is part of the definition of chronic kidney disease – mineral and bone disorder (CKD-MBD). It is also a surrogate parameter of cardiovascular and all-cause mortality risk in the CKD population. However, vascular calcification is not a homogenous entity, but a rather complex manifestation influenced by derangements of calcium and phosphate homeostasis, by dysregulated calcification inhibitors and promoters, and by the type of arterial disease (atherosclerosis *vs* arteriosclerosis). Despite the clear-cut risk association between the presence of vascular calcification and mortality, it is currently not well defined, how this knowledge about calcification should be translated into active clinical management. Further, the choice of the appropriate imaging test is a matter of debate. This article attempts to provide an update on insights into the pathophysiology of vascular calcification processes and a subjective view of the clinical consequences of management of CKD patients at risk.

KEY WORDS: **bone turnover, calcium, fetuin-A, MGP 3, vascular calcification.**

VASCULAR DISEASE IN CHRONIC KIDNEY DISEASE

Accelerated calcifying athero- and arteriosclerosis, as well as valvular heart disease, are hallmarks of cardiovascular disease manifestations in patients with chronic kidney disease (CKD). The different roles of intimal (atherosclerotic) and medial (arteriosclerotic) manifestations are well described in this population. While plaques obstruct vessels and, thus, cause ischaemic events, medial sclerosis, or in most instances more correctly medial calcification, manifests as arterial stiffening as detected by an increased pulse wave velocity (PWV). Increased PWV is a key contributor to left ventricular (LV) stress and dysfunction because premature reflection of the arterial wave invisibly elevates the LV pressure load. However, there are only very few autopsy or biopsy studies proving in humans that both manifestations are indeed caused by completely different pathomechanisms and that their appearance patterns differ between normal and CKD populations.[1–4] Gross and colleagues demonstrated that coronary arteries from dialysis patients, in contrast to patients with normal renal function, may develop some areas of medial sclerosis, on top of classical coronary atherosclerosis. Vessels from children with CKD mostly show medial sclerosis which appears to follow an accelerated course in association with time on dialysis treatment. Epigastric arteries which were obtained from dialysis patients at the time of transplantation display a mixed athero- and arteriosclerotic calcification pattern. In this context, different vascular beds may show very distinct manifestations of vascular disease and calcification.

While intimal plaque calcification is the key feature of genuine atherosclerosis and probably the exclusive coronary artery calcification manifestation in the normal population, medial calcification seems to predominantly manifest in peripheral and small arteries. Whether larger arteries and the aorta may develop 'pure' arteriosclerosis, or whether large artery calcification may be a mixture of intimal and medial, or mostly pure intimal calcification, is a matter of debate. Certainly, the magnitude of vascular calcification is exorbitantly high in CKD, and further aggravated by diabetes and age. Based on registry data and cross-sectional analyses, hyperphosphataemia and/or hypercalcaemia (derived from a positive calcium balance, e.g. calcium-containing phosphate binders) are also thought to be causal factors of both cardiovascular mortality and progression of undesirable calcifications in uraemia.[5]

Finally, cardiovascular calcifications certainly develop prematurely in CKD when compared to normal populations, as three studies focussing on young dialysis patients with childhood-onset renal disease indicated.[6–8] By different

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imaging techniques it was demonstrated that cardiovascular calcifications are highly prevalent in children and young adults, associated with an increased calcium × phosphorus product, high parathyroid hormone (PTH) levels, increased calcium intake and inflammation.

IMAGING OF CARDIOVASCULAR CALCIFICATION IN CKD

Most of the available studies on the prevalence and natural history of cardiovascular calcification in CKD used computed tomography (CT)-based techniques (electron-beam computed tomography (EBCT), multi-slice computed tomography (MSCT)). EBCT and MSCT are currently regarded as the most sensitive methods for detection of cardiovascular calcification and, thus, as the gold standard among imaging techniques. Of note, CT-based techniques cannot differentiate between intimal and medial calcification, however, coronary calcification mostly reflects atherosclerosis even in the dialysis population.[1,2] One study explicitly evaluated the sensitivity and specificity of several imaging tests and functional/haemodynamic measures for detecting vascular calcification compared to the detection of coronary artery calcium (CAC) by EBCT.[9] This analysis focussed on pulse pressure measurements, valvular calcification (by echocardiography) and abdominal aortic calcification (by lateral abdominal X-ray) in comparison to the severity of CAC scores as assessed by EBCT scores of 30–99, 100–399, 400–999 and 1000 or more. Pulse pressure is a function of heart rate and blood pressure, and increased pulse pressure is characterized by increased systolic and normal or decreased diastolic pressures; thus, this measure only roughly and sometimes inaccurately reflects the vascular calcification burden. In this regard, no meaningful correlation was found between pulse pressure and CAC scores, however, a good correlation was detected between abdominal aortic calcification by plain radiograph and CAC scores. Valvular calcification, as detected by echocardiography, was another feasible predictor of CAC.

Some additional studies performed correlation analyses comparing CT-based imaging techniques of assessing CAC with other measures of calcification. Pulse pressure, abdominal aortic calcification by lateral X-ray, PWV, echocardiography (valvular calcifications), intima-media thickness (IMT) of the carotid arteries, and MSCT of the thoracic and abdominal aorta were tested in this regard.[8,10–14] Abdominal aortic calcification again appeared to be a good predictor of CAC scores, as did PWV measurements. PWV, however, is not always available and a method which is limited in its widespread use by being dependent on the appropriate training of the individual investigator. The value of IMT, valvular calcification, and especially pulse pressure, appeared to be quite limited, but these studies in general were not explicitly designed to systematically evaluate test sensitivity and specificity. It has to be pointed out that even the currently available cardiac CTs are not capable of detecting early vascular calcification manifestations, as was recently quantitatively demonstrated in arteries from children when compared to imaging readouts.[3]

Table 1 Available imaging methods to detect cardiovascular calcification[3,9–16]

Method	Clinical importance
Multi-slice spiral computed tomography	Gold standard
Electron-beam computed tomography	Gold standard (limited availability)
Plain lateral abdominal x-ray	Good correlation with CT
Valvular calcification by echocardiography	Good correlation with CT
Pulse wave velocity	Good correlation with CT (investigator-dependent; limited standardization and availability)
Pulse pressure	Very limited correlation with CT
Intima-media thickness (carotid IMT)	Limited correlation with CT (questionable surrogate of calcification)
Aortic calcification by CT	Probably good parameter (validation limited)
Fistula calcification	Possibly good parameter (validation limited)

CT, computed tomography; IMT, intima-media thickness.

The majority of the calcification imaging data were obtained in CKD stage 5D populations, while some studies included patients in earlier CKD stages (10.14). Despite EBCT and MSCT techniques remaining the gold standard, especially plain X-rays and, possibly, the detection of valvular calcification by echocardiography may become practical alternatives to evaluate the calcification status in CKD patients (Table 1).

CARDIOVASCULAR CALCIFICATION AS A RISK FACTOR FOR INCREASED MORTALITY

Cumulative data on calcification manifestations in more than 4000 CKD patients are currently reported in the published work. The cross-sectional prevalence of cardiovascular calcification in CKD is clearly dependent upon age and time on dialysis and varies among different populations. In prevalent haemodialysis patients, coronary artery calcification is found to be present in up to 90% of this population. In incident dialysis patients and patients in CKD stages 4–5, significant vascular calcification is detected in approximately 50–60%.

Most information on the risk prediction of cardiovascular calcification was derived from studies in dialysis patients. However, there is also some information on renal transplant recipients and patients in CKD stages 4–5.[12,17] EBCT, MSCT, ultrasound, echocardiography and several conventional X-ray techniques were used as diagnostic tests. Uniformly, cardiovascular calcification or progression of calcification were identified as independent risk predictors for cardiovascular and all-cause mortality. Risk associations between the development and progression of calcification,

and epidemiological and biochemical parameters were analyzed in most of these studies. Age was the most consistent risk factor for severe or progressive calcification, while diabetes, time on dialysis, male sex, high serum intact PTH and/or alkaline phosphatase levels, inflammation (C-reactive protein levels), calcium intake, hyperphosphataemia and increased calcium × phosphate product were identified in some but not in all studies. Consistent information on the relationship between cardiovascular calcification and bone outcomes in CKD patients is currently not available.

INSIGHTS INTO PATHOMECHANISMS OF EXTRAOSSEOUS CALCIFICATION: INDUCERS AND INHIBITORS

Historically, extraosseous calcification in dialysis patients was interpreted as a sole result of prolonged supersaturation of serum with Ca and P ions, namely passive precipitation. Serum is, indeed, a 'metastable' Ca and P solution, however, precipitation is actively prevented by a number of systemic and local factors. Three key pathophysiological pathways contribute to unwanted extraosseous calcification in CKD: (i) 'true' passive precipitation of Ca and P in the presence of excessively high extracellular concentrations; (ii) presence or upregulation of promoters/inducers of cellular osteogenic transformation and hydroxyapatite formation; and (iii) deficiencies of calcification inhibitors.

Hyperphosphataemia is one of the most potent active inducers of vascular calcification. *In vitro* and *in vivo* studies document that the key process in vascular, in particular medial, calcification is an active, cell-mediated event driven by increases in intracellular phosphate uptake and concentration inducing a phenotypic switch of vascular smooth muscle cells (VSMC) into osteoblast-like cells.[18,19] Blocking intracellular phosphate entry by inhibitors of the sodium-phosphate co-transporter, PIT-1 immediately prevents osteogenic differentiation and hydroxyapatite formation, despite the presence of a high extracellular phosphate milieu.[18] In this context, high calcium exposure *in vitro* acts synergistically with hyperphosphataemia in inducing this osteoblast-like transformation of VSMC.[20]

Fetuin-A (α2-Schmid Heremans glycoprotein, AHSG) is a hepatocyte-derived serum protein (molecular weight, ~60 kD).[5] Serum concentrations are relatively high with levels between 0.5–1.0 g/L in average populations. The dominant biological function of fetuin-A is inhibition of calcification, thus potently limiting hydroxyapatite crystal formation, as has been conclusively demonstrated in fetuin-A gene knockout animals.[21] It is estimated that fetuin-A is responsible for approximately half of the precipitation inhibitory properties within the extracellular space. Fetuin-A molecules have been shown to form stable colloidal spheres with Ca and P, so-called calciprotein particles (CPP).[22,23] Such CPP possibly fulfil extracellular clearance functions for small calcification nuclei, analogous to the function of lipoproteins. *In vitro*, fetuin-A is not produced but taken up by VSMC. Presence of fetuin-A in the culture medium subsequently inhibits formation and the intracellular calcification of so-called matrix vesicles prior to their extracellular release.[24]

Fetuin-A deficiency is both a mortality and calcification predictor in dialysis patients.[3,25–27] In part, these associations are related to chronic inflammation, because fetuin-A is a negative acute-phase reactant. In patients not on dialysis, the data concerning fetuin-A-associated risk relationships is less clear. There are putative relationships between increased fetuin-A levels and the likelihood of metabolic syndrome, which are based on limited experimental data and need to be confirmed in the future.[28,29] It currently appears probable that the dialysis state reflects a breakdown of several cardioprotective systems, and, thus, the down-regulation of one additional (calcification inhibitory) system may translate into outcomes.

A second key factor in calcification protection is matrix Gla protein (MGP).[5] MGP belongs to a family of N-terminal γ-carboxylated (Gla) proteins which require a vitamin K-dependent γ-carboxylation for biological activation. MGP is a pivotal local inhibitor of cartilage and arterial calcification. MGP gene knockout mice are characterized by severe medial calcification of the aorta leading to lethal ruptures of the bone-like aorta within a few weeks after birth.[30] There are three incidental pieces of evidence connecting vitamin K deficiency to the risk of vascular calcification. The Rotterdam study group identified that a low vitamin K2 (menaquinone) intake was associated with coronary artery disease-related and all-cause mortality and with the severity of aortic calcification.[31] Further, long-term use of vitamin K-antagonist-based oral anticoagulation (warfarin) in patients with aortic valve disease was found to be associated with higher coronary and valvular calcium scores as compared to a cohort without anticoagulation.[32] Finally, calciphylaxis, a disastrous ulcerating syndrome characterized by extensive and progressive calcification of small cutaneous arterioles, is associated with a high coincidence of warfarin treatment.[33,34] Experimentally, vascular calcification can actively be induced by warfarin in rats: withdrawal of warfarin and subsequent vitamin K1 or K2 replacement can partially reverse the induced calcification in this model.[35] Data from registries and prospective clinical studies (e.g. evaluation of the therapeutic impact of menaquinone on the progression of cardiovascular calcification) is needed to explore the potential therapeutic impact of vitamin K replacement strategies. Of note, even high-dose vitamin K supplementation is harmless, because it will never cause a pro-coagulatory state; thus, vitamin K supplementation appears to have the potential of an attractive form of treatment.

There are several potent additional systems of calcification protection of the body, including pyrophosphates and the RANK-ligand/osteoprotegerin system. Further, healthy bone turnover certainly contributes to protection from cardiovascular calcification by serving as a buffer reservoir for excess calcium and phosphate. Therefore, deranged bone metabolism (high-turnover or adynamic states) may trigger and maintain extraosseous calcification processes. However, in 2005, a seminal experimental publication by Murshed

and colleagues painted a very convincing integrative picture summarizing many of the known mechanisms; their key results were as follows:[36] (i) extracellular phosphate availability rather than calcium regulates bone mineralization (*in vitro*); (ii) extracellular phosphate is a key modifier of extraosseous calcifications; extraosseous calcifications are prevented by hypophosphataemia and actively induced by a high phosphate load in gene knockout models of calcification inhibitor deficiency; (iii) the co-presence of increased levels of phosphate, low levels of calcification inhibitors and an appropriate bone-like matrix permits proactive calcification at any tissue site.

MANAGEMENT OF CARDIOVASCULAR CALCIFICATION

There is no definite or conclusive evidence available on how to prevent or treat progressive calcification in CKD. There are, however, five studies comparing the effects of different phosphate binder therapies on the progression of CAC scores in CKD population (four in haemodialysis patients, one in CKD stage 3–5 patients).[37–41] The Treat-to-Goal (TTG) study analyzed the progression of CAC and aortic calcification (EBCT) in prevalent haemodialysis patients over 1 year comparing the impact of sevelamer-HCl to calcium-containing phosphate binders.[37] In this study, sevelamer-HCl use was associated with an overall lack of calcification progression. In the Renagel in New Dialysis Patients (RIND) study, a similar design was used and similar results were obtained in incident haemodialysis patients randomized within 90 days after starting dialysis treatment.[38] In contrast, the Calcium Acetate Renagel Evaluation-2 (CARE 2) study showed equal progression rates of CAC with both compounds when atorvastatin was added to lower low-density lipoprotein (LDL) cholesterol to comparable levels.[39] The CARE 2 population however included a large percentage of cardiovascular high-risk patients (smokers, >50% diabetics) which may have caused a blunted responsiveness to modifications of phosphate binder treatments or calcium loads. The BRIC study longitudinally investigated CAC progression and bone histomorphometry in haemodialysis patients comparing calcium acetate *versus* sevelamer-HCl.[40] The major conclusions were that there was no difference in CAC progression or changes in bone remodelling between the calcium and the sevelamer groups. This study had a major confounder, because high dialysate calcium concentrations (1.75 mmol/L) were used in most patients. Thus, a generally positive calcium balance may have neutralized the potential advantage of the calcium-free phosphate binder.

The study by Russo and colleagues is the only available study to date evaluating CAC progression in pre-dialysis patients.[41] Patients were stratified to treatment with low phosphate diet alone, low phosphate diet plus calcium carbonate or low phosphate diet plus sevelamer-HCl. CAC progression was halted only in the sevelamer-HCl treated group, while CAC progression was still less pronounced with calcium carbonate than with low phosphate diet alone. Taking together the results from epidemiological trials and from these prospective studies on the surrogate outcome 'CAC progression', it appears quite possible, but not proven, that a high calcium load may be an undesired factor in the pathogenesis of CKD-related calcification in subjects with pre-existing calcification. The threshold of a tolerable *versus* a harmful calcium load is currently not defined, and probably depends on the coexisting degree of hyperphosphataemia and the bone turnover state.

No studies have systematically investigated the effect of parathyroidectomy or the impact of calcimimetics and vitamin D-analogues on calcification progression or regression. Experimental data *in vitro* and in animal models of renal insufficiency with secondary hyperparathyroidism suggest differential effects of calcimimetics, calcitriol, doxercalciferol and paricalcitol on vascular calcification.[42–44] Calcitriol consistently and doxercalciferol mostly induced arterial calcification in these models, while these effects were associated with profoundly high calcium and/or phosphate levels. While paricalcitol showed no or less pronounced arterial calcification, calcimimetics were completely neutral and even able to actively prevent vitamin D-induced calcifications in these models. A recent translational study by Hruska's group in uraemic LDL-receptor knockout mice suggested that vitamin D-analogues (calcitriol, paricalcitol) may have differential effects depending on employing either low (protective) or high (inducing) doses.[45] The protective effects may be related to the down-regulation of Runx2 (cbfa-1), the key transcription factor of osteogenic differentiation. Calcification protective properties of calcimimetics in humans are currently tested in the prospective ADVANCE study evaluating CAC progression by MSCT in haemodialysis patients.

CONCLUSION

Vascular and soft-tissue calcifications are not a random process of passive Ca and P precipitation, but involve active cellular processes influenced by the presence or absence of inhibitors and inducers. Cardiovascular calcification is also a strong risk predictor of death in the CKD population. High phosphate may be the key element in this context, therefore, limiting excess phosphate exposure and regulating phosphate metabolism currently appears to be the most prudent and intriguing therapeutic approaches. Unanswered questions include the optimal choice, dose and combination of drugs to tackle the different features of CKD mineral and bone disorder, including phosphate binders, vitamin D analogues and calcimimetics. In the future, modifying vitamin K status, pyrophosphate or fetuin-A release may become additional targets in calcification-prone individuals.

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