Vascular calcification in chronic kidney disease: a review

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ABSTRACT

Vascular calcification (VC), an independent and strong predictor of cardiovascular risk, is often found in CKD patients. The degree of VC is providing incremental prognostic value over traditional risk markers. There is interest in improving our understanding of mechanisms, establishing diagnostic methods and effective prevention and treatment modalities. The abnormal mineral metabolism of CKD is known to facilitate the progression of VC, in concert with altered activities of VC inhibitors. Possible measures to prevent VC include the control of serum calcium and phosphate as well as other factors involved in its progression, including vitamin D sterols, parathyroid hormone, fibroblast growth factor-23, klotho, and VC inhibitors. In addition, we discuss new possible therapeutic approaches to halt VC or reverse its progression. The principal aim of this review is to provide an updated overview of VC in patients with CKD, with particular focus on pathophysiology, diagnosis, prevention and treatment.

Keywords: chronic, renal insufficiency; review; vascular calcification.

INTRODUCTION

Based on the *Health Science Descriptors* definition,[1] vascular calcification (VC) is a pathologic process characterized by thickening and loss of elasticity of the muscular arteries walls due to calcification of the tunica media and/or intima.

The majority of authors refer to VC as a general term for both intima and media calcification. However, there are differences between them. Intima calcification, which typically occurs within atherosclerotic plaque in the aorta, coronary arteries, and other large arteries, is an indicator of advanced stages of atherosclerosis. Media calcification, which is often found in patients with metabolic syndrome, diabetes and/or chronic kidney disease (CKD), is characterized by diffuse mineral deposition along elastic fibers in both elastic-type conduit arteries and muscle-type resistance arteries.

VC is a common problem among CKD patients. Its prevalence increases with the progressive decrease of kidney function.[2] The association between soft-tissue calcification and uremia has been recognized more than 100 years ago. At that time, Rudolf Virchow (1821-1902) described the presence of extraosseous calcifications in a group of patients with non-metabolic bone disease, uremia, primary hyperparathyroidism and vitamin D intoxication. Virchow proposed an interesting hypothesis to explain this phenomenon: he supposed that calcium salts were dissolved from bone and carried by the blood stream to be deposited at some distant site to form "calcium metastases", a process analogous to the dissemination of malignant cells from a primary neoplasm.[3] Contemporaneously, Karl Rokitansky (1804-1878) made another important contribution by describing the presence of atherosclerotic lesions.[4] Some years later, in 1903, Johann Georg Mönckeberg described the medial type of VC which he named calcific sclerosis.[5]

Although the first descriptions of media calcification were made a long time ago, it became a widely recognized problem only after the introduction of long-term dialysis and renal transplantation. In the

past decades, studies in CKD patients showed that the degree of VC was an independent and strong predictor of death in association with vessel stiffness, arterial hypertension, left ventricular hypertrophy and cardiomyopathy.[6,7]

Therefore, great efforts have been made to improve our understanding of the pathogenesis, diagnosis, prevention and treatment of VC. The purpose of this review is to provide an update of our present insight into the VC related to CKD.

Pathogenesis

General mechanisms of vascular calcification

VC is a complex process, involving not only simple precipitation of supersaturated phosphate and calcium concentrations in the extracellular milieu (mineral step) but also a tightly regulated, cell mediated process including apoptosis, osteochondrogenic differentiation, and elastin degradation (cellular step). The time course of these two steps *in vivo*, in particular to know which one appears first, remains poorly defined. The main pathogenetic events are summarized below.

Apoptosis

Vascular smooth muscle cells (VSMC) apoptosis is regarded as an important contributor in the initiation of VC. Of note, VSMC within atheromatous plaques exhibit increased sensitivity to apoptosis compared with those in normal vessel wall.[8,9] Apoptotic bodies derived from VSMC are thought to play a role as nucleating structures for calcium crystal formation such as matrix vesicles in the initiation of calcification.[10,11]

Osteochondrogenic differentiation

VSCM phenotype change to osteochondrogenic cells is characterized by the appearance of matrix vesicles containing apatite and calcifying collagen fibrils on the surface of VSMC. As in bone, these vesicles act as early nucleation sites for calcification. Moreover, VSMC synthesize bony-associated proteins and promote crystal formation and deposition.[12] *In vitro* studies have demonstrated this phenotypic change, featured by the expression of bone-associated proteins including alkaline phosphatase (ALP), osteocalcin, and osteopontin. Runt-related transcription factor 2 (Runx2) and Msh homeobox 2, which are obligate transcription factors in normal bone development, also have been shown to be associated with VSMC osteochondrogenic differentiation.[13]

The phenotypic change of VSMC has also been demonstrated in *in vivo* studies. Apolipoprotein-E and matrix Gla protein (MGP) knockout mice exhibit osteochondrocyte-like cells flanking the calcium deposits in the vessel wall.[14,15] In human calcified arteries, the expression of collagen II and sex determining region Y related high-mobility group box 9 (sox9), which are keys transcription factors for chondrogenesis, has been documented as well.[16]

The VSMC nature of osteochondroblast-like cells in VC has recently been questioned by Tang Z *et al*.[17] These authors claimed that in response to vascular injuries, multipotent vascular stem cells, not mature VSMC, differentiate into osteochondrogenic cells and thereby initiate VC. This hypothesis has been immediately refuted by Nguyen *et al*.[18] who stated that there is compelling evidence that VSMC are not terminally differentiated and are capable of transition into a phenotype characterized by cell proliferation and loss of differentiation markers.

Elastin degradation

Elastic lamellae consist mainly of amorphous elastin, the major component of the aortic medial layer, in addition to the concentric layers of helically arranged smooth muscle cells. Elastocalcinosis is characterized by deposition of hydroxyapatite on the elastic lamellae of the arteries. Elastin degradation is known to play an important role in the initiation and progression of VC. It is induced by elastase, metalloproteinases and other proteases including cysteine and serine. Degraded elastin has a high affinity for calcium, facilitating growth of hydroxyapatite along the elastic lamellae. Moreover, elastin derived peptides binding to elastin laminin receptors on the surface of VSMC and through transforming growth factor-β signaling can increase proliferation and upregulate Runx-2, resulting in osteochondrogenic differentiation.[19,20]

Molecular mechanisms of vascular calcification

In addition to the mechanisms described above, it has become increasingly clear that there are several inhibitory proteins and promoters involved in the process of VC (Table 1).[21-25] A complex interplay between promoters including bone morphogenic protein 2 (BMP-2) and receptor activator of nuclear factor-*kappa* B ligand (RANKL), and inhibitors

including MGP, BMP-7, osteoprotegerin, fetuin-A, and osteopontin, regulates this process. Actually, it has also been speculated that promoter molecules can act via microRNA (miR) regulation. Recently, Balderman *et al.*[26] shown that human VSMC treated by BMP-2 downregulate miR-30b and miR-30c to increase Runx2 expression and promote mineralization.

Table 1 Summary of the most common inhibitory and stimulatory factors involved in the pathogenesis of vascular calcification

Inhibitors	Promoters
Matrix Gla protein (MGP)	Hyperphosphatemia
Osteopontin (OPN)	Hypercalcemia
Bone morphogenic protein 7 (BMP-7)	Bone morphogenic protein 2 (BMP-2)
Magnesium	Receptor activator of nuclear factor-*kappa* B ligand (RANKL)
Fetuin-A	
Osteoprotegerin (OPG)	
Pyrophosphate (PPi)	
Klotho	
FGF-23 (?)	FGF-23 (?)

The following paragraphs describe the main molecular mechanisms involved in VC.

Phosphate metabolic pathway

Phosphate homeostasis is maintained by the hormonal control of its transport in intestine, bone, and kidney. The most active form of vitamin D, 1,25-dihydroxyvitamin D [1,25 diOH D], which is synthesized in the kidney, increases the intestinal absorption of phosphate and stimulates osteoclastogenesis in bone, leading to an increase in extracellular phosphate concentration. PTH acts on the kidney to stimulate both 1,25 diOH D synthesis through activation of 1α-25OH D hydroxylase, and urinary phosphate excretion.[27] In addition to PTH and 1,25 diOH D, FGF23 and Klotho have been discovered more recently as novel factors involved in phosphate metabolism. The importance of the Klotho - FGF23 axis will be discussed below.

Phosphate is a well-known inducer of VSMC apoptosis and osteochondrogenic differentiation. Increased phosphate levels suppress both the expression of growth arrest specific gene 6 (Gas6) and its receptor in VSMC.[28] This inhibition leads to the suppression of phosphatidyl inositol-3 kinase PI3K/Akt survival pathway, and thereby favors VSMC apoptosis.[29]

Phosphate transport into the cell is primarily mediated by sodium-dependent phosphate (Na/Pi) co-transporters. Its uptake into VSMC occurs mainly via phosphate transporter 1 (Pit-1), a member of the type III Na/Pi co-transporters, leading to osteochondrogenic differentiation.[30] Of note, Pit-1 knockdown has been shown to suppress phosphate-induced calcification and to block induction of Runx2 and osteopontin.[31]

Pyrophosphate (PPi)

PPi is a major physiological inhibitor of hydroxyapatite formation. It also potently inhibits VC. PPi is generated by hydrolysis of ATP induced by the enzyme ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (Enpp1), an extracellular membrane-bound glycoprotein. When the Enpp1 gene is mutated and the enzyme is inactive, like in the rare clinical syndrome called "idiopathic infantile arterial calcification", severe calcification of the internal elastic lamina of muscle arteries ensues. This represents one among the numerous demonstrations of the important role of PPi in preventing VC.[32] In contrast, ALP, whose expression is induced by VSMC osteochondrogenic transformation, promotes vessel wall matrix mineralization. One of the main roles of ALP is to hydrolyze PPi, which in turn generates phosphate ions and thereby favors the development of VC.

Klotho - fibroblast growth factor-23 axis

In addition to its well-known anti-aging action, Klotho serves as a co-factor of FGF23 in conferring FGF23 specificity for FGF receptor activation. The two together play an essential role in the control of phosphate and vitamin D metabolism, by enhancing urinary phosphate excretion and suppressing renal 1α-25OH vitamin D hydroxylase activity. Klotho-deficient mice and FGF23-null mice exhibit a similar phenotype, characterized by an accelerated aging process with shortened life span, atherosclerosis, and soft-tissue calcification including VC.[33,34] Interestingly, Klotho overexpression in CKD mice strikingly reduces aortic calcification, as compared to Klotho-deficient and wild-type mice with similar renal function impairment. Klotho also is capable of directly inhibiting phosphate-induced calcification of VSMC *in vitro*.[35] Regarding FGF-23, its role in VC is less clear in the context of CKD. As an apparent

paradox, high serum FGF23 were also found to be associated with the severity of atherosclerosis[36] and VC in patients with CKD.[37] Curiously, other study involving non-CKD patients observed a negative association between FGF-23 and the presence of atherosclerotic lesion.[38] Several recent studies, but not all, showed an involvement of both Klotho and FGF23 in VC, both in interaction and separately from each other, in addition to calcium, phosphate, PTH and 1,25 diOH D.[39,40] However, their exact and respective roles in this process require further study.

Calcium-sensing receptor

The CaR is a G protein-coupled receptor. It is expressed in tissues which are involved in the regulation of calcium metabolism, including the parathyroid, thyroid, kidney, bone and intestine.[41] In addition, the CaR has also shown to be expressed in blood vessels, both in endothelial cells[42] and VSMC.[43] Alam *et al*.[44] showed that a reduction in CaR expression in VSMC is associated with increased mineralization, and that calcimimetics can attenuate mineral deposition. Ivanovski *et al*.[45] showed a protective effect of calcimimetics against the progression of VC and atherosclerosis in uremic apolipoprotein-E knockout mice. They also showed a direct inhibition by calcimimetics of phosphate-induced human VSMC calcification *in vitro*. This inhibitory effect could be abolished by suppression of CaR expression. Koleganova *et al*.[46] reported that calcimimetics increase MGP expression and decrease Pit1 expression in uremic rats, preventing vessel wall remodeling. These observations suggest that calcimimetics play an important role in VC not only by indirect effects, such as improved control of serum PTH, calcium and phosphate levels, but also by a direct effect on vascular cells. These findings underline the importance of research into the role of CaR in the pathogenesis of of VC.

Oxidative stress

Oxidative stress can be defined as a disturbance of normal cellular and molecular function caused by an imbalance between the excessive production of reactive oxygen species (ROS) and the natural defense ability of cells against oxidation.[47] As an example, oxidative modifications of proteins by ROS can induce nitrotyrosine expression by endothelial cells and high-level expression of receptors for uremic toxins such as advanced glycation end products (AGE). In the cardiovascular system, AGE accumulation contributes to arterial stiffening due to its binding to collagen and elastin in a disorderly way. Moreover, uremic toxicity leads to an impairment of endothelial nitric oxide (NO) synthesis, which plays a crucial role in vascular protection since NO inhibits the proliferation and migration of VSMC, expression of adhesion molecules, and platelet aggregation.[48]

The precise relationship of oxidative stress with VC is not yet well established. Yamada *et al*.[49] observed in rats with CKD induced by adenine-rich diet a progressive development of arterial medial calcification, which was accompanied by a time-dependent increase in both aortic and systemic oxidative stress. Time-course studies indicated that both oxidative stress and hyperphosphatemia were correlated with arterial medial calcification. Our group showed a significant increase in nitrotyrosine immunostaining in aortas of uremic as compared to non-uremic apoE knock-out mice,[50] and Guilgen *et al*.[51] reported same finding in arteries from CKD patients as compared to healthy donors, demonstrating the effective participation of oxidative stress in VC.

Inflammation

Chronic systemic inflammation is a common feature in CKD, caused both by the accumulation of pro-inflammatory compounds related to the markedly decreased glomerular filtration rate, and enhanced production and release of inflammatory cytokines.[52]

Previous *in vivo* and *in vitro* studies showed that inflammation induced intracellular lipid accumulation and foam cell formation by disrupting low-density lipoprotein receptor feedback regulation, exacerbating the progression of atherosclerosis and VC.[53,54] Another interesting classical observation was reported by Ketteler *et al*. in a group of 312 stable patients on hemodialysis the authors observed that serum fetuin-A concentration was lower than in healthy controls and was inversely associated with serum C-reactive protein, and that both were associated with enhanced cardiovascular and all-cause mortality.[55]

These observations exemplify the essential role of inflammation in vascular calcification and its links with mortality in patients on CKD patients.

Pathophysiologic links between bone and vascular calcification

In recent years many clinical and experimental studies have demonstrated an association between osteoporosis and VC.[56-61] Schulz *et al*.[62] prospectively

demonstrated an association between the progression of aortic calcification and decreased bone mineral density. The authors also found that the risk of vertebral and hip fractures was greater in women with aortic calcification. Several studies have extended these findings to patients with CKD in whom coronary and aortic calcification is apparently even more closely associated with reduced bone volume and disturbances in bone remodeling, especially in those with low bone turnover.[60,63-65]

It has long been known that aging, diabetes mellitus,[66] dyslipidemia,[67] smoking, and alcohol abuse contribute to both decreased bone mineral density and VC increase.[68] However, the association persists after adjusting for some of these factors, suggesting the existence of other mechanisms that have not yet been fully elucidated.

Among the mechanisms proposed some point to various vascular pathologies as the underlying cause. Others have suggested that changes in bone cells could affect the vascular tissue. A third mechanism would include metabolic disorders common to a variety of diseases, either alone or in conjunction with inflammation, such as diabetes mellitus and dyslipidemia which would stimulate both bone resorption and vascular disease.

All organs are endowed with a vascular tree assuring the entry of nutrients and oxygen. Bone tissue is no exception to this rule. In addition, the blood transports bone cell precursors which are involved in bone remodeling and thereby contribute to the integrity of the skeleton. Ischemia caused by intraosseous atherosclerosis can compromise vascularization and favor osteoporosis.[69] An association has also been shown between decreased bone mineral density and the presence of peripheral arterial disease.[70] A study in women showed that the rate of bone perfusion was markedly reduced in those with osteoporosis as compared to osteopenic women or those with normal bone mass.[71]

Recently, novel functions of bone tissue have been discovered in addition to already well-known functions such as locomotion, protection of internal organs and participation in the regulation of mineral metabolism. Osteoblasts, through the production of osteocalcin, have been found to participate in the regulation of fat metabolism, energy homeostasis, and insulin secretion and sensitivity, all of which are essential for proper functioning and integrity of the cardiovascular system.[72] Osteocalcin regulates insulin gene expression, β-cell proliferation, and adiponectin expression and secretion in adipocytes.[73] Both in general population and in patients with CKD serum osteocalcin has been shown to be positively correlated with serum adiponectin.[74,75] Moreover, both correlate inversely with arterial stiffness and progression of coronary calcification.[74] Leptin, a hormone that regulates adipose tissue mass, is a potent inhibitor of bone formation and also promotes VC.[76] In advanced stages of CKD serum leptin levels are generally high. Coen *et al*.[77] demonstrated that uremic patients with high serum leptin levels and low serum PTH levels were prone to develop VC.

The identification of circulating bone cells with potential for VC raised questions as to whether this could be another link.[78-80] Both mesenchymal cells from bone marrow and those of hematological lineage can give rise to circulating bone cells with osteogenic potential that could, for example, also home in atherosclerotic lesions and contribute to intima calcification. The ability of these cells to promote VC has as yet only been demonstrated *in vitro* but not *in vivo*.[81]

Recently, two comprehensive reviews about the bone-vascular axis were published by Fadini *et al*.[81] and London.[82]

Diagnosis

The clinical diagnosis of media calcification is practically impossible by physical examination alone. Its presence is suggested when palpable arteries are detectable when the sphygmomanometer is inflated to a higher level than the true systolic blood pressure. This propaedeutic maneuver is known as the Osler's sign.[83]

At present, there is no reliable, sufficiently sensitive and specific biomarker for the diagnosis of VC. As described above, in the "*Molecular mechanisms of vascular calcification*" section, several biomarkers have been shown to be associated with the initiation and/or development of VC. However, it remains unproven that any of these markers reflects calcium phosphate deposition in the arterial wall. It is therefore unclear at present whether they are of any use for clinical practice.

Non-invasive imaging methods are the gold standard. Among the methods available for the detection and quantification of arterial calcification, electron-beam computed tomography (CT) and the more widely accessible multislice CT technique

are the most frequently used methods for a precise assessment of the severity of VC and its progression. Results of coronary artery calcification (CAC) are typically reported using the Agatston CAC score, which is based on the product of calcified plaque area and density coefficient. This score has been shown to be predictive of cardiac events. A limitation is the inability to distinguish between intima and media calcification. Moreover, Kristanto *et al*.[84] pointed out that the early onset of calcium deposition remains invisible even with these quantitative techniques and a zero Agatston score does not exclude the presence of incipient coronary calcification.

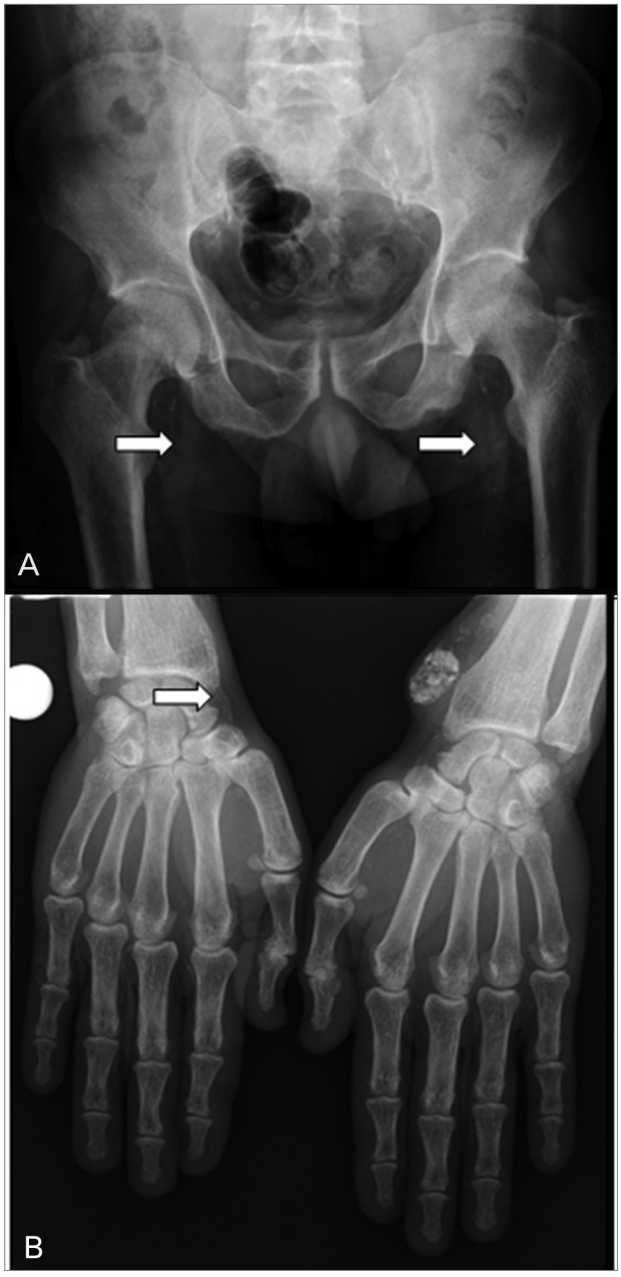
The Kidney Disease Improving Global Outcomes (KDIGO) 2009 guideline suggests to use cheap lateral abdominal radiography for the semiquantitative assessment of VC and an echocardiogram for the detection of valvular calcification in patients with CKD stages 3 to 5. In clinical routine this technique is a reasonable alternative to costly CT-based imaging methods.[85]

Kauppila *et al*.[86] developed an abdominal calcification severity scoring system, using lateral lumbar films. The severity of calcified deposits is graded from 0 to 3, separately for the posterior and anterior wall at each vertebral segment, from the first to the fourth lumbar vertebra. Adragao *et al*.[87] developed another scoring system, using pelvis films for iliac and femoral arteries, and hand films for radial and digital arteries. They divided pelvis and hand films into eight sections and graded from 0 to 8 (maximum), depending on the presence or absence of VC (Figure 1). Although these semi-quantitative methods have limited ability to distinguish between the extension and degree of severity of calcification, they are more widely available and less expensive and can be used for cardiovascular risk stratification.

Prevention and Treatment

To date, none among a variety of available treatments has been definitively proven to prevent or reverse VC and it is widely admitted that this complication, once established, is irreversible. However, some drugs, including phosphate binders and cinacalcet, have been shown to halt or at least slow the progression of the disease in patients with CKD. Whether this translates into an improvement of clinical outcomes has yet to be demonstrated.[85] Therefore, major efforts should be directed at prevention as the main option.

Figure 1. A: Arterial calcifications of pelvic arteries of a chronic hemodialysis patient; B: Arterial calcifications of hand arteries of a chronic hemodialysis patient. White arrows show extended vascular calcification areas (images from Dr. V. Jorgetti's personal archive).



In observational studies hyperphosphatemia, hypercalcemia and extremely high as well as low serum PTH values have been associated with poor outcomes in CKD. As to serum phosphate, even when its serum levels are in the high normal range, it may be associated with VC and mortality, as shown in patients with no known kidney disease and in CKD stage 3 patients.[88,89]

In CKD stage 4-5 and 5D patients it is recommended at present to bring serum phosphate, calcium and PTH concentrations to target ranges which

have been found to be associated with lower rates of VC (Table 2)[85] and risks of mortality (Figure 2).[90] However, in early stages of CKD these parameters are in the normal range the majority of patients, in contrast to what is observed in later stages.[91] Nevertheless, some groups have examined the effects of reducing phosphate overload and/or bringing PTH levels towards the range of normal values in CKD stage 3-4 patients.[92]

The following paragraphs describe the main options which are presently available for the prevention of VC, with particular focus on phosphate control, as well as the supposedly protective role of some drugs in current use for the treatment of CKD-associated mineral and bone disorder (CKD-MBD). In addition, we discuss therapeutic modalities for new potential targets.

Control of serum phosphate

Currently, the options available to lower serum phosphate in CKD patients with hyperphosphatemia are (i) limiting dietary phosphate intake (while ensuring adequate protein intake), (ii) increasing the frequency or duration of dialysis in CKD stage 5D, and (iii) using phosphate binders and calcimimetics.

Diet

The KDIGO guideline suggests, in patients with CKD stages 3-5D, to limit dietary phosphate intake in the treatment of hyperphosphatemia alone or in combination with other treatments but this suggestion is mainly based on expert opinion, not on hard evidence.[85] In patients with CKD stage 5, phosphate intake should not exceed 1.000 mg per day. Recently, increasing attention has been paid not only to the amount of phosphate in the diet, but also to its quality. Inorganic phosphate in food additives is frequently found in processed food and "fast food". In contrast to organic phosphate, inorganic phosphate is more effectively absorbed and therefore leads to phosphate overload more easily.[93] Therefore, the multiprofessional clinical staff who has in charge the treatment of CKD patients should have two goals in mind with respect to recommendations on phosphate intake: avoid inorganic phosphate contained in food additives and limit daily phosphate intake to less than 1.000 mg/day. Note that these goals should be attained without inducing protein malnutrition.

Phosphate removal by dialysis

Intradialytic plasma phosphate kinetics obeys to a 2-compartment model, thus differing from urea kinetics. The elimination mode of phosphate appears to resemble more that of typical middle molecules than that of small molecules such as urea.[94] Phosphate clearance during hemodialysis is affected by several factors, including blood and dialysate flow rate, dialyzer membrane surface area, ultrafiltration rate, and dialysis session frequency and length.

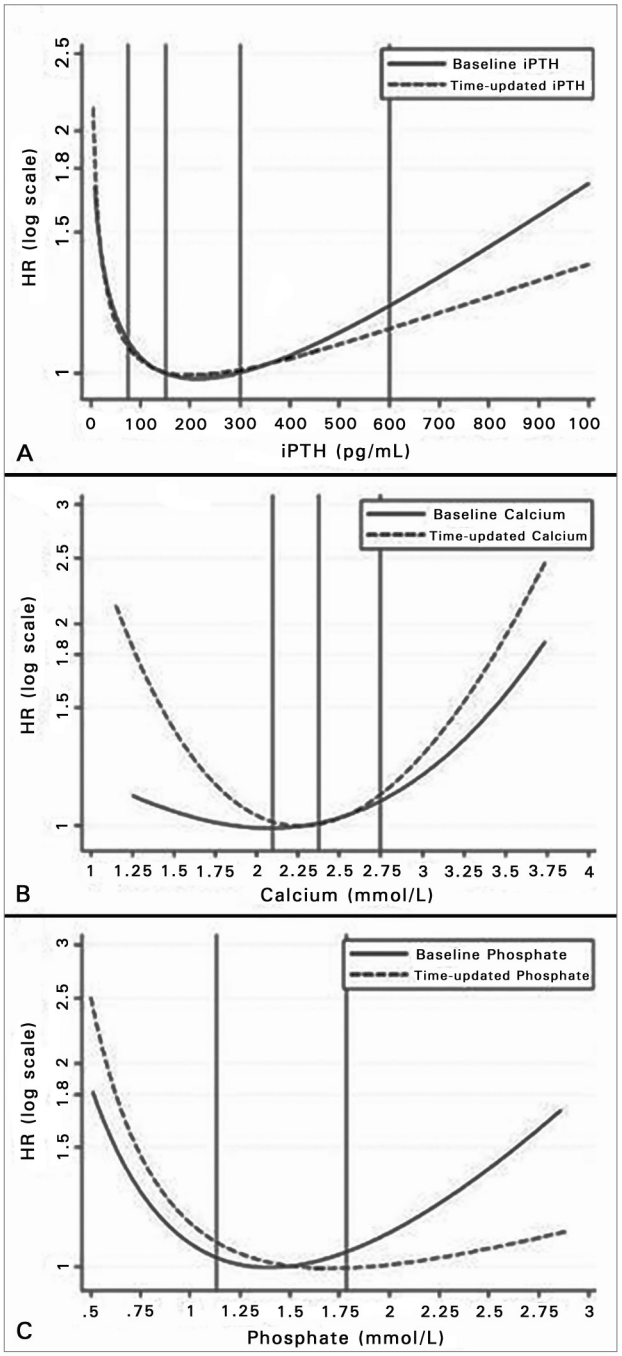
Increasing dialysis frequency (e.g., short daily dialysis, 6 times a week, for 2.5-3 hr each session) or dialysis length (e.g., nocturnal hemodialysis, 6 times a week, 8 hr each session) are helpful strategies to treat hyperphosphatemia in CKD stage 5D.[95,96] Hemodiafiltration can increase phosphate mass removal further.[97] In peritoneal dialysis (PD), a cross-sectional comparative study between automated PD and continuous ambulatory PD showed that weekly total phosphate mass removal was similar with both methods.[98] Mass transfer could be increased

Table 2 Optimal range recommendations for serum concentrations of total calcium, phosphate and parathyroid hormone (PTH), according to CKD stages[§]

	CKD stages 3 to 5	CKD stage 5D	Normal range*
Phosphate (mg/dl)	Normal range	Lowering elevated P levels toward the normal range	2.7-4.5
Total calcium (mg/dl)	Normal range	Normal range	8.6-10.2
[Cad] (mEq/l)	NA	2.5 to 3.0	NA
PTH (pg/ml)	< Upper normal range (for range, see +)	2-9 x Upper normal range** (for range, see +)	10-65

Cad: Dialysate Ca concentration; NA: Not applicable; [§] Slightly modified from text of reference 85 by the authors; * Normal range can show variations according to measurement assay; + Stages 3-5 CKD patients should first be evaluated for hyperphosphatemia, hypocalcemia, and vitamin D deficiency. When serum PTH is progressively raising and/or is persistently outside the upper limit of normal for the assay used despite correction of modifiable factors, treatment with calcitriol or vitamin D analogs should be considered; ** In dialysis patients, calcimimetic treatment is an additional option.

Figure 2. Relative risk (RR) of all-cause mortality in chronic hemodialysis patients as a function of three different serum parameters comparing baseline versus time-dependent Cox regression using fractional polynomials. A: RR for serum intact parathyroid hormone (iPTH). Patients with iPTH levels outside the KDOQI target range (150-300 pg/mL) had a higher risk of death compared to those within target range; B: RR for total serum calcium (calcium values < 1.15 mmol/L and > 3.74 mmol/L, not shown). Patients with total calcium > 2.75 mmol/L had a higher risk of death than those who were in the normal range. For low total calcium values (< 2.10 mmol/L) there was no effect on the risk of death in baseline adjusted Cox analysis, but a slightly higher risk in time-dependent analysis; C: RR for serum phosphate. Patients with phosphate levels outside the KDOQI target range (1.13-1.78 mmol/L) had a higher risk of death compared to those within target range in baseline adjusted Cox analysis. The adjusted time-dependent analysis was consistent with the baseline-adjusted analysis for low phosphate, but not for high phosphate levels. (Reproduced with permission from reference 90: Floege *et al.* Nephrol Dial Transplant 2011;26:1948-55).



by increasing total daily peritoneal fluid infusion.[99] An interesting review on phosphate removal using various hemodialysis and PD treatment modalities was recently published by Kuhlmann.[100]

Oral phosphate binders

Phosphate binder choice should be individualized depending on patients' preference and tolerance. The greatest problem with all phosphate binders is not lack of efficacy, but lack of patient compliance. All currently available phosphate binders, such as calcium or magnesium salts, sevelamer hydrochloride or carbonate, and lanthanum carbonate are effective in lowering serum phosphate.[85] Of note, there is insufficient evidence that any specific phosphate binder significantly impacts patient-level outcomes.

Although there is some published evidence suggesting that sevelamer compared with calcium-based phosphate binders attenuates the progression of VC in patients with CKD stages 3-5[101] and 5D,[102,103] other studies failed to confirm these results.[104,105] Sevelamer's effects on VC could be both direct and indirect. This phosphate binder exerts pleiotropic effects, including an attenuation of oxidative stress and inflammation and a decrease in circulating levels of uremic toxins.[106,107]

Regarding calcium-based phosphate binders, they can lead to calcium overload when taken in excessive amounts.[108,109] They should be restricted to no more than a total of 1.500 mg elemental Ca intake per day, especially in presence of VC, hypercalcemia and/or adynamic bone disease,[85] the two latter conditions being associated with VC. Magnesium/calcium-based phosphate binders have recently been shown to be an acceptable alternative.[110]

Calcimimetics

Both experimental and clinical evidence indicates that the calcium-sensing receptor (CaR) is not only expressed in kidney and parathyroid tissue, but also in vascular cells, and that plays a role in VC. In a mouse model of CKD, calcimimetics delayed the progression of aortic calcification and atherosclerosis,[111] and in a uremic rat model calcimimetics attenuated media calcification and proliferation of VSMC.[112]

There is more limited evidence for a positive effect of calcimimetics on VC in the clinical setting. The administration of calcimimetics to dialysis patients with secondary hyperparathyroidism, in addition to lowering

serum PTH, calcium and phosphate,[113] can also reduce the progression of VC, as shown in the recent ADVANCE study. The authors examined the effect of cinacalcet plus low-dose vitamin D on coronary artery and cardiac valve calcification in 360 prevalent HD patients with secondary hyperparathyroidism, as compared to placebo with optimal standard therapy and flexible doses of vitamin D. In the cinacalcet group there was a 24% increase in Agatston CAC score, as compared to a 31% increase in placebo group ($p = 0.073$), with corresponding changes in the more recently developed volume CAC score of 22% and 30%, respectively ($p = 0.009$).[114]

One of the mechanisms by which a calcimimetic may slow VC progression is its serum PTH, calcium and phosphate lowering action. Other potential mechanisms are a direct stimulation of the CaR expressed in VSMC and an increase in MGP expression in the arterial wall, as shown both in *in vivo* and *in vitro* experiments.[45,115]

Vitamin D

Both native vitamin D and active vitamin D sterols, also called vitamin D receptor activators (VDRA), may be useful to treat secondary hyperparathyroidism, a condition associated with VC. However, pharmacological doses can result in undesirable effects such as the development of adynamic bone disease,[116] hypercalcemia, and/or hyperphosphatemia,[117] which all favor the development of VC. Newer VDRAs, such as paricalcitol, have been said to be more selective in suppressing PTH secretion and to be less hypercalcemic and hyperphosphatemic.[118]

Some experimental studies provided evidence in favor of this claim.[119-121] An interesting observation came from the experimental study of Lim *et al*.[40] The authors observed that CKD induces vascular klotho deficiency. They further demonstrated that both calcitriol and paricalcitol significantly upregulated klotho and restored FGF receptor-1 mRNA expression in VSMC of arteries from patients with CKD, which were cultured in procalcific media. They proposed that klotho restoration by vitamin D receptor activation confers VSMC FGF23 responsiveness and unmasks FGF23 calcification inhibitory effects.

Despite these exciting news from experimental models, there is so far no convincing study demonstrating that any vitamin D derivative is less prone than the native parent compounds to induce VC in patients with CKD.

New potential treatment modalities

Pyrophosphate (PPi)

PPi is a potent calcification inhibitor *in vitro* and an inhibitor of arterial media calcification *in vivo*, exerting its effects through direct physicochemical inhibition of hydroxyapatite crystal formation.[122,123] Despite its known protective effects against the progression of VC, intravenous administration has been considered to be problematic due to short-half life and complications such as skin necrosis.[124] Renewed interest in the calcification inhibitory effects of PPi has been raised by recent experimental studies.[125,126] Since hemodialysis patients have low circulating PPi levels,[127] an interesting therapeutic option could be to deliver PPi into the peritoneal cavity from where PPi would be slowly transported into the circulation. Riser *et al*.[125] and O'Neill *et al*.[126] have recently shown that daily administration of sodium PPi by peritoneal route was able to prevent the development of aorta calcification in two different animal models of CKD. The next step could be exploratory studies in PD patients.

Vitamin K

MGP, which is synthesized by VSMC in the arterial media, is a vitamin K-dependent inhibitor of calcium and phosphate precipitation and crystal formation in the vessel wall. In addition, it suppresses the activity of bone morphogenetic proteins 2 and 4.[128-130] Vitamin K deficiency affects the activation of MGP by gamma-carboxylation of glutamic acid residues. Undercarboxylated and/or nonphosphorylated MGP loses its inhibitory action on the development and progression of VC and associates with mortality risk in CKD patients.[131,132] A recent study showed that the majority of HD patients have a poor vitamin K status and low vitamin K intake compared with healthy subjects.[133] Another recent study showed that vitamin K deficiency was associated with fractures and VC in general population.[134] In patients with CKD, low vitamin K intake may be related, at least in part, to the dietary regimen generally prescribed, which is restricted in green vegetables and other foods containing large amounts of potassium, but also vitamin K.

To improve vitamin K status and thereby MGP protein activity in CKD patients, vitamin K supplementation may be indicated.[135] Thus, Westenfeld *et al*. observed in stable long-term hemodialysis patients

that inactive MGP concentration can be markedly decreased by daily vitamin K supplementation during 6 weeks.[136]

To date, there are some trials registered on the *U.S. National Institutes of Health* website (www.clinicaltrials.gov) that are related with vitamin K, CKD and VC. A search with the input keywords "Vitamin K" AND "Chronic kidney disease" resulted in three ongoing trials: "Warfarin and Coronary Calcification Project (WACC)", "Vitamin K to Attenuate Coronary Artery Calcification in Hemodialysis Patients (iPACK HD)", and "Vitamin K2 and Vessel Calcification in Chronic Kidney Disease Patients (CACSK2)". Also, we should mention the European study VITAVASC. We expect to have, in the near future, more information on potentially beneficial effects of vitamin K supplementation on VC risk and outcomes in patients with CKD.

General conclusion

VC is a complex disease process. It involves not only the local precipitation of calcium and phosphate in the vessel wall, but it also is a regulated cell mediated process, which is under the control of both inhibitory and stimulatory proteins and nonpeptidic factors. This normal, physiological equilibrium is disturbed by CKD, favouring the initiation and progression of VC in parallel with the progressive decline in kidney function.

Based on available experimental and clinical evidence, several drugs currently used for the treatment of CKD-MBD, including sevelamer and calcimimetics, appear to exert at least partially protective effects against the VC process associated with CKD. Drugs such as PPi and vitamin K may represent new avenues for a hopefully more efficient prevention and treatment of VC and its dramatic cardiovascular complications. However, the protective effects of these latter drugs have yet to be demonstrated by future randomized, controlled trials in patients with CKD.

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Conflict of interest statement

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