Chapter 3

Mechanisms and Clinical Implications of Vascular Calcifications in Chronic Kidney Disease

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Additional information is available at the end of the chapter

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Abstract

Chronic kidney disease (CKD), a major public health problem that affects up to 10–13% of the general population worldwide, imposes considerable socio-economic burden due to both the need for renal replacement therapy and, even more important, the negative influence on the overall patients' health status. Cardiovascular (CV) diseases are the main cause of death in CKD patients and are triggered not only by the traditional CV risk factors but also by specific, uremia-related, factors. Among these, calcium-phosphate and bone metabolism disorders play a central role. Abnormalities of mineral metabolism occur early, evolve silently as the kidney function deteriorates, and are associated with CV morbidity and mortality, mainly by the development of valvular and vascular calcifications. This chapter aims to summarize the recent knowledge on the types and mechanisms of arterial calcifications, as well as their clinical implications, in the setting of CKD. The issue is significant for both nephrologists and cardiologists and could be an example of the requirement for interdisciplinary collaboration in the medical practice.

Keywords: atherosclerosis and arteriosclerosis, arterial stiffness, calcifications, cardiovascular morbidity, chronic kidney disease

1. Introduction

The prevalence of chronic kidney disease (CKD) is constantly growing [1] largely due to the shift in age distribution of the population toward individuals older than 60 years, in which CKD is more common, accounted for by the combined effect of physiologic decline in glomerular filtration rate (GFR) and systemic atherosclerosis, and also due to increasing prevalence of arterial hypertension, diabetes mellitus, and obesity, all risk factors for CKD. Notably, the mortality of CKD patients is higher than their non-CKD counterparts, predominantly with respect to cardiovascular mortality. Abnormalities of arterial and left ventricular functions,

such as arterial stiffness, atherosclerosis and arteriosclerosis, left ventricular hypertrophy, and systolic and end-diastolic stiffness, which are common in CKD patients, were incriminated [2]. The pathophysiology of cardiovascular disease (CVD) in CKD is complex, with both traditional and uremia-related risk factors being involved. Among the latter, calcium-phosphate metabolism anomalies are more and more debated, and the concept of chronic kidney disease-mineral and bone disorder (CKD-MBD) has been adopted. It is a broad term that refers to a systemic disorder of mineral metabolism due to the kidneys' failure to maintain homeostasis of calcium (Ca), phosphate (PO_4), and active vitamin D, which leads to maladaptive alterations in related hormones, namely fibroblast growth factor-23 (FGF23) and parathyroid hormone (PTH), and results in defective bone architecture and extraskeletal calcifications [3, 4]. CKD-MBD occurs early in the course of CKD, progresses as kidney function declines, and it is manifested by three separate, but interrelated, components that are not necessarily present concurrently in all patients, any combination of these component being possible [4]:

1. Changes in biochemistry profile (Ca, PO_4, vitamin D, PTH, FGF23, and alkaline phosphatase—ALP), which reflect mineral and hormonal abnormalities;
2. Bone abnormalities regarding turnover, mineralization, volume, linear growth, or strength; and
3. Soft tissue (vascular, valvular, and periarticular) calcifications.

The vascular calcifications at least partially account for increased cardiovascular (CV) risk in CKD patients, so it is worth to draw attention on the mechanisms involved in their development.

2. Types and characteristics of vascular calcification in chronic kidney disease

Even at early ages, CKD patients develop vascular calcifications at all the levels (large vessel arteries such as the aorta, medium arteries like the coronary arteries, as well as small-caliber arteries of the skin), in a much greater proportion than the general population, and the prevalence and severity of arterial and valvular calcifications increase as kidney function decreases [5].

The main types of arterial calcifications, both commonly seen in CKD, are distinguished by their location in the structure of the arterial wall (**Figure 1**) and their association with atherosclerotic plaque formation:

1. *Atherosclerosis* consists in the calcification of the intimal layer in association with cellular necrosis, inflammation, atherosclerotic plaques, and lipid deposition [6]. This type of calcification is related to traditional risk factors such as arterial hypertension and dyslipidemia (**Table 1**). The vessel lumen is eccentrically reduced and deformed due to patchy calcification of the atherosclerotic plaques [7]. It produces arterial stenosis which accounts for tissular ischemia and infarction and may predispose to plaque rupture generating life-threatening thrombi.
2. *Arteriosclerosis*, which represents the calcification of the medial layer, occurs in the elastic lamina of large-caliber and medium- to small-size arteries. It seems to be independent of atherosclerosis, although both can coexist [6, 7]. Medial calcification was known initially as

Mönckeberg's sclerosis, and it has radiographically been described as "railroad tracks" on the peripheral arteries of upper and lower limbs [6, 8]. This type of calcification is related to non-traditional risk factors such as hyperphosphatemia, excess PTH, and cytokines of chronic inflammation (**Table 1**), and it is more prevalent in patients with CKD and diabetes [6]. The vessel lumen is reduced concentrically due to amorphous mineral that forms circumferentially along or within one or more elastic lamellae of the media [7]. It induces arterial stiffness, which contributes to increased pulse pressure and, consequently, to left ventricular hypertrophy and altered coronary perfusion [9, 10].

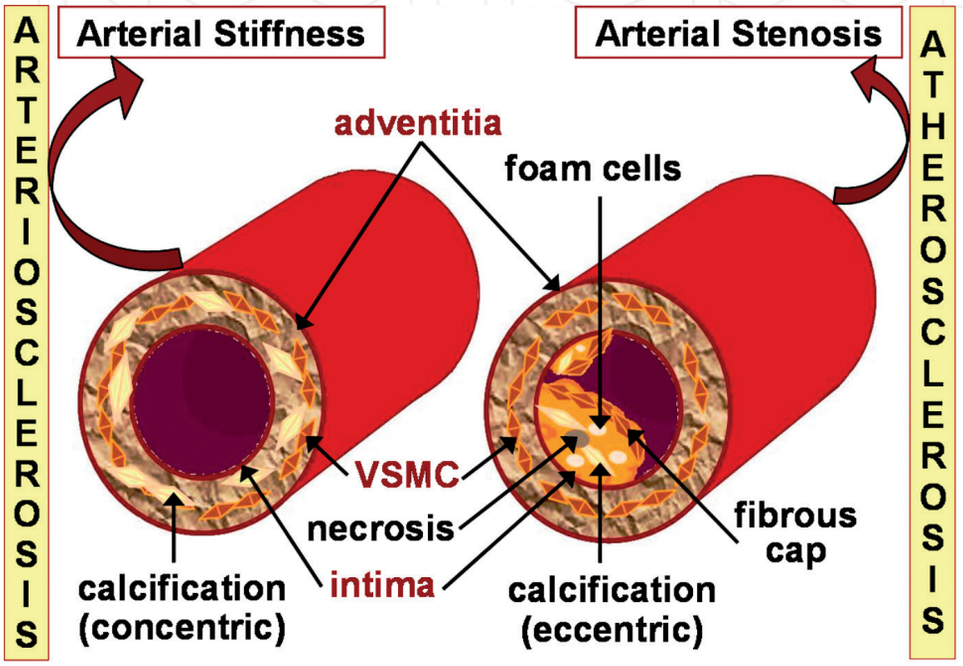

Figure 1. Main types of arterial calcifications and their consequences. VSMCs, vascular smooth muscle cells.

Traditional risk factors	Non-traditional (CKD-related) risk factors
Arterial hypertension	Hyperphosphatemia, high calcium-phosphate product
Dyslipidemia	Hyper- or hypoparathyroidism
Diabetes mellitus	High dosage of vitamin D metabolites
Smoking	Chronic inflammation
Old age	Oxidative stress
Family history of premature coronary heart disease	Metabolic abnormalities: hypoalbuminemia, hyperhomocysteinemia
	Decrease of calcification inhibitors (Fetuin-A)
	Anemia

CKD: chronic kidney disease.

Table 1. Risk factors for vascular calcification in chronic kidney disease patients (modified from Román-García et al. [5]).

These two types of calcifications encountered in CKD also vary based on their localization on the arterial tree. Intimal calcifications are found more proximally, while medial ones have a predilection for distal sites [10].

Etiologically, vascular calcifications may be categorized as metastatic calcifications, those which arise from systemically high calcium and phosphate product, or dystrophic calcifications, which take place under pathologic conditions of cell death or apoptosis [9]. *Metastatic calcifications* occur when the calcium-phosphate product exceeds its solubility in serum resulting in its deposition in healthy, extraskeletal tissue such as the arterial wall, the viscera, the conjunctiva, articulations, or tumors [8]. In contrast, *dystrophic calcifications* result from the de novo deposition of calcium and phosphate in diseased or damaged tissue. This occurs when cells die as a result of direct injury or apoptosis and release their intracellular calcium contents which can serve as a foundation for further calcium deposition [8].

3. Pathogenesis of vascular calcifications in chronic kidney disease

3.1. Overview on the molecular basis of mineralization and vascular calcifications

Although not yet entirely elucidated, the process of vascular calcification was extensively studied and the bulk of its steps were unveiled. The common feature to almost all physiologic mineralization mechanisms, either inside the bone or in extra-osseous tissues, involves matrix vesicles, which form the nidus for hydroxyapatite crystals nucleation [11]. These matrix vesicles are membrane-bound particles of 20–200 nm where mineral crystals are arranged by interaction with specific regulators, like membrane transporters and enzymes, with crucial roles in the influx of calcium and phosphate ions into the vesicles [9]. For example, tissue nonspecific alkaline phosphatase hydrolyzes pyrophosphate and generates inorganic phosphate, which is further transported through the vesicle membrane by the sodium-phosphate cotransporter type III [12]. On the other hand, annexins function as ion channels and provide a way for calcium to enter inside the matrix vesicle, where the accumulation of both divalent ions induces crystalline nucleation [9, 12].

In the bone, matrix vesicles bud off from the plasma membrane of chondrocytes or osteoblasts, at the epiphyseal plate of growing bone and are released into the premineralized organic matrix where they serve as a vehicle for the interaction of calcium and phosphate ions to form hydroxyapatite and initiate mineralization of the organic substance [11]. Hydroxyapatite crystals that are released from vesicles serve as templates for subsequent crystal formation, creating the lattice of the bone [9, 13]. Therefore, matrix vesicles have an osteogenic role.

Growing body of evidence supports significant resemblance between bone and vascular calcifications, leading to the belief that ectopic calcifications and normal osteogenesis are driven alike. Indeed, many cellular and molecular signaling processes are identical in vascular calcification and osteogenesis. Among these, matrix vesicle release and expression of mineralization-regulating proteins by vascular smooth muscle cells (VSMCs) are seen in the vessel wall [14]. Consequently, vascular calcification is also considered a regulated biomineralization process.

The balance among promoters and inhibitors of calcification plays the key role during mineralization (**Figure 2**).

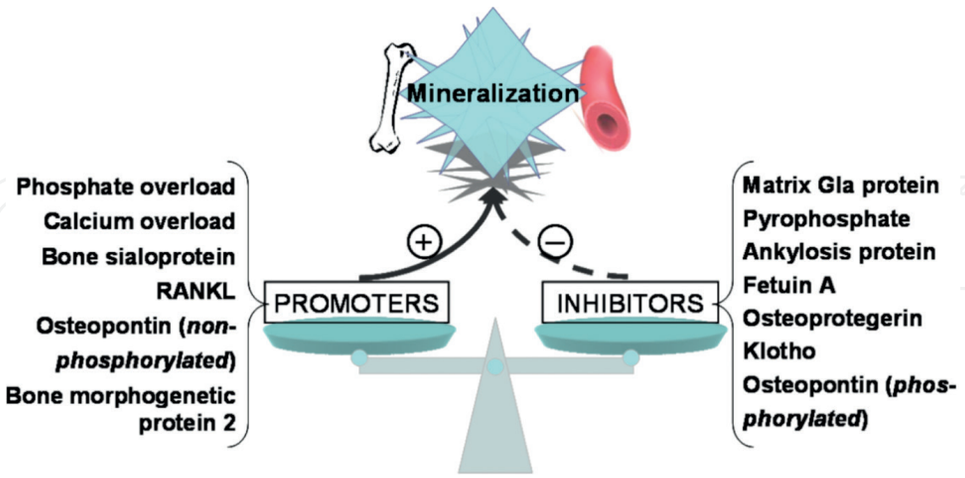

Figure 2. Regulating molecules of the mineralization/calcification processes. RANKL, receptor activator of nuclear factor-kB ligand; (+), stimulation; (−), inhibition.

The main known inhibitor molecules involved in both bone and extra-osseous sites calcification, are:

1. *Matrix GLA protein* (MGP, matrix γ-carboxyglutamate protein), an extracellular protein has roles in normal bone formation as well as inhibition of vascular calcification [15, 16]. The inactive MGP (desphospho-uncarboxylated MGP, dp-ucMGP) needs two subsequent modifications (serine phosphorylation and glutamate carboxylation) in order to exert its function [17]. Circulating levels of dp-uc MGP are considered a biomarker associated with cardiovascular risk and mortality, severity of the vascular damage, and all-cause mortality [17]. MGP is able to bind calcium and hydroxyapatite, thanks to its vitamin K-dependent γ-carboxylation, inhibiting their precipitation and mineralization [16]. MGP synthesis has been detected in cartilage, lung, heart, kidney, arteries, and calcified atherosclerotic plaques attesting to MGP's role in inhibition of soft tissue calcifications [18]. In addition, recent works suggested a link between MGP and renal microvasculature, and argued in favor of a possible renoprotective action of activated MGP and, consequently, emphasized the importance of having adequate vitamin K stores [17].

2. *Osteoprotegerin* (OPG) is a soluble cytokine and tumor necrosis factor (TNF) receptor-like molecule that acts as an inhibitor of osteoclast differentiation by binding the receptor activator of nuclear factor κB-ligand (RANKL), thus blocking RANKL-mediated activation of osteoclasts [11, 19]. OPG is present in many human tissues: bone (osteoblasts), vessels (endothelial and vascular smooth muscle cells), lung, heart, liver, kidney, hypothalamus, lymphoid organs and B-cells, bone marrow, articular chondrocytes, and breasts [19, 20]. Its expression in bone is regulated by osteoblasts through the same pathway that regulates bone formation, indicating RANKL/OPG ratio is a major determinant of bone mass and OPG has an osteoprotective role [21]. However, its functions in the vascular system are still a matter of debate. While experimental studies sustain an anti-calcification role (due to

inhibition of apoptotic passive calcification and the alkaline phosphatase-mediated osteogenic differentiation of vascular cells), elevated serum levels of OPG were found in various cardiovascular diseases and were hypothesized as a promoter of atherosclerosis progression [19]. Osteoprotegerin expression was significantly lower and RANKL was identified in calcified valves of human aortic stenosis, indicating that in the absence of inhibition by OPG, RANKL may promote matrix calcification and induce the expression of osteoblast-associated genes (bone alkaline phosphatase and osteocalcin) [22].

3. *Extracellular pyrophosphate* (PPi) is a small molecule made of two phosphate ions linked by an ester bond, which regulates cell differentiation and serves as an essential physiologic inhibitor of calcification by negatively interfering with hydroxyapatite formation and crystal growth [11]. PPi is produced from the hydrolyses of extracellular adenosine-5′-triphosphate by the enzyme ectonucleotide pyrophosphatase/phosphodiesterase [23]. On the other hand, alkaline phosphatase (ALP) catalyzes the hydrolysis of phospho-monoesters (including PPi) with release of inorganic phosphate (P_i) in order to avoid accumulation of this mineralization inhibitor, thus ensuring normal bone mineralization [11, 24]. However, through this action, ALP also acts as a powerful inducer of vascular calcification partially as a result of increased PPi degradation [23].

4. *Fetuin-A*, a circulating glycoprotein from the cystatin superfamily of proteins, produced by the liver, functions as a potent inhibitor of de novo hydroxyapatite formation from supersaturated mineral solutions, and it also acts as a negative acute phase reactant, thus being downregulated in acute and chronic systemic inflammation [25–27]. In experimental and clinical studies, it was shown that serum containing fetuin-A inhibited precipitation of calcium salts in a dose-dependent manner, and its serum concentrations were inversely correlated to C-reactive protein, calcifications, and cardiovascular and all-cause mortality, even when the serum calcium-phosphate product was close to the normal range [26, 28]. Hence, it was assumed that a major link between low fetuin-A levels and mortality consists of promoting accelerated cardiovascular calcification [26].

Other main factors with essential contribution to the processes of mineralization and calcification are those involved in the signaling pathways, like:

1. *Bone morphogenetic proteins* (BMPs) are cytokines with multiple functions, which modulate gene expression through phosphorylation of regulatory Smad transcription factors [16, 27]. Smad6 and Smad7 proteins act as negative regulators and thus are crucial to limit the osteogenic vascular response induced by BMPs [27]. For example, BMP 2—a protein that belongs to the transforming growth factor-β (TGF-β) superfamily of cell regulatory proteins—is involved in both osteogenic and chondrogenic differentiation of multipotent mesenchymal progenitors and drives the formation of cartilage and bone [29]. It also participates in vascular calcification probably through inducing osteoblastic differentiation of VSMCs. Conversely, BMP 7, primarily expressed in the kidney where it is required for the normal development of the organ, was found to restore the bone anabolic balance, reduce serum phosphate levels, and reduce vascular calcification [27].

2. *Core-binding factor alpha 1* (Cbfa1), also known as runt-related transcription factor 2 (Runx2), is a nuclear protein essential for osteoblastic development and skeletal morphogenesis, and it is believed to be the switch that turns a mesenchymal cell into an osteoblast [11, 13, 30]. It acts

as a scaffold for the interaction of nucleic acids and regulatory factors that are involved in the expression of a number of downstream proteins essential for osteoblastic differentiation, such as type I collagen, osteocalcin, and osteopontin [13].

3. *Type I collagen* makes up over 90% of the organic component of bone where it forms the framework necessary for mineralization [13, 31]. It was shown that ex vivo cells grown on type I collagen were found to mineralize three times faster and incorporate two times more calcium than cells grown in plastic media. Moreover, rapidly mineralizing cells generate a matrix that contains three times the amount of collagen type I and fibronectin but 70% less collagen type IV than their non-mineralizing counterparts. These findings indicate a regulatory role of the matrix composition on arterial calcification development [31].

4. *Osteocalcin* is a protein secreted by active osteoblasts into the extracellular matrix where it binds hydroxyapatite via 3 γ-carboxylated glutamic acid residues during bone mineralization. For this reason, it is often used as a marker for bone formation [32].

5. *Osteopontin*, also known as secreted phosphoprotein 1 or bone sialoprotein 1, is an extracellular structural component of bone (of the non-collagenous organic bone matrix) and an important modulator of bone mineralization, which can either promote or inhibit hydroxyapatite formation, depending on its post-translational modifications [11, 15]. Non-phosphorylated osteopontin shows a stimulatory effect on calcification, while phosphorylation of osteopontin converts it into a potent inhibitor of ectopic calcifications, proportional to the number of phosphorylated sites [33]. Overexpression of osteopontin was found in human atherosclerotic plaques, in calcified smooth muscle cells, in medial layers of arteries of diabetic patient, and calcified heart valves, suggesting it intervenes in the development of ectopic calcifications [34].

In conclusion, mineralization and calcification processes are tightly regulated through the complex interactions of various tissular and circulating molecules, many of which suffer profound changes in chronic kidney disease.

3.2. How does chronic kidney disease favor vascular calcifications?

3.2.1. Imbalance between pro- and counter-calcification factors

Vascular calcifications in CKD patients are thought to arise due to disruptions in the balance between promoters and inhibitors of calcification, leading to osteoblastic transformation of vascular smooth muscle cells (VSMCs) [5, 35]. Because VSMCs and osteoblasts derive from a similar mesenchymal cell precursor, VSMCs can be induced to differentiate along osteoblastic lines. The process involves an increase in calcification promoters, decrease in calcification inhibitors, and formation of calcification vesicles culminating with the induction of a cellular phenotypic change from VSMCs to osteoblast-like cells [5].

Concerning *promoters of calcification*, it is recognized that the osteoblastic differentiation, which is the initial step in vascular calcification, is revealed by the expression of pro-calcification factors such as Cbfa1 and BMP on vascular cells [13, 15]. In vitro experiments showed that changes in serum composition like those that occurred in the course of CKD may upregulate expression of Cbfa1, while in vivo studies found higher expression of Cbfa1 in both the

media and intima of calcified arteries compared to non-calcified arteries of the same patients, thus emphasizing the important role of Cbfa1 in vascular calcifications [5, 36]. In addition, since positive immunostaining for bone matrix proteins (like osteonectin, osteopontin, bone sialoprotein, alkaline phosphatase, and type I collagen) were more common than overt calcifications but were proportional with their extent, it appears that the deposition of these proteins precedes calcification [36]. Another modulator of calcification—osteocalcin—has been detected in VSMCs where it may potentially regulate their glucose utilization, promoting a phenotypic change in these cells [32]. Furthermore, an inverse correlation between osteopontin plasma levels and glomerular filtration rate (GFR) was reported, suggesting that reduced renal excretion due to impaired kidney function may lead to increased circulating levels [37]. Increased osteopontin and other promoters of calcification in CKD can be accounted for by different mechanisms also. For example, in experimental settings, high concentrations of phosphorus, uremic serum, oxidized lipids, cytokines, and high glucose (abnormalities commonly seen in CKD patients as well) were able to stimulate the VSMCs and vascular pericytes to produce bone-forming transcription factors and proteins [36]. Taken together, these findings suggest that biochemical changes that occur during the progression of CKD (hyperphosphatemia, hypercalcemia, accumulating uremic toxins, cytokines, oxidized lipoproteins, and advanced glycation end products) tip the balance in favor of promoters of vascular calcification.

On the other hand, abnormalities of *calcification inhibitors* can also contribute to the pathogenesis of vascular calcifications in CKD. For example, lower levels of matrix Gla protein were associated with decreased kidney function, probably because metabolic abnormalities due to CKD, such as vitamin D deficiency, may suppress MGP production. Alternatively, MGP may be lost from circulation as it binds to hydroxyapatite crystals in vascular calcifications. Regardless of the mechanism, reduced plasma MGP has been suggested as a marker for the presence and severity of vascular calcifications in patients with CKD [38]. Also, lower levels of circulating fetuin-A were described in CKD and were associated with coronary artery calcification, valvular calcifications, and increased mortality in dialysis patients [36].

These changes in the levels of both promoters and inhibitors of vascular calcification, that occur in CKD patients, ultimately culminate in the *transdifferentiation of VSMCs to an osteoblast phenotype* through an active, cell-mediated, osteogenic process, with the release of calcium matrix vesicles that can nucleate hydroxyapatite and form the first nidus for calcification [11, 30]. The process is driven by upregulation of bone-forming transcription factors and proteins on VSMCs, such as Cbfa1 and bone morphogenetic protein 2, which control the expression of osteogenic proteins (osteocalcin, osteonectin, alkaline phosphatase, collagen type I, and bone sialoprotein). Exposure to high levels of calcium, phosphate, cytokines, and so on, along with the deficit of calcification inhibitors (such as fetuin-A, matrix Gla protein, pyrophosphate) are required for the cells' phenotypic switch [39]. The transformed cells deposit collagen and non-collagenous proteins in the arterial wall and incorporate calcium and phosphorus into matrix vesicles to initiate mineralization and crystal growth. The overall positive calcium and phosphorus balance from CKD patients supports both the cellular transformation and the generation of matrix vesicles [36].

3.2.2. Mineral metabolism abnormalities and vascular calcifications

Elevated calcium levels have long been implicated in the vascular calcifications observed in CKD patients. Early on, these patients are usually hypocalcemic as a result of calcitriol deficiency,

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but treatment with calcium salts and vitamin D derivatives can induce a positive calcium balance or even overt hypercalcemia [30]. In this context, it is possible that in patients with advanced kidney disease, calcium that is absorbed from the gastrointestinal tract cannot be excreted by the failing kidneys nor can it be deposited in bones with altered turnover (either high or low turnover is detrimental) and is therefore deposited at extra-osseous sites, such as the vascular bed [5, 6]. Calcium changes in the external milieu have a direct effect on the nearby cells. Normally, VSMCs recognize these changes via the membrane such as calcium sensing receptor (CaR) and a G-protein-coupled receptor, which was shown to be downregulated in calcified arteries from CKD patients, suggesting that calcium sensing is disrupted in these patients [6, 40]. In response to elevated extracellular calcium, VSMCs release calcium-laden vesicles, as an attempt to prevent intracellular calcium overload. When the vesicles do not contain enough calcification inhibitors (as in CKD), this adaptive response in fact promotes extracellular matrix calcification by serving as a site of origin for propagated calcification [35].

Besides calcium, *hyperphosphatemia* that is so common in advanced CKD, has emerged as a major culprit of vascular calcifications [41]. Increased serum levels of phosphate induce osteoblastic transformation of VSMCs, while the decrease of phosphatemia reduces the expression of proteins responsible for active bone mineral deposition in vascular cells [15, 35]. As suggested by in vitro studies, phenotypic transformation of VSMCs in response to hyperphosphatemia is mediated by Pit-1 (a type III sodium-phosphate cotransporter), which allow the influx of phosphate into VSMCs and predisposes the cells to undergo mineralization. It was observed that the first step of vascular calcification requires an increased uptake of calcium and phosphate by the VSMCs [42].

In addition to sodium-phosphate cotransport, alkaline phosphatase is necessary for the uptake of phosphorus into the cell and the subsequent induction of osteopontin. Moreover, VSMCs treated with pooled uremic sera from CKD patients also increased expression of osteopontin and mineral deposition, suggesting that uremic serum plays a role in vascular calcifications [43].

Clinical data also support the link between elevated phosphate and vascular calcifications. For example, in a population-based cohort without CKD, serum phosphate levels at the upper end of normal range were associated with aortic valve sclerosis and mitral annular calcification, independent of PTH or calcium values [44]. Moreover, each 1 mg/dL increase in serum phosphate appears to predict higher risk for de novo coronary artery calcification (CAC) over time, with an impact similar to traditional cardiovascular risk factors, in relatively healthy subjects [45]. As in general population, phosphate serum concentration correlated with a greater risk of ectopic calcification in patients with moderate CKD (stage 3), as each 1 mg/dL increase in phosphatemia, even within normal laboratory ranges, was associated with a 21, 33, 25, and 62% higher prevalence of coronary and thoracic arteries, aortic and mitral valves calcifications, respectively [46].

Furthermore, in the presence of increased phosphate, even modest increases in calcium can substantially exacerbate calcification, by inducing nucleation of basic calcium-phosphate and, consequently, the growth of nascent vesicles that are released from both viable and apoptotic VSMCs [47]. The dominant role of phosphate is further supported by experimental studies which showed that dietary phosphate restriction in FGF23-null mice (an animal model characterized by hyperphosphatemia, markedly elevated circulating calcitriol levels, extensive vascular calcifications, and early mortality) yielded complete resolution of ectopic calcifications, a result which was not obtained with the vitamin D-deficient diet [48].

The relationship between *vitamin D* and vascular calcification appears to follow a biphasic dose-response curve, with adverse effects associated with very high and very low calcidiol levels [49]. At certain levels, vitamin D promotes bone formation by increasing the expression of critical matrix proteins in osteoblasts, leading to the incorporation of calcium into bone, thus taking it away from the vasculature. In addition, vitamin D may also prevent vascular calcifications through modulation of inflammatory responses [50]. Indeed, in dialysis patients, serum levels of calcidiol were inversely correlated with the extent of coronary calcifications [51], and clinical observations revealed that vitamin D receptor agonists were associated with decreased deposition of calcium, improved therapeutic outcomes, and survival benefits, independent of baseline levels of calcium, phosphate, parathyroid hormone, measured comorbidities, and kidney function [6, 15, 52].

However, vitamin D excess was associated with medial calcification and arterial stiffness [49]. Indeed, high doses of vitamin D may actually increase the risk of vascular calcification in CKD owing to its effects on increasing intestinal calcium and phosphate absorption, as well as the mobilization of these minerals from bone, leading to hypercalcemia and hyperphosphatemia, especially in patients already taking calcium-based phosphate binders [13, 50, 52]. Besides its indirect effects due to interactions with the other major factors involved in osteoblastic transformation of VSMCs, vitamin D appears to directly induce the phenotypic switch through the vitamin D receptors on VSMCs resulting in upregulation of proteins involved in calcium transport and mineralization such as osteopontin and osteocalcin [35, 53].

Taken together, these data suggest that excess calcitriol can promote vascular calcifications through several interrelated mechanisms, while moderate physiological or pharmacological doses are beneficial (by suppressing the expression of osteoblastic genes in VSMCs). Debate also exists concerning the potential differential effects and benefits of native vitamin D as compared to active vitamin D receptor agonists, with an assumption that early administration of nutritional supplementation in CKD patients may prevent vascular calcification [54]. However, this remains to be proven by future research.

Secondary hyperparathyroidism may also be involved, indirectly, in the osteoblastic transformation of VSMCs since its excessive action on bone resorption results in hypercalcemia and hyperphosphatemia [55]. Also, arterial hypertension which may result from persistently increased parathyroid hormone (PTH), through the stimulation of renin-angiotensin-aldosterone and sympathetic nervous systems, is another indirect pathway to endothelial dysfunction and arterial calcification [56]. Despite these pathogenetic links, the exact contribution of PTH on vascular calcification is not known yet. In various clinical trials, therapies directed to decrease PTH (parathyroidectomy and calcimimetics) provided discordant results on prevention or regression of vascular calcifications [57–59]. Moreover, both hyperparathyroidism (which induce high bone turnover and activation of osteoclasts with calcium and phosphorus release into the circulation) and suppressed PTH (which induce adynamic bone disease with low bone turnover and reduce uptake of calcium and phosphate into the bone) were associated with extensive arterial calcifications [60]. Consequently, it was hypothesized that parathyroid hormone does not exert a direct intervention in the pathogenesis of vascular calcification in CKD, so its exact role on this matter remains to be elucidated.

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The relationships of *Fibroblast growth factor 23* (FGF23) and its receptor—*Klotho*—with calcifications were also investigated, but conflicting results were reported. Some authors found an association of increased FGF23 with carotid artery calcification in stages 3 and 4 CKD patients [61], and with abdominal aortic calcifications in hemodialysis patients [62], while others observed contrary findings [63]. To date, it is not clear whether FGF23 can directly act on vascular cells to promote or inhibit matrix calcification. It is possible that the involvement of FGF23 in vascular calcification would be only indirect, through the related calcium-phosphate metabolism disturbances [64]. Alternatively, since FGF23 needs Klotho as mandatory co-receptor and Klotho (which controls the dedifferentiation of VSMCs by blocking the expression sodium-phosphate cotransporters) decreases from the early stages of CKD, the ability of FGF23 to interact with vascular cells is consequently altered [64, 65]. Despite the fact that experimental data are congruent to suggest that the effect of Klotho is protective against vascular calcifications, it still remains unknown whether or not Klotho is expressed in the vessel wall [64]. Thus, no definitive conclusions regarding the direct effects of FGF23 or Klotho on VSMCs functions can be drawn based on the current state of knowledge.

4. Clinical consequences of vascular calcifications in chronic kidney disease

Observational studies point to cardiovascular disease (CVD) as the leading cause of morbidity and mortality in CKD patients. The annual 2014 report of the United States Renal Data System estimates that, in patients with CKD, the prevalence of CVD is 69.8% compared to 34.8% in patients without renal disease, and these numbers increase with decline in kidney function [66]. In fact, the risk of any cardiovascular (CV) event seems to increase as estimated glomerular filtration rate (eGFR) decreases, ranging from a 43% increase in risk with an estimated GFR of 45–59 mL/min/1.73 m^2 to a 600% increase in cardiovascular (CV) risk at an estimated GFR of less than 15 mL/min/1.73 m^2 [67].

The burden of CVD in patients with CKD is, at least in part, accounted for by the presence of non-traditional risk factors, which are much more prevalent in this group. Among these, mineral metabolism abnormalities and vascular calcifications are commonly seen. For example, Russo et al. reported that 40% of patients with stage 3 CKD had coronary artery calcification compared with only 13% of the control subjects with no renal impairment [68]. Similar data were found in our own experience: a cross-sectional, unicentric study that enrolled 110 stable CKD patients not on renal replacement therapy, and 34 age- and gender-matched patients without CKD showed higher prevalence of coronary artery disease (defined as past myocardial infarction, angor pectoris associated with electrocardiographic or ultrasound indices, coronary angioplasty or bypass) in CKD (49% vs. 19%, $p = 0.001$). In addition, more CKD patients than Controls had valvular (38% vs. 17%, $p = 0.02$), and vascular calcification (carotid plaques 60% vs. 29%, $p = 0.02$ and abdominal aorta calcifications 54% vs. 26%, $p = 0.003$), irrespective of the CKD stage [69].

4.1. Arterial stiffness

Clinical consequences of vascular calcifications in CKD include loss of arterial elasticity with resultant rise in arterial stiffness due to reduced compliance of large arteries, lower delivery of oxygen to the tissues, and endothelial dysfunction. Arterial stiffness represents the functional disturbance of vascular calcification and predominantly results from greater medial calcification. The main consequence of arterial stiffness is increased pulse pressure, which contributes to left ventricular hypertrophy and impaired coronary perfusion by increasing ventricular afterload and reducing coronary blood flow during diastole [70]. In response to higher pressure or flow, the arterial wall undergoes a remodeling process, which consists of either reorganization of cellular and noncellular elements (eutrophic remodeling) or increased muscle mass (hypertrophic remodeling), both with significant impact on altered arterial function, that is, the reduced ability to buffer pressure, and pulsatile flow oscillations [71].

Aortic pulse wave velocity, an accurate and reproducible parameter of arterial stiffness and a marker of cardiovascular dysfunction, is linked to several other CV risk factors such as microalbuminuria and proteinuria, vascular calcifications, and left ventricular hypertrophy [72]. Wang and coworkers, in a study on 102 non-dialysis CKD patients, found an inverse relation between pulse wave velocity and estimated glomerular filtration rate, with a significant stepwise increase in pulse wave corresponding to the advance in CKD from stage 1 to 5 [73], suggesting that arterial stiffness increases with decreased kidney function. Contrary to this result, but in line with others which did not detect independent associations between eGFR and aortic stiffness [74, 75], in a cross-sectional, single-center study on 135 stable patients (79% with CKD), we found increased cardio-ankle vascular index (CAVI, a stiffness marker less influenced by blood pressure than pulse wave velocity) in 73% subjects, irrespective of chronic kidney disease presence and severity [76].

It is largely accepted that arterial stiffness is a powerful independent predictor of mortality and CVD in advanced CKD, as well as in general population [70].

More debatable is the influence of arterial stiffness on kidney function. In theory, besides the effects on myocardium, the decreased compliance of the large arteries would be followed by the transmission of cyclic blood flow from the aorta to peripheral microcirculations in various organs (including the kidneys) because its transformation in the physiological continuous capillary flow fails. Consequently, the protective autoregulatory mechanisms of the glomerular microcirculation are overpassed, and renal tissue becomes more vulnerable to the high blood pressure–related damage, favoring the decline in glomerular filtration [71]. Despite these pathogenetic explanations, clinical studies yielded conflicting results, as mentioned earlier. The majority of large population-based studies (adult or elderly cohorts) seem to support an independent association of aortic stiffness (measured by carotid-femoral pulse wave velocity) with the risk of incident CKD, but not with the risk of CKD progression (even if, the latter is not a unanimously reported result) [71].

The presence of arterial stiffness in CKD patients is important also from the therapeutic point of view, since numerous trials investigating the efficacy of anti-hypertensive drugs in cardiologic cohorts showed significant differences among various therapeutic regimens with regard

to central hemodynamic parameters. Thus, it was found that calcium channel blockers but not beta-blockers, lower the central pulse pressure [77], so the presence of arterial stiffness could impact the choice of blood-pressure-lowering medication in CKD patients.

4.2. Atherosclerotic cardiovascular disease

Atherosclerotic lesions, which refer to intimal deposition of material with consecutive occlusive consequences, are highly prevalent in CKD patients mainly due to traditional CV risk factors. Specific features of atherosclerosis in chronic kidney disease comprise a higher proportion of calcified plaques among atherosclerotic plaques and a greater intervention of inflammatory stimuli than in general population [71]. Atherosclerosis represents one link between serum calcium and CVD with the content of coronary artery calcium emerging as a predictor of coronary heart disease [78]. Indeed, Budoff et al. showed a graded relationship between decreased kidney function in CKD patients and higher coronary artery calcification scores [79], connecting calcium and kidney function with the development of cardiovascular disease, in particular ischemic heart disease.

Even in the general population, lower level of kidney function was associated with increased 5-year probability of atherosclerotic cardiovascular disease [80]. Many studies found an inverse association between the glomerular filtration rate and the risk of occurrence or progression of atherosclerosis. For example, a cross-sectional retrospective study on almost 450 subjects with moderate to severe CKD (eGFR below 60 mL/min) and acute coronary syndrome suggested that estimated kidney function is an independent risk factor for atherosclerotic multivessel cardiovascular disease, as the decreased eGFR independently predicted a three-vessel coronary stenosis, with a magnitude dependent on the severity of renal impairment. The risk was seven times higher in patients with CKD stages 4–5 than in those with stage 1 CKD [81].

However, it should be mentioned that a significant proportion of cardiovascular death among CKD patients is not strictly related to atherosclerosis (i.e., it is not due to myocardial infarction, stroke, and heart failure), as the main event is sudden cardiac death which has a multifactorial causation [82].

Atherosclerotic lesions are usually accompanied by impairment of the endothelium. Endothelial function is often abnormal in CKD patients, who have diminished endothelium-dependent dilatation compared with controls and increased von Willebrand factor, regardless of the stage of renal disease and coexisting risk factors, suggesting that atherosclerosis may develop early in the progression of chronic kidney disease [83]. Besides common factors like age, hypertension, diabetes, smoking, dyslipidemia, and atherosclerosis, endothelial dysfunction is also accounted for by retention of uremic toxins, fluid overload, anemia, phosphate load, increased FGF23, increased homocysteine, enhanced oxidative stress, impaired nitric oxide metabolism (accumulation of asymmetrical dimethyl L-arginine), accumulation of advanced glycation end products, proinflammatory cytokines, and impaired angiogenesis [84].

Vascular calcifications of the large arteries, like abdominal aorta (assessed by the lumbar aortic calcification score—ACS) is not only a predictor of the cardiovascular morbidity and mortality, but it could also provide an indirect estimation of the intrarenal vascular status, as we

found in a cross-sectional study that enrolled 77 stages 2–5 non-dialysis CKD patients, older than 50 years, and with known atherosclerotic disease. This study described increased aortic calcification as eGFR declines and found that higher lumbar aortic calcification score was independently associated with lower ankle-brachial index and higher intima-media thickness, suggesting a relationship of abdominal calcifications with the extension of atherosclerosis in other territories [85]. In addition, the novel finding of the study was the ability of an aortic calcification score >5 to predict with 65% sensitivity and 68% specificity a pathologic (<0.7) renal resistive index (marker of intrarenal atherosclerotic lesions on Doppler ultrasound) [85].

4.3. Calcific uremic arteriolopathy

Previously referred to as *calciphylaxis*, this is another form of vascular calcification almost exclusive to chronic kidney disease patients with kidney failure, although some cases were scarcely reported in non-CKD patients. Female gender, hyperphosphatemia, high alkaline phosphatase, and low serum albumin are among the risk factors of calcific uremic arteriolopathy [86]. It is typically found in end-stage kidney disease, obese, diabetic females, often associated with secondary hyperparathyroidism, hypercalcemia, hyperphosphatemia, malnutrition, chronic warfarin therapy, or hypercoagulability [87].

Calcific uremic arteriolopathy involves diffuse medial calcification of small- to medium-sized subcutaneous arteries and arterioles of up to 50-μm diameter, with intimal fibroproliferative occlusions that lead to necrosis. Histological abnormalities include intimal hyperplasia, inflammation, obliterative endovascular fibrosis, arteriolar medial calcification, and thrombotic cutaneous ischemia. The result is dermal, subdermal, and adipose tissue necrosis with subsequent skin ulceration. Calciphylaxis occurs independent of osteogenic activity, when the physiological calcium phosphate solubility threshold exceeds 60 mg^2/dL^2 [13, 86].

Overt clinical signs include livedo reticularis advancing to patches of ischemic necrosis and painful skin ulcers, especially on the legs, thighs, abdomen, or breasts. Often, the initial presenting complaint is a dull deep dermal pain with periods of neuritic-type dysesthesia associated with palpable subcutaneous nodules or dermal plaques, which evolve to livedo reticularis and then nonhealing ulcerations [7, 88]. These lesions predispose the patients to life-threatening skin necrosis or acral gangrene susceptible to supra-infectious complications. Dermal fat, lung, and mesentery are most commonly affected [7, 86].

Sepsis, which is also the main cause of death due to calcific uremic arteriolopathy, and amputation are among the severe morbidities associated with this obliterative disease.

5. Conclusions

In chronic kidney disease, even in non-dialysis stages, the prevalence of atherosclerotic lesions, vascular calcifications, and arterial stiffness are significantly higher as compared to patients of same-age without kidney damage. Because the interplay of multiple factors is responsible for the arterial disorders in CKD, the exact mechanism involved is still a matter of debate. Therefore, the best therapeutic approach to minimize the adverse impact of CKD-related

mineral and bone disorder on the patients' outcome is not yet known and controversies exist especially regarding the influence of intestinal phosphate binders and vitamin D receptor activators on arterial calcifications.

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