Chapter

Vascular Calcification and Cardiovascular Risk in Chronic Kidney Disease: A Problem That Is Here to Stay

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Abstract

Cardiovascular disease is the primary cause of morbidity and mortality in chronic kidney disease (CKD) population, particularly in end stage renal disease (ESRD). This could be explained in part due to the presence of traditional cardiovascular risk factors, such as older age, hypertension, dyslipidemia and diabetes, but is also associated with nontraditional cardiovascular risk factors related to CKD, like inflammation, anemia, abnormal calcium and phosphate metabolism and extracellular fluid volume overload, which may contribute to intimal or medial wall arterial calcification. Vascular calcification (VC) is a dynamic process, resulting from the dysregulation of the balance of molecules that promote and those that inhibit this course. It is important for clinicians to both acknowledge and recognize the pathways and risk factors of VC in order to improve cardiovascular health in CKD patients. This chapter will focus on the biology of VC, the association with CKD, risk factor modification, screening and prevention of VC and cardiovascular disease in CKD patients.

Keywords: vascular calcification, cardiovascular disease, chronic kidney disease, mineral metabolism

1. Introduction

Vascular Calcification (VC) refers to the ectopic deposition of calcium phosphate crystals in arterial walls [1, 2]. This is a process that can be seen in all arteries with a distinction made based on the offended layer of the wall – medial or intimal – with different explaining mechanisms [3]. Besides vessel walls, other tissues like cardiac valves can also show calcifications [1, 4, 5].

In 1855, Virchow described the VC as "artery ossification", precisely in patients with renal disease [3, 6]. More recent, studies showed the same findings in Egyptian mummies [7].

In fact, with current molecular knowledge, we understand that mineralization of the tunica media, the type of VC associated with CKD and its mineral disturbances [8], occurs with trans-differentiation of muscle cells to bone cells, in a process one could call ossification [9, 10]. On the origin of those cellular changes,

several factors interact to put in motion a series of events that will result in an active promotion of mineral deposition [1, 11, 12].

VC increases with age [13] and it is associated with many diseases such as diabetes [14], cardiovascular diseases (CVD) [8, 15], some genetic diseases [2, 16] and particularly CKD [8, 17–19]. In the latter, VC is observed in early stages, progresses with the renal impairment and affects almost every dialysis patient [20], even in the absence of traditional risk factors of hypertension, obesity, dyslipidaemia and smoking [21, 22]. For calcification to happen, an unregulated state of bone ossification induction and the loss of many molecules that are believed to be osteogenesis inhibitors, must occur [11, 12]. In CKD, the culprit appointed by many recent studies is another of its complications – the mineral disturbance [11].

Cardiovascular (CV) events are tightly related to the CKD population, with high incidence of sudden cardiac death, arrhythmia, congestive heart failure and stroke, corresponding to the most common cause of death, especially in dialysis patients [2, 20, 23]. VC progression and severity [24] is directly associated with these CV events and its related mortality [21] due to arterial calcification and its consequences, like ventricular hypertrophy or micro-embolic disease [10, 23, 25]. This explains the high prevalence of cardiac events and mortality even in patients without the traditional cardio-vascular risk factors [8, 10].

Until now, no study came to the conclusion that VC directly causes CV events [26]. Moreover, VC has some features in common with arterial aggression leading to CVD [26, 27]. For that reason, there are still some who argue that VC is a consequence and not the cause for CVD [28–30].

This chapter will focus on the biology of vascular calcification and the association with chronic kidney disease.

2. The biology of vascular calcification

The calcification of the vasculature refers to the pathological deposition of phospho-calcium minerals in the arteries, leading to its stiffness [19] and thickening, as a result of a complex interaction of factors [4, 23]. Regardless of that, phospho-calcium can spontaneously precipitate [31]. Oversaturation increases of those ions concentrations and acidic environment favors their precipitation with a formation of various intermediates minerals, gradually bigger, until the least soluble, **hydroxyapatite** (HA) which will then crystalize [32]. The deposition of this crystal, or **nucleation process**, occurs chiefly in the apoptotic cells [9, 17, 33] or in mineral containing vesicles [34], which will then release more calcium to extracellular matrix (ECM), promoting more apoptosis when entering the neighboring cells, in a vicious cycle [32].

2.1 Calcification types

The histological location of that deposition and the pathobiology has made a distinction between types of calcifications [4, 35]. This phenomenon can arise in: i) the inner layer of endothelial and connective subendothelial tissue of arteries, ii) the medial muscular layer, iii) in the cardiac valves (resulting in valve sclerosis and stenosis) and iv) calcific uremic arteriolopathy, previously known as calciphylaxis [3]. These are the calcifications referring to the vascular system, of which we will focus on the first two. Besides VC, other extra-skeletal calcifications may happen [3, 36].

Atherosclerosis, an accumulation of calcium in the intimal layer of large and medium sized arteries, is observed following a long inflammatory process and as

a last stage of that series of events [23, 37, 38]. It involves the infiltration of macrophages in the epithelial and subepithelial connective tissue and the formation of a lipid plaque [3, 23]. Hence, it is related to dyslipidaemia, arterial hypertension and tabagism, but also with age [8].

In **medial calcific sclerosis** (MCS) or **Mönckeberg's medial arteriosclerosis**, the target of calcification is the muscular medial layer of elastic or muscular arteries, and posteriorly, fibrosis, stiffness and thickening ensues [36, 37, 39]. This form is widely related to mineral imbalance, and it is seen in diabetes, some rare genetic diseases [40] and strongly related to CKD [17, 37]. This is the form to which we will be referring to.

It is important to point out that atherosclerosis is due to inflammation and lipid deposition and for that depends on the so-called traditional CV factors (dyslipidaemia, arterial hypertension and tabagism) while medial VC does not [23, 41, 42]. In atherosclerosis, plaque calcification creates areas of different compliance [23, 39, 43, 44]. It ultimately leads to plaque rupture and acute vessel obstruction and is more closely associated with an increase in mortality [23, 43, 45, 46].

The association of MCS and mortality was brought up by many studies but not yet proven to be a cause-effect [28, 29] and some opinions still diverge, as we will discuss ahead. Research efforts have improved our knowledge on the molecular mechanisms involved, showing a passive process of physicochemical reaction resulting in vesicles [17, 47]. As stated earlier, apoptotic debris are good mineral nucleation sites but extracellular vesicles (EV) are regarded as having much higher mineralization potential [48].

Cells capable of osteo-transdifferentiation include not only vascular smooth muscle cells (VSMC) in the media, but also miofibroblasts in the adventitia, pericytes under endothelial cells (related to atherosclerosis), multipotent vascular mesenchymal cells and cardiac valve interstitial cells [42, 49, 50]. EVs, originally termed matrix vesicles, are nanoparticles of cellular origin, heterogeneous in size, shape and content, with two origins: membrane budding or in an endosomal pathway where multivesicular bodies fuse with the plasma membrane and then releases to the ECM by the regulation of sphingomyelin 3 [1, 17, 47, 51]. These EVs are designated exosomes [1, 52]. These vesicles are known for many decades to be related to bone mineralization but only recently were identified in VC. Indeed, EV's membrane have affinity to matrix proteins and, contrary to non-calcifying vesicles, contain phosphatidilserin (acidic phospholipid) and calcium-channel annexin family molecules in the membrane, responsible for the vesicle's release. Annexin-6 has been described as a regulator of VC in vivo [1, 37, 47, 53].

EV's release is promoted by high levels of calcium and their calcifying potential depends on its altered content compared to normal vesicles, with lower calcification modulating proteins – which we will analyze in detail later –, higher calcium and phosphate content, lipids, microRNAs, matrix metalloproteinases (MMP) for matrix digestion and alkaline phosphatase (ALP), which normally is not present in vascular tissue. ALP releases free inorganic phosphate (Pi) – enhancing more crystal formation [47].

The nucleation of HA begins inside the vesicles, with molding crystals' size and shape, and continues with the of in the ECM. Even tough origin and release of EVs are still poorly understood, vesicles are regarded as nucleating foci for VC [52, 54].

2.2 Transdifferentiation of VSMC

The unregulated osteogenesis is owed to the influence of external and internal factors (age, inflammation, toxins, CKD or diabetes) that induce

transformation of VSMC to osteogenic cells responsible for a pathological mineralization [23, 37, 54, 55].

Gene silencing lead to the loss of contractile properties by the underexpression of α-smooth muscle actin (SMA) and smooth muscle protein 22 (SM2) – the de-differentiation of VSMCs – whereas the upregulation of several genes coding for bone-like cells, give VSMCs the **osteogenic characteristics** [48, 49, 56]:

- **ALP** – enzyme responsible for the increasing Pi availability needed for crystal formation. ALP in soft tissues is a marker of ectopic calcium deposits [49, 57].

- **Collagen I** – implicated in the ECM remodeling and HA deposition as described [58].

- **Osteopontin (OPN)** – it is a binding-calcium particle (preventing HA formation) and a pro-inflammatory agent. It is thought that the cleavage of OPN under osteogenic circumstances by MMPs, may disrupter its equilibrium rendering an inflammatory and angiogenic role and involved in proliferation and migration of calcifying VSMCs [59].

- **Osteocalcin and Osteonectin** – increased in calcifying cells but with role on VC yet to be demonstrated [3, 25].

- **RANK-L** – Receptor activator of nuclear factor KB ligand. RANK is a member of tumor necrosis factor (TNF) receptor family, expressed by osteoclasts and increased in medial VC where it promotes VSMC calcification (demonstrated in vitro). The mechanism is the upregulation of a pathway of transdifferentiation. It also promotes osteoclast activity, responsible for osteoporosis [13].

2.3 Loss of calcification inhibitors

Along with the aforementioned change in phenotype, the loss of calcification inhibitors must take place concomitantly for the end-result of VC [58] These are referred as the calcifying protein inhibitors preventing HA harm to ECM and cells [37].

They are herein described:

- **Matrix Gla Protein (MGP)** – Vitamin K dependent protein (VKDP), expressed in several tissues and in VSMCs. The activated molecule has a glutamic γ-carboxylation [55]. In normal states, the carboxylate MGP binds and inhibits crystal formation and prevents osteoblastic transformation. The unbalance toward the uncarboxylated inactive form is present in VC and is assumed to be a procalcifying agent [47]. Because of its vitamin K dependence for the γ-carboxylation of glutamic residues, anti-cumarinic medications (anti-vitamin K) may disrupt its normal quantities [58, 60].

- **Gla-Rich Protein (GRP)** – GRP is the newest member of the vitamin K-dependent protein (VKDP) family, first identified in sturgeon calcified cartilage and characterized by the presence of an unprecedented 15 putative calcium-binding Gla residues in human [61]. It is considered a negative regulator of osteogenic differentiation [62], a modulator of calcium availability in the ECM [61, 63], and an inhibitor of calcification in the CV [64] and articular systems [65]. GRP calcium binding properties [61, 66] and association to calcification processes [61, 63–67] indicates that its function might be

associated with prevention of calcium-induced signaling pathways and direct mineral-binding to inhibit crystal formation/maturation. GRP is also involved in the mineralization-competence of VSMCs derived EVs and possibly associated with the fetuin-A/MGP calcification inhibitory system [64].

- **Fetuin-A (Fet-A)** – also known as α2-Heremans-Schimd glycoprotein, is a potent anti-calcification glycoprotein in circulation [34]. Binds crystals therefore preventing its growth and the formation of insoluble nanoparticles [68, 69]. The complexes formed with crystals – primary calciprotein particles (CPP-1) – are more easily eliminated by the reticuloendothelial system than normal calciparticles and with less toxicity to those cells [70]. The re-arrangement of the CPP-1 in terms of form and crystal features will make a particle believed to be more cytotoxic and VC-inducing, called secondary CPP (CPP-2) [37]. Fet-A is linked with reduction of inflammatory response and oxidative stress produced by calcification. As seen, it is diminished in EVs responsible for VC [49]. Studies shown Fet-A to be decrease in serum of CKD patients, with age, restrictive diet, low weight and aerobic exercise [58]. Despite being phospho-calcium containers impeding its growth and deposition, CPPs are cytotoxic particles and in case of impaired elimination it is believed to be responsible for the inflammation state and premature aging seen on CKD.

- **Osteoprogerin (OPG)** – binds RANK-L preventing its binding to RANK and its effects: osteoclastic differentiation and medial calcification [58, 71].

- **Pyrophosphate (PPi)** – prevents the formation of phospho-calcium crystals in the extracellular environment [58, 71].

2.4 Regulation: Signaling pathways

Different, complex and not fully understood molecular pathways activate the changes described in the pathobiology of VC [4]. Many of those factors have more than one implication in VC and a lot of them interact with each other. There is a "perfect storm" [25, 49] behind VC. Hereby we analyze the mechanism and the signals involved with the onset of VC.

- **Runx2/Cbaf** – The transcriptional promoter activity of Runt-related **transcriptor factor-2,** also known as Core-binding factor subunit *α*1, is responsible for the underexpression of muscle fibers causing the de-differentiation process of VSMCs [72]. Together with the downstream **Osterix gene** it is responsible for the upregulation of the aforementioned Osteocalcin, Osteopontin (OPN), Osteonectin, ALP, Collagen I, RANK-L and also sclerostin, since all of them have a binding site for cbaf in their genes/promoters [49, 58]. These promoters regulate genes involved with the cell cycle for lineage determination, interacting in order to enhance osteoblastic/osteochondrogenic protein characteristics and osteogenic lineage commitment of the VSMCs [73]. This mechanism is related with age, CKD, diabetes type 2, inflammation and with toxins like uremic products [37, 72].

- **Wnt pathway** – in this signal transduction pathway, several glycoproteins (Wnts) are capable of activating specific membrane receptor complexes, responsible for the dephosphorylated stabilization of ß-catenin that leads to the intracellular signaling of transcription factors activation [13, 74]. Different receptors are involved but low-density related protein receptors (LRP) 5 and

6 were described as involved in the pathway resulting in activation of Runx2 and Osterix by ß-catenin, in a way not yet clarified [13, 58]. There are many proteins to regulate Wnt cascade like sclerostin, which binds to the membrane receptor thus inhibiting it, and **MSX-2** homeobox that promotes paracrine Wnt signals [13, 49]. It is also implied in the cell-cycle, proliferation, lineage commitment and regulation of apoptosis and its activation is related to TNF-α, oxidative stress and hyperphosphatemia [13, 58].

- **The Bone Morphogenetic Protein-2 (BMP-2)** – a cytokine of TGF-ß family and a mediator of VC. By activating its membrane receptor can activate (by phosphorylation) co-regulatory proteins of the Smad family and will put, together with Runx2, the cascade of events in motion [42, 58]. Both BMP-2 and BMP-4 receptors can phosphorylate and activate that cascade toward osteoblastic/chondrocyte's formation and prevent mesenchymal cells transformation in (normal) VSMCs or adipocytes. It is crucial for VC to happen, since neither BMPs nor Runx2 are able to put the osteogenesis machinery in motion alone [13, 58].

- **TGF-ß** – upregulated when elastosis develops, like BMPs, can promote the transducing of osteogenic genes with Runx2 activation [58].

- **RANK/RANK-L/OPG pathway** – the already mentioned TNF-α family receptor, RANK activation results in the activation of NF-***κ***B transcription factor. Involved in cellular responses to aggressions like free radicals, oxidized LDL cholesterol or some cytokines and it was linked to VC in some studies. It also increases BMP-4 expression and so, activates Runx2 [58]. As discussed, RANK-L is the activator of receptor RANK and OPG competes with RANK-L, preventing the pathway activation. So, RANK signaling depends on RANK-L:OPG ratio but also indirectly of the regulatory factor Runx2, capable of promoting RANK-L expression [25, 71].

- **ENPP1/PPi/ALP axis** – as discussed, PPi opposes crystal formation whereas ALP is a crystal-promotor by hydrolysing PPi and making it a source of phosphor (Pi) [47]. In a complex interaction of several enzymes, PPi is formed by Ectonucleotide Pyrophosphatase/Phosphodiesterase-1 enzyme (ENPP1) from adenosine triphosphate (ATP). Another membrane enzyme – Progressive Ankylosis Protein (ANK) – makes PPi available to the extracellular environment where it can prevent Pi to mineralize. An overexpression of ENPP1, ANK and ALP gives the continuous exaggerated production and release of PPi to the ECM, for posterior destruction by ALP [36, 47, 57, 58].

Vitamin D can induce such activity. ATP Binding Cassette Subfamily C member 6 (ABCC6) and ecto-5′-nucleotidase (NT5E) genes encode for the intermediary enzymes in the formation and degradation of ATP to AMP and ultimately, adenosine; These several enzymes are related to genetic calcifying diseases [58].

- **Phosphate and calcium status** – increased concentrations of these ions result in the formation of either crystals or nanoparticles with proteins, as referred. Transient or continuous elevated extracellular phosphate, in any form, has cytotoxicity consequences: it can lead to apoptosis because of ROS burden, an important calcifying event; and it can activate the digesting MMPs responsible for elastolysis [4, 13, 75]. Elevated calcium concentrations facilitate phosphate entry and is linked to calcification even in normal phosphoric quantities. Both calcium and Pi are related with Runx2 activation [13, 17, 47]. HA crystals,

nanoparticles or free phosphate can render the same results. In fact, it has been proposed that cell-internalized CPPs with low fetuin-A are the most common event and that they can be detected circulating in CKD patients' blood, as they do not in healthy individuals [32].

- **MicroRNAs (miRNAs)** – regulate gene expression by targeting translated mRNAs and altering its traducing to proteins. EVs are known to have miRNAs and there have been new studies on this matter [47].

On the other hand, it is of notice that for the installation of VC, there is a role to be played by the concomitant loss of several osteogenesis antagonizing molecules, such as [58]:

- **MGP, GRP and Fet-A** – the carboxylated forms not only bind calcium but also inhibit BMPs impeding BMPs-BMP receptor activation.

- **BMP-7** – apart from the other BMPs, it does not promote Runx2 but the expression of smooth muscle fibers, antagonizing the VSMC differentiation. Because of that, the loss of its action will possibly increase VC risk [58, 72].

- **Sclerostin** – a glycoprotein with expression promoted by Runx2 and OSX is not consistently associated with VC and its role is to be fully explained, but it was however identified as an inhibitor of the Wnt cascade in its signal activation by ß-catenin and therefore inhibiting osteogenesis [58].

- **Adiponectin** – an adipocyte's hormone, restricts osteoblastic differentiation by 1/p38 receptors signaling pathway [40].

A complex myriad of factors may offend the vessel wall's cells such as: elastolysis, cytokines, ROS, oxidized lipids, glucose, uremic molecules, BMPs and circulating calciprotein particles (CCPs). These will ignite signaling pathways like the Wnt pathway, resulting in the trans-differentiation of VSCM by the action

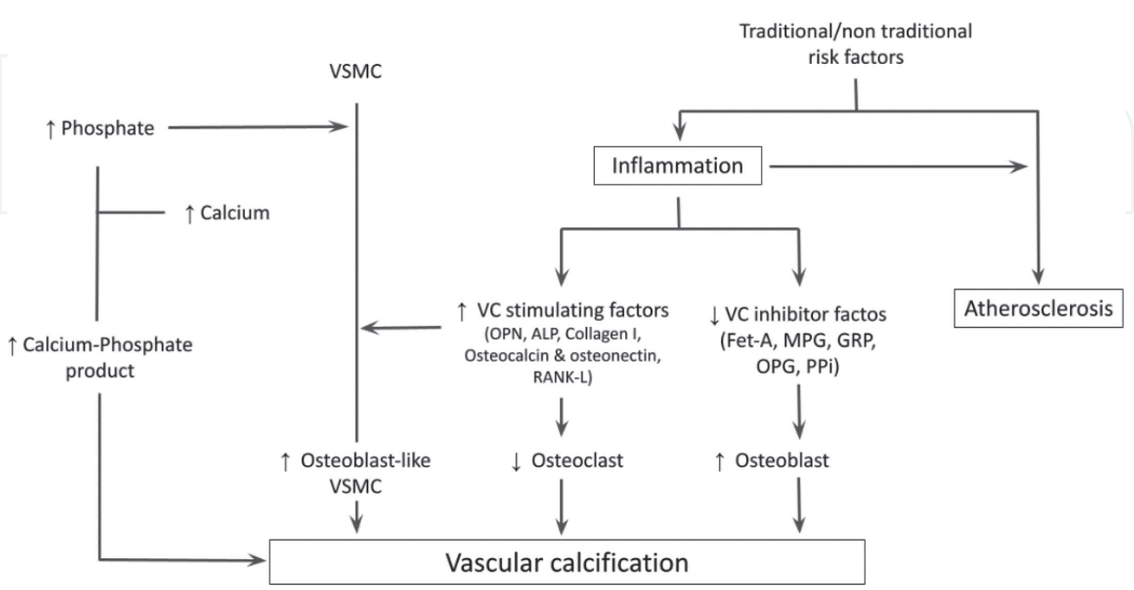

Figure 1.
Mechanisms of vascular calcification. ALP: Alkaline phosphatase; Fet-A: Fetuin-A; GRP: Gla-rich protein; MGP: Matrix Gla protein; OPN: Osteopontin; OPG: Osteoprotegerin; PPi: Pyrophosphate; RANK-L: Receptor activator of nuclear factor KB- *ligand; VC: Vascular calcification; VSMC: Vascular smooth muscle cells. (Adapted from Dellegrottaglie et al. [28].)*

of transcription factor Runx2. Deposition of minerals is promoted in the matrix, either in vesicles or apoptotic bodies. As this occurs, a loss of calcification inhibitors will pose no obstruction to the process.

Ultimately, the stiffness caused by this remodeling of the medial layer will result in ventricular hypertrophy [19, 49].

Figure 1 resumes the before mentioned pathways and mechanisms implied in the vascular calcification process.

3. Vascular calcification in chronic kidney disease

Cardiovascular disease is the leading cause of death in patients with CKD, especially among those with end stage renal disease (ESRD) [76, 77]. This high cardiovascular risk may be in part due to excess VC [78, 79].

The prevalence of vascular calcification in CKD patients increases with progressively decreasing kidney function and is greater than the general population [21, 80]. In patients with estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 m2 and not on dialysis, VC is known to be present in 47 to 83%. In dialysis adult patients, coronary artery calcification (CAC) has been detected in 51–93% and valvular calcification in 20–47% [2, 5, 81].

Increased CV risk may be due to CAC, with remarkably high prevalence in patients undergoing dialysis [8, 76, 82, 83]. It can be said that 20-year-old patients in dialysis have the same CV mortality risk as 80–90 year-old non-diabetic and non-uraemic subjects [82]. This may be associated with dialysis vintage, the intake of supplemental calcium, particularly with calcium-containing phosphate binders, and the mean calcium-phosphorus ion product.

3.1 Clinical significance

The diagnosis of CKD–Mineral Bone Disease (MBD) includes the detection of extra-osseous calcification, such as arterial, valvular, and myocardial calcification [5].

The clinical significance of vascular calcification depends on the site (ie, medial or intimal) and type of the affected arteries. Intimal calcification is associated with the formation and progression of atherosclerotic lesions and is associated with the development of plaques and occlusive lesions as in coronary artery disease, cerebrovascular disease, and peripheral vascular disease [9].

Tunica media calcification was initially considered to be clinically non-significant [9]. However, it was later demonstrated in CKD and ESRD patients that was associated with decreased vascular distensibility and increased vessel stiffness and pulse pressure with consequent progression of intimal lesions [9, 11, 84–86]. London et al. conducted a study including 202 HD patients which showed that medial calcification had major impact on clinical outcome, being an independent prognostic marker for all cause and CV mortality in chronic HD patients independently of classical atherogenic factors, with close association to time on HD [8].

3.1.1 Coronary artery calcification

Coronary artery calcification (CAC) is common and progressive in young adults with ESRD who are undergoing dialysis [21]. Some studies reveal an association between CAC and CVD in this population [80, 83].

Coronary artery calcification score, measured noninvasively by electron-beam computed tomography (EBCT), was found to be an independent predictor of overall

mortality in dialysis patients [87]. In one study with 39 patients undergoing HD, those with CAC had higher serum phosphorus concentrations, higher calcium–phosphorus ion product in serum, and their daily intake of calcium-containing phosphate-binding agents was nearly two times greater than those without calcification [21].

3.1.2 Large-vessel calcification

Calcification of aorta and other large arteries is associated with increased arterial stiffness. This will cause lack of distensibility, leading to hypertension and increased pulse pressure, both risk factors for left ventricular dysfunction and heart failure among CKD patients [85, 88].

3.2 Risk factors

The higher prevalence of traditional CV risk factors, such as older age, hypertension, dyslipidemia and diabetes, and the presence of nontraditional CV risk factors related to CKD (anemia, abnormal calcium and phosphate metabolism, extracellular fluid volume overload, electrolyte imbalance) may explain the high occurrence of CVD and contribute to intimal or medial wall calcification in patients with kidney failure [28].

3.2.1 Demographic features and time on dialysis

Vascular calcification is associated with the increasing age and time on dialysis [21, 89, 90]. A study with 134 patients on HD, peritoneal dialysis and with stage 4 CKD demonstrated that age and male gender were important determinants of VC [77, 91].

3.2.2 Mineral metabolism

Disorders of mineral metabolism may promote CV calcification, contributing to higher CVD and mortality in patients with kidney failure or ESRD. Several observational studies have noted an association between mineral and bone disorders with adverse outcomes, most notably with increased phosphate levels (increased risk of VC, cardiomyopathy, and mortality) [11, 22, 92, 93].

Hyperphosphatemia, uremia, hyperglycemia and other metabolites may initiate the process of VC by transforming vascular smooth muscle cells to a chondrocyte or osteoblast-like cell. In dialysis patients, this process is accelerated in the setting of the common presence of high calcium, high phosphorus, and abnormal bone remodeling [91].

3.2.2.1 Hyperphosphatemia and hypercalcemia

Epidemiological studies have shown that hyperphosphatemia is associated with unexpectedly high rates of CV events and death in ESRD patients [94].

Hyperphosphatemia is a strong inducer of VC by inducing smooth muscle cells to undergo an osteochondrogenic phenotype change through a mechanism requiring sodium-dependent phosphate cotransporters [11]. In a group of 43 patients receiving peritoneal dialysis studied by MDCT at baseline and after 1 year of follow-up, Stompor and colleagues reported a significant correlation between changes in coronary calcium score and mean values of phosphate and calcium–phosphate product [95]. A study with uremic rats fed with high phosphate diet showed that aortic medial calcification could be blocked by treatment with the phosphate binder, sevelamer [96].

Lowering serum phosphate levels with a non-calcium containing phosphate binder slows progression of VC in pre-dialysis and ESRD patients [97–99]. In pre-dialysis patients, treatment with calcium carbonate did not enhance the progression of CAC, as it has been observed in patients on dialysis [97].

3.2.2.2 Oral calcium intake

In dialysis patients, the use of calcium-based phosphate binders is strongly associated with development and progression of CAC, due to the ingestion of large amounts of calcium and the consequent hypercalcemia [97]. Therefore, a positive calcium balance may increase the risk of calcium overload and CV calcification and it should be taken into account when calcium salts are prescribed. Discontinuation or dose reduction of calcium-based phosphate binders is suggested in the presence of hypercalcemia, CV calcification, adynamic bone disease, and/or low serum PTH levels [90].

Phosphate binding and lowering of serum phosphate can be achieved with calcium-based or non-calcium-based binders. A meta-analysis including 11 randomized trials (4622 patients) showed that patients assigned to non-calcium-based binders had a 22% reduction in all-cause mortality compared with those assigned to calcium-based phosphate binders in patients with CKD [100].

3.2.2.3 Dialysate calcium

The exposure to high calcium concentrations may influence the development of low–turnover bone disease and CAC in HD patients. A randomized controlled study showed that lowering Ca exposure through dialysate (dialysate Ca concentration of 1.25 mmol/L vs. 1.75 mmol/L) attenuates progression of CAC and improves low bone turn-over in HD patients with baseline PTH levels ≤300 pg./ml [101].

3.2.2.4 Secondary hyperparathyroidism and adynamic bone disease

Both secondary hyperparathyroidism (SHPT) high-turnover renal osteodystrophy and adynamic bone disease have been associated with VC. In a group of 58 ESRD patients on HD, bone-histomorphometry characteristics were compared with the arterial calcification (AC) scores. High AC scores were associated with bone histomorphometry values, suggestive of low bone activity and adynamic bone disease. This indicates that therapeutic interventions associated with excessive decrease of parathyroid activity favors lower bone turnover and adynamic bone disease that, in combination with interventions that increase the Ca balance, could influence the development and progression of AC. [102]

3.2.2.5 Vitamin D and calcimimetic agents

A major complication of SHPT is renal osteodystrophy, in association with alterations in calcium and phosphorus metabolism leading to CV calcification. Active vitamin D compounds and calcimimetic agents are used to treat SHPT in dialysis patients. Untreated vitamin D deficiency has been associated with increased VC, in part due to accelerated development of atherosclerosis [103, 104]. In these patients, vitamin D supplement may have a protective benefit against VC by decreasing endothelial injury, inactivating renin-angiotensin-aldosterone system, decreasing insulin resistance, lowering cholesterol, inhibiting foam cell and cholesterol efflux in macrophages, and modulating vascular regeneration [105]. Excessive administration of vitamin D has been also associated with increased VC, possibly related to hypercalcemia and an elevated calcium-phosphate product. Cinacalcet

has the ability to simultaneously lower PTH, calcium, phosphorus, and CaxP in patients with SHPT [106]. In a study, Cinacalcet plus low-dose active vitamin D derivatives attenuated vascular and cardiac valve calcification [107].

3.2.2.6 Hypomagnesemia and FGF-23

Hypomagnesemia has been associated with increased VC [23, 108, 109]. In a population with diabetes mellitus type 2 and mild to moderate CKD, hypomagnesemia was found to be an independent predictor of mitral valve calcification and intima media thickness [110].

3.2.2.7 Oral vitamin K antagonists

The vitamin K-dependent MGP, despite not being related to blood coagulation cascade, is affected by Vitamin K antagonists (VKA) and is considered a strong inhibitor of calcification of arterial vessel wall and cartilage [111]. Warfarin (a VKA) is a risk factor for calcific uremic arteriolopathy, necrotizing skin condition observed in dialysis patients, associated with extremely high mortality rates [11, 112, 113].

3.2.3 Diabetes

Many studies have shown that diabetes is a risk factor for VC in patients without CKD. In patients with eGFR <60 mL/min/1.73 m2 who were not on dialysis, diabetes also increased the risk of VC from 3.5 to 55.7% [114].

3.2.4 Dyslipidemia

Dyslipidemia increases the risk of VC in patients without CKD, via induction of inflammation and endothelial/vascular smooth muscle cell damage by oxidized lipids [115, 116]. Beneficial effects of sevelamer on VC may be related to its lipid-lowering effects [99].

3.2.5 Other molecules

Serum Gla-Rich Protein (GRP) levels were found to progressively decrease from stage 2 to stage 4 CKD. A multivariate analysis study identified that decreased eGFR, low levels of GRP, and high levels of fibroblast growth factor-23 (FGF-23) were associated with higher VC score and pulse pressure. These results indicate an association between GRP, renal dysfunction and CKD-mineral and bone disorder. The relationship between low levels of GRP and VC suggests a future potential utility for GRP as an early marker of vascular damage in CKD [117]. Once uremic CPPs and EVs are important players in the mechanisms of widespread calcification in CKD, GRP could have a role as an inhibitory factor, preventing calcification at systemic and tissue levels. Possible future approaches targeting the increase of γ-carboxylated GRP bioavailability could represent promising therapeutics [118].

3.3 Detection

The recognition of vascular or valvular calcification in patients with CKD stages G3a–G5D places them with highest cardiovascular risk [119]. The early diagnosis of VC and the identification of its cause raises hope for therapeutic intervention that might reduce CVD in patients with CKD.

Various noninvasive methods, such as ultrasound, fluoroscopy, and digital subtraction angiography, have been used to detect and measure VC [28]. Currently, EBCT and multidetector computed tomography (MDCT) are excellent methods to detect and quantify VC [28], with their results being stronger predictors of a cardiovascular event in normal population [28, 119]. Bursztyn and colleagues reported a twofold greater progression in coronary calcium score (measured by MDCT) in hypertensive patients with CKD, than in hypertensive patients with normal renal function [120].

A number of noninvasive methods, which are more easily and available techniques, can also be used to detect the presence or absence of valvular calcification, like lateral abdominal radiograph and echocardiogram [119]. The simplest technique is plain radiography, which demonstrates pipe-stem calcification of the tunica media and more irregular, patchy calcifications of the internal elastic lamina. However, this is an insensitive method and does not quantify the severity of VC [8].

Several scores, based on plain radiographic imaging or CT scans, are used in clinical studies to calcium quantification and scoring:

- **Agatston score** - quantifies CAC detected by an unenhanced low-dose cardiac CT scan. The Agatston score allows for early risk stratification for a major adverse cardiac event.

- **Adragão score** – the Adragão score quantifies calcification of the iliac, femoral, radial and digital arteries observed on plain radiographs of the hands and pelvis. The final value ranges between 0 and 8 points (0 to 4 in the pelvis and 0 to 4 in the hands). VC score ≥ 3 had an almost fourfold higher risk of cardiovascular mortality, representing a simple and inexpensive tool for assessing the cardiovascular risk related to VC in HD patients [121].

In the Study of Mineral and Bone Disorders that included 742 patients with nondialysis CKD stages 3–5 from 39 centers in Spain, VC assessment using Adragão Score was independently associated with all-cause and cardiovascular mortality as well as a shorter hospitalization event–free period [122].

- **Kauppila score** – This score quantifies the severity of lumbar aortic calcifications observed on a lateral abdominal radiograph ranging from the T-10 vertebra to the first two sacral vertebrae. A score of 1 to 3 is assigned based on extent of calcification (ie, one-third, two-thirds, or more than two-thirds of the vertebra).

3.4 Treatment

The approach to decrease the progression of VC can be influenced by treatment. Given that VC is associated with increased cardiovascular risk and the pathogenesis seems to be related to CKD-MBD abnormalities and atherosclerosis, the treatment should focus on the prevention of arterial lesions, correcting the several traditional and non-traditional pro-atherogenic risk factors responsible for arterial injury, mainly calcium and phosphate balance [5, 29].

Some experimental studies have suggested that the administration of magnesium prevents VC, not only reducing the deposition of calcium, but also inhibiting osteogenic transdifferentiation, and may be considered an important and realistic approach to potentially reduce the risk for VC and subsequent cardiovascular complications in CKD patients. Clinical trials are warranted to further assess the clinical relevance of magnesium in this regard [123, 124].

In patients with CKD G3a–G5D, decisions about phosphate-lowering treatment should be based on progressively or persistently elevated serum phosphate, as mentioned earlier [119].

In the management of moderate to severe secondary hyperparathyroidism, current treatment options consist of the oral administration of intestinal phosphate binders, oral or intravenous calcitriol or active vitamin D analogs, and the oral or intravenous calcimimetic agents (cinacalcet and etacalcetide) [125].

4. Conclusions

The high incidence of multiple traditional and non-traditional risk factors predisposes advanced CKD and dialysis patients to a considerable burden of CVD. This chapter has summarized the biology of VC, highlighting the processes of mineralization, the effects of local inflammation, and the available evidence about risk factors modification, prevention and screening in CKD. Clinicians must understand the limitations of the current evidence and adapt specific therapeutic strategies to the individual patient. Future research into modifiable, non-traditional risk factors with emphasis on mineral bone metabolism is warranted and we look forward to their clinical application and improvement of CVD outcomes in CKD patients.

Conflict of interest

The authors declare no conflict of interest.

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