Vascular Calcification in Patients with Chronic Kidney Disease: Types, Clinical Impact and Pathogenesis

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Key Words

Vascular calcification • Chronic kidney disease • Secondary hyperparathyroidism • Bone loss vascular-bone links

and not yet fully understood. Phosphorus plays a major role, while other factors related to bone formation have been recently identified.

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Abstract

Vascular calcification plays a major role in cardiovascular disease, which is one of the main causes of mortality in chronic kidney disease patients. Vascular calcification is determined by prevalent traditional and uraemia-related (non-traditional) risk factors. It occurs mainly in the arteries, which are classified into three types according to their size and structural characteristics. In addition, vascular calcification has been associated with bone loss and fractures in chronic kidney disease patients and the general population, stressing the fact that both disorders can share pathogenetic pathways. The strategies to control vascular calcification involve several measures, chief among them the control of hyperphosphataemia. Furthermore, it has been recently described that strategies that reduce bone resorption and increase bone mineralization may decrease the risk of vascular calcifications; however, this approach still remains controversial. The mechanisms involved in vascular calcification are complex

Vascular Calcification in Chronic Kidney Disease: Types and Risk Factors

In recent years, the main cause of morbidity and mortality in chronic kidney disease (CKD) patients has been judged to be cardiovascular disease. Recent studies suggest that vascular calcification plays a major role in cardiovascular disease in dialysis patients. Following the recommendations of the Kidney Disease: Improving Global Outcomes Foundation [1, 2], kidney damage is defined as structural or functional kidney abnormalities, which are accompanied by other abnormalities such as vascular calcification, bone loss and fractures, parathyroid dysfunction and several biochemical abnormalities, especially in serum PTH, calcium, phosphorus and alkaline phosphatase. All together are today known as CKD bone and mineral disorders [2].

Table 1. Risk factors associated to vascular calcification in CKD patients

Traditional risk factors

Hypertension
Dyslipidaemia
Diabetes mellitus
Smoking
Older age
Family history of premature coronary heart disease

Uraemia-related and non-traditional risk factors

Time on dialysis
Hyperphosphatemia
High calcium-phosphorus product
Hyperparathyroidism and hypoparathyroidism
High dosage of vitamin D metabolites
Low fetuin-A
Anaemia
Poor nutrition (low albumin)
Chronic inflammation (CRP, IL-1, IL-6, TNF- α)
Hyperhomocysteinaemia
Advanced glycated end-products

The predisposition of patients with CKD towards developing vascular calcification was mentioned for the first time back in the 19th century; since then, many studies have addressed this important aspect. Vascular calcification occurs mainly in the arteries, which can be classified into three types according to their size and structure: elastic or large-calibre arteries, muscular or medium-calibre arteries and small-calibre arteries.

Elastic or large-calibre arteries show a relatively thin wall in proportion to their diameter. The tunica media is rather thick and contains more elastic fibres than smooth muscle; the adventitia tends to be fairly thin. Through the elastic arteries, blood is conducted from the heart to the distribution arteries. Large vessels like the aorta, subclavia and common carotid arteries are included in this group.

Muscular or medium-calibre arteries have a tunica media which contains a great proportion of smooth muscle fibres; they are capable of withstanding further vasodilatation and vasoconstriction to adjust the volume of blood to accommodate perfusion requirements. Medium-calibre arteries include the axillary, brachial, radial, coronary, femoral and tibial arteries.

Finally, small-calibre arteries are less than 2 mm thick and their tunica media contains only smooth muscle fibres. In these vessels, luminal size variations, caused by vasoconstriction and vasodilatation of smooth muscle

cells, are responsible for regulating the local blood flow and perfusion pressure. This group includes the palmar arch and the digital arteries, among others.

The classical description of arterial calcification specifies it may occur in two locations: the intima and the media layers [3]. Nevertheless, this classical concept is not fully accepted by all authors [4, 5].

Intimal calcification begins and progresses under the influence of both genetic and lifestyle circumstances throughout a person's lifetime. Intimal calcification is associated with a sequence of atherosclerotic events that include endothelial dysfunction, intimal edema, lipid cell formation and the migration of leukocytes and macrophages that can in turn cause a plaque rupture, thus leading to the formation of the thrombus [6]. Atherosclerotic lesions have a patchy distribution along the length of the artery and may cause local stenoses and occlusions. Furthermore, it is characterized by chronic arterial inflammation exacerbated by alterations in lipid metabolism [7] and other well-characterized risk factors, including hypertension, diabetes, dyslipidemia [8, 9], obesity, smoking and a family history of premature coronary heart disease.

Calcification of the media occurs in the elastic lamina of large-calibre and medium- to small-size arteries; it seems to be independent of atherosclerosis, but both can coexist. This type of calcification was known initially as Monckeberg sclerosis and it can be seen radiographically as railroads [10]. It typically affects arteries such as visceral abdominal, thyroid and lung [10], but it is also extremely common in the aorta, limb and femoral arteries. Calcification of the media increases linearly with age. It is frequently observed in patients with metabolic abnormalities such as hypervitaminosis D, CKD and diabetes [11].

Table 1 summarizes the most prevalent traditional, uraemia-related and non-traditional risk factors for vascular calcification in CKD patients. Like in the general population, traditional cardiovascular risk factors, present in a large proportion of patients with CKD, are responsible to a great extent for the progression of vascular calcifications. Among non-traditional cardiovascular risk factors, including uraemia-related risk factors, hyperphosphatemia and the dialysis vintage are the risk factors more strongly associated with increased vascular calcification and mortality [12]. Elevated CRP and IL-6, as expression of chronic inflammation, have also been frequently associated with vascular calcification.

Clinical Impact of Vascular Calcification

As previously mentioned, CKD patients exhibit a very high percentage of vascular calcifications [13–16], leading to cardiovascular disease, decreased life expectancy and mortality [17] even in the earliest phases of CKD. Russo et al. [18] showed that 40% of patients with CKD (mean glomerular filtration rate 33 ml/min/1.73 m²) suffered from calcification of the coronary arteries compared with 13% of controls within a similar age range but with normal renal function. Kramer et al. [19] found a significant positive association between the presence of coronary calcifications and renal failure, an association which increased dramatically in CKD diabetic patients.

CKD patients develop vascular calcification even at early ages [16] and almost in all localizations in a greater proportion than the general population. They are frequently localized in high-calibre arteries, such as the aorta (79%), medium arteries (70.5%), including coronary arteries [11], and also in small calibre arteries (20.2%) [20]. These differences may reflect the heterogeneity of the three categories of arteries studied, which also imply relevant functional changes in the vasculature [21]. The calcification of the cardiac valves involves also a high risk of cardiovascular dysfunction [22] in both general and haemodialysis (HD) populations.

In a recent study, prevalent aortic calcifications were significantly higher in HD patients (79%) than in a random-based general population of the same age, sex and region (37.5%) [20]. Other reports have shown similar results in HD patients [23] in which age was positively associated with vascular calcification in large and medium-calibre arteries. Time on HD and total time on renal replacement therapy have been positively associated with vascular calcification, particularly in medium-calibre arteries: each new year on renal replacement therapy increased the risk of having vascular calcifications by approximately 15% [24]. Therefore, the time spent on dialysis is an important risk factor for medial and intimal arterial calcifications in CKD patients [25].

In addition to information available for the HD patients, the Framingham study has shown that vascular calcification is also an independent predictor of vascular morbidity and mortality in the general population [26].

Links between Vascular Calcification and Bone Disorders

Vascular calcification, bone loss and fragility fractures are very common disorders associated with aging, both in patients with CKD [16, 27, 28] and in the general pop-

Table 2. Strategies to reduce vascular calcification

Balanced diet, with low salt and saturated fats intake
Regular physical exercise
Smoking abstinence
Low alcohol intake
Control of serum phosphorus and calcium levels
Use of physiologic doses of vitamin D
Treatment of secondary hyperparathyroidism
Lipid-lowering therapy with statins
Treatment of hypertension
Rigorous control of diabetes mellitus
Caution with acenocumarol or warfarin treatments

ulation [29–32]. In recent years, several epidemiological studies have drawn attention to the relationship between vascular calcification and bone health [31–34]. Even though the pathogenetic factors linking vascular calcifications and bone fragility are not fully understood, recent studies have shown that vascular calcification in some localizations were associated with an increased risk of fragility fractures in both general and HD populations [20, 35, 36].

Strategies to Reduce Vascular Calcifications

Any strategy designed to reduce the impact of vascular calcifications has to begin with primary prevention measures to control cardiovascular risk factors. In the particular case of CKD, it is imperative to avoid further kidney damage. In this respect, it is crucial to promote a healthy lifestyle, with a balanced diet, regular physical exercise, smoking abstinence and a low alcohol intake. Once vascular calcifications appear, secondary prevention must aim to reduce their complications, intensifying previous measures and initiating the appropriate drug therapy. Particular aspects of the pharmacological approach are discussed below.

Theoretically, any kind of intervention aiming to reduce vascular calcification should curtail the influence of factors that promote calcifications and/or augment the effects of factors that may inhibit calcifications [37]. Most strategies to reduce vascular calcifications have focused on the most common modifiable risk factors such as hyperphosphataemia, hypercalcaemia, the CaxP product, hyperparathyroidism, smoking, dyslipidaemia or hypertension (table 2).

Control of Hyperphosphataemia, Hypercalcaemia and CaxP Product

Disturbances in serum phosphorus, calcium and the calcium-phosphorus product are frequently seen in CKD patients and are implicated in the promotion of vascular calcification as well as in an increased death risk [37]. Because dietary restriction of phosphorus and intermittent dialysis are not usually effective in controlling serum phosphorus, most patients with CKD stage 5 show a high prevalence of hyperphosphataemia with its known implications in the pathogenesis of secondary hyperparathyroidism, cardiovascular alterations and mortality. As mentioned before, *in vivo* and *in vitro* studies shed light on the role of phosphorus as a promoter of vascular calcification, demonstrating that the control of phosphorus should be a priority in clinical practice.

Calcium-based phosphate binders such as calcium acetate and calcium carbonate have replaced aluminium hydroxide as the most widely prescribed phosphate binders. The possible negative role of calcium overload from these binders on the progression of vascular calcifications has led to the progressive but not total reduction of calcium- and aluminium-based phosphate binders in favour of new calcium- and aluminium-free phosphate binders (sevelamer hydrochloride, sevelamer carbonate and lanthanum carbonate). These new compounds have been shown to have reduced hypercalcaemic adverse events in comparison to calcium-based phosphate binders [38].

An experimental study demonstrated that treatment with sevelamer in rats decreased renal calcification compared to rats that received calcium carbonate and untreated rats [39]. In addition, a clinical trial showed that sevelamer reduced the progression of both coronary and aortic calcifications compared to calcium carbonate [40]. However, the mechanism of the beneficial effect of sevelamer on the progression of calcification is still not fully understood. One possible mechanism is based on the reduction of the calcium load; however, reduced vascular calcification may also result from reductions in total and LDL cholesterol, which occur during treatment with sevelamer [38]. In fact, two recent studies have shed doubts on the role of phosphate binders in the progression of calcification [41, 42].

Control of Secondary Hyperparathyroidism: Vitamin D and Calcimimetics

The use of vitamin D metabolites is a challenging subject that still remains controversial. The current treatment of secondary hyperparathyroidism in dialysis pa-

tients includes suppression of PTH with supraphysiologic doses of vitamin D or its analogues. Although it is widely known that a high dosage of vitamin D metabolites favours the onset and progression of vascular calcifications, several studies have paradoxically demonstrated a long-term beneficial effect of vitamin D on vascular calcifications. Low vitamin D status is associated with a higher prevalence of vascular calcifications, bone and mineral disturbances, susceptibility to some infections, higher risk of autoimmune diseases, some malignancies and many other complications [43].

Observational studies in patients on HD and in the general population have also demonstrated a lower morbidity and a cardiovascular survival advantage in patients who are treated with vitamin D receptor activators [44, 45].

A major breakthrough in the management of calcium and phosphate metabolism in dialysis patients was achieved recently with the introduction of calcimimetics. These compounds were the first agents introduced to lower PTH with advantageous effects on serum calcium and phosphate. It has been demonstrated experimentally that the calcimimetic R568 reduces aortic calcifications and mortality in rats in which aortic calcifications were induced using a high dose of calcitriol [46]. Moreover, another experimental study showed that calcimimetics may even favour the regression of vascular calcification [47].

Control of Dyslipidemia

Dyslipidemia, particularly increased LDL cholesterol, has been implicated in the progression of vascular calcifications. In addition, in the general population, the beneficial effect of lowering LDL cholesterol levels on the progression of calcification has been reported by several groups [48, 49]. As mentioned previously, patients who were treated with sevelamer showed a significant decrease in LDL cholesterol levels [40], which may explain the beneficial effects in the progression of cardiovascular calcification. It is known that the rapid progression of coronary arterial calcification in HD patients is associated with higher triglycerides and lower HDL cholesterol levels [50].

Control of Blood Pressure

Hypertension is a modifiable risk factor for vascular calcifications in both the general population and CKD patients. Several studies in patients with end-stage renal disease and essential hypertension have shown that arterial stiffening is an independent predictor of mortality.

As arteries become stiffer, the pulse wave velocity increases and it is responsible for a rapid return of wave reflections from the periphery to the ascending aorta during systole, which causes an abnormal rise of aortic systolic blood pressure with decreased diastolic blood pressure and high pulse pressure. Increased wave reflections and high pulse pressure are independent risk factors for mortality in end-stage renal disease patients [51].

Diabetes

Diabetes is a disease that is known to be complicated by heterogeneous metabolic risk factors, such as hyperglycemia, dyslipidemia, insulin resistance, glycation, oxidative and carbonic stress, and tissue hypoxia. In the non-uremic population, vascular calcification occurs more frequently in diabetics. In CKD patients, vascular calcification in diabetics has been reported to be more prevalent and more advanced than in non-diabetics [52]. Several studies emphasize the importance of glycaemic control in the prevention of the development and progression of vascular calcification in diabetic CKD patients [53].

Factors That Decrease Vascular Calcification

Although vascular calcification is very common in patients with CKD, it is absent in a non-negligible percentage of patients (close to 20%) despite a similar exposure to the known factors that promote calcification [54]. As mentioned before, inhibitors of the precipitation of calcium and phosphate must be playing a major role in preventing extra-osseous calcification. Unfortunately, the therapeutic potential of these inhibitors of calcification has not been explored in clinical trials. Because MGP requires vitamin K for γ -carboxylation, an acquired vitamin K deficiency by the use of warfarin or acenocumarol may predispose towards vascular calcification [55].

Clinical and experimental studies have consistently established a positive association between arterial calcification and bone resorption [56, 57]. Consequently, it can be hypothesized that treatment strategies that simultaneously reduce bone resorption and increase bone mineralization may decrease the risk of vascular calcifications.

Bisphosphonates, used as standard therapy for osteoporosis, inhibit the experimentally induced vascular calcification, offering perspectives for the treatment of vascular calcification. The exact mechanism by which bisphosphonates inhibit arterial calcification is not entirely understood. One possibility is an indirect effect through inhibition of bone resorption, which would re-

duce the efflux of calcium and phosphate out of the bone, resulting in a decreased performance of the substrates required to form hydroxyapatite in the arterial wall [58].

Bisphosphonates have been demonstrated to reduce vascular calcification in experimental models [59], but also in CKD in a reduced group of HD patients [60]. Nevertheless, the use of bisphosphonates, particularly in CKD patients with underlying renal osteodystrophy, should be carefully considered as they are still in the research phase [58]. It has been recently described that in uremic rats treated with bisphosphonates, there was a strong correlation between inhibition of aortic calcification and bone mineralization, suggesting that bisphosphonates may not be able to prevent vascular calcification without inhibiting bone formation [61].

Even though new strategies may improve the management of vascular diseases and, more specifically, may have a positive impact on the high prevalence of vascular calcifications, the more effective approach is still that involving the best possible control of mineral and bone metabolism and inflammatory parameters [11].

We need more experimental, epidemiological and randomised clinical studies designed to ascertain the effects of the newly available bone-vascular active drugs on the bone and cardiovascular systems.

Molecular Aspects Related to Vascular Calcification

Until recent years, vascular calcification was considered the result of a simple precipitation of the circulating calcium and phosphate. However, the mechanisms by which the process of vascular calcification is produced are complex; it does not consist of a simple precipitation of calcium and phosphate, it involves active and modifiable processes that will be discussed later in this review. The final result is the formation of bone structures inside the artery wall [62, 63]. This regulated process involves several changes, such as the decrease in vascular calcification inhibitors [64], increase in vascular calcification promoters, formation of calcification vesicles [65], and, as a result, the induction of a cellular phenotypic change: from vascular smooth muscle cells (VSMCs) to bone-like cells [66, 67]. Interestingly enough and in line with several epidemiological studies, the increase in bone-like cells in the vessels has been reported to be associated to a decrease in bone mass and mineralization [11, 35, 68].

Thanks to the advances in molecular biology, a great number of mechanisms have been extensively investigated, and several inhibitors and promoters of vascular cal-

Fig. 1. Promoters and inhibitors of vascular calcification.

cification have been described (fig. 1). Among the latter, phosphorus and calcium play a relevant role. In humans and other mammals, serum concentrations of calcium and phosphate exceed the calcium-phosphate solubility product; thus, the likelihood of precipitation is high. Nevertheless, in both young and adult populations, intra-vessel precipitation is uncommon, clearly stressing the important role played by the vascular calcification inhibitors in preventing calcium/phosphate precipitation and deposition.

Promoters of Vascular Calcification: Phosphorus, Bone Morphogenetic Protein-Msx-2-Wnt Axis, Inflammation and Oxidative Stress

High serum phosphorus is the most important uremia-related, non-traditional risk factor associated with vascular calcification in CKD patients and the general population [17]. It is well known that high serum phosphorus levels stimulate parathyroid activity, decreasing the levels of calcium-sensing receptor and vitamin D receptor; it also lowers the activity of 1- α -hydroxylase, consequently decreasing serum calcitriol levels.

In addition, phosphorus is also capable of acting as a secondary intracellular messenger, activating several molecular pathways related to bone formation. It reaches the intracellular space via a specific Na-dependent channel called Pit-1 and exerts some interesting actions; in fact, the blockade of Pit-1 prevents vascular calcification [69].

In vitro experiments have demonstrated that elevated intracellular phosphate levels may directly increase an important bone-specific transcription factor core-binding factor α (Cbfa-1), resulting in the activation of several osteogenic pathways in the VSMCs, which leads to phenotypic changes of VSMCs into bone-like cells [70, 71].

Focusing on the downstream actions of Cbfa-1, it promotes the expression of osteocalcin and alkaline phosphatase in the vasculature. In addition, one of the most important families of proteins involved in mineralization and vascular calcification, the bone morphogenetic proteins (BMPs) are activated by Cbfa-1. The BMP family, especially the 2 and 4 members, have been described as potent promoters of vascular calcification, as they can re-

cruit other bone-related players such as Msx-2 and Wnt-related proteins [72–74]. Inflammation is also an important factor involved in the pathogenesis of vascular calcification development [75].

Oxidative stress has also been related to vascular calcification. VSMCs cultured with H₂O₂ developed calcification via stimulation of Cbfa-1 [76]. In addition, in vivo studies have shown that some antioxidants can prevent vascular calcification [77]. In agreement with these experimental analyses, clinical studies have shown that serum levels of oxidized LDL, advanced oxidation protein products and urine levels of F-2 isoprostanes (biomarkers of oxidative stress) may all be considered as risk factors for vascular and valvular calcification [25, 78].

Inhibitors of Vascular Calcification: Pyrophosphates, Fetuin A and Osteoprotegerin

Pyrophosphates (PPi) are located in the vascular matrix and they are supposed to preserve the aortic VSMC phenotype, thanks to the inhibition of calcium carbonate formation, hence inhibiting calcium phosphate crystal formation; PPi inhibits the change of VSMCs into bone-like cells [79, 80].

In serum, the most abundant inhibitors of vascular calcification are fetuin-A (α 2-Heremans-Schmid glycoprotein), osteoprotegerin (OPG) and matrix-gla protein. Fetuin-A is a known inhibitor of osteogenesis [81], capable of inhibiting vascular calcification [82]. Fetuin-A knockout mice spontaneously develop widespread soft tissue calcification, including significant myocardial calcification, findings associated to the upregulation of the profibrotic factor TGF- β [83].

OPG inhibits osteoclast differentiation, modulating bone resorption through its action as a decoy receptor of RANKL. OPG-null mice develop early-onset osteoporosis and severe medial layer calcification [84], suggesting that OPG acts as an inhibitor of in vivo vascular calcification. OPG was shown to inhibit ALP activity in aortic tissue and prevent the progression of medial layer vascular calcification [85]. The importance of the OPG/RANKL axis in vascular calcification has been recently shown; the increase in vascular calcium content was parallel to an increase in the RANKL and BMP4 expression [86].

Other Players

Klotho, a co-receptor of fibroblast growth factor 23 (FGF-23), among other functions, has a phosphaturic function [87, 88]. The knockout mice for the Klotho gene showed accelerating aging with widespread ectopic calcification, including vascular calcifications. The mecha-

nisms by which fibroblast growth factor 23/Klotho affect vascular calcification may involve phosphate excretion as well as vitamin D and PTH leading to vascular calcification and bone loss [89–92].

Advanced glycation end-products (AGEs) are chemical modifications of proteins and lipids that become non-enzymatically glycosylated after contact with carbohydrates [93]. The generation of AGEs is a continuous in vivo process and their accumulation increases with aging and diseases, specially diabetes [94]. AGEs accumulate in the vessel wall and contribute to the development of atherosclerosis through the formation of cross-links between molecules in the basement membrane of the extracellular matrix, involving different cell-surface receptors, especially RAGE [95]. Furthermore, recent studies have shown a correlation between the accumulation of AGEs in bone and increased fracture risk, even with normal bone mineral density [96–98]. AGEs may induce abnormal cross-links in the collagen proteins causing bone fragility [99, 100]. Furthermore, they may decrease osteoblast function and increase osteoclast activity, alter the function of the osteoblasts and increase the activity of the osteoclasts [101].

In summary, vascular calcification, an established highly prevalent finding of CKD, known since the beginning of renal replacement therapy, has recently re-emerged as a key complication of CKD, and great efforts are currently underway to clarify the morphological, functional and molecular aspects of this disorder.

Acknowledgments

The authors wish to thank the Fondo de Investigaciones Sanitarias, REDinREN del ISCIII (Redes Temáticas de Investigación Cooperativa en Salud; RD06/0016/1013), FYCIT (Fundación para el Fomento en Asturias de la Investigación Científica Aplicada y la Tecnología) and Fundación Renal Iñigo Álvarez de Toledo (Spain). The authors also would like to extend their appreciation to Marino Santirso for language editing of this article.

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