Controversies in Nephrology

Media Calcification and Intima Calcification Are Distinct Entities in Chronic Kidney Disease

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Calcification of the vascular tree is common in physiologic and pathologic conditions, i.e., aging, diabetes, dyslipidemia, genetic diseases, and diseases with disturbances of calcium metabolism. In chronic kidney disease, vascular calcification is even more common, develops early, and contributes to the markedly increased cardiovascular risk in this particular population. Pathomorphologically, atherosclerosis (*i.e.*, plaque-forming degenerative changes of the aorta and of large elastic arteries) and arteriosclerosis (*i.e.*, concentric media thickening of muscular arteries) can be distinguished. Increasing knowledge about calcification together with improved imaging techniques provided evidence that also vascular calcification has to be divided into two distinct entities according to the specific sites of calcification within the vascular wall: Patchy calcification of the intima in the vicinity of lipid or cholesterol deposits as present in plaque calcification and calcification of the media in the absence of such lipid or cholesterol deposits, known as Mönckeberg-type atherosclerosis. The two types of calcification may vary according to the type of vessel (large elastic *versus* smaller muscular type artery) and proximal *versus* distal sites of the arterial tree. Furthermore, clinical studies showed that it is not purely academic to distinguish between intimal and medial calcification but rather relevant for the clinical presentation, treatment, and prognosis because each type leads to different clinical consequences. *In vivo* studies in animal models provided evidence in favor of common pathomechanisms between vascular calcification and atherosclerosis; however, there is other, strong experimental and clinical evidence that pleads for the continued distinction between intimal and medial calcification.

Cardiovascular calcification is frequent in elderly patients or populations with a particularly high atherogenic risk profile (*e.g.*, diabetes, chronic kidney disease [CKD]). It has become evident that the atherosclerotic calcification burden is a potent risk marker of cardiovascular events. Cardiovascular calcification may affect the arterial media of the aorta and muscular arteries, the intima of the aorta and its large descendents and the coronary arteries, the myocardium, and the heart valves, respectively.

Morphologically, in some animal models of atherosclerosis and calcification as well as in most patients, calcification of the media can be distinguished from intimal calcification (*i.e.*, calcification of advanced atherosclerotic plaques in the vicinity of lipid and cholesterol depositions). This may be important in view of the different clinical consequences of medial and intimal calcification. Whereas media calcification causes arterial stiffness, increased pulse pressure, and left ventricular hypertrophy, the consequences of intimal or plaque calcification are less clear, since the determinants of plaque rupture with consecutive thrombosis are still under debate. Because of these differences in clinical presentation and also from a morphologic perspective, we and others are convinced that even if medial and intimal calcification may share some common pathomechanisms and can occur together in patients, it is reasonable to keep a clear-cut distinction between the two of them. The following review provides several arguments in favor of this postulate.

Types and Mechanisms of Calcification: General Considerations

Physiologic calcification or biomineralization of extracellular matrices is a normal developmental process that is essential for the proper formation and functioning of various tissues (*e.g.*, skeletal bone, teeth, growth plate cartilage). Ectopic calcification (*i.e.*, calcification of tissues or organs that do normally not calcify) is usually prevented by local and systemic inhibitors of calcification; however, when pathologic calcification occurs in tissues that do not normally mineralize (*e.g.*, in the vasculature or soft tissue), it may lead to serious adverse consequences. Vascular calcification is a pathologic process that occurs in response to dysregulated or inappropriate environmental stimuli (*e.g.*, advancing age, atherosclerosis, certain metabolic disorders [*e.g.*, CKD, diabetes, chronic inflammatory disease such as systemic lupus erythematosus and other rheumatic diseases]) and in rare genetic diseases (*e.g.*, Keutel syndrome, a rare autosomal recessive condition characterized by abnormal cartilage calcification) (1,2). Pathologic soft tissue calcification, in particular vascular calcification, is often triggered by active processes involving inflammatory cytokines (*e.g.*, TNF-α, C-re-

active protein, CD40-CD154) or by disordered calcium/phosphate (Ca/P) metabolism (3,4) inducing vascular smooth muscle cell (VSMC) damage and subsequent phenotypic switch with activation of bone forming programs.

From a pathomorphologic perspective, four types of calcification can be distinguished on the basis of location, association with plaque, and mode of formation (2): (*1*) Dystrophic (passive) calcification (minor form of widespread nonspecific organ and soft tissue calcification as a result of abnormal Ca/P products) and three morphologic types of actively regulated calcification (in the absence of raised Ca/P levels): (*2*) calcification of cardiac valves; (*3*) calcification in arterial intimal layers in association with macrophages, lipids, and VSMC as in classical atherosclerosis (Figure 1, A and B); and (*4*) calcification in arterial medial layers as a result of elastin fiber mineralization, VSMC degeneration, and upregulation of osteogenic programs as in CKD or diabetes (Figure 1, C through E and G).

According to this concept, the tree last types of vascular calcification are no longer regarded a passive influx of Ca into the arterial wall or media as a consequence of Ca overflow or tissue degeneration but rather an actively regulated mechanisms. This process requires complex regulatory networks that involve (*1*) positive and negative regulators; (*2*) temporal expression or activation of modulators; and (*3*) multiple amplification or suppressive feedback loops that orchestrate cell recruitment, differentiation, function, survival, and interactions with other cells or matrix molecules. Major roles for osteopontin, osteoprotegerin (inhibitor of osteoclastogenesis), matrix-gla protein, or fetuin-A that prevent calcification and for receptor activator of NF-κB (induces maturation of osteoclast progenitors) or receptor activator of NF-κB ligand that promote calcification have been identified (2).

As shown by Abedin *et al.* (1), there are some differences but also marked similarities between soft tissue calcification, atherosclerotic (*i.e.*, intimal or plaque calcification), and medial calcification. Whereas inflammation and cytokine production is common to all three types, intimal calcification is characterized by subintimal lipid deposition and macrophage accumulation, whereas in medial calcification, metabolite-induced (toxic) vascular changes in the absence of lipid deposits are regarded as specifically leading to upregulation of osteogenic regulatory genes that then induce osteogenic differentiation of mesenchymal cells with subsequent matrix mineralization, bone, and cartilage formation (1). Doherty *et al.* (5) also pointed out the similarities between bone formation and vascular calcification with tightly regulated mechanisms for mineral deposition and mineral resorption. Of note, osteogenic differentiation with metaplastic bone formation is only rarely seen in intimal calcification, whereas it is often seen in medial calcification of peripheral arteries (Figure 1F). Studies from our own group showed that in patients, in contrast to mice and rats, chondrogenic differentiation does not play a major role in human intimal and medial calcification (6).
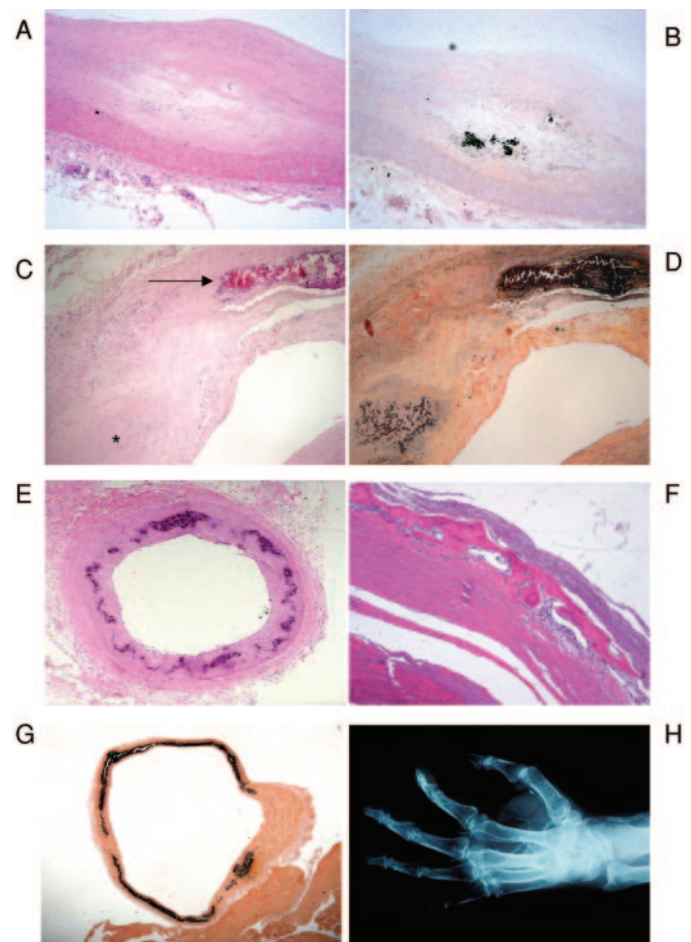

Figure 1. (A and B) Coronary intima plaque with subtle calcification in hematoxylin and eosin (H&E) stain (A) and Kossa stain (B). Note that the arterial media is completely free of calcification. (C and D) Coronary artery of a patient with chronic kidney disease (CKD) showing intimal (∗) as well as medial calcification (arrow). (C) H&E stain. (D) Kossa stain. (E) Medial calcification of a peripheral artery in the absence of any intimal change. Note that no lipid or cholesterol depositions are visible. (F) Peripheral muscular type artery with metaplastic bone formation in the arterial media (H&E stains). (G) Heavy calcification of the aortic media with rupture of elastic fiber in a subtotally nephrectomized rat with moderate chronic renal failure of 12 wk duration (Kossa stain). (H) X-ray of a patient with CKD and heavy medial calcification of the cubital and radial arteries and their branches as shown by the so-called tram track phenomenon (courtesy of Dr. Nonnast-Daniel, Department of Nephrology, University of Erlangen, Germany).

What Is Specific about Atherosclerosis and in Particular Vascular Calcification in CKD?

The high prevalence of coronary and noncoronary atherosclerotic lesions as well as the high occurrence of coronary events in patients with CKD has been documented in clinical registers and numerous autopsy studies (7–10). The prevalence and extent of cardiovascular calcifications are strong predictors of cardiovascular disease and all-cause mortality in hemodial-

ysis and peritoneal dialysis patients (11). In young adults who had CKD since childhood, advanced coronary arterial sclerosis and calcification was found, which starts before the initiation of dialysis treatment and progresses rapidly thereafter (12). This finding clearly argues against an age-related degenerative process as is mostly the case in general atherosclerosis. There is increasing evidence that vascular calcification in CKD can be divided on a morphologic and clinical basis into intimal (*i.e.*, plaque calcification) and medial calcification, both of which are associated with increased morbidity and mortality in CKD. In the majority of reported cases with advanced chronic renal failure, however, it was impossible to distinguish medial from intimal calcification on the basis of light microscopy examination because of the generally advanced age of the patients and the long duration of vascular lesion development.

Morphology and Pathomechanism of Intimal (Plaque) Calcification

Classical atherosclerosis is pathomorphologically defined by intimal or subintimal lipid deposits forming fatty streaks as the initial lesion or plaques of variable size that lead to eccentric lumen obliteration with thinning of the underlying media. The stability of the atherosclerotic plaque is nowadays regarded to be dependent on the presence of a thick fibrous cap and only a small lipid core. Other factors that are discussed as being involved in plaque stability are plaque inflammation, vascularization, and calcification. In patients with CKD, the burden of coronary atherosclerotic plaques is increased, and conspicuous differences in coronary plaque morphology were found compared with matched nonrenal patients (8). Clearly more advanced stages of atherosclerosis, such as fatty degeneration and plaque formation in the intima, were seen in patients with CKD. Coronary plaques in CKD showed the classical features apart from the fact that above all type VII lesion, the calcified atherosclerotic plaque was significantly more frequent. Plaque rupture is thought to be finally caused by angiogenesis in the adventitia of the coronary arteries, which leads to an intramural hematoma formation and rupture of the fibrous cap (13). Although this point is still controversial, model calculations support an increased mural tension in the transition area between the calcified plaque and the circumferential nonatherosclerotic endothelium (13). This transition area can for instance rupture, if a paradoxic catecholamine-mediated vasoconstriction occurs. Atherosclerotic vascular segments either are lacking endothelium or show endothelial dysfunction and are thus predestined to paradox vasoconstriction. In addition, patients with CKD show an increased sympathetic tone with high levels of catecholamine concentrations during dialysis sessions. The paradox and catecholamine-mediated vasoconstriction could thus contribute to plaque rupture and may favor the malignant character of calcified plaques that are present in CKD. This concept, however, is not universally accepted, because Lin *et al.* (14) found that calcification does not seem to increase plaque vulnerability when testing *in vitro* the mechanical response of a calcified plaque model to fluid stress. Most likely, the final answer to this question will be possible only pending further

investigation. Unfortunately, even using the most highly performing imaging techniques presently available, it is impossible or at least extremely difficult to distinguish between intimal and medial calcification in the coronary arteries. The whole issue becomes even more difficult when we take into account that the site of calcification depends very much of the anatomic location and structure of the vessel (*i.e.*, coronary artery *versus* peripheral artery and proximal *versus* more distal parts).

Using x-ray diffraction analysis, two autopsy studies revealed deposits of hydroxyapatite crystals (Ca and P) in coronary plaques of patients with CKD (8,15). In addition, smaller crystalline granules were found in the plaques but not consistently in the vascular media. This finding obviously differs from the findings in muscular arteries, where both granular and well-formed, patchy media calcification can be seen (16). When Gross *et al.* (15) investigated more distal parts of the coronary arteries using backscatter imaging analysis, they found significantly more medial calcification in patients with CKD compared with nonrenal control subjects as well as significantly more deposition of Ca but not of P only in the arterial media. The discrepancies with respect to findings made in a previous autopsy study (8) could be explained by the striking heterogeneity between different vascular regions; therefore, it is almost impossible to draw conclusions pertaining to the changes in the coronary arteries on the basis of findings in peripheral arteries.

Morphology and Pathomechanisms of Medial Calcification

Autopsy and clinical studies provided evidence for more advanced and more heavily calcified coronary plaques in patients with end-stage CKD compared with age- and gender-matched nonrenal patients with coronary heart disease (8,17). In addition, a study in the epigastric artery of patients with CKD at the time of kidney transplantation (16) found a high percentage (44%) of media calcification in CKD in the absence of classical atherosclerosis together with expression of bone-associated proteins and the osteoblast differentiation factor core binding factor α-1 (Cbfa1). In 11 of 12 patients, pure medial calcification was present and only in one of 12 additional intima calcification was seen. Complementary *in vitro* data in VSMC confirmed that uremia induces the osteoblast differentiation factor Cbfa1 and the expression of other osteogenic proteins indicative for the phenotypic switch of VSMC in medial calcification of CKD (18). Increased calcification of the media of smaller elastic and of muscular type arteries in CKD was also seen in clinical studies and was documented to be of functional and prognostic relevance (19,20). In a recent analysis of arteries taken at the time of cardiac surgery, we found significant calcification of the arterial media but not the intima already in patients with only moderately increased serum creatinine (<1.5 mg/dl) compared with matched control patients (unpublished data). Of note, in this study, lipid or cholesterol deposits were not observed in the vicinity of the medial calcification. Additional immunohistochemical studies showed medial calcification to be paralleled by significant higher *in situ* expression of

proinflammatory markers (C-reactive protein [CRP], CD40, and CD154). These data indicate that media calcification (1) occurs earlier in the course of CKD in the absence of lipid and cholesterol deposition as signs of classical atherosclerosis, (2) occurs at least in some arteries in part independent of changes in Ca × P, and (3) may be associated with local inflammation of the vascular wall. Medial calcification of muscular arteries has been known as so-called Mönckeberg sclerosis (MS) since its first description in 1903. MS was described as sheet-like calcification of the media and assumed to be an age-related phenomenon that does not involve the arterial intima. Its pathogenesis has been linked to epinephrine excess; hypervitaminosis D; autonomic nervous system overstimulation; and recently also to conditions such as diabetes, CKD, and osteoporosis (21). The histologic definition of MS sometimes differs in that some authors also included calcification of the internal lamina elastica (22). In patients with CKD, however, MS-type calcification restricted to the arterial media has been documented in coronary arteries and peripheral arteries (epigastric and radial arteries) (15,16,23). Using plain x-ray analysis, heavy medial calcification of peripheral arteries (the cubital and radial arteries and their branches) can also be demonstrated in patients with CKD and in particular in those with hyperparathyroidism (Figure 1H) (24). It is interesting that these calcifications can entirely regress after surgical parathyroidectomy, at least in some patients.

Pannier *et al.* (19) showed in patients with CKD that increased stiffness of capacitive arteries such as the aorta as a result of media calcification is an independent strong predictor of cardiovascular mortality, whereas stiffness of peripheral conduit arteries had no prognostic value. In patients with type 2 diabetes, medial calcification was associated with a four-fold increased risk for lower extremity amputation and two-fold enhanced cardiovascular mortality (25). London *et al.* (26) investigated the possible mechanisms responsible for increased medial calcification in CKD with particular emphasis on disturbances of mineral metabolism and active expression of various mineral-regulating proteins. This is important because an inverse relationship between arteriosclerosis and bone density has been documented in patients with CKD. The group found that a high arteriosclerosis score was associated with bone histomorphometry suggestive of low bone activity and adynamic bone disease. These results suggest that therapeutic interventions associated with excessive lowering of parathyroid activity (parathyroidectomy, excessive Ca, or aluminum load) favor lower bone turnover and adynamic bone disease, which could then influence the development and progression of arteriosclerosis.

Another rare but life-threatening clinical condition that is associated with heavy medial calcification of cutaneous arterioles and ulcerating tissue necrosis is calciphylaxis. Here, the typical vascular changes have been given the name "calcific uremic arteriolopathy"; it is assumed to be associated with disturbances in mineral metabolism in CKD (*i.e.*, hyperparathyroidism and elevated serum P) but possibly also to reduced levels of the calcification inhibitor fetuin-A (27).

Which Common Pathomechanisms Are Shared by Intimal and Medial Calcification?

Various clinical and experimental studies have clearly demonstrated that in patients with CKD, in addition to traditional (classical) risk factor for atherosclerosis, nontraditional (nonclassical) risk factors are operative. In particular, increased oxidative stress and subsequent systemic and local inflammation in CKD have been associated with intimal and medial vascular calcification (28–30). *In vitro*, CRP and other proinflammatory cytokines such as TNF-α directly or indirectly act on VSMC, thereby inducing the aforementioned switch in VSMC phenotype that favors calcification (31,32). In patients, the presence of chronic inflammation can be demonstrated using highly sensitive assays for CRP. Increased CRP concentrations were found to be a predictor for all-cause mortality and specifically for cardiovascular mortality, not only in the general population (33) but especially in patients with CKD (34). In coronary arteries of patients with CKD, *in situ* expression of markers of inflammation were found to be elevated and correlated to increased vascular calcification (17). In hemodialysis patients, CRP is a significant predictor of both intimal and medial calcification in the aorta and in peripheral arteries (35). Against this background, also genetic factors have to be taken into consideration because polymorphisms of the inflammatory adhesion molecule E-selectin have been associated with coronary calcification in young women (36).

Disturbances in Ca and P metabolism were shown to be important in the development of cardiovascular calcification in CKD. Calcification of the vessel wall, however, is not just a passive process due to Ca and P precipitation but rather a highly regulated active process involving differentiation of VSMC toward osteoblasts. This phenotypic modulation of VSMC can be induced by increased extracellular Ca and P content as well as by various other factors, such as mineralocorticoid receptor activation by aldosterone, calcitriol, and proinflammatory mediators (37,38). During this process, osteoblast-specific genetic programs are initiated with expression of osteopontin, bone morphogenetic protein (BMP), isoforms, osteocalcin, and Cbfa-1-RUNX2 (39), which lead to the formation of mineralized matrix, cartilage, and bone. By electron microscopy, electron-dense areas containing hydroxyapatite (*i.e.*, Ca and P deposits) were documented in calcified areas of the human arterial media. These basic calcium phosphate crystals could interact and activate monocytes and macrophages that then produce proinflammatory cytokines that further enhance calcification. Work from Shanahan and co-workers (40) provided novel insights into how VSMC regulate calcification in response to various insults (*i.e.*, the uremic milieu). Here, damage to the VSMC leads to subsequent vesicle release from viable and dying cells and apoptosis, which then creates an environment permissive for the nucleation of basic Ca and P minerals. This, combined with the aforementioned osteogenic conversion of VSMC and consequent loss of their normal inhibitory processes/pathways, results in calcification. Following this concept calcification can happen only at sites of VSMC existence, which is the case in the arterial media. Calcification in the arterial intima or the plaque, respectively, would then require influx of VSMC as a prerequisite

(which is not the case in all plaque stages). Of note, *in vivo*, the previously described calcification process is counteracted by circulating or local inhibitors of calcification, such as fetuin-A or matrix-Gla protein, whose concentrations may be decreased or whose functions may be impaired in CKD (41). A major pathway by which chronic inflammation in CKD may promote vascular calcification probably involves downregulation of fetuin-A, the most potent circulating inhibitor of extraosseous calcification. In cross-sectional studies, dialysis patients with low fetuin-A serum levels showed a significantly poorer survival than those with normal values (42). Apart from fetuin-A, various other inhibitors probably counteract unwanted vascular and soft-tissue calcification. Among those, leptin, matrix-gla protein, BMP (*e.g.* BMP-2, -7), osteoprotegerin, and inorganic pyrophosphate, an inhibitor of hydroxyapatite crystal growth, have been related to vascular calcification in CKD. The complexity of vascular calcification in CKD was recently elegantly reviewed by Shroff and Shanahan (43) and Moe and Chen (44) integrating pathophysiologic aspects of both the underlying disease and its various therapeutic aspects.

To What Extent Can We Use Data from Animal Studies to Solve the Issue of Intimal and Medial Calcification?

Because the issue of the separate occurrence of advanced atherosclerosis and in particular the pathomechanisms of vascular calcification are difficult to investigate in patients with CKD, experimental models were widely used. Most animal species, such as mice, rats, rabbits, and dogs, do not spontaneously develop atherosclerosis with age, in contrast to the human situation. Using the rabbit model with CKD induced by subtotal nephrectomy, in the absence of a high-cholesterol diet, the earliest morphologic changes were seen in the aortic media with rupture of elastic lamellae and increased extracellular matrix. After 3 mo of CKD, media degeneration with calcification, but no intimal calcification was seen; after 8 mo, the aorta and all major systemic arteries including the coronary arteries were transferred into stiff, calcified tubes with still no evidence of lipid deposition (45). These experimental findings argue for differences in the genesis and sequence of media and intima calcification in CKD and are in line with our findings in patients.

At present, the apolipoprotein E knockout mouse (ApoE$^{-/-}$), a model of spontaneous atherosclerosis without dietary manipulations, is widely used. The mice spontaneously develop atherosclerotic lesions in the aorta and the large arteries that are very similar to human lesions. This model has been used by several research groups to study vascular changes caused by CKD (46–52). Induction of CKD by either uni- or subtotal nephrectomy (*i.e.*, five-sixths nephrectomy) produced larger atherosclerotic plaques, indicating that CKD speeds up the growth of the existing plaques, causing larger lesions rather than producing *de novo* plaque development. Initially, in the aorta, intimal plaques arise containing macrophages loaded with lipid but presenting only very few inflammation cells. Plaques are characterized, however, by a proinflammatory phenotype, as indicated by expression of several markers of inflammation (CD40, CD154, intercellular adhesion molecule, vascular cellular adhesion molecule), increased oxidative stress, en-

dothelial cell activation (receptor for advanced glycation end products), cell proliferation, and increased matrix production (osteopontin, collagen IV) (46,50,51). In most studies, however, calcification was not observed. Using a somewhat different approach to induce CKD in the ApoE$^{-/-}$ with a presumably higher inflammatory stimulus, Massy *et al.* (52) documented both increased intimal and medial calcification. Another animal model that was recently used for analysis of plaque formation and vessel calcification under the conditions of CKD is the LDL receptor knockout mouse (LDLR$^{-/-}$) (53), which in addition requires a high-cholesterol diet. In this study, CKD together with high-fat diet also increased both intimal and medial calcification. In another study of LDR$^{-/-}$ mice that were fed an atherogenic diet, treatment with recombinant osteoprotegerin reduced the calcification area and the expression of osteocalcin, a marker of mineralization, without affecting size or number of aortic atherosclerotic lesions (54). In an animal model of type 2 diabetes, TNF-α was shown to induce the MSX-Wnt osteogenic program that regulates arterial calcification (32). In diabetic LDLR$^{-/-}$, upregulation of an ectopic BMP2-Msx2 gene regulatory program was demonstrated in the tunica media of coronary arteries and the aorta (55). *In vivo* studies in five-sixths nephrectomized animals with renal failure clearly showed that vitamin D and its analogs induced different degrees of vascular calcification in the absence of classical atherosclerosis (56,57). Obviously, these animal data provide a nice proof of concept.

These experiments provide clear evidence that under specific conditions, calcifying programs other than the classical atherosclerotic pathway are operative in the arterial media, inducing medial but not necessarily intimal calcification. We recently learned from animal knockout models that lack of matrix-gla protein, osteoprotegerin, smad6, carbonic anhydrase isoenzyme II, fibrillin-1, and klotho gene product favors varying degrees of arterial calcification in the absence of lipid deposition (58). In addition, also hyperlipidemia, vitamin D, nicotine, and warfarin, alone or in various combinations, have been shown to produce arterial calcification in animal models. In these studies, however, very rarely intimal and medial calcification were distinguished.

Conclusions

The extent of atherosclerosis and arteriosclerosis is undoubtedly exceedingly high in patients with CKD and the consequences (cardiovascular events) represent a major clinical problem in these patients. Experimental findings confirmed an acceleration of atherosclerosis, which seems to starts very early in the course of CKD and is characterized by marked medial and intimal calcification. The two types of calcification can occur independent of each other with media calcification already being present in early stages of CKD and show some differences in pathogenesis and clinical outcome. Although in advanced stages of CKD both types of calcification may often be present, one should try to distinguish clearly between these two aspects of vascular calcification because increased knowledge about their pathogenesis, location, and frequency may open the possibility for specific prevention of lesion formation and adequate treatment.

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Disclosures

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