

## Arterial stiffness, vascular calcification and bone metabolism in chronic kidney disease

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cation and we evaluate their connection to impaired arterial stiffness in the mirror of recent scientific results.

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### Abstract

Patients with chronic kidney disease (CKD) have an extremely poor cardiovascular outcome. Arterial stiffness, a strong independent predictor of survival in CKD, is connected to arterial media calcification. A huge number of different factors contribute to the increased arterial calcification and stiffening in CKD, a process which is in parallel with impaired bone metabolism. This coincidence was demonstrated to be part of the direct inhibition of calcification in the vessels, which is a counterbalancing effect but also leads to low bone turnover. Due to the growing evidence, the definition of "CKD mineral bone disorder" was created recently, underlining the strong connection of the two phenomena. In this review, we aim to demonstrate the mechanisms leading to increased arterial stiffness and the up-to-date data of the bone-vascular axis in CKD. We overview a list of the different factors, including inhibitors of bone metabolism like osteoprotegerin, fetuin-A, pyrophosphates, matrix Gla protein, osteopontin, fibroblast growth factor 23 and bone morphogenic protein, which seem to play role in the progression of vascular calcifi-

### ARTERIAL STIFFNESS IN CHRONIC KIDNEY DISEASE

Cardiovascular disease is the major cause of death in patients with chronic kidney disease (CKD)<sup>[1]</sup>. The cardiovascular mortality of patients on maintenance hemodialysis is more than 10-fold higher compared with the normal population<sup>[2]</sup>.

In patients with CKD, increased aortic stiffness, measured as aortic pulse wave velocity (PWV), is a strong independent predictor of cardiovascular mortality<sup>[3]</sup>. A tendency for arterial stiffness to increase in parallel with the progression of decrease in kidney function is present in CKD. Wang *et al*<sup>[4]</sup> showed a stepwise increase in PWV (i.e. arterial stiffness) from CKD stage 1 to stage 5. Although there was a high prevalence of cardiovascular risk factors and cardiovascular disease in that patient population, estimated GFR and systolic BP were the major determinants of arterial stiffness in patients with CKD, independent of conventional risk factors for cardiovascular disease<sup>[4]</sup>. Later, the connection between the progression of CKD

and arterial stiffening was confirmed by another study, where the role of diabetes and CRP was also evaluated<sup>[5]</sup>.

Many pathophysiological mechanisms are present, leading to increased arterial stiffness associated with CKD. To highlight the complexity of the question, we demonstrate some of these processes.

Alterations of the extracellular matrix (ECM) have been proven in subtotaly nephrectomized rats, where the aortic wall thickness was significantly greater than in controls. ECM volume was increased, elastic fibers were smaller and collagen “islands” were evident<sup>[6]</sup>. The role of matrix metalloproteinases (MMPs) have been suggested as increased MMP production, present in CKD<sup>[7]</sup>, enhances collagen and elastin turnover through enzymatic cross-link degradation, causing weakening of the ECM<sup>[8]</sup>. These data highlight the potential use of future therapeutic interventions with MMP inhibitors.

Accumulation of advanced glycation end-products (AGE) also result in arterial stiffening. Collagen modified with AGEs is stiffer and less susceptible to slow hydrolytic degradation and glycation can also cause arterial stiffening through generation of reactive oxygen species and nitric oxide deactivation<sup>[9]</sup>. Direct connection is present between the levels of circulating AGE and the serum creatinine in patients with CKD<sup>[10,11]</sup>. AGE cross-link breaker treatment can lead to significant reductions in arterial stiffness and endothelial dysfunction in hypertensive and older patients<sup>[12,13]</sup>. More data are needed but the future therapeutic use of AGE cross-link breakers to reduce arterial stiffness in CKD can also be possible.

Endothelial dysfunction is strongly associated with increased arterial stiffness in healthy individuals<sup>[14]</sup>. We previously demonstrated that endothelial dysfunction is remarkable in CKD, where both endothelium-dependent and -independent vasodilations are impaired<sup>[15]</sup>. This may reflect the high oxidative stress and the presence of risk factors like hypertension, diabetes and the reduced clearance of uremic toxins, such as asymmetrical dimethylarginine, an effective inhibitor of nitric oxide synthase<sup>[16,17]</sup>. A study of cultured endothelial cells *in vitro* showed that stiff arteries themselves further reduce nitric oxide bioavailability through diminished expression of endothelial nitric oxide synthase<sup>[18]</sup>, suggesting that arterial stiffness can be a self-perpetuating process.

Endothelin, a powerful vasoconstrictor produced by endothelial cells is also implicated in the pathogenesis of several cardiovascular conditions and the progression of CKD<sup>[19]</sup>. Short-term administration of endothelin-receptor antagonist in non-diabetic CKD reduces proteinuria and arterial stiffness independently of blood-pressure lowering<sup>[20]</sup>, data which suggest beneficial effects in the treatment of the vascular complications of CKD in the near future.

A clear association between chronic inflammation and arterial stiffness has been shown in different studies involving patients in a chronic inflammatory state, like rheumatoid arthritis and CKD<sup>[21,22]</sup>, as well as studies of inflammatory markers and aortic PWV (aPWV) in

healthy populations<sup>[23]</sup>. Accelerated arterial calcification has been shown in animal models due to inflammatory degradation of ECM elastin<sup>[24]</sup>. According to these data, the exploration of the role of immunosuppression and the inhibition of elastase enzymes as therapeutic possibilities in arterial stiffness reduction would be required<sup>[25]</sup>.

The renin-angiotensin-aldosterone system (RAAS) is also involved in the process of arterial stiffening. Angiotensin II stimulates vascular smooth muscle cells (VSMCs) to generate intracellular superoxides and inflammatory cytokines and induced vascular remodeling through VSMC hypertrophy and proliferation, increased collagen synthesis and increased production of MMP<sup>[26,27]</sup>. Inhibition of the RAAS with angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers is associated with reduction of arterial stiffness, but it is accompanied with blood pressure reduction<sup>[28,29]</sup>. In hemodialyzed patients, treatment with ACEI blood pressure lowering combined with decreased aPWV was associated with reduced all-cause and cardiovascular mortality<sup>[30]</sup>.

Aldosterone levels are correlated with arterial stiffness in hypertensive men, independent of blood pressure<sup>[31]</sup>. Aldosterone increases arterial stiffness independently of wall stress in subtotaly nephrectomized rats given high-salt diets and these effects are inhibited by the mineralocorticoid receptor (MR) blocker eplerenone<sup>[32]</sup>. There are limited data demonstrating the influence of MR antagonism on arterial stiffness in human CKD. The addition of the MR inhibitor spironolactone to ACEI/ARB treatment in stage 2 and 3 CKD patients significantly reduced arterial stiffness and left ventricular mass, supporting the hypothesis that aldosterone is a major mediator of arterial stiffness and left ventricular hypertrophy in CKD<sup>[33]</sup>.

High salt diets also have important role in the development of hypertension and arterial stiffness<sup>[34]</sup>. Dietary sodium promotes VSMC hypertrophy and increases VSMC tone; it also increases collagen cross-linking and facilitates aldosterone-induced oxidative stress and inflammation<sup>[34]</sup>. The restricting of dietary sodium in hypertensive patients can effectively reduce arterial stiffness<sup>[35]</sup>. The influence of accumulating dietary sodium and other dietary compounds, like bioavailable phosphate, AGEs and oxidants, on arterial stiffness in CKD is not totally understood and will be an area of future research.

## VASCULAR CALCIFICATION IN CKD

The dramatically increased cardiovascular risk of death of uremic patients is directly associated with the magnitude of vascular calcification (VC)<sup>[36]</sup>. VC can either take place in the intima or in the media of the vessel wall. Calcification of the intima is a part of atherosclerosis, while medial calcification is the hallmark of arteriosclerosis. Both forms are prominent in CKD but arteriosclerosis primarily has an important role in the development of arterial stiffness<sup>[37]</sup>.

A cross-sectional study assessed characteristics of dialysis patients with predominant intimal vs medial calcifi-

cations. Intimal calcification patients were about 20 years older and characterized by a history of traditional risk factors (e.g. smoking, dyslipidemia) prior to the start of dialysis, while medial calcification patients were characterized by a long history of dialysis and higher incidence of disturbances of calcium x phosphate metabolism, despite being younger<sup>[37]</sup>. Key risk factors associated with progressive cardiovascular calcification are age and diabetes, but many others are present in CKD and ESRD, like hyperphosphatemia, hypercalcemia, a high intake of calcium (by calcium-containing phosphate binders) and inflammation<sup>[38-41]</sup>. Amongst these risk factors, hyperphosphatemia is an especially strong and independent predictor of cardiovascular mortality in uremic patients<sup>[39]</sup>. Treatment of secondary hyperparathyroidism and hyperphosphatemia with calcimimetics might have beneficial effects for the vascular mineralization and mortality in CKD, an effect that can be at least be partly mediated through the reduction of arterial stiffness<sup>[42]</sup>.

For many years, VC in CKD patients was thought to occur predominantly by unregulated, purely physiochemical mechanisms<sup>[43]</sup>. Recently, VC was hypothesized to be an active process in which vascular cells may acquire osteoblastic functions<sup>[44]</sup>. Indeed, an increasing body of evidence suggests that atherosclerotic calcification shares features with bone calcification.

## CONNECTIONS BETWEEN VC AND BONE METABOLISM IN CKD

The first observations suggesting the existence of the bone-vascular axis were the frequent associations of osteoporosis and atherosclerotic VC observed in postmenopausal women<sup>[45-47]</sup>. Longitudinal population-based studies revealed a relationship between the progression of VC and bone demineralization, and others were identified between bone mineral density and aortic or central artery calcifications<sup>[46]</sup>, or coronary arteries in type 2 diabetes<sup>[48]</sup>.

A relationship between bone and vascular modifications was also observed in CKD and ESRD. In dialysis patients, coronary artery calcification score was found to be inversely correlated with vertebral bone mass<sup>[49,50]</sup>. High systemic calcification score combined with bone histomorphometry suggestive of low bone activity was observed in hemodialysis patients<sup>[51,52]</sup>.

Disorders of the mineral metabolism associated with CKD were found to be key factors contributing to the excess mortality observed in this patient population<sup>[53,54]</sup>. Moreover, the skeletal remodeling disorders caused by CKD contribute directly to the disordered mineral metabolism and heterotopic mineralization, especially the VC in CKD<sup>[55]</sup>. Finally, CKD impairs skeletal anabolism, decreasing osteoblast function and bone formation<sup>[56]</sup>. Due to these pathophysiological discoveries, the term "CKD mineral bone disorder" (CKD-MBD) was created by the Kidney Disease Improving Global Outcomes Foundation (KDIGO)<sup>[57]</sup>.

## REGULATORS OF BONE METABOLISM WHICH HAVE AN ACTIVE ROLE IN VC AND THEIR ASSOCIATION WITH ARTERIAL STIFFNESS IN CKD

### Osteoprotegerin

Osteoprotegerin (OPG) inhibits the activation of osteoclasts and promotes osteoclast apoptosis *in vitro*. The mechanism of this action is that OPG serves as a decoy receptor for the receptor activator of nuclear factor  $\kappa$ B-ligand (RANKL) and thereby inhibits osteoclastogenesis and osteoclast activation by blocking RANK activation<sup>[58]</sup>. Mice deficient for OPG (OPG<sup>-/-</sup>) develop calcifications of the aorta and renal arteries, together with osteoporosis<sup>[59]</sup>. From these data, an inverse relationship between OPG levels and outcomes would have been expected; however, the opposite observations were made in clinical studies. In dialysis patients, serum OPG levels were independently associated with VC<sup>[60]</sup> and showed that OPG levels can, in part, explain the association between coronary artery calcification and CKD<sup>[61]</sup>. In our previous study, we found a significant positive relationship between serum OPG levels and aPWV in patients on hemodialysis and demonstrated an inter-relationship of these parameters on their effect on cardiovascular mortality<sup>[62]</sup>. These findings are compatible with the hypothesis that the prognostic significance of high OPG levels is, at least in part, mediated by higher PWV in these patients. Whether this relationship is dependent on the degree of aortic calcification and whether the same relationship also holds true in other populations needs further study.

### Pyrophosphate

Pyrophosphate (PP), a ubiquitous small-molecule inhibitor of mineralization abundantly present in the extracellular environment, binds to calcium and mineral surfaces to inhibit crystal growth<sup>[63,64]</sup>. The local PP concentration depends on three regulatory factors: tissue nonspecific alkaline phosphatase (TNAP) degrades PP into phosphate ions, while ectonucleotide pyrophosphate phosphodiesterase (ENPP1) and the transporter protein ANK increases local PP concentration and thus facilitates a local defense against calcification in several tissues<sup>[65]</sup>. Idiopathic infantile arterial calcification, a severe syndrome causing deleterious calcifications in vessels of very young children, is caused by loss-of-function mutation of the ENPP1 gene<sup>[66]</sup>.

Hemodialysis patients have reduced plasma PP levels; possibly these low molecular weight solutes are effectively removed by the dialysis procedure<sup>[67]</sup>. Low PP levels seem to be connected with increased arterial stiffness in patients with end-stage renal failure, as was found in a study by Eller *et al.*<sup>[68]</sup>, where patients heterozygous for ENPP1 K121Q polymorphism had higher coronary calcification scores and arterial stiffness. The impact of this mutation for the survival of CKD patients and the possible benefit of their intense treatment need further investigation.



Bisphosphonates share the chemical structure of PP and, especially the first generation of bisphosphonates, offer therapeutic hope for treatment of calciphylaxis and coronary calcification in dialysis patients<sup>[69,70]</sup>. However, bisphosphonate treatment may aggravate pre-existing hyperparathyroidism, so caution is advised in uncritical use in CKD<sup>[71]</sup>. The future solution may be direct supplementation of PP, as it was found in animal studies to reduce aortic calcification in experimental kidney failure<sup>[63]</sup>, or the direct inhibition of TNAP<sup>[72]</sup>, but further studies are needed before human use<sup>[64]</sup>.

### Matrix Gla protein

Among the ECM proteins that have been reported to regulate osteoblast-dependent mineralization, matrix Gla protein (MGP) is one of the most potent inhibitors of calcification<sup>[73,74]</sup>. MGP is a member of the N-terminal  $\gamma$ -carboxylated protein family. MGP requires vitamin K-dependent  $\gamma$ -carboxylation for biological activation. Mice deficient for MGP (MGP<sup>-/-</sup>) show severe medial calcification of the aorta and die a few weeks after birth because of the rupture of the bone-like aorta<sup>[74]</sup>. It has been shown that undercarboxylated MGP (ucMGP) is associated with intimal and medial calcification, indicating local or systemic vitamin K depletion is a potentially important confounder in the development of arterial calcification<sup>[75]</sup>. The potential clinical importance of vitamin K-dependent  $\gamma$ -carboxylation is underlined by a study by Koos *et al.*<sup>[76]</sup>, showing that patients on oral anticoagulant therapy had increased coronary and valvular calcifications compared to patients without anticoagulation treatment, presumably due to less active MGP. In the case of calciphylaxis, a severe disease of CKD patients characterized by extensive arteriolar calcifications, warfarin was shown to be a risk factor for the manifestation of this life-threatening disease<sup>[77]</sup>.

Data about the association between MGP and arterial stiffness are controversial. Significantly lower ucMGP levels were found in dialysed patients compared to age-matched controls. In this dialyzed patient population, inverse correlation was found between augmentation index, an arterial stiffness parameter, and serum ucMGP levels, but no association was demonstrated with PWV. Besides, ucMGP had an inverse association with phosphate and positive association with fetuin-A levels, suggesting that low ucMGP can be a marker of active calcification and impaired arterial stiffness in dialysis<sup>[78]</sup>. This study was performed with SphygmoCor, one of the gold standard equipments of arterial stiffness measurements.

In contrast, in patients with CKD in stages 1-4, no correlation was found between serum MGP levels and arterial stiffness, although the stiffness was assessed by contour analysis of digital volume pulse, which is not a gold standard method<sup>[79]</sup>. In a recent study performed on renal transplant recipients, where arterial stiffness was measured with SphygmoCor, serum MGP level was not found to be predictor of carotid-femoral PWV<sup>[80]</sup>.

### Fetuin-A

Fetuin-A is a mineral carrier protein and a systemic inhibitor of pathological mineralization, complementing local inhibitors that act in a cell-restricted or tissue-restricted fashion. Fetuin-A deficiency is associated with soft tissue calcification in mice and humans<sup>[81]</sup>. The relevance of relative fetuin-A deficiency in humans was first demonstrated in a cohort of > 300 prevalent hemodialysis patients<sup>[82]</sup>. Patients within the lowest tertile of fetuin-A serum levels had a significantly increased all-cause and cardiovascular mortality. Sera from patients with low fetuin-A concentrations had a significantly impaired ability to inhibit calcium x phosphate precipitation compared to sera with normal fetuin-A concentrations<sup>[82]</sup>. Another study confirmed these data in incident dialysis patients, where hypoalbuminemia and CRP were strongly correlated to fetuin-A deficiency, suggesting an association with the malnutrition-inflammation-atherosclerosis (MIA) syndrome<sup>[83]</sup>. Patients on peritoneal dialysis with low fetuin-A levels showed a clear association with the MIA syndrome as well as with mortality and cardiovascular events<sup>[84]</sup>.

Both coronary and aortic calcifications were found to be related to low fetuin-A levels and in patients with diabetic nephropathy, a positive association between fetuin-A levels and coronary calcification score was demonstrated<sup>[85-87]</sup>.

The theory, that fetuin-A up-regulation may serve as a systemic defense mechanism to counteract early VCs, is supported by a study, where immunohistochemistry showed fetuin-A depositions around areas of VC, correlating well with the degree of calcification<sup>[85]</sup>. This calcification inhibitor effect of fetuin-A probably progressively fails with the development of uremia due to yet unidentified mechanisms<sup>[71]</sup>.

Studies examining the role of fetuin-A in arterial stiffening in CKD have shown varied results. Among non-diabetic children receiving renal replacement therapy, fetuin-A was an independent predictor of baseline aortic PWV<sup>[88]</sup>. In a recent prospective observational study performed in peritoneal dialysis patients, inverse correlation was found between the serum fetuin-A concentration and heart-to-femoral PWV and fetuin-A was found to be an independent determinant of aortic stiffness<sup>[89]</sup>. However, in a study of elderly dialysis patients, the relationship between fetuin-A and arterial stiffness lost significance after correction for age, gender, mean arterial pressure and diabetic status<sup>[90]</sup>. Another study of heterogeneous CKD stage 4 and dialysis population found no association between fetuin-A and change in VC<sup>[91]</sup>. In contrast, low fetuin-A was shown to be an independent risk factor for change in arterial stiffness in non-diabetic patients with CKD stages 3 and 4<sup>[92]</sup>. The relationship between fetuin-A and arterial stiffening was found to be different in diabetic and non-diabetic patients, which underlines the concept that in diabetic CKD patients, arterial stiffening may be driven by different processes than in the non-diabetic population. These may include deposition of advanced glycation products, calcification of more exten-

sive atherosclerotic plaque or development of additional *de novo* atherosclerosis, processes yet to be proven<sup>[92]</sup>.

### Osteopontin

Osteopontin (OPN) is an acidic phosphoprotein normally found in mineralized tissues such as bones and teeth, and it is involved in regulation of mineralization by acting as an inhibitor of apatite crystal growth, as well as promoting osteoclast function through the  $\alpha_v\beta_3$  integrin<sup>[93]</sup>. OPN is abundant at sites of calcification in human atherosclerotic plaques<sup>[94]</sup> and smooth muscle cells deficient for OPN display enhanced susceptibility to calcification *in vitro*<sup>[95]</sup>. In a study by Speer *et al.*<sup>[96]</sup>, OPN-null mice (OPN-/-) that have no overt vascular phenotype were bred to MGP-/- mice in which VC spontaneously develops. Mice deficient in both MGP and OPN (MGP-/-OPN-/-) showed accelerated and enhanced VC compared with mice deficient in MGP alone (MGP-/-OPN+/+).

Studies indicate that OPN is an inducible inhibitor of VC *in vivo* and may play an important role in the adaptive response of the body to injury and disease. In light of previous *in vitro* findings, part of the inhibition of arterial calcification in MGP-/- mice may be accounted for by the potent apatite inhibitory activity of phosphorylated OPN<sup>[97]</sup>.

Elevated plasma OPN levels are found to be associated with CRP and increased arterial stiffness in patients with rheumatoid arthritis, suggesting that this protein might represent a bridge between inflammation and the consequent joint damage and cardiovascular risk in rheumatoid arthritis patients<sup>[98]</sup>. To date, there is no data about the connection between serum OPN levels and arterial stiffness in CKD.

### Bone morphogenic proteins

Bone morphogenic proteins (BMPs) are members of the transforming growth factor  $\beta$  superfamily of cytokines and consist of a group of at least 15 morphogenes involved in intracellular messaging<sup>[99]</sup>.

BMP-2 expression is up-regulated in human atherosclerotic plaques isolated from abdominal aorta and associated with specific immunostaining for MGP, osteocalcin and bone sialoprotein that are absent in normal aorta and early atherosclerotic lesions<sup>[100]</sup>. *In vitro* studies show that cultured cells isolated from aortic wall express BMP-2 and produce calcified nodules similar to those found in bone cell cultures<sup>[43]</sup>. Chen *et al.*<sup>[43]</sup> reported a higher BMP-2 protein concentration in a pooled uremic serum than in normal human serum and demonstrated that BMP-2 could induce calcification of phosphate-treated bovine smooth muscle cells *via* up-regulation of Cbfa1<sup>[101]</sup>. The connection between BMP-2 and arterial stiffening has been proven recently, as Dalfino *et al.*<sup>[102]</sup> found direct correlation between brachial-ankle PWV and BMP-2 in a population of 85 CKD (stage 2 or higher) patients.

BMP-7 is a crucial element for the development of kidneys, eyes and bones<sup>[103]</sup>. In the adult, BMP-7 main-

tains a role in osteoblast function, suggesting a hormonal role in bone metabolism. Interestingly, BMP-7 expression decreases early in the course of renal failure<sup>[104]</sup>. BMP-7 deficiency can have important consequences in the pathogenesis and treatment of chronic renal insufficiency<sup>[105]</sup>, but is also very interesting in the pathogenesis and treatment of VCs. Indeed, BMP-7 maintains VSMC differentiation and prevents their transformation into cells with an osteoblastic phenotype<sup>[106,107]</sup>. Thus, the state of BMP-7 deficiency, characteristic of chronic renal failure, could favor VC, especially within the context of atherosclerotic lesions. The possible connection between BMP-7 and arterial stiffness still needs to be evaluated.

### Fibroblast growth factor 23

Fibroblast growth factor 23 (FGF-23) is a recently discovered regulator of phosphate and mineral metabolism. FGF-23 is a 251 amino acid protein that is predominantly synthesized and secreted by cells from an osteoblast lineage<sup>[108,109]</sup>. FGF induces phosphaturia by reducing the number of Na-P co-transporters on renal tubular cells, as well as mitigating the effects of calcitriol on intestinal absorption<sup>[110]</sup>. The biological effects of FGF-23 are exerted through activation of FGF receptors (FGF-R). Klotho is a trans-membrane protein originally described in mice with a phenotype of accelerated aging and atherosclerosis<sup>[109]</sup>. Klotho directly interacts with FGF-R, allowing it to bind FGF-23 with a higher affinity and increased specificity<sup>[111,112]</sup>. The activation of FGF-23 therefore occurs in a Klotho-dependent manner<sup>[112]</sup>.

The main known physiological role of FGF-23 is to regulate urinary phosphate excretion and maintain a stable serum phosphate<sup>[113]</sup>. An important secondary role is the counter-regulation (against PTH) of vitamin D biosynthesis. The main stimuli for increased expression of FGF-23 are high dietary phosphate, calcitriol and persistent hyperphosphatemia<sup>[114-116]</sup>. In CKD, recently reported clinical studies support a phosphate-centric, FGF-23 mediated pathogenesis of secondary hyperparathyroidism and findings suggest that FGF-23 plays an active role in CKD-MBD<sup>[117]</sup>.

A prospective cohort study of 219 dialysis patients demonstrated an association between FGF-23 levels and mortality, independent of serum phosphate<sup>[118]</sup>. In another study in dialyzed patients, FGF-23 levels were shown to predict 1 year mortality, also independent of phosphate levels<sup>[119]</sup>. Although FGF-23 levels in these two studies did not demonstrate additional prognostic information when compared with phosphate levels, the possibility of using FGF-23 as a biomarker in patients with CKD and normal phosphate levels is of interest and needs to be assessed.

There is also growing evidence about the association of cardiovascular disease and FGF-23 levels. In an observational study of 833 patients with early CKD and stable coronary artery disease, elevated FGF-23 was independently associated with mortality and cardiovascular events<sup>[120]</sup>. Association between arterial stiffness and

FGF-23 has also been demonstrated once in a cohort of 967 patients with early CKD, where arterial stiffness was measured with ShygroCor<sup>[121]</sup>.

## FUTURE DIRECTIONS - NEW THERAPEUTIC PATHWAYS

With the growing amount of data about the pathophysiological role of bone metabolism regulators in VC, new possible therapeutic targets have emerged, such as denosumab, a human monoclonal antibody which binds RANKL with high specificity, mimicking the effect of endogenous OPG. In postmenopausal osteoporosis it decreases bone resorption but its effect on VC needs further studies<sup>[122]</sup>. Theoretically, the modification of the effects of FGF-23 could improve vitamin-D homeostasis<sup>[72]</sup>. Amongst the endogenous inhibitors, administration of fetuin-A and BMP-7 can have beneficial effects in the future<sup>[122]</sup>. Besides the direct supplementation of PP, as it was found to reduce aortic calcification in experimental kidney failure in animal studies<sup>[63]</sup>, the inhibition of TNAP can also have perspectives in the increasing of PP levels and improvement of cardiovascular outcomes<sup>[72]</sup>.

Certainly, the effect of these future interventions for arterial stiffening is an open question but there are already some results about present medications which are beneficial for arterial stiffness and that may partly act through the modification of the inhibitors of bone metabolism. The non-calcium-containing phosphate binder sevelamer has been linked to slowing the progression of aortic calcification in hemodialysis patients<sup>[123]</sup>. We demonstrated its beneficial effect for arterial stiffness parameters in dialysis patients previously<sup>[124]</sup>. A recent study in non-diabetic CKD patients demonstrated a significant increase in fetuin-A after treatment with sevelamer<sup>[125]</sup>. According to these results, sevelamer may attenuate the progression of arterial pathology in CKD, at least partly through the elevation of fetuin-A.

The AGE-cross-link breaker alagebrium did improve endothelial dysfunction and carotid augmentation index in patients with isolated systolic hypertension<sup>[13]</sup>. Its beneficial effect for the osteoporosis in patients with rheumatoid arthritis is hypothesized<sup>[126]</sup> but the influence of alagebrium on bone metabolism inhibitors has not been evaluated yet.

## CONCLUSION

Increased arterial stiffness is associated with structural and functional changes of the vasculature and the poor cardiovascular outcome of patients with CKD. In these patients, a huge number of deleterious factors are present, like endothelial dysfunction, cumulation of AGEs, chronic inflammation, the impaired RAAS and processes leading to arterial media calcification. Recently, there is growing evidence about the active involvement of some of the bone and phosphate metabolism regulators in increased medial calcification and stiffening. Evaluation

of their exact pathophysiological role and their correlation with mortality in CKD could lead to their use as biomarkers or new therapeutic targets, and may slow the progression of arterial stiffening and improve cardiovascular outcome.

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