Up to date IN NEPHROLOGY

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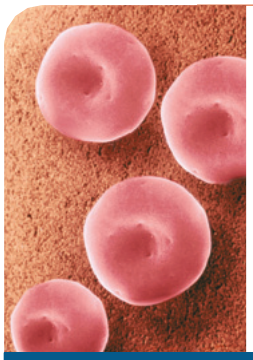

VASCULAR CALCIFICATIONS IN DIALYSIS PATIENTS

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Abbreviations

ALP:	alkaline phosphatase
BMP-2:	bone morphogenic protein-2
CAC:	coronary artery calcification
CaR:	calcium sensing receptor
CaxP:	calcium phosphate product
CKD:	chronic kidney disease
CKD-MDB:	chronic kidney disease – mineral bone disorder
CRP:	C-reactive protein
CV:	cardiovascular
Dp-uc-MGP:	dephosphorylated uncarboxylated matrix Gla protein
EBCT:	electron-beam-computed tomography
ESRD:	end-stage renal disease
Fet-A:	fetuin-A
FGF-23:	fibroblast growth factor 23
HD:	hemodialysis
HDL:	high-density lipoprotein
LDL:	low-density lipoprotein
MGP:	matrix Gla protein
MSCT:	multi-slice computed tomography
OC:	osteocalcin
ON:	osteonectin
OPG:	osteoprotegerin
OPN:	osteopontin
PPi:	pyrophosphate
PTH:	parathyroid hormone
sHPT:	secondary hyperparathyroidism
uc-MGP:	uncarboxylated matrix Gla protein
VSMC:	vascular smooth muscle cells

1 Introduction

In the two last decades, vascular calcifications have been identified as a major cardiovascular (CV) risk factor in chronic kidney disease (CKD) patients, especially in patients at the end-stage renal disease (ESRD) stage. In the last century [1], calcifications were already known (and feared) by nephrologists but they stressed on "soft-tissue" calcifications [1;1-5] or on calciphylaxis [6] which can be considered as an epiphenomenon of vascular calcifications (very serious but relatively rare). Actually, there were relatively few articles on vascular or valvular calcifications [7].

The interest on vascular calcifications has been highlighted since the following observations: firstly, dialysis patients have an exceptionally high CV mortality compared to the general population and this risk is not simply linked to traditional CV risk factors [8]. Secondly, epidemiological studies have underlined the relationship observed between CV mortality and mineral metabolism markers (especially, phosphorus, calcium, calcium-phosphorus product and, for some authors, PTH) [9-14]. Lastly, it has been demonstrated that a strong relationship does exist between several of these mineral metabolism markers (the term "renal osteodystrophy" is replaced by "CKD-MDB" for Chronic Kidney Disease-Mineral Bone Disorder [15]) and the vascular calcifications [7;16-27]. Studies establishing the link between mortality and vascular calcifications are more difficult to do. However, even though data are relatively limited and methodologies sometimes questionable, several studies have suggested such a relationship between mortality (notably CV mortality) and vascular calcification [26;28-35]. Nevertheless, we have to insist on the fact that no direct proof of the "causal" link between vascular calcifications and CV mortality exists. More pre-

cisely, we have no proof that decreasing vascular calcifications is either possible [36] or even beneficial from a CV point of view. Nevertheless, indirect proofs are advanced. Vascular calcifications even became important "endpoints" in clinical trials including patients receiving dialysis and are considered as a useful "surrogate marker" instead of CV mortality.

Compared to the general population (where vascular calcifications are, for example, present in older subjects), coronary calcifications in dialysis patients are more prevalent (up to 90 % of dialysis patients [16]) and more severe (calcium score 2.5 to 5-fold higher [20]) [16;20;23;25;37-40]. They are also early (often present in very young patients) [16;18-20;25;26;41;42] and more rapidly progressive [16;20;41;43;44]. The same conclusions could be made for cardiac valve calcifications [20;23;24;45-47].

We still have to find out with more precision in what way vascular calcifications are harmful. Currently, there are some arguments to say that vascular calcifications in the intima, which are strongly linked to atherosclerosis (see below), could actually stabilize this atherosclerosis plaque [5;48-51].

It seems that aorta calcifications (calcifications of the media, see below) could be more important, from a pathophysiological point of view, to explain the rather high CV mortality in dialysis patients. Indeed, aorta calcifications induce vascular stiffness, increasing aortic pulse wave velocity (and pulse pressure) [22;28;35;52;53] and inducing left ventricular hypertrophy and diastolic dysfunction that both could explain the high prevalence of cardiac-related death [28;53-57]. Of course, cardiac valve calcification can induce *per se* functional abnormalities like rapid development of aortic stenosis [24;46]. There are also some data suggesting that the intensity of valve calcification could predict CV mortality [58] although others do not find such an association after adjusting for other risk factors [31;59].

This "Up to Date in Nephrology" brochure reviews the recent literature on the types of vascular calcifications, the pathophysiological mechanisms responsible for vascular calcifications, the detection methods and the impact of CKD-MBD treatment options.

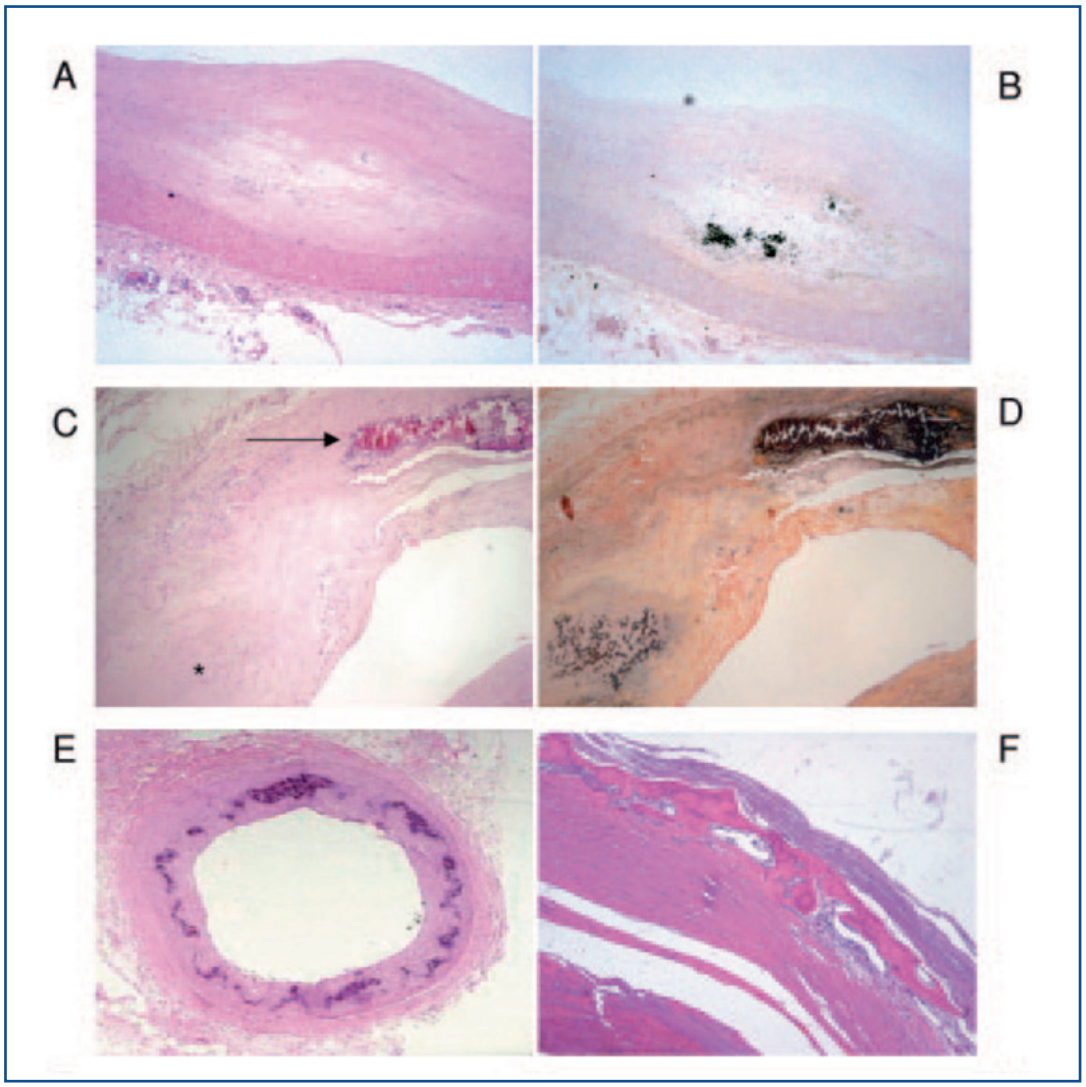


Figure 1. (A) and (B) Coronary intima plaque with subtle calcification in hematoxylin and eosin (H&E) stain (A) and Kossa stain (B). The arterial media is completely free of calcification.(C and D) Coronary artery of a patient with CKD showing intimal (*) as well as medial calcifications (arrow). (C) H&E stain. (D) Kossa stain.(E) Medial calcification of a peripheral artery in the absence of any intimal change. No lipid or cholesterol depositions are visible.(F) Peripheral muscular type artery with metaplastic bone formation in the arterial media (H&E stains). (Dr. Nonnast-Daniel, Department of Nephrology, University of Erlangen, Germany) ***(Amann. Clin J Am Soc Nephrol 2008, 3, 1599-1605).***

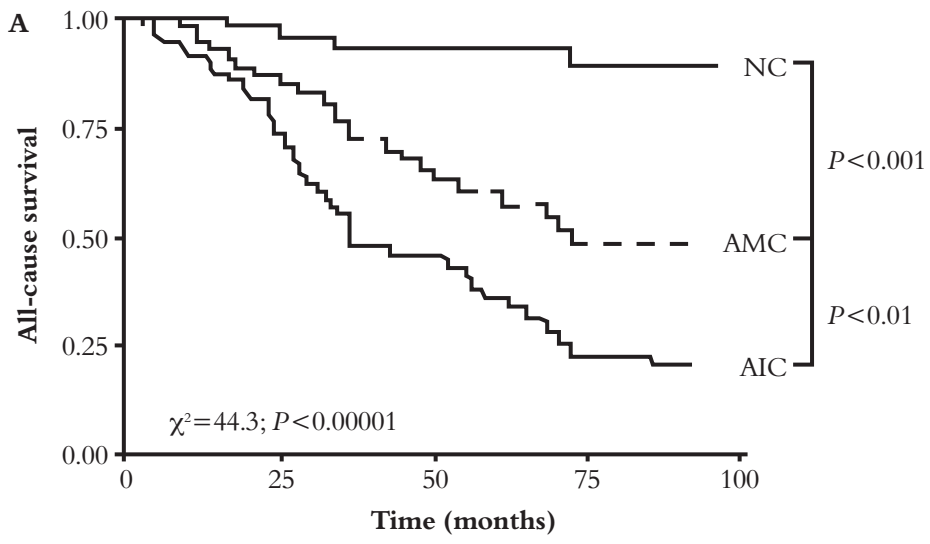
2 TYPES AND MECHANISMS OF VASCULAR CALCIFICATIONS

2.1 Medial versus intimal calcifications

(Figure 1) [60]

Two types of vascular calcifications are classically described [60]. One type is linked to atherosclerosis lesions (in the vicinity of lipid depositions) and is located in the intima of the vascular wall. The other type is located in the media and is also known as Monckerberg sclerosis [60]. This type of calcification is highly prevalent in ageing, in CKD [61] and in diabetic patients. The medial calcifications are thought to be important to explain that calcifications are early and severe in dialysis patients compared to general population. It is probable that molecular mechanisms involved in these two types of calcifications are different (notably the role of lipids), but some mechanisms could be common (local inflammation, calcium and phosphate balance disturbances, calcification inhibitors and activators balance disturbances, see below) [60]. In the same view, pathological mechanisms explaining soft-tissue and cardiac valve calcifications are little known [60;62]. In the CKD context, most of animal or *in vitro* studies have been realized with media (or both media and intima) calcifications models [60;63]. The impact on CV mortality is also difficult to apprehend but one study suggests that the risk of CV mortality is higher with intima calcifications. It should be noted that in this report, calcifications were only assessed by standard radiography, (see below) [26] *(Figure 2)*. However, as intima calcification is strongly linked to atherosclerosis, this statistical association with mortality does not imply that calcification *per se* is the most harmful in this context [48;60]. This topic is difficult and subject of debate. This is, at least in part, due to the lack of an easy method to make differential diagnosis between intimal and medial calcification (see below) [60]. This debate is also illustrated by the poor correlation observed between coronary vascular calcifications by electron-beam computed tomography (EBCT), which cannot distinguish intimal and medial calcifications and lesions observed by coronary angiography in one study [64]. However, medial calcifications are very frequent in dialysis patients and their pathogenic power is not linked to vascular obstruction. So, the results of this last study do not imply that calcium score measurement is without interest from a CV point of view [65].

Figure 2. All-cause (A) and CV mortality (B) of ESRD patients as a function of their calcification status. NC: non-calcified, AMC: arterial medial calcification, AIC: arterial intimal calcification *(London et al. Nephrol Dial Transplantation, 2003, 18,1731-1740).*



2.2 Calciphylaxis

Calciphylaxis is a calcification syndrome associated with ischemic cutaneous necrosis [66] *(Figure 3)* [67]. Association between vascular calcification and cutaneous gangrene has already been described in 1899 [68] and the term "calciphylaxis" has been proposed by Hans Selye in 1962 [69]. Calciphylaxis or "calcific uremic arteriolopathy" is histologically characterized by vascular calcification (especially calcification in the media), intimal proliferation, endovascular fibrosis and intravascular thrombosis in small or medium arteries [66;70]. The lesions are usually located in two distinct patterns: distal with lesions of the lower extremities or proximal with the lesions on the abdomen, inner thighs and buttocks [66;70;71]. These calcifications induce painful skin lesions progressing to ischemic necrosis. Calciphylaxis is a severe syndrome with a high mortality rate (45 to 80%) [66;70-72;72]. The details of the pathogenesis are still unknown: is it simply a "sur-acute" form of vascular calcification? [70;73]. It seems that hypercalcemia/hyperphosphatemia, low- or high bone turnover, steroids, warfarin and vitamin D therapies could be implicated [17;66;70-73]. Female gender, Caucasian race, diabetes, and obesity are often proposed as risk factors [70;71;73]. Additional to wound care (notably with hyperbaric oxygen [70]), the optimal therapy for calciphylaxis is not standardized. Calcium and phosphate control by non-calcium phosphate binders, parathyroidectomy [66;74], sodium thiosulphate [75], bisphosphonates [76;77] and cinacalcet (see below) have been proposed [70]. Sodium thiosulphate (potent antioxidant as well as chelator of calcium) and cinacalcet could be the most interesting therapies but we need more trials to proof this.

Figure 3. Extensive cutaneous necrosis of the thighs with liveloid contour *(Prey et al. Rev Med Interne, 2009, 30, 186-189).*

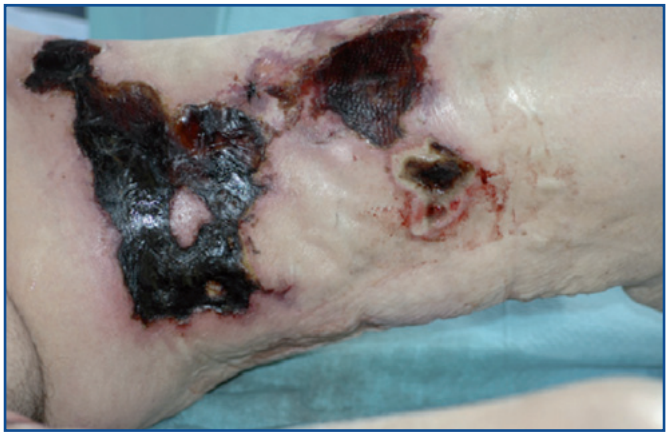
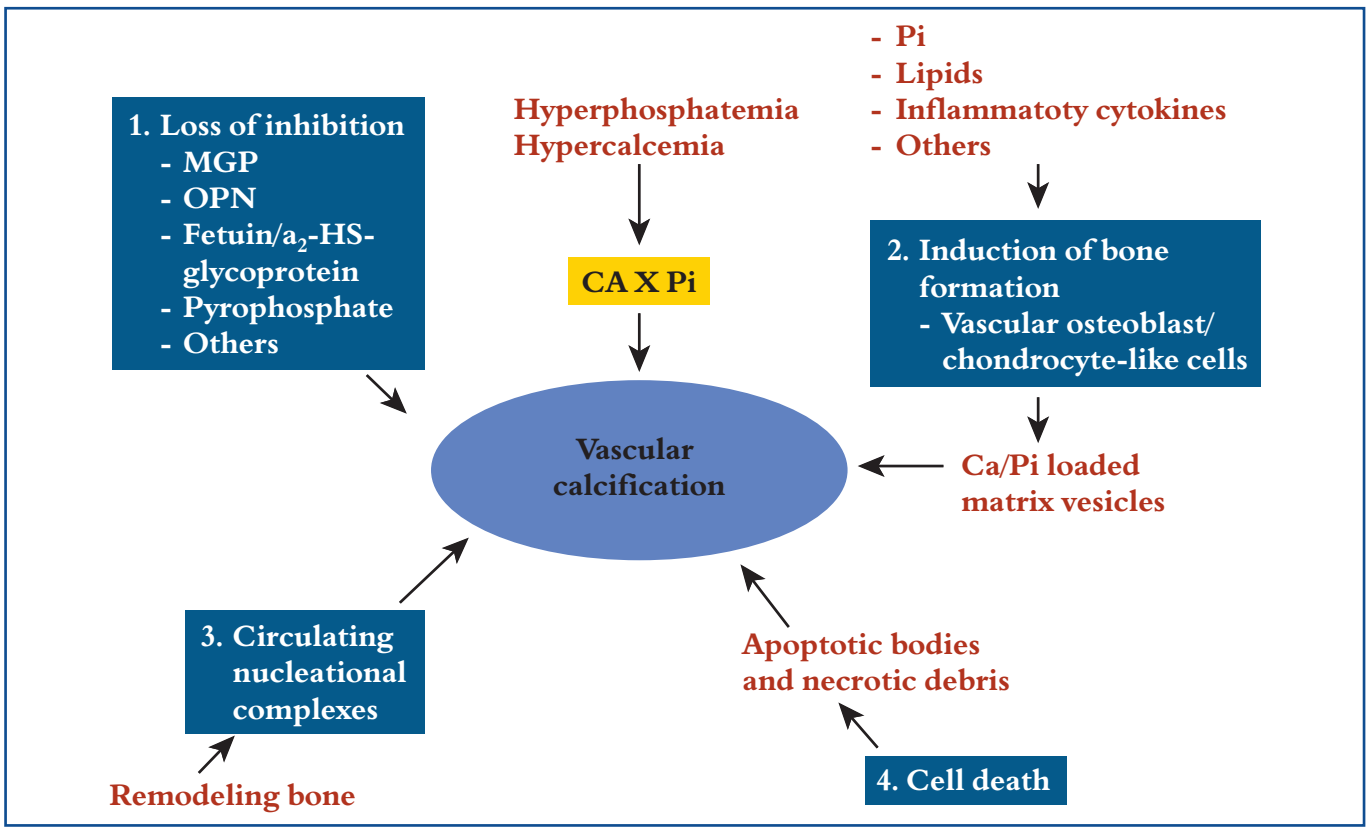


Figure 4. Four non-mutually exclusive theories for vascular calcification. (1) Loss of inhibition as a result of deficiency of constitutively expressed tissue-derived and circulating mineralization inhibitors leads to default apatite deposition. (2) Induction of bone formation resulting from altered differentiation of vascular smooth muscle or stem cells. (3) Circulating nucleational complexes released from actively remodeling bone. (4) Cell death leading to release of apoptotic bodies and/or necrotic debris that may serve to nucleate apatite at sites of injury *(Giachelli. J Am Soc Nephrol, 2004, 15, 259-2964).*



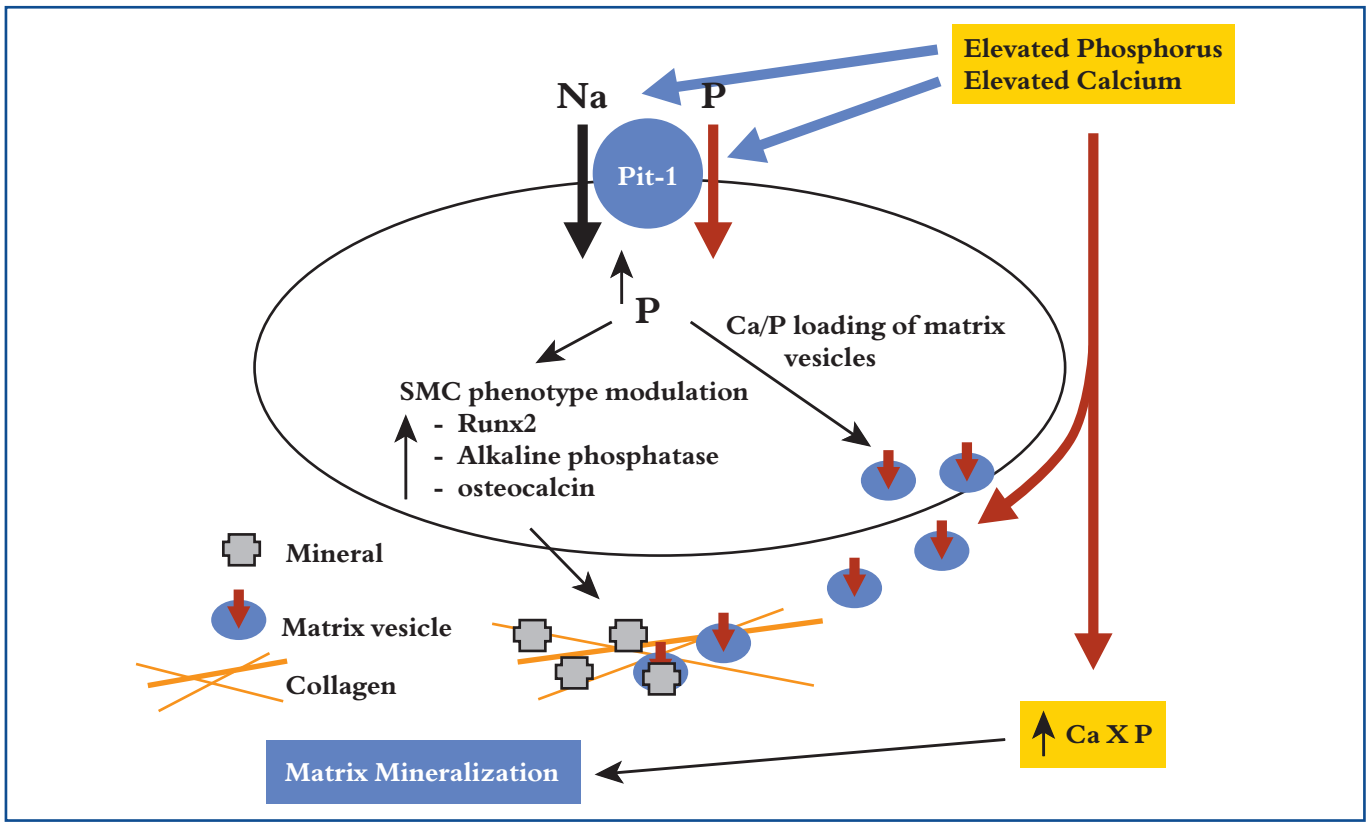
2.3 Vascular calcifications in CKD: an active and complex process

Occurrence of vascular calcification is not new. Arterial calcification has been discovered in the "Iceman" who lived 5000 years ago [78] and scientists had already paid attention to this phenomenon – and to its relation with renal disease - in the 19th Century [79]. However, this pathology has only been studied since the last two decades. Today, vascular calcification is considered as an actively regulated and complex process that remains not completely understood. We will describe hereafter some of the pathophysiological mechanisms. It is important to underline that these different mechanisms are not mutually exclusive *(Figure 4)* [80].

2.3.1 The role of calcium and phosphate and induction of bone formation

One major mechanism in the development of vascular calcifications is similar to that of bone formation. Indeed, vascular smooth muscle cells (VSMC) undergo osteogenic differentiation into phenotypically distinct osteoblast-like cells [80-82]. In this mechanism, phosphate has the most important role [82;83]. Actually, *in vitro,* high extracellular phosphate concentrations induce a rise in intracellular phosphate concentration which is actively mediated by Pit-1, a sodium dependent phosphate co-transporter [83;84]. This increasing phosphate concentration into the VSMC will induce a phenotypic switch of VSMC into osteoblast-like cells [80;83;85]. The protein Cfba1/Runx2 is a specific and indispensable transcriptional regulator for this osteoblastic differentiation. Its expression is also enhanced in the presence of high extracellular phosphate [83;85;86]. These "new" cells will express alkaline phosphatase (ALP), secrete bone-associated proteins (such as osteopontin [87], collagen type 1, and bone morphogenic protein-2 and osteocalcin [83;88]) under the control of Cfba-1, and release mineralization-competent matrix vesicles in the extracellular matrix [82;83;89]. All these modifications will favour for an optimal microenvironment for hydroxyapatite formation and calcification. Similar osteogenic differentiation is also observed, *in vivo,* in animal and human uremic models [81;85;90] *(Figure 5)* [80].

Figure 5. Proposed model for the effects of elevated Ca and P on vascular smooth muscle cell (VSMC) matrix mineralization. Elevated Ca and P are proposed to stimulate vascular matrix mineralization in two ways. First, both Ca and P increase the activity of Pit-1: elevated P stimulates P uptake via Pit-1, and elevated Ca induces expression of Pit-1 mRNA; Both mechanisms are proposed to enhance P uptake into VSMC as well as matrix vesicles. Elevated intracellular P then leads to VSMC phenotypic modulation, which includes upregulation of osteogenic genes (Runx2, osteocalcin, and alkaline phosphatase), and generation of a mineralization-competent extracellular matrix. In addition, increased Pit-1 in matrix vesicles promotes P loading of matrix vesicles, promoting nucleation of mineral within the extracellular matrix. Second, elevated Ca and/or P lead to increased Ca X P ion product, thereby promoting growth of apatite crystals in the matrix via thermodynamic mechanisms *(Giachelli. J Am Soc Nephrol, 2004, 15, 259-2964).*



In vitro exposure of VSMC to hypercalcemia also induced overexpression of Pit-1 receptor and thus enhanced intracellular phosphate concentration [89;91]. Elevated intracellular calcium is also associated with an alteration of calcification inhibitors such as matrix Gla protein (MGP) and fetuin A (see below) [89]. If calcium may participate to the pathogenesis of vascular calcification, phosphate has, once again, the pivotal role [80;82;83;89;92].

2.3.2 The role of bone turnover

As calcium and phosphorus have a pivotal role in the pathogenesis of vascular calcification, it is not surprising that bone-turnover disturbances can enhance vascular calcification because bone is the most important reserve of calcium and phosphorus and could act as a buffer [93]. Some authors have written about the "bone-vascular" axis. From our point of view, the link between "low bone turnover" and risk of vascular calcifications is better illustrated by the current literature. However, we also think that the two "theories" of low and high turnover are not excluding each other in a context of "bone as a calcium-phosphorus buffer" [5].

Low turnover: In the first article measuring vascular calcifications, *Braun et al.* have already shown that coronary calcifications are inversely correlated with bone mass [20]. Probably the most interesting study on this topic has been published in 2004 by *London et al.* These authors have actually compared vascular calcifications with bone histomorphometry by plain radiography, in 58 dialysis patients. They found that a high calcification score is independently associated with histomorphometry suggestive of low bone turnover (or adynamic bone) [94]. One complementary study from the same group underlined that calcium load is particularly deleterious, in terms of vascular calcifications, for these patients with low bone turnover [95]. Studying both coronary calcifications and bone histomorphometry in 101 dialysis patients, *Barreto et al.* also found an inverse correlation between calcium score and bone trabecular volume and trabecular thickness in an univariate analysis [38]. In 2009, *Adragao et al.* studied the relationship between bone histomorphometry and coronary calcifications in 38 dialysis patients. Contrary to precedent trials, low bone turnover was not associated with calcifications. However, lower bone volume was a risk factor for coronary calcification [96].

High turnover: We have already mentioned the epidemiological link between PTH and CV mortality in dialysis patients, observed by some authors [10;11;13;14]. *Coen et al.* have demonstrated a relationship between high PTH levels (especially very high levels) and coronary calcifications [12;97]. Even though specific PTH fragments (1-34 PTH) have been shown to inhibit vascular calcifications in an animal model [98], *Neves et al.* have shown, in a model of parathyroidectomized CKD rats, that perfusion of PTH, *per se*, could induce vascular calcifications [99].

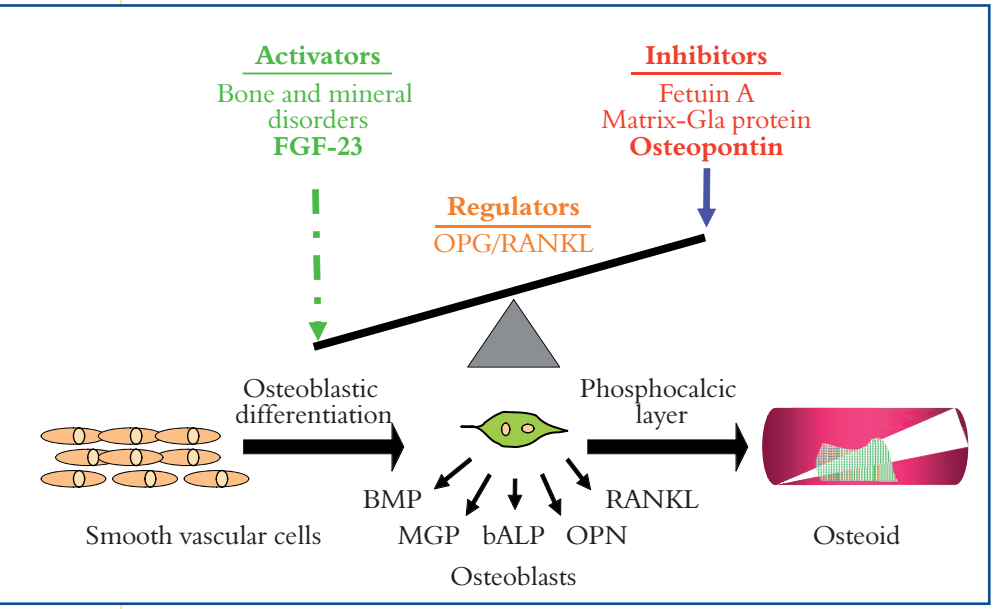
2.3.3 Balance between calcification inhibitors and promoters *(Figure 6)*

In water, calcium and phosphate directly form insoluble precipitates. This is not the case in serum, suggesting the existence of calcification inhibitors [100]. In the last years, several inhibitors have been actually described with a potential role in vascular calcifications. Chondrocytes, osteoblasts and osteoclasts have been identified in calcified atherosclerotic plaques [101;102]. These cell types can locally express calcification activating proteins (osteonectin, osteocalcin and bone morphogenic protein-2), inhibiting proteins (osteopontin, Matrix-Gla protein, pyrophosphate) and regulatory factors (osteoprotegerin system) [103]. Circulating proteins (Fetuin-A) could also participate in the vascular calcification process. We will summarize the most recent findings for some of the most important proteins.

Matrix Gla protein (MGP)

MGP is a 10-kDa protein expressed by chondrocytes and VSMC. Its role as calcification inhibitor has been illustrated by MGP knock-out mice who develop extensive aortic calcifications [104]. In 2002, *Moe et al.* demonstrated a correlation between vascular MGP expression and the calcifications of epigastric arteries in dialysis patients [90;105]. Of importance is the fact

Figure 6. Balance between calcification inhibitors and promotors *(with the permission of Prof. Cristol, Department of Biochemistry, Montpellier).*



that MGP requires vitamin K for its activation by γ-carboxylation [80;89;104;106]. It has been shown that non fully γ-carboxylated (but not γ-carboxylated MGP) is associated with vascular calcification [80;106;107]. MGP would bind and inactivate a pro-mineralization factor, BMP-2 [108]. MGP also binds calcium crystals, inhibits crystal growth and plays a role in the normal phenotype of VSMC in preventing the osteoblastic differentiation [86;109]. MGP is vitamin K dependent for its carboxylation and its activation. This is the actual explanation for the observations that warfarin leads to extensive vascular calcifications in animal and human studies [110-112]. It is interesting to note that vitamin K therapy partially reverses warfarin-induced vascular calcifications in rats [112].

Osteoprotegerin (OPG)

OPG is a regulatory factor produced by bone marrow-derived stromal cells. OPG has a pivotal role in the regulation of the bone turnover inhibiting osteoclast differentiation and acting like a decoy receptor for the receptor activator of NF-χB ligand (RANKL system) [113]. OPG-deficient mice will also develop both severe aortic calcifications and osteoporosis [114]. However, the role of OPG in vascular calcification remains unclear [115]. On one hand, OPG is considered to prevent vascular calcification as it blocks the bone remodeling process in the vascular tissue following the interaction between RANK (expressed by osteoclast-like cells) and RANKL (expressed by osteoblast-like cells). It is also a neutralizer of the pro-apoptotic actions of TRAIL (TNF-related apoptosis-inducing ligand), which strongly activates vascular cell apoptosis [116]. OPG is also thought to inhibit ALP activity and to prevent vascular calcifications [117]. On the other hand, the inhibition of the bone remodeling process by OPG could induce a calcium shift in vascular cells.

Osteopontin (OPN)

OPN is a phosphoprotein expressed in the mineral tissues which inhibits mineralization by blocking hydroxyapatite formation and activating osteoclast function [118]. OPN is present in calcified vessels. OPN knock-out mice do not develop vascular calcification but, when these mice are bred with MGP knock-out mice, the vascular calcifications are more important than in simple MGP knock-out mice [119]. OPN must be phosphorylated to act as a calcification inhibitor [120]. OPN inhibits mineralization of VSMC by binding to the mineralized crystal surface [121]. Contrary to the fully phosphorylated OPN, cleaved OPN could act as a proinflammatory cytokine and a proangiogenic factor facilitating vascular mineralization [118;122].

Pyrophosphate (PPi)

PPi is a small molecule made of two phosphate ions. It acts as a calcification inhibitor by inhibiting hydroxyapatite crystal formation [123]. Once again, knock-out mice (in fact, knock-out mice for a precursor) develop vascular calcifications [124]. Absence of PPi will promote VSMC differentiation but the mechanism is not fully understood [125;126]. Absence of PPi combined with high phosphate levels and presence of type 1 collagen could facilitate the development of calcification [92;127]. It has been shown that dialysis patients exhibit low serum PPi and that these are lowered further during a hemodialysis session [128].

Fetuin-A (Fet-A)

Fet-A (60 kDa) is a potent calcification inhibitor produced by the liver. Contrary to other factors acting locally, Fet-A action is systemic. Its calcification inhibitory action is powerful and illustrated by knock-out mice developing severe extraosseous calcifications [129]. Even though the aorta remains free from calcifications in this model, CKD and high phosphate diet will induce severe aorta calcifications in these knock-out mice [130]. Increasing Fet-A expression is found in calcified arteries from dialysis patients [105]. Fet-A is thought to inhibit calcification by binding early calcium phosphate crystals and by inhibiting crystal growth and mineral deposition. This could be facilitated by the formation of large calciprotein particles [131;132]. Fet-A could also prohibit matrix vesicle calcification of the VSMC which take up circulating Fet-A in an extracellular calcium-dependent way [133;134]. Serum Fet-A concentration is decreased in dialysis patients [135]. The exact mechanism is still hypothetical. It is probably related to chronic inflammation, as Fet-A is a negative acute phase reactant [136].

Fibroblast Growth Factor 23 (FGF-23)

FGF-23 is a 30 kDa bone-derived protein that promotes renal phosphorus wasting and inhibits the conversion of 25-hydroxy-vitamin D to the active 1,25-dihydroxy-vitamin D form [137]. In CKD patients, when the glomerular filtration rate decreases below 25-30 ml/min, the maintenance of normal phosphate levels is presumably accomplished by a compensatory rise in FGF-23 [137;138]. The action of FGF-23 on its specific receptor is mediated through the type-1 membrane-bound alpha-Klotho (Klotho). Indeed, Klotho is a FGF-23 receptor cofactor that directly interacts with the FGF-23 receptor. The importance of Klotho for FGF-23 activity is such that supraphysiological concentrations of FGF-23 have no impact on mineral metabolism without the presence of Klotho [139]. In mice, high levels of FGF-23 have been shown to reflect a response to dietary phosphorus burden and thus can serve as a marker of arterial calcification [140]. It is also evident that Klotho knock-out mice will develop vascular calcifications (this model is considered as a model of ageing) [141]. Interestingly, Klotho has recently been described as a regulator of calcium homeostasis, notably influencing the calcium transport across the cell membrane [142;143]. However, as Klotho is neither expressed in the myocardium, nor in the blood vessels, its role as a promoter of vascular calcification has to be clarified in future studies. In the same way, knock-out mice for FGF-23 also develop vascular and visceral calcifications [137;144;145]. However, this deletion is also associated with an increased serum phosphate concentration, an increased expression of renal 1α-

hydroxylase and an increased serum 1,25 vitamin D concentration [137;144;145]. Double knock-out mice (for FGF-23 and 1α-hydroxylase) develop neither hyperphosphatemia nor calcifications, underlining the potential role of vitamin D in vascular calcifications (see below) [146]. So, a "direct" (phosphate and vitamin D independent) role of FGF-23 in the pathogenesis of vascular calcifications remains to be proven and a question still to be answered is: Is FGF-23 an inducer or a marker of vascular calcifications? [34;137;140;147].

Alkaline phosphatase (ALP)

ALP is a phenotypic marker of osteoblasts and is thought to be essential for vascular calcifications [148]. Its expression in calcification seems to be under the control of Cfba-1 [80]. ALP is expressed on the surface of differentiated cells and could hydrolyze PPi, a calcification inhibitor [149].

Bone morphogenic protein-2 (BMP-2)

BMP-2 is an important molecule in the regulation of bone formation as well as vascular calcification. In bones, it promotes osteoblast differentiation and mineralization [150]. Inhibition of BMP-2 inhibits osteoblast differentiation and bone formation *in vivo* and *in vitro* [151] and protects against atherosclerosis and vascular calcification [152].

Osteocalcin (OC)

OC, a vitamin-K dependent matrix protein that inhibits calcium salt precipitation *in vitro* [153], shows a strong affinity for hydroxyapatite and inhibits crystal growth [154]. Even though its role remains unclear, OC limits bone formation [155] and it has been found in calcified atherosclerotic plaques and calcified aortic valves [156]. The role of OC in the pathogenesis of vascular calcification clearly remains to be determined.

Osteonectin (ON)

ON, also called SPARC or BM40, is a calcium binding protein involved in bone development that demonstrates affinity for hydroxyapatite and collagen [157]. ON has been found in association with large calcifications in atherosclerotic plaques [158].

2.3.4 The role of inflammation, lipids and oxidative stress

The uremic state is also characterized by increased oxidative stress. Oxidative stress has been shown to enhance ALP in VSMC [159] and to promote differentiation of VSMC via an activation of the Cfba-1/Runx2 protein [160;161]. As oxidative stress is also the result of inflammation, inflammatory cytokines have also been implicated in the pathogenesis of vascular calcification. Actually, tumour-necrosis factor-α (TNFα) has been shown to induce differentiation of VSMC and expression of ALP [162-165]. Notably, oxidized LDL and other lipid peroxidation products induce osteoblastic differentiation in a dose-dependent manner [166] although HDL inhibits it (but oxidized HDL promotes differentiation, too) [167]. Fet-A is both a negative acute-phase protein and a calcification inhibitor and could thus be one link between inflammation and calcification [163].

2.3.5 Other factors

The concentration of leptin is increased in CKD patients [168]. Leptin has been shown to regulate osteoblastic differentiation and calcification of VSMC, which are known to express leptin receptors [169].

In summary, there are two types of vascular calcifications; one type is located in the intima of the vascular wall, the other type is located in the media. Medial vascular calcifications are highly prevalent in patients with CKD. Calciphylaxis is a calcification syndrome associated with ischemic cutaneous necrosis and is histologically characterized by vascular calcification, intimal proliferation, endovascular fibrosis and intravascular thrombosis in small and medium arteries. Calcium and phosphate control by non-calcium phosphate binders, parathyroidectomy, sodium thiosulphate, bisphosphonates and cinacalcet have been proposed as therapy for calciphylaxis but more trials are needed. Vascular calcification is associated with CV mortality in CKD patients. However, the pathogenesis of vascular calcifications is not fully understood. It is a rather complex process influenced by derangements of calcium and phosphate homeostasis, by a dysregulated balance between calcification promoters (ON, OC, and BMP-2) and calcification inhibitors (OPN, MGP, and PPi), by regulatory factors (OPG system) and by circulating proteins such as Fet-A, a calcification inhibitor with systemic action. A direct role of FGF-23 (phosphate and vitamin D independent) remains to be proven. ALP, a phenotypic marker of osteoblasts is thought to be essential for vascular calcification. Inflammatory cytokines have been involved in the pathogenesis of vascular calcification. Fetuin-A (Fet-A) is also a negative acute-phase protein and could be a link between inflammation and calcification. Oxidized LDL and other lipid peroxidation products induce osteoblastic differentiation and vascular calcification while HDL regulates it. Bone turnover disturbances can enhance vascular calcification. Leptin is increased in CKD patients and enhances calcification of VSMC.

3 DETECTION AND QUANTIFICATION OF CALCIFICATIONS

3.1 Clinical Chemistry

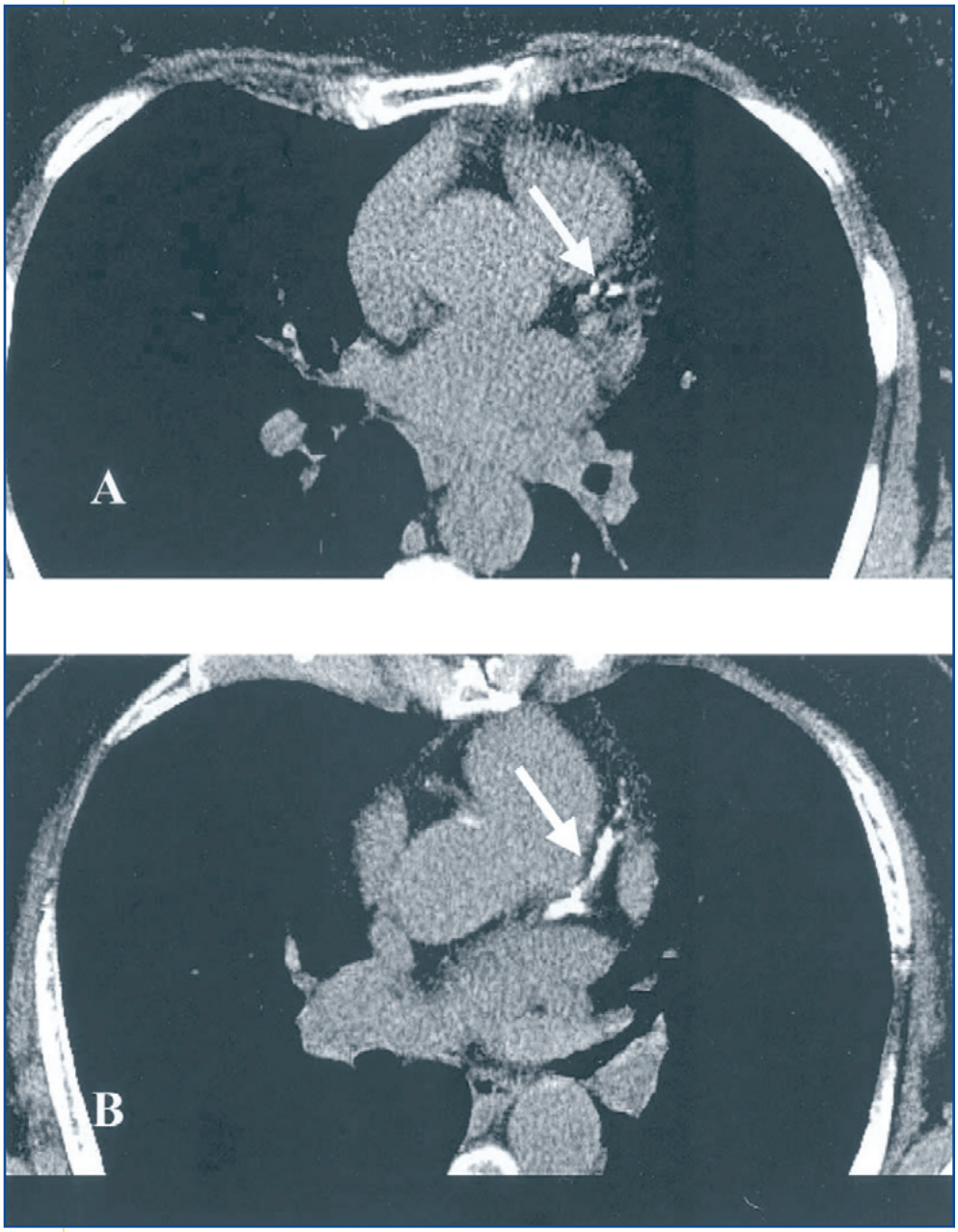
According to their role in the pathogenesis of vascular calcifications, several biomarkers have been proposed to predict either vascular calcifications or CV mortality. In *Table 1* we have compiled some of the most important trials on this topic in dialysis patients. However, interesting results have been recently published for CKD (non dialysis) patients [170;171].

Table 1. Studies evaluating biomarkers for vascular calcifications or CV mortality

First author	Ref.	Biomarker	Methods	Main results
Nitta K	172	OPG	Transversal study on 102 HD patients Calcification score by MSCT Patients classified in four groups according to their calcification score	OPG, CRP, iPTH and systolic blood pressure are significantly greater in patients with higher aortic calcification scores (but not CaxP product, albumin, total cholesterol, triglyceride and diastolic blood pressure).
Stenvinkel P	173	Fet-A	Prospective study on 258 ESRD patients shortly before starting renal replacement therapy	All-cause and cardiovascular mortality associated with low Fet-A levels independently of age, smoking, diabetes, albumin, cardiovascular diseases and inflammation
Hermans M	174	Fet-A	Cross-sectional study on 131 HD patients. Stiffness measured by pulse wave velocity or aortic augmentation index	Fet-A cannot be identified as an independent predictor of aortic stiffness in a population of HD patients with relatively low levels of inflammation activity
Shroff R	175	Fet-A OPG uc-MGP	Cross-sectional study 61 children on dialysis. uc-MGP, (not dp-uc-MGP) Carotid intima media thickness measured by B-mode ultrasound of both common arteries. Measure of pulse wave velocity. Calcification score measured by MSCT	Patients with calcifications had lower Fet-A and higher OPG than those without calcifications. Fet-A independently predicted aortic pulse wave velocity. Fet-A and OPG predicted cardiac calcification. No correlation with uc-MGP and clinic or vascular scores
Guttierez O	27	FGF-23	Prospective cohort of 10.044 patients who were starting HD. In this cohort, nested case-controls study of sample of 200 individuals who died versus 200 survivors in the first year of HD treatment	FGF-23 is significantly higher in cases versus controls and is also associated with higher risk of mortality among patients who are starting HD treatment. Serum phosphate in the higher quantile (>1.8 mmol/L) associated with a 20% increase in the multivariable adjusted risk of death, as compared with normal levels (1.1 to 1.4 mmol/L).
Jean G	34	FGF-23 OPG	219 HD patients	2-year mortality rate significantly higher for HD patients with FGF-23 in the higher quartile versus first quartile. OPG assayed but not discussed
Cianciolo G	115	OPG MGP iFGF-23 OPG	Transversal study in 253 HD patients MSCT	OPG is an independent predictor of coronary artery calcification; MGP is a protective factor. Fet-A and CAC associated in univariate, but not multivariate analysis (due to low grade inflammation found in the study?). FGF-23 showed a significant inverse correlation with CAC in univariate analysis, but is not in an independent predictive factor for CAC in multivariate analysis.
Morena M	103	OPG	185 HD patients followed for 2 years	OPG is a strong predictor of mortality
Schurgers L	176	dp-uc-MGP	Prospective study in 107 HD patients MSCT and lateral X-ray radiography of abdominal aorta	Dp-uc-MGP is positively and independently associated with aortic calcification score
Schlieper G	31	Fet-A Uc-MGP	Prospective study in 212 HD patients Calcification of vascular access assessed by plan X-Ray. Calcification of the aortic and mitral heart valves was performed by electrocardiography. Carotid intima-media thickness performed by untrasonography	Male gender, diabetes mellitus and dialysis vintage are independent risk factors for vascular access calcification, but neither biochemical parameters (ca, P, CaxP, PTH, Fet-A or ucMGP) nor age. The presence of vascular access calcification is an important risk factor for mortality.
Ketteler M	135	Fet-A	Cross-sectional study in 312 HD patients Coronary-artery and vascular calcification assessed by MSCT	Fet-A is lower in HD patients than in healthy controls. This is associated with vascular calcification, raised amounts of CRP and enhanced cardiovascular and all-cause mortality
O'Neill WC	177	PPi	Transversal study in 54 HD, 23 peritoneal dialysis and 38 patients with stage 4 CKD Uc-MGP	Plasma PPi is negatively associated with vascular calcification in ESRD and CKD but is not affected by dialysis mode, inflammation and nutritional status.
Nasrallah MM	178	FGF-23	Transversal study in 65 non diabetic HD patients (46 prevalent and 19 incidents) Aortic calcification index assessed by abdominal aorta CT scan	FGF-23 is independently correlated to aortic calcification.

Even though the results observed in these studies are important, one should admit that they are sometimes contradictory. The discrepancies could be linked to several factors: the use of different assays and different techniques to evaluate the calcification scores and the presence of different study designs

Figure 7. Electron-beam computed tomography (EBCT) of the coronary artery. A. Calcification in middle left coronary artery (68-year-old female volunteer). B. Extensive calcification in middle and distal left anterior coronary artery (70-year-old women with ESRD) ***(Raggi et al. J Am Coll Cardiol 2002, 39, 695-701).***



3.2 Imaging

3.2.1 Electron-beam computed tomography (EBCT) and multislice CT-Scan (MSCT)

EBCT and MSCT are often considered as the reference methods for detecting and quantifying vascular (and especially coronary) calcifications [23;179-181] *(Figure 7)*. EBCT uses a gun of electrons to generate a beam focused on a tungsten ring target. The beam then sweeps from side to side along the tungsten ring generating a fan of X-rays. This allows for excellent temporal resolution [182]. EBCT was the first CT scanner with sufficient time resolution to image the moving heart [181]. *Braun et al.* were the first authors to study coronary calcifications with EBCT in dialysis patients [20]. Quantification of calcifications is based on a score published by *Agatston et al.* [183]. Electrocardiographically triggered slices (ECG gating) with a distance of 3 mm are made usually starting approximately 2 cm below the carina and extending to the inferior margin of the heart [16;182]. The "calcium score" was determined by multiplying the area of calcification by a weighted density score: 1=130 to 199 Hounsfield units (HU), 2=200 to 299 HU, 3=300 to 399 HU and 4>400 HU. Individual scores are calculated for the left main coronary artery, the descending branch of the left coronary artery, the circumflex branch of the left coronary artery and the right coronary artery. The total coronary score is the sum of these individual scores [20;183]. *Sensu stricto*, this score must be considered as semi-quantitative because the calcification areas are to be obtained, "by hand", by radiologists. Nevertheless, compared to the other semi-quantitative scores with simple X-ray radiography (see below), the scores obtained with CT scans are obviously the most quantitative.

In the Agatston study, the inter-observers difference between scores was 2.5±5.5% [183]. Still in the normal population, the good intra- and interobserver variability was confirmed thereafter (5-8 %) [184;185]. Maybe more intriguing is the reproducibility of coronary calcifications observed in some studies (two dif-

ferent scores at two different times in the same patient by the same observer) [185]: 7.2% [184], from 28% (for scores > 100) to 72% (for scores < 10) [186] (the higher the "calcium score", the lower the variability, which is important in dialysis patients with high scores in the majority) and 35% [187]. So, interpreting EBCT results may not be easy in longitudinal studies. Comparing to EBCT, MSCT technology is well known and more available. Moreover, its use is not limited to the quantification of vascular calcifications [182]. Using both multi-detector row CT data and volumetric score (relying on isotropic interpolation for better quantification) will improve precision and reproducibility [182;185;187-189]. The data regarding MSCT reproducibility are relatively poor, especially in dialysis patients. One study with 50 non-dialysis subjects described a mean variability of 12% with the Agatston score and of 7.5% with the volumetric score [189]. One study has specifically studied variability of calcium score measures by MSCT in 15 dialysis patients. The mean intra-observer variability was 0.9% after correction for the lowest (< 10) Agatston scores (27% if the 3 scores below 10 are included) [182].
Besides coronary calcifications, valves and aortic calcification can also be detected and quantified by EBCT or MSCT [20]. However, the quantification methodology, although based on the Agatston score, is less established and systematized than for the quantification of coronary artery calcifications [23;182].
If these methods seem the best, both to detect and quantify vascular calcifications, it does not allow making distinction between medial and intimal calcifications [2;21;181]. Moreover, these methods are relatively costly. EBCT is not easy available, at least in Europe [21;180;181]. We also have to keep in mind that the irradiation dose is not negligible, especially with MSCT (total irradiation dose is 3- to 4-fold higher in MSCT compared to EBCT [180]) [181]. These techniques are thus still not recommended in clinical practice and are eventually limited to clinical trials [190].

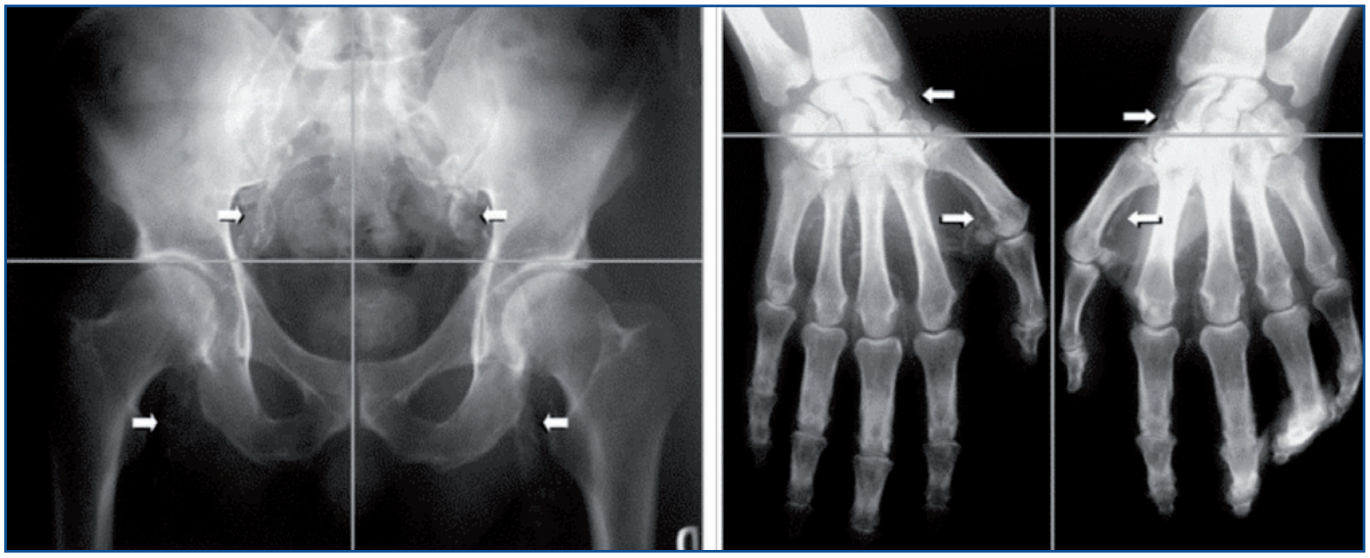
3.2.2 Ultrasonography

Echocardiography is a sensitive method for detecting cardiac valve calcification, although sensitivity seems less than for EBCT or MSCT [59]. This technology is widespread over the world, relatively cheap and free of ionizing radiation [21;45;180;181]. The quantification of calcification however is more problematic. Highly echogenic plaques producing bright white echoes with shadowing were considered to be calcifications. Some authors only differentiate patients with or without calcifications [58;59;191;192]. Interobserver variability is estimated at 4% [31].
Vascular ultrasonography has also been proposed by the London's team to semi-quantify calcifications [22;28]. Calcifications are researched in four sites: common carotid artery, abdominal aorta, iliofemoral axis and in the legs. Once again, highly echogenic plaques producing bright white echoes with shadowing were considered to be calcifications. The score for each site was 1 (calcification) or 0 (no calcifications) with a possible maximum score of 4. The score is very simple to obtain and among the 120 patients included in the study, only 5 have different scores when obtained by two different observers. With this methodology, the authors have well described the relationship between CV mortality and vascular calcifications [22]. Ultrasound technologies require a skilled and consistent operator. Moreover, data derived from ultrasound are qualitative. It is not known if this technique is sufficiently sensitive to track changes over time [180]. This technology theoretically permits the distinction between intima and media [2;5] but making distinction does not appear to be so simple [180;181]. One advantage for ultrasound is the possibility to give some additional "functional" parameters (like elasticity) and to study the uncalcified plaque [180;181].

3.2.3 Standard radiography

Standard radiography is cheap, accessible and poorly irradiating. However, the quantification remains, at best, semi-quantitative and observer-dependent. Comparing to EBCT and MSCT, the sensitivity is logically less performing. Different ways to apply standard radiography have been proposed. *London et al.* proposed pelvic and thigh radiography with patients in recumbent position [26]. Calcifications were then classified as discrete intimal-like plaques with irregular and patchy distribution or uniform linear railroad track-type in the media. The goal of these authors was to propose a simple method to distinguish intimal and medial calcifications. Two different observers analyzed the 202 patients included and the interobserver concordance was 92%. In this article, the authors showed higher mortality linked to intimal calcification [26].
Although cheap, these calcification scores were not quantitative, so limiting its interest, notably in trials studying the impact of therapies on vascular calcifications [26;180].
Okuno et al. proposed a very simple score only based on the presence (or not) of aorta calcifications observed on lateral abdomen radiography (at levels of the first four lumbar vertebrae). The presence of calcification was predictive of CV mortality in this cohort of 515 dialysis patients [33].
In 2004, *Adragao et al.* proposed a simple calcification score based on plain radiography of pelvis and hands. The pelvic radiography is divided into four sections by two imaginary lines (horizontal one over the upper limit of both femoral heads and a vertical one through the median of the vertebral column). Hand radiographies are divided by a horizontal line over the upper limit of the metacarpal bones. The presence of linear calcification (no distinction done between medial and intimal calcifications) is counted as one in each section and the

Figure 8. Plain radiography of pelvis and hands. A. Calcification score is the sum of the presence (1) or absence (0) of parallel linear calcifications in each section. In this example, pelvis score = 1 + 1 + 1 + 1 = 4. B. Hands score in this example is 4; total score is the sum of pelvis and hands score *(Adragao et al. Nephrol Dial Transplant, 2004, 19, 1480-1488).*

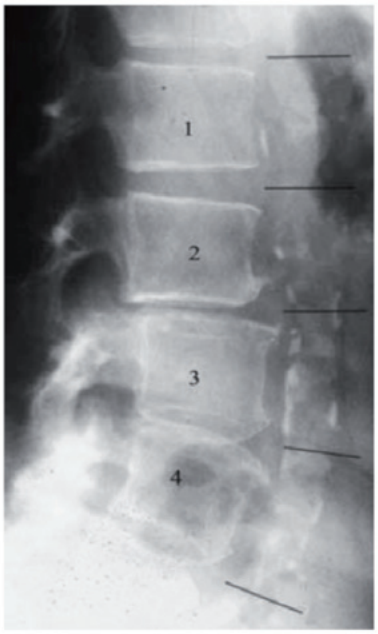


maximum score is thus 8 *(Figure 8)* [32]. With this technique, the authors found a statistically higher CV mortality for patients with scores higher than 3 [32]. These results will be confirmed in 2009 [35].

In 2009, *Jean et al.* proposed another semi quantitative score (1 to 3) based on 8 plain radiographies (front pelvis, profile lumbar and knee, right hand and arm, chest, skull and orthopantogram). Score 1 is associated with light aortic or iliac calcification, score 2 with major aortic and iliac and femoral calcifications and score 3 with severe diffuse aortic, iliac, femoral, popliteal and arm calcifications. In this study, the authors found significantly higher 1 year mortality for score 3 compared to score 1 [34].

In 2008, *Schlieper et al.* proposed simple plain radiogra-

Figure 9. Abdominal aorta calcification (AAC) scoring. Grading of calcification was assessed at the anterior and the posterior walls of the abdominal aorta adjacent to vertebrae L1-L4 and the composite score determined *(Honkanen et al. Nephrol Dial Transplantation, 2008, 23, 4009-4015).*



GRADING

0: no calcific deposits in front of the vertebra

1: small scattered calcific deposits filling less than 1/3 of the longitudinal wall of the aorta

2: 1/3 – 2/3 of the wall calcified

3 : 2/3 or more of the wall calcified

Level	Affected segment	Scores for individual segments		Composite score (AAC)
		Posterior Wall Range 0-3	Anterior Wall Range 0-3	Anterior-Posterior severity Range 0-6
L1	1	1	0	1
L2	1	2	1	3
L3	1	3	2	5
L4	1	3	3	6
Total	4	9	6	15
Maximum	4	12	12	24

phy of the vascular access to assess the presence of calcification (or not). This score interestingly and independently predicts mortality [31]. In all these studies, only one observer calculates the scores and we have thus no idea of intra- and interobservers variability [31-35].
A more "quantitative" and interesting method consists of a lateral X-ray of the lumbar abdominal aorta [21;191;192]. This technique has been proposed for the general population by *Kaupilla et al.* in 1997. This "calcium score" has been shown to be predictive for CV morbidity and mortality in the general population [193;194]. A lateral X-ray is obtained that includes the last two thoracic and the first two sacral vertebrae. The aorta is identified as the tubular structure coursing in front of the anterior surface of the spine. Only the segments of the aorta in front of the first to the fourth lumbar vertebra are considered. Points are assigned from 1 to 3 according to the length of each calcified plaque along the anterior and the posterior profile of the aorta in front of each vertebra. The score could theoretically vary to a maximum of 24 [193] *(Figure 9)* [39]. This technique has been used in a large epidemiological trial about vascular calcifications (the CORD study) including 933 dialysis patients [39]. The calcium score has been calculated in 64 patients by two different observers with an excellent interobserver agreement (χ=0.9). This technique was also used by the London's group in the trial studying correlation between vascular calcification, histomorphometry and calcium load [95].
Both lateral X-ray of the lumbar aorta and echocardiography have been shown to correlate well with EBCT results [191;192]. However, the ability of this combination to predict EBCT coronary calcification is less impressive [191;192]. Additional studies seem necessary before concluding about the interest of abdominal X-ray, notably in clinical practice.

In summary, several biomarkers (OPG, OPN, Fet-A, MGP, uc-MGP, dp-uc-MPG, FGF-23, PPi) have been proposed to predict either vascular calcifications or CV mortality. EBCT and MSCT are currently regarded as the most sensitive methods for detection and quantification of vascular and especially coronary calcifications. However, they do not allow making distinction between medial and intimal calcifications. Moreover, they are costly and the irradiation dose, especially with MSCT is not negligible. As such, these methods are limited to clinical studies. The following methods are cheaper but less sensitive and less quantitative. Echocardiography is used for the detection of cardiac valve calcification. Vascular ultrasonography has been proposed to semi-quantify calcifications. Standard radiography is a more simple method that allows distinguishing intimal and medial calcifications. However, the calcification scores are generally not very quantitative. In 2004, a simple calcification score (1 to 8) based on plain radiography of pelvis and hands and in 2009, a semi quantitative score (1 to 3) based on 8 plain radiographies have been proposed. A more "quantitative" method is lateral X-ray of the lumbar abdominal aorta (score 1 to 24). Both, echocardiography and lateral X-ray of the lumbar aorta have been shown to correlate well with EBCT results. Additional studies are needed.

4 IMPACT OF CKD-MBD TREATMENT OPTIONS

4.1 K-DOQI and KDIGO guidelines

In August 2009, the new Kidney Disease: Improving Global Outcome (KDIGO) guidelines [190] replaced the former Kidney Disease Outcomes Quality Initiative (K-DOQI) guidelines [195;196]. In these new guidelines, a complete chapter is dealing with vascular calcification. Other changes with a potential direct or indirect impact on vascular calcifications are also summarized in *Table 2*.

4.2 Role of phosphate binders

In epidemiological studies, phosphorus is one of the most important mineral parameters associated with CV mortality in dialysis patients [9-11;197]. Moreover, in *in vitro* or animal models, high phosphate and calcium concentrations are often required to induce vascular calcifications [82;89;198]. So, the impact of phosphate lowering therapies on vascular calcifications is of interest. This is especially the case for phosphate lowering therapies that contain no calcium. Actually, calcium-based phosphate binders have been "accused" to participate to the occurrence and progression of vascular calcifications in several (but not all) trials [16;18;22;26;44;94]. Moreover, non calcium-based chelators (i.e. sevelamer) have been shown to prevent ectopic calcifications (and atherosclerosis) in animal models [199;200]. Four important randomised trials have been published comparing the effect of calcium-based phosphate binders versus sevelamer on the development of vascular calcifications: the Treat to Goal [41], the RIND [30], the BRIC [201] and CARE-2 trials [202]. The conclusions of these studies are contradictory (two favouring for sevelamer [30;41] and two showing no difference between sevelamer or calcium-based phosphate binders [201;202]). *Sensu stricto*, due to differences in method-

Table 2. Summary of KDOQI and KDIGO guidelines with potential impact on vascular calcifications

Parameter	K-DOQI (195;196)	KDIGO (190)
Imaging	Bone radiographs are not indicated for the assessment of bone disease of CKD (EVIDENCE), but they are useful in detecting several peripheral valvular calcifications (OPINION). New techniques, like electron beam computed tomography will likely become standard tools to monitor valvular calcification and its therapy.	In patients with CKD stage 3-5D, we suggest that a lateral abdominal radiograph can be used to detect the presence or absence of vascular calcification, and an echocardiography can be used to detect the presence or absence of valvular calcification, as reasonable alternatives to computed tomography based imaging (2C)
Phosphorus	In CKD patients at stage 5 and those treated with hemodialysis or peritoneal dialysis, serum levels of phosphorus should be maintained between 1.13 to 1.78 mmol/L (EVIDENCE)	In patients with CKD stage 5D, we suggest lowering elevated phosphorus levels towards the normal range (2C)
Calcium	In the normal range of the laboratory (but for CKD stage 5, preferably toward the lower end, 2.10-2.37 mmol/L)	In the normal range of the laboratory (2C)
CaXP product	<55 mg^2/dL^2 (EVIDENCE)	In patients with CKD stages 3–5D, we suggest that individual values of serum calcium and phosphorus, evaluated together, be used to guide clinical practice rather than the mathematical construct of calcium–phosphorus product (2D).
PTH	150-300 pg/ml (EVIDENCE)	In patients with CKD stage 5D, we suggest maintaining PTH levels in the range of 2x-9x upper normal limit for the assay (2C). Marked changes in PTH levels in either direction within this range prompt an initiation or change in therapy to avoid progression to levels outside of this range (2C).
Alkaline phosphatase	No recommendation	We recommend monitoring serum levels of calcium, phosphorus, PTH, and alkaline phosphatase activity beginning in CKD stage 3 (1C). In patients with CKD stages 3–5D, we suggest that measurements of serum PTH or bone-specific alkaline phosphatase can be used to evaluate bone disease because markedly high or low values predict underlying bone turnover (2B).
Native Vitamin D	No recommendation	In patients with CKD stages 3–5D, we suggest that 25(OH)D (calcidiol) levels might be measured, and repeated testing determined by baseline values and therapeutic interventions (2C). We suggest that vitamin D deficiency and insufficiency be corrected using treatment strategies recommended for the general population (2C).

ologies and protocols, these four studies are difficult to compare. The fact that sevelamer also decreases LDL plasma levels could also explain the positive effect on cardiac calcifications [185;203;204]. So, polemic between supporters of calcium-based versus non calcium-based phosphate binders is ferocious and not closed today [1;93;205-208]. However, one open label trial comparing sevelamer with calcium-based phosphate binders in 2013 dialysis patients found no difference in overall and CV mortality except in a subgroup analysis of overall mortality for patients over 65 years [209].
However, it seems reasonable to avoid hypercalcemia potentially induced by calcium-based phosphate binders [210].

4.3 Role of vitamin(s)

The role of vitamin D in the pathogenesis of vascular calcifications is difficult to evaluate [54]. Actually, both the vitamin D receptor and the 1α-hydroxylase are expressed in VSMC and cardiac myocytes [86]. It also seems evident from animal and *in vitro* studies that high doses of active vitamin D will induce or favour vascular calcifications [54;63;211-216]. We have already talked about the role of vitamin D in FGF-23 knock-out mice (see above) [137;144-146]. However, it is not clear if this vascular calcification development is dependent, or not, on vitamin D-induced hyperphosphatemia and/or hypercalcemia [54;86;212-214;217]. Moreover, low "physio-

logical" doses of active vitamin D seem to inhibit vascular calcification in some (but not all [216;218]) animal models [219]. The dose [216;218;219] and the type of active vitamin D (calcitriol or analogs) could be of some importance [86;217]. Large studies, although retrospective, have also shown that active vitamin D therapies could be associated with a reduction in mortality [220-222].

The role of native vitamin D could be different from active vitamin D in the vascular calcifications context. One recent retrospective study showed that patients (both CKD and non CKD were included) with low serum 25-OH-vitamin D were more likely to develop coronary artery calcifications [223]. *Wolf et al.* had already shown that lower baseline 25-OH vitamin D levels were significantly associated with increased mortality in a cohort of 825 incident dialysis patients but there was no data neither on CV mortality nor on vascular calcifications [224]. In a sample of 52 dialysis patients, *London et al.* did not find any correlation between 25-OH vitamin D levels and calcification scores (ultrasonography and X-ray radiography [22]) but 25-OH vitamin D was negatively correlated with aortic pulse wave velocity [225]. In 233 dialysis patients (47 % treated with low doses of active vitamin D), *Matias et al.* described an association between 25-OH vitamin D levels and calcification scores [32] in multivariate analysis [226].

Additional and interventional studies seem necessary before concluding about the role of active and native vitamin D on vascular calcifications.

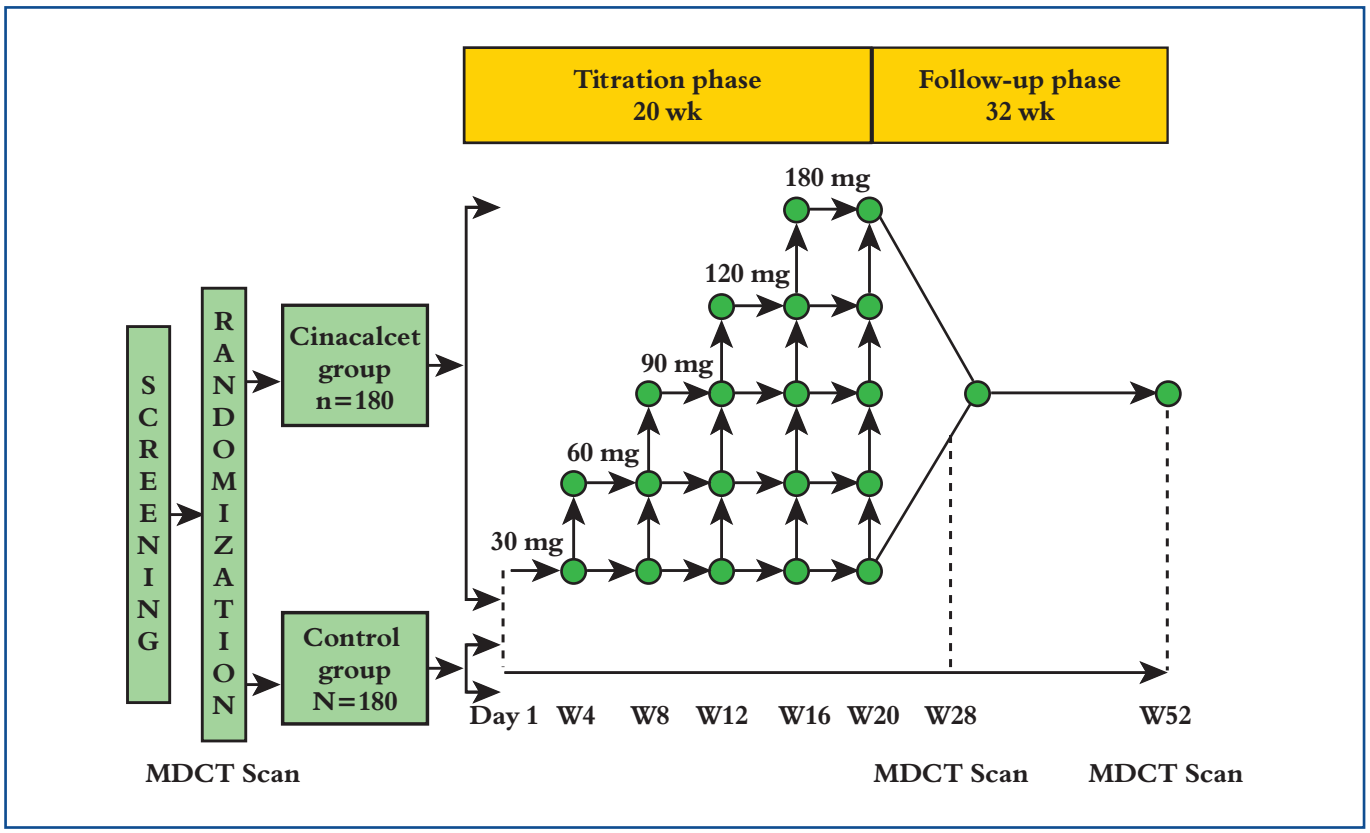
In conclusion, the use of vitamin D must be reasonable. Yet, this therapy must induce neither hypercalcemia nor hyperphosphatemia. It could be also of interest not to induce low bone turnover with these therapies (see above) [67;94].

4.4 Role of calcimimetics

Calcimimetics (such as cinacalcet, Mimpara®) form a new therapeutic class that modulates the calcium sensing receptor (CaR) in the parathyroid glands in order to diminish PTH secretion [227;228]. Contrary to other therapies for sHPT (such as vitamin D), cinacalcet simultaneously reduces PTH, serum calcium and phosphorus. Moreover, very high PTH levels have been proposed as predictive of coronary calcifications in dialysis patients [12]. Lastly, it has been shown that CaR is present in human VSMC and is probably down regulated in VSMC from dialysis patients which could be related to the process of calcification [229-231]. So, the potential interest of cinacalcet for the treatment of vascular calcification does well exist. Several *in vitro* and animal studies have actually underlined the role of calcimimetics in such a context [230]. The *in vitro* study published by *Alam et al.* suggested that calcimimetics may inhibit the development of VSMC calcifications [229]. One study in a CKD rat model has shown that both calcimimetic and calcitriol reduce PTH secretion. The effects on serum calcium were logically opposite. Rats treated with calcitriol developed aorta calcifications although rats treated with calcimimetics did not. Maybe the most interesting are the results on rats treated with combined therapy (calcimimetic and calcitriol). Indeed, the hypercalcemia but not the calcifications are prevented by such a combination [212]. The same authors confirmed these results in another rat model with lower vitamin D doses [218]. These results will be confirmed by other authors with the difference that combination therapy here leads to lower vascular calcifications than under calcitriol therapy alone (but higher than with a calcimimetic alone) [232]. Another author using a model of CKD rats with high phosphate diet (thus without vitamin D) showed that vascular and cardiac calcifications could be prevented by both calcimimetic and parathyroidectomy [233]. In another model of uremia-enhanced vascular calcifications (apoE knock-out mice), *Ivanovski et al.* confirmed that calcimimetics reduce the progression of vascular calcifications both in the intima and media. They also showed *in vitro* the direct inhibitory effect on the process of both vascular calcification and atherosclerosis. This last observation was unexpected and mechanisms are unknown [234]. *Moe et al.* studied the effect of calcium (in the diet) and calcimimetics on vascular calcification occurrence. If both calcium and calcimimetics show efficacy in lowering PTH levels, both the combination (calcium+calcimimetics) and the calcium only group will favour for cardiac and vascular calcifications whereas the group only treated with calcimimetic will show benefit on extraosseous calcifications [235]. *Koleganova et al.* also showed that calcitriol (with non hypercalcemic doses) increases medial calcification and proliferation of VSMC compared to calcimimetics [216].

In humans, several case reports have been published suggesting the potential benefit of cinacalcet in calciphylaxis [67;236-238]. A retrospective analysis of four randomised, double blind and placebo controlled clinical trials in patients with secondary hyperparathyroidism suggested cardiovascular benefit with cinacalcet. Indeed, this treatment led to a 39 % risk reduction in CV events leading to hospitalization (infarctus, unstable angina or heart failure). There was no effect on global mortality [239]. Recently, a prospectively designed observational study, including 19186 patients followed from November 2004 up to 26 months, found a significant all-cause CV survival benefit that was associated with cinacalcet prescription in hemodialysis patients receiving IV Vitamin D [240]. One limited Japanese, not randomised, not placebo controlled trial including 8 patients treated with cinacalcet and 60 controls suggested that cinacalcet is effective in preventing the progression of coronary calcifications [241]. Regarding this topic, the definitive results of the ADVANCE trial are awaited with impatience. In this randomised study, 360 hemodialysis subjects were treated for 52 weeks with cinacalcet and low doses of active vitamin D

Figure 10. ADVANCE study: study design, treatment schema and inclusion criteria. MDCT = multi-detector computed tomography *(Floege et al. Nephrol Dial Transplantation, 2010, 25, 1916-1923).*



- On hemodialysis for ≥ 3 months
- iPTH > 300 pg/mL (31.8 pmol/L) OR
- iPTH ≥ 150 pg/mL and ≤ 300 pg/mL (15.9 – 31.8 pmol/L)
  — *and* receiving treatment with vitamin D analogs at time of PTH assessment
  — *and* corrected serum Ca x P > 50 mg^2/dL^2 (3.9 $mmol^2/L^2$)
- Corrected serum Ca ≥ 8.4 mg/dL (2.1 mmol/L)
- Screening CAC score ≥ 30
  — Subjects were subsequently stratified: ≥ 30–399, ≥ 400–999, and ≥ 1000

(for example, calcitriol at 0.125 μg/d) in one group and with variable doses of active vitamin D in the other group. Study design and inclusion criteria are presented in *Figure 10* [40]. To avoid interferences in calcification progression, all patients were treated with calcium-based chelators and lipid lowering therapy should not be initiated. The primary objective was the progression of coronary calcifications measured by MSCT. Secondary objectives are resumed in *Figure 11*. Preliminary results didn't show any statistical difference for the primary objective (24% CAC progression in the cinacalcet group versus 31% in the flexible Vitamin D group; p = 0.073). However, after adjustment for the initial phosphorus concentrations (which were higher in the cinacalcet group), the difference in the primary objective was significant in favour of the cinacalcet group (+26% CAC progression versus +42%, p=0.031) [242]. Furthermore, a consistent trend toward less progression of CV calcification was observed at all sites evaluated in the cinacalcet group compared to the control group *(Figure 12)*. It has to be noted that the Vitamin D sterol levels in the arm with cinacalcet plus low dose Vitamin D remained continuously above the initially predefined low doses of Vitamin D sterols (e.g. at least 0.2 μg/d calcitriol instead of 0.125 μg/d). Although the ADVANCE results suggest that treatment with cinacalcet may attenuate the progression of vascular calcifications, only the ongoing EVOLVE trial could provide a conclusive answer to the question whether cinacalcet treatment improves CV outcome and mortality in dialysis patients compared to treatment without cinacalcet [243]. Finally, we will only cite some therapies recently proposed for vascular calcifications: vitamin K [244], sodium thiosulfate [245] and bisphosphonates [246]. Although of some interest in some case reports or even in preliminary trials, more trials seem necessary before clinical use.

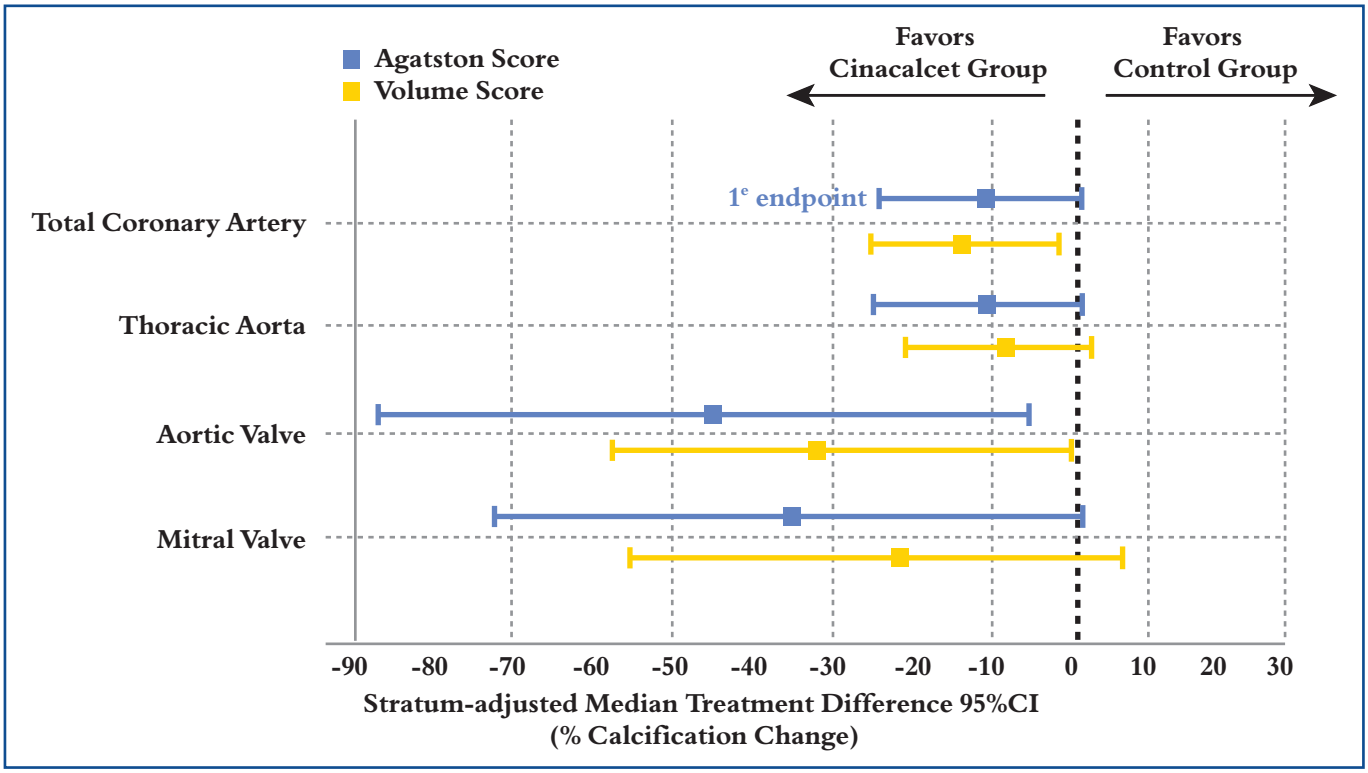
Figure 11. Secondary endpoints of the ADVANCE study *(Floege et al. Nephrol Dial Transplant, 2010, 25, 1916-1923).*

Secondary Endpoints

- Absolute change in CAC score at week 52
- Absolute and percentage change from baseline in
  - Aortic calcification at week 52
  - Aortic valve calcification at week 52
  - Laboratory parameters at end of study (weeks 44 through 52)
- Proportion of patients achieving >15% progression of CAC at week 52
- Safety

In summary, high phosphate is one of the key elements associated with vascular calcification and CV mortality in dialysis patients. Several randomised controlled trials compared the effects of calcium-containing phosphate binder therapies and sevelamer, a non-calcium based phosphate binder, on the development of vascular calcifications in CKD patients. Conclusions were contradictory (two favouring sevelamer, two showing no difference between sevelamer and calcium-based phosphate binder). An open label study in dialysis patients found only a difference in overall mortality in a subgroup of patients over 65 years. It is reasonable to avoid hypercalcemia potentially induced by calcium-based phosphate binders. Vitamin D has been considered a corner stone in sHPT treatment because decreases in vitamin D concentrations parallel increases in PTH as kidney function declines. However, the dose and the type of active vitamin D (calcitriol or analogs) could be of some importance in the contribution to vascular calcification. Studies examining the role of vitamin D on vascular calcifications have given contradictory results. What is clear is that the use of vitamin D must be reasonable and must induce neither hypercalcemia nor hyperphosphatemia. Moreover, low-bone turnover should not be induced. In vitro and animal studies revealed that the use of calcimimetics reduced calcifications in these models. Preliminary results from the ADVANCE trial suggest that a cinacalcet-based treatment regimen might attenuate the progression of CV calcifications in sHPT patients. Another randomised trial, the EVOLVE study, will evaluate the effect of cinacalcet on CV events and mortality in patients with sHPT receiving dialysis. Some therapies such as vitamin K, sodium thiosulfate and bisphosphonates have been recently proposed for vascular calcifications but need to be assessed in more trials before clinical use.

Figure 12. ADVANCE study: median treatment differences, all sites *(Raggi et al. Poster presented at the 2010 Clinical Meeting of the National Kidney Foundation, Orlando, FL, April 13-17, 2010).*



CONCLUSIONS 5

Vascular calcification, a recognized systemic complication of CKD, has become an important surrogate marker of high CV mortality. Its pathogenesis is complex. Many improvements have been made in this field for understanding the mechanisms of medial calcifications.
More trials are needed before proposing efficient preventive measures and therapeutic tools.

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* Mimpara® plus low-dose vitamin D and phosphate binders, if prescribed. Superior biomarker control is versus a regimen without Mimpara®.[5]

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MIMPARA® **Name of the medicinal product:** MIMPARA® 30 mg, 60 mg and 90 mg film-coated tablets. **Qualitative and quantitative composition:** MIMPARA®30 mg: Each tablet contains 30 mg cinacalcet (as hydrochloride). MIMPARA® 60 mg: Each tablet contains 60 mg cinacalcet (as hydrochloride). MIMPARA® 90 mg: Each tablet contains 90 mg cinacalcet (as hydrochloride). Excipients: Each 30 mg tablet contains 2.74 mg of lactose. Each 60 mg tablet contains 5.47 mg of lactose. Each 90 mg tablet contains 8.21 mg of lactose.Tablet Core: Pre-gelatinised starch (maize), Microcrystalline cellulose, Povidone, Crospovidone, Magnesium stearate, Colloidal anhydrous silica. Tablet Coat: Carnauba Wax, Opadry II green: (Lactose monohydrate, hypromellose, titanium dioxide (E171), glycerol triacetate, FD&C Blue (E132), iron oxide yellow (E172)), Opadry clear: (Hypromellose, macrogol). **Therapeutic indications:** Treatment of secondary hyperparathyroidism (HPT) in patients with end-stage renal disease (ESRD) on maintenance dialysis therapy. Mimpara may be used as part of a therapeutic regimen including phosphate binders and/or Vitamin D sterols, as appropriate. Reduction of hypercalcaemia in patients with: parathyroid carcinoma, primary HPT for whom parathyroidectomy would be indicated on the basis of serum calcium levels (as defined by relevant treatment guidelines), but in whom parathyroidectomy is not clinically appropriate or is contraindicated. **Posology and method of administration: Secondary hyperparathyroidism:** Adults and elderly (> 65 years): The recommended starting dose for adults is 30 mg once per day. Mimpara should be titrated every 2 to 4 weeks to a maximum dose of 180 mg once daily to achieve a target parathyroid hormone (PTH) in dialysis patients of between 150-300 pg/ml (15.9-31.8 pmol/l) in the intact PTH (iPTH) assay. PTH levels should be assessed at least 12 hours after dosing with Mimpara. Reference should be made to current treatment guidelines. PTH should be measured 1 to 4 weeks after initiation or dose adjustment of Mimpara. PTH should be monitored approximately every 1-3 months during maintenance. Either the intact PTH (iPTH) or bio-intact PTH (biPTH) may be used to measure PTH levels; treatment with Mimpara does not alter the relationship between iPTH and biPTH. During dose titration, serum calcium levels should be monitored frequently, and within 1 week of initiation or dose adjustment of Mimpara. Once the maintenance dose has been established, serum calcium should be measured approximately monthly. If serum calcium levels decrease below the normal range, appropriate steps should be taken, including adjustment of concomitant therapy. Children and adolescents: Mimpara is not indicated for use in children and adolescents due to a lack of data on safety and efficacy. **Parathyroid carcinoma and primary hyperparathyroidism:** Adults and elderly (> 65 years): The recommended starting dose of Mimpara for adults is 30 mg twice per day. The dose of Mimpara should be titrated every 2 to 4 weeks through sequential doses of 30 mg twice daily, 60 mg twice daily, 90 mg twice daily, and 90 mg three or four times daily as necessary to reduce serum calcium concentration to or below the upper limit of normal. The maximum dose used in clinical trials was 90 mg four times daily. Serum calcium should be measured within 1 week after initiation or dose adjustment of Mimpara. Once maintenance dose levels have been established, serum calcium should be measured every 2 to 3 months. After titration to the maximum dose of Mimpara, serum calcium should be periodically monitored; if clinically relevant reductions in serum calcium are not maintained, discontinuation of Mimpara therapy should be considered. Children and adolescents: Mimpara is not indicated for use in children and adolescents due to a lack of data on safety and efficacy. **Hepatic impairment:** No change in starting dose is necessary. Mimpara should be used with caution in patients with moderate to severe hepatic impairment and treatment should be closely monitored during dose titration and continued treatment. **Method of administration:** For oral use. It is recommended that Mimpara be taken with food or shortly after a meal, as studies have shown that bioavailability of cinacalcet is increased when taken with food. Tablets should be taken whole and not divided. **Contraindications**: Hypersensitivity to the active substance or to any of the excipients. **Undesirable effects: Secondary hyperparathyroidism:** Data presented from controlled studies include 656 patients who received Mimpara and 470 patients who received placebo for up to 6 months. The most commonly reported adverse reactions were nausea and vomiting, occurring in 31% Mimpara and 19% placebo treated patients, and 27% Mimpara and 15% placebo treated patients, respectively. Nausea and vomiting were mild to moderate in severity and transient in nature in the majority of patients. Discontinuation of therapy as a result of undesirable effects was mainly due to nausea (1% placebo; 5% cinacalcet) and vomiting (< 1% placebo; 4% cinacalcet). Adverse reactions, considered at least possibly attributable to cinacalcet treatment based on best-evidence assessment of causality and reported in excess of placebo in double-blind clinical studies are listed below using the following convention: very common (> 1/10); common (> 1/100 to < 1/10); uncommon (> 1/1,000 to < 1/100); rare (> 1/10,000 to < 1/1,000); very rare (< 1/10,000). *Immune system disorders*: Uncommon: hypersensitivity reactions. *Metabolism and nutrition disorders:* Common: anorexia. *Nervous system disorders*: Common: dizziness, paraesthesia. Uncommon: seizures. *Gastrointestinal disorders*: Very common: nausea, vomiting. Uncommon: dyspepsia, diarrhoea. *Skin and subcutaneous tissue disorders*: Common: rash. *Musculoskeletal, connective tissue and bone disorders:* Common: myalgia. *General disorders and administration site conditions:* Common: asthenia. *Investigations:* Common: hypocalcaemia, reduced testosterone levels. **Parathyroid carcinoma and primary hyperparathyroidism:** The safety profile of Mimpara in these patient populations is generally consistent with that seen in patients with Chronic Kidney Disease. The most frequent ADRs in these patient populations were nausea and vomiting. **Post-marketing experience:** The following adverse reactions have been identified during postmarketing use of Mimpara, the frequencies of which cannot be estimated from available data: There have been reports of isolated, idiosyncratic cases of hypotension and/or worsening heart failure in cinacalcet-treated patients with impaired cardiac function in post marketing safety surveillance. Allergic reactions, including angioedema and urticaria. **Marketing authorisation holder:** Amgen Europe B.V., Minervum 7061, NL-4817 ZK Breda, The Netherlands. **Marketing authorisation numbers:** EU/1/04/292/001-012. **Date of first authorisation:** 22 October 2004. Date of renewal of the authorisation: 23 September 2009. **Date of revision of the text:** 31 August 2009. **Classification of the medicine:** Medicinal product subject to restricted non-renewable medical prescription **More information available at:** Amgen n.v/s.a; Arianelaan, 5, Avenue Ariane, B-1200 Brussel-Bruxelles, tel: 02/775 27 11.

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