

Mineral and bone disorder and vascular calcification in patients with chronic kidney disease

Authors

Luis Alberto Batista Peres^{1,2}

Pedro Paulo Verona Périco^{1,2}

¹ State University of Western Paraná - UNIOESTE.

² Gurgacz Assisi Foundation.

ABSTRACT

Vascular calcifications has been associated with bone and mineral disorders. The alterations in the serum level of calcium concentrations and phosphate are important factors implicated in the arterial calcification in chronic kidney disease. The pathogenesis of vascular calcification is a complex mechanism and not completely clear, being able to correspond to an active process of cellular transformation and heterotopic ossification. Beyond the hypercalcemia and hyperphosphatemia, they are involved in this process changes in the metabolism of inhibitors and promoters of calcification such as fetuin A, osteopontin, osteoprotegerin, and matrix gla protein. For the diagnosis of the calcified arterial injury are available several complementary methods, a method of estimate of the cardiovascular risk based on plain radiographs of the lumbar column and another method based on simple x-rays of the pelvis and hands. Below, we will present a review approaching the link between vascular calcifications and mineral disorders.

Keywords: bone diseases, metabolic; cardiovascular abnormalities; renal insufficiency, chronic.

INITIAL REMARKS

Increasing evidence has suggested that bone metabolism disorders (BMD) in chronic kidney disease (CKD) are associated with cardiovascular calcification, which is a major cause of death; however, the mechanisms of such association are not completely known.¹ High serum concentrations of calcium and phosphorus are considered to be major contributors to arterial calcification.² In bone remodeling disorders, such as increased calcium

and phosphorus absorption from the bone tissue into the blood stream; or in low remodeling - in which the bone loses the ability to buffer these minerals and not incorporating them into its tissue - leading to a serum overload, which favors extraosseous calcifications, including vascular calcification (VC).^{3,4}

CKD patients are 20 times more likely to die from cardiovascular causes when progressing to stage five - a stage in which the patient is about to start or already is in renal replacement therapy.⁵ Go *et al.*⁶ found, in a nonlinear relationship, an increased risk of death, cardiovascular events and hospitalization when the glomerular filtration rate (GFR) was lower than 60 ml/min/1.73 m², being even higher at GFR lower than 45 ml/min/1.73 m². Patients in stages 4 and 5, without dialysis, have mortality rates of 11.4% to 14.1% per year.

Coronary artery calcification (CAC) is more prevalent and more severe in CKD patients than in the general population. More than 50% of CKD patients not on dialysis treatment and 70% to 90% of patients on dialysis have significant CAC.⁷

PATHOPHYSIOLOGY OF VASCULAR CALCIFICATION

There are two VC patterns: atherosclerosis and arteriosclerosis. Atherosclerosis occurs in the intima layer of the artery and is associated with inflammation, the development of atheromatous plaque, resulting in an occlusive lesion. It is localized and the vessel areas adjacent to the plaque remain normal. In turn, arteriosclerosis involves the tunica media layer

Submitted on: 12/08/2012.

Approved on: 02/07/2013.

Correspondence to:

Luis Alberto Batista Peres.
UNIOESTE - State University
of Western Paraná - Campus
Cascavel
Rua Vicente Machado, nº 2687,
Jardim Vitória, Cascavel, PR,
Brazil. CEP: 85813-250.
E-mail: peres@certto.com.br

DOI: 10.5935/0101-2800.20140031

of the artery and is characterized by the diffuse deposition of mineral throughout the vessel. It may be considered a physiological phenomenon of aging. Its effects are dilation, diffuse thickening and hardening of the vessel, causing hemodynamic changes such as isolated increase in systolic pressure and/or diastolic pressure reduction, thus increasing pulse pressure.^{8,9} The tunica media layer involvement has been classically described as Monckeberg calcification, and it is the most common pattern in CKD.⁷

VC starts with matrix vesicles, similarly to what physiologically happens to the skeletal tissue, also detected in smooth muscle cells.¹⁰ They are apparently made by the degradation products originated from autophagic cytoplasmic vacuoles. Hydroxyapatite crystals are generated inside these rupturing vesicles, exposing its contents to the extracellular medium.¹¹

The risk factors for VC are divided into traditional: involving advanced age, hypertension, diabetes, smoking, dyslipidemia, and others; and the non-traditional ones: including inflammation, oxidative stress and mineral and bone disorders (MBD) of CKD, among other factors.⁹

VASCULAR CALCIFICATION PATHOPHYSIOLOGY IN THE BONE AND MINERAL DISORDER OF CHRONIC KIDNEY DISEASE

VC pathogenesis in CKD is complex and instead of happening through a simple precipitation of calcium and phosphate in the vessel wall due to hypersaturation of these ions, it is the result of an active process of transformation of smooth muscle cells into osteoblast-like cells.⁹ Smooth muscle cells and osteoblasts share a common stem cell.¹² Structures identical to bone tissue, occasionally found in atherosclerotic lesions, suggest that the VC is an actively regulated process in which the vascular cell acquires osteoblast-like cell functions, secreting osteoid matrix.^{7,13} In other words, this VC pattern equals that of a heterotropic ossification.¹⁴

The presence of osteoclast differentiation factors (RANK/RANKL system) and precursors in calcified plaques suggests osteoclastic activity on the arterial wall. The coexistence of osteoblasts and osteoclasts reinforces the hypothesis that the calcification process is similar to that occurring in bone tissue. The imbalance in this process, such as increased differentiation into osteoblast and decreased osteoclast differentiation, may lead to calcification, but the exact role

played by the reduction in osteoclast differentiation remains to be investigated.¹⁵

FACTORS INVOLVED IN VASCULAR CALCIFICATION OF BONE MINERAL DISORDERS

There are several factors involved in the relationship between BMD and VC. The most significant among them are bone remodeling abnormalities, changes in serum levels of minerals and the very treatment for BMD.¹⁶ CKD patients have their homeostasis severely compromised, leading to adaptive changes in serum calcium, phosphorus, alkaline phosphatase, PTH, vitamin D, and fibroblast growth factor 23 (FGF23).¹⁷ Among these, hyperphosphatemia and hypercalcemia may be the most important in VC pathogenesis.⁹

HYPERPHOSPHATEMIA AND VASCULAR CALCIFICATION

Several studies have associated hyperphosphatemia with VC and vascular disease.¹ Serum phosphate levels may directly induce vascular injury, or indirectly stimulate the osteoblastic differentiation of muscle cells from the arterial tunica media layer.² Kambay *et al.*,³ in a systematic review of studies published between 1985 and 2008, found a gradual and independent association between serum phosphate concentration and cardiovascular events, mortality and CKD progression, defining phosphorus as a true vascular toxin. However, they did not find the same association in relation to calcium. Jono *et al.*⁴ investigated human vascular smooth muscle cells in culture, obtained from fetal autopsy or from heart transplants, and found higher calcium deposition in cells exposed to phosphorus in a dose-and-time-dependent relationship, mediated by sodium and phosphorus co-transport.

HYPERCALCEMIA AND VASCULAR CALCIFICATION

Regarding calcium, Yang *et al.*⁵ studied human smooth muscle cells in culture, obtained from autopsy, and concluded that when these cells were exposed to calcium concentrations equivalent to hypercalcemia and phosphorus concentrations equivalent to normophosphatemia, there was increased mineralization. When this exposure was associated with high concentrations of phosphorus, there was an increase in speed and in the extent of mineralization. The same study reported a reduction in mineralization by exposure to phosphonoformic acid, an inhibitor of sodium and phosphate cotransport, suggesting that the mineralization

caused by hypercalcemia happens through this type of cell transport, in the same way as with phosphate.

Secondary hyperparathyroidism (SHPT), because of its high rate of bone remodeling, causes high calcium uptake in cases of hypercalcemia, through deposition in the newly formed non-mineralized bone. However, this ability can be overcome when osteoblast activity is reduced by vitamin D or other toxic effects of uremia, resulting in an imbalance between bone formation and resorption. In low turnover disorders, the inability to incorporate excess calcium by this same mechanism results in further hypercalcemia, favoring the development of extraosseous calcifications.⁶

CONSEQUENCES OF TREATING BONE AND MINERAL METABOLISM DISORDERS

For the treatment of BMD in the development of VC, we use calcium-based phosphate scavengers and vitamin D supplementation. Phosphate scavengers used to fight the deleterious hyperphosphatemia, when calcium based they provide even more of the latter mineral and can cause hypercalcemia.⁷ Vitamin D receptors are present in vascular smooth muscle cells and, *in vitro* studies have shown that supraphysiological doses of this vitamin induced mineralization of these cells.⁸ Moreover, Vitamin D analogues for the treatment of SHPT may cause an exaggerated PHT suppression with a consequent gradual change in the BMD pattern for an adynamic disease of reduced bone remodeling, which has implications on the calcium, phosphorus, and calcification of soft tissues.^{9,10}

CALCIFICATION PROMOTERS AND INHIBITORS

Substances involved in the regulation of bone formation are also involved in the process of VC in BMD and CKD, acting as physiological promoters and inhibitors of soft tissue calcification. Phosphorus is the most significant and studied VC promoter and, moreover, bone morphogenetic proteins (BMPs) and some transcription factors have also been described as VC promoters. Fetuin A, osteopontin, osteoprotegerin, and matrix GLA protein (MGP) are physiological substances able to inhibit soft tissue calcification, which explains why there is no spontaneous mineralization even in tissues exposed to fluids supersaturated with calcium and phosphate.^{11,12}

Osteopontin is a bone matrix protein with physiological inhibitory action on calcification. It is a phosphorylated acid glycoprotein that was first discovered

in bone. It is not found in most normal tissues; nonetheless, it is abundant at sites of ectopic calcification as CKD VC. The mechanism by which mineralization is inhibited is not entirely clear, but it is likely to be by inhibition of physical deposition and buildup of hydroxyapatite.^{13,14}

Although physiologically it is an inhibitor of calcification, high levels of osteopontin are associated with cardiovascular risk, especially in CKD patients. Lorenzen *et al.*¹⁵ were the first to describe the association of circulating plasma levels of osteopontin with renal function in an inverse correlation with GFR, suggesting that this increase is partially due to a reduced renal excretion caused by renal failure. High plasma concentrations of osteopontin are also associated with hyperphosphatemia and high calcium-phosphate binding, underscoring its association with VC development.⁵ Giachelli *et al.*,¹⁶ in a study with rats comparing groups with and without arterial injury, considered osteopontin as a new component of human atherosclerosis, especially when associated with calcium deposition.

Osteoprotegerin, a glycoprotein member of the tumor necrosis factor superfamily, inhibits osteoclast maturation, protecting bone tissue from the resorptive activity of these cells.¹⁷ Along with the MGP and fetuin A, it is an important VC inhibitor. Bucay *et al.*,¹⁸ evaluating mice with artificially-caused genetic deficiency of osteoprotegerin, found a reduction in bone density characterized by severe osteoporosis and high incidence of fractures, as well as calcification of the aorta and renal arteries.

Paradoxically, Moe *et al.*¹⁹ found high levels of osteoprotegerin associated with increased coronary and aortic calcification in assessing a group of 30 hemodialysis patients and a group of 38 patients undergoing renal transplantation. They explained that the reason for the association between high levels of osteoprotegerin and increased VC would be the primary effect of osteoprotegerin on bone turnover. When osteoprotegerin is high, there is low bone turnover, caused by inhibition of osteoclastic activity, reducing bone ability to uptake calcium and phosphate, leading to ectopic and vascular calcifications.

Kazama *et al.*,²⁰ compared patients with healthy kidneys, patients in pre-dialysis and those in chronic hemodialysis for more than five years and found elevated serum osteoprotegerin in the dialysis group. They suggested that the increased levels of osteoprotegerin was due to reduced renal clearance caused by

kidney failure, corroborating these results with the study from Sato *et al.*, in which there was a regression occurred in osteoprotegerin serum levels two weeks after transplantation. As another possible explanation for the osteoprotegerin elevation in CKD, these same authors considered the increase in its production being stimulated by SHP.

Inducers of smooth muscle cell differentiation into osteoblast-like cells (including inorganic phosphate) seem to act through the transcription factors core binding factor 1 (Cbfa1), a transcription factor expressed by mesenchymal precursor cells in the bone marrow. This process can be enhanced by reducing calcification inhibitors such as fetuin A, MGP, osteopontin and osteoprotegerin.²¹

The Cbfa1 is one of the keys regulating the differentiation of osteoblasts and the production of bone matrix components such as collagen type I, osteocalcin and osteopontin, making up a pro-mineralization matrix.^{4,22} Komori *et al.*²³ studied mice with induced genetic deficiency of Cbfa1, and found complete blockage in the skeletal ossification of these specimens that died soon after birth, without even having breathed, due to the fragility of their non-ossified ribs.

Jono *et al.*⁴ speculated that increasing the phosphorus within the smooth muscle cells increases Cbfa1 expression. Moe *et al.*²² suggested that other toxins, yet unknown, and hyperphosphatemia, are connected to the up-regulation of Cbfa1, with subsequent increased expression of bone matrix in the vascular tissue, resulting in calcification in dialysis patients.

MGP is a matrix protein that inhibits the extracellular matrix mineralization. It is produced by smooth muscle cells and chondrocytes, two cell types which produce non-calcified extracellular matrix. Luo *et al.*²⁴ observed that MGP-deficient mice had extensive calcification of the aorta and its branches, as well as inadequate calcification in growing cartilage. These specimens had early death due to hemorrhage caused by rupture of the thoracic aorta. They then considered MGP as the first inhibitor of calcification of arteries and cartilage *in vivo*.

The MGP exerts its effect directly inhibiting the formation of calcium crystals, together with other inhibitors of calcification, such as fetuin A or, indirectly, influencing the transcription of other factors that inhibit the differentiation of vascular cells into osteoblast-like cells, in a vitamin K-mediated carboxylation process.²⁵

Parker *et al.*,²⁵ evaluated 842 patients with RF ranging from normal to moderate CKD, and found an association between decreased RF and low serum levels of non-carboxylated MGP (the precursor of active MGP). They submitted that the causes of this association were: vitamin D deficiency, reduced MGP precursor production, and reduction of RF leading directly to the VC that when abundant would reduce non-carboxylated MGP levels because of its high affinity for the hydroxyapatite deposited within the vessels in a consumption mechanism.

Fetuin A was originally described as the largest fetal and neonatal globulin in calves. The human homologue, alpha 2-Heremans-Schmid glycoprotein or fetuin-A temporarily inhibits the formation and precipitation of hydroxyapatite, being able to inhibit undesirable VC without inhibiting bone mineralization.²⁶

Ketteler *et al.*²⁷ found reduced serum levels of fetuin A in patients on dialysis. They submitted that this reduction was due to a state of chronic microinflammation found in CKD, indicated by increased C-reactive protein in patients undergoing dialysis, since fetuin is negatively regulated by inflammation. Schafer *et al.*²⁶ found severe calcifications in various organs of rats with fetuin A genetic deficiency, when compared to the control group.

In CKD patients, FGF23 levels rise up as RF declines. In a cohort of 142 patients with CKD, elevated levels of FGF23 were associated with severe coronary and aortic calcifications, being thus considered an important VC marker in CKD patients.²⁸ Despite these known data, the mechanisms by which FGF23 promotes VC are not exactly clear to the present.²⁹

CELL DEATH AND VASCULAR CALCIFICATION

The smooth muscle cell apoptosis is another mechanism that initiated VC, triggered by the interaction of these cells with inflammatory cells, which express surface death ligands or secrete proapoptotic cytokines such as, for example, tumor necrosis factor alpha. The apoptotic bodies of these cells are similar to the matrix vesicles in cells of the long bone epiphysis cartilage that are part of the physiological process of skeletal ossification.³⁰ Reynolds *et al.*³¹ evaluated cell cultures of human aortas and found that high levels of calcium and phosphorus, such as in CKD, substantially increased the calcium-phosphate-nucleation-induced calcification of live smooth muscle cells matrix

vesicles and also in the apoptotic bodies of dead cells. They also observed that when these cells were exposed to non-uremic human serum containing MGP and fetuin A, calcification was inhibited.

ARTERIAL INJURY DIAGNOSIS

There are invasive methods for atherosclerosis imaging diagnosis, such as coronary angiography and intravascular ultrasound; and non-invasive methods such as ultrasound, multidetector computed tomography (CT), MRI and scintigraphy.³² In order to investigate calcified vascular lesions, especially coronary lesions, the gold standard test is cardiac CT by electron beam, or the multislice heart CT.³³ However, both techniques are expensive, require substantial doses of radiation and cannot be easily performed in an outpatient basis.³⁴ The Kauppila and Adragão methods have been used for VC radiographic assessment.

Moldovan *et al.*³⁵ found a significant relationship between the presence of radiological signs of BMD and VC when they used plain radiographs to assess a cohort of 81 patients on dialysis. In this study, BMD patients had a higher Adragão score.

London *et al.*³⁶ compared histomorphometric data, obtained by bone biopsy and CV evaluated by ultrasound and found an association of these and low bone remodeling disease.

KDIGO study group, in its practical clinical guide to diagnosis, evaluation, prevention and treatment of BMD in CKD, suggests plain radiography in lateral view of the lumbar spine is a reasonable alternative to CT for VC evaluation.³⁷

METHOD KAUPPILA

Kauppila *et al.*³⁸ developed a classification index for the location, severity and progression of AAC (Abdominal Aortic Calcification), assessed by plain radiography on a lateral view of the lumbar spine, evaluating 617 individuals from the Framingham study with a 25-year follow-up. Subsequently, they investigated the association between this index and cardiovascular disorders in 2515 Framingham Study participants followed up for more than 20 years. They concluded from this study that the AAC investigated by means of lumbar lateral view is a subclinical marker of atherosclerosis and an independent predictor of subsequent cardiovascular morbidity and mortality.³⁹

Honkanen *et al.*⁴⁰ used this method to investigate AAC in 913 patients on dialysis. They found calcifications in 81% of the individuals surveyed and a significant direct relationship with dialysis duration.

Bellasi *et al.*³⁴ compared the Kauppila *et al.* method using the lateral view spine radiography with the CT scan - considered the gold standard, and they found very good correlation between the two methods in the evaluation of coronary calcification.

Since abdominal aortic calcification (AAC) correlates with calcification in other sites, such as the coronary arteries, and it has been shown to be significant in predicting cardiovascular events and mortality, this inexpensive and easy to perform method can be a useful alternative to the CT techniques used in CKD patients.⁴⁰

ADRAGÃO METHOD

Adragão *et al.*⁴¹ developed a grading system which they termed Simple Vascular Calcification Score, based on plain radiographs of the hands and pelvis. They assessed such score in 123 patients on dialysis during a time interval of 37 months, finding a VC incidence of 61% in some of these sites. They compared their data with cardiovascular mortality, hospitalization for cardiovascular causes, vascular disease diagnosis, fatal and nonfatal cardiovascular events, and they found a frequent association between these and a score greater than 3.

Insofar as we are concerned, there are no reports of comparative studies between the Adragão method and the coronary CT, which could provide important insight into methods which are less expensive and free of risks associated with radiological contrast in the evaluation of peripheral VC - coronary VC predictors, in the assessment of cardiovascular risk.

FINAL REMARKS

Being VC a prevalent consequence of CKD and an important cause of morbidity and mortality in patients on dialysis, the search for an assessment method that is fast, simple, inexpensive and easily applied in routine treatment of chronic renal failure patients on dialysis, but proven safe and effective, remains the objective of research on this subject.

Understanding the link between BMD and VC will certainly bring new perspectives to address these disorders and thereby reduce cardiovascular risk in advanced CKD patients.

REFERENCES

1. Alfrey AC, Ibels LS. Role of phosphate and pyrophosphate in soft tissue calcification. *Adv Exp Med Biol* 1978;103:187-93. PMID: 213949
2. Covic A, Kanbay M, Voroneanu L, Turgut F, Serban DN, Serban IL, et al. Vascular calcification in chronic kidney disease. *Clin Sci (Lond)* 2010;119:111-21.
3. Kanbay M, Goldsmith D, Akcay A, Covic A. Phosphate - the silent stealthy cardiorenal culprit in all stages of chronic kidney disease: a systematic review. *Blood Purif* 2009;27:220-30.
4. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, et al. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res* 2000;87:E10-7.
5. Yang H, Curinga G, Giachelli CM. Elevated extracellular calcium levels induce smooth muscle cell matrix mineralization in vitro. *Kidney Int* 2004;66:2293-9. PMID: 15569318
6. Kurz P, Monier-Faugere MC, Bognar B, Werner E, Roth P, Vlachojannis J, et al. Evidence for abnormal calcium homeostasis in patients with adynamic bone disease. *Kidney Int* 1994;46:855-61.
7. Cozzolino M, Dusso AS, Liapis H, Finch J, Lu Y, Burke SK, et al. The effects of sevelamer hydrochloride and calcium carbonate on kidney calcification in uremic rats. *J Am Soc Nephrol* 2002;13:2299-308.
8. Moe SM. Vascular calcification and renal osteodystrophy relationship in chronic kidney disease. *Eur J Clin Invest* 2006;36 Suppl 2:51-62.
9. Lewis R. Mineral and bone disorders in chronic kidney disease: new insights into mechanism and management. *Ann Clin Biochem* 2012;49:432-40.
10. González EA, Martin KJ. Renal osteodystrophy. *Rev Endocr Metab Disord* 2001;2:187-93.
11. Speer MY, Giachelli CM. Regulation of cardiovascular calcification. *Cardiovasc Pathol* 2004;13:63-70.
12. Mejía N, Roman-García P, Miar AB, Tavira B, Cannata-Andía JB. Chronic kidney disease-mineral and bone disorder: a complex scenario. *Nefrología* 2011;31:514-9.
13. Speer MY, McKee MD, Guldberg RE, Liaw L, Yang HY, Tung E, et al. Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: evidence for osteopontin as an inducible inhibitor of vascular calcification in vivo. *J Exp Med* 2002;196:1047-55. PMID: 12391016
14. Giachelli CM, Speer MY, Li X, Rajachar RM, Yang H. Regulation of vascular calcification: roles of phosphate and osteopontin. *Circ Res* 2005;96:717-22. PMID: 15831823
15. Lorenzen J, Krämer R, Kliem V, Bode-Boeger SM, Veldink H, Haller H, et al. Circulating levels of osteopontin are closely related to glomerular filtration rate and cardiovascular risk markers in patients with chronic kidney disease. *Eur J Clin Invest* 2010;40:294-300.
16. Giachelli CM, Bae N, Almeida M, Denhardt DT, Alpers CE, Schwartz SM. Osteopontin is elevated during neointima formation in rat arteries and is a novel component of human atherosclerotic plaques. *J Clin Invest* 1993;92:1686-96. PMID: 8408622
17. Ossareh S. Vascular calcification in chronic kidney disease: mechanisms and clinical implications. *Iran J Kidney Dis* 2011;5:285-99.
18. Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, et al. osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 1998;12:1260-8.
19. Moe SM, Reslerova M, Ketteler M, O'Neill K, Duan D, Koczman J, et al. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). *Kidney Int* 2005;67:2295-304. PMID: 15882271
20. Kazama JJ, Shigematsu T, Yano K, Tsuda E, Miura M, Iwasaki Y, et al. Increased circulating levels of osteoclastogenesis inhibitory factor (osteoprotegerin) in patients with chronic renal failure. *Am J Kidney Dis* 2002;39:525-32.
21. Chillon JM, Mozar A, Six I, Maizel J, Bugnicourt JM, Kamel S, et al. Pathophysiological mechanisms and consequences of cardiovascular calcifications: role of uremic toxicity. *Ann Pharm Fr* 2009;67:234-40. PMID: 19596096
22. Moe SM, Duan D, Doehle BP, O'Neill KD, Chen NX. Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. *Kidney Int* 2003;63:1003-11. PMID: 12631081
23. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* 1997;89:755-64.
24. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature* 1997;386:78-81. PMID: 9052783
25. Parker BD, Ix JH, Cranenburg EC, Vermeer C, Whooley MA, Schurgers LJ. Association of kidney function and uncarboxylated matrix Gla protein: data from the Heart and Soul Study. *Nephrol Dial Transplant* 2009;24:2095-101.
26. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, et al. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest* 2003;112:357-66. PMID: 12897203
27. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, et al. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. *Lancet* 2003;361:827-33. PMID: 12642050
28. Desjardins L, Liabeuf S, Renard C, Lenglet A, Lemke HD, Choukroun G, et al. FGF23 is independently associated with vascular calcification but not bone mineral density in patients at various CKD stages. *Osteoporos Int* 2012;23:2017-25. PMID: 22109743
29. Chen NX, Moe SM. Vascular calcification: pathophysiology and risk factors. *Curr Hypertens Rep* 2012;14:228-37.
30. Kockx MM. Apoptosis in the atherosclerotic plaque: quantitative and qualitative aspects. *Arterioscler Thromb Vasc Biol* 1998;18:1519-22. PMID: 9763521
31. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, et al. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. *J Am Soc Nephrol* 2004;15:2857-67.
32. Ibañez B, Badimon JJ, García MJ. Diagnosis of atherosclerosis by imaging. *Am J Med* 2009;122(1 Suppl):S15-25. PMID: 19110084
33. Muntner P, Ferramosca E, Bellasi A, Block GA, Raggi P. Development of a cardiovascular calcification index using simple imaging tools in haemodialysis patients. *Nephrol Dial Transplant* 2007;22:508-14.
34. Bellasi A, Ferramosca E, Muntner P, Ratti C, Wildman RP, Block GA, et al. Correlation of simple imaging tests and coronary artery calcium measured by computed tomography in hemodialysis patients. *Kidney Int* 2006;70:1623-8. PMID: 16955104
35. Moldovan D, Moldovan I, Rusu C, Racasan S, Patiu IM, Brumboiu A, et al. Vascular calcifications and renal osteodystrophy in chronic hemodialysis patients: what is the relationship between them? *Int Urol Nephrol* 2011;43:1179-86. PMID: 20862543

36. London GM, Marty C, Marchais SJ, Guerin AP, Metivier F, de Vernejoul MC. Arterial calcifications and bone histomorphometry in end-stage renal disease. *J Am Soc Nephrol* 2004;15:1943-51.
37. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int Suppl.* 2009;S1-130. PMID: 19644521
38. Kauppila LI, Polak JF, Cupples LA, Hannan MT, Kiel DP, Wilson PW. New indices to classify location, severity and progression of calcific lesions in the abdominal aorta: a 25-year follow-up study. *Atherosclerosis* 1997;132:245-50. PMID: 9242971
39. Wilson PW, Kauppila LI, O'Donnell CJ, Kiel DP, Hannan M, Polak JM, et al. Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. *Circulation* 2001;103:1529-34. PMID: 11257080
40. Honkanen E, Kauppila L, Wikström B, Rensma PL, Krzesinski JM, Aasarod K, et al. Abdominal aortic calcification in dialysis patients: results of the CORD study. *Nephrol Dial Transplant* 2008;23:4009-15.
41. Adragao T, Pires A, Lucas C, Birne R, Magalhaes L, Gonçalves M, et al. A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. *Nephrol Dial Transplant* 2004;19:1480-8.