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PERSPECTIVES

Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD)

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Abstract

Chronic kidney disease-mineral bone disorder (CKD-MBD) is a new term to describe the complex interplay of abnormal mineral metabolism, increased bone fragility and impaired linear bone growth, and vascular calcification in patients with CKD. These abnormalities are more common, and the natural history accelerated, in the setting of CKD. All components of CKD-MBD are associated with increased morbidity and mortality. The pathophysiology of CKD-MBD is just beginning to be understood, offering hope for new therapies to reduce the burden of fractures and cardiovascular disease in patients with CKD. *IBMS BoneKEy*. 2010 December;7(12):447-457.

Chronic kidney disease (CKD) is a worldwide health problem, affecting 13-25% of the population (1-3). Disturbances in mineral metabolism are common complications of CKD, beginning early in the course of progressive CKD. CKD staging is based on estimated glomerular filtration rate (eGFR): CKD stage 3 = eGFR of 60 to 30 ml/min; CKD stage 4 = eGFR of 30-15 ml/min; CKD stage 5 = eGFR < 15 ml/min; and CKD stage 5D are patients on dialysis (4). Beginning in stage 3 CKD, the damaged kidney is unable to fully excrete a phosphorus load nor can it convert vitamin D into its active metabolite $1,25(OH)_2D$ (calcitriol), leading to a compensatory secondary hyperparathyroidism. Elevated PTH and decreased calcitriol levels are found in 40% of patients with an eGFR between 40 and 50 ml/min and in 80% of patients with an eGFR below 20 ml/min (5). In addition, an elevation in FGF23 is also apparent early in the course of CKD (6;7). These mineral and endocrine functions disrupted in CKD are critically important in the regulation of bone remodeling. As a result, bone abnormalities (altered remodeling and loss of bone volume) are found almost universally in patients with CKD requiring dialysis and in the majority of patients with CKD stages 3-5 (8-10). These skeletal changes result in an increased prevalence of hip fracture compared to the general population across the entire range of CKD stages 3-5 and in dialysis patients (11-20). Dialysis patients in their 40s have a relative risk of hip fracture 80-fold that of age- and sex-matched controls (13). In patients with stage 4 CKD, the risk of hip fracture was nearly 4-fold that of the general population without CKD even at advanced ages (17). Furthermore, a hip fracture in patients with a GFR < 45 ml/min or on dialysis is associated with a doubling of the mortality observed in non-dialysis patients with a hip fracture (14;19;21).

Derangements in mineral metabolism are also associated with cardiovascular disease and all-cause mortality (22-26). Cardiovascular disease accounts for 70% of all deaths in patients with CKD, with an overall mortality of 20% per year in patients on dialysis (27). In individuals with kidney failure on dialysis, cardiovascular mortality rates are 10- to 500-times higher than in the general population, even after adjustment for gender, race, and the presence of diabetes (12). Importantly, individuals at earlier stages of CKD not yet on dialysis (stages 3-4) are up to 17-times more likely to die of cardiovascular disease than they are of progressing to dialysis (25). Multiple cross-sectional studies in dialysis patients

have found that disordered mineral metabolism, including hyperphosphatemia and hyperparathyroidism, increases the risk of cardiovascular and all-cause mortality (22; 28;29). One mechanism by which abnormal mineral metabolism may increase cardiovascular risk is by inducing or accelerating arterial and valvular calcification. Patients on dialysis have 2- to 5-fold more coronary artery calcification than age-matched individuals with angiographically-proven coronary artery disease (30). In patients not yet on dialysis, there is also increased coronary artery calcification compared to matched controls (31;32). Peripheral artery calcification can lead to claudication and systolic hypertension, and this in turn can lead to increased cardiac afterload resistance and left ventricular hypertrophy. Coronary artery calcification can lead to cardiac ischemia and sudden death and the latter is the leading cause of cardiovascular death in patients on dialysis (33).

In patients with CKD, there is emerging evidence to support an interrelationship between bone, vascular disease and disordered mineral metabolism. These advances in science led to an initiative in 2006 by KDIGO (Kidney Disease: Improving Global Outcomes) to clarify and update nomenclature. As a result, the new term "Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)" was put forth to describe a clinical syndrome composed of mineral metabolism abnormalities, renal osteodystrophy and extra-osseous calcification including vascular calcification (Table 1) (34). The older term, renal osteodystrophy, was then defined to specifically indicate abnormal bone resulting from impaired kidney function. Furthermore, a new classification for renal osteodystrophy, the TMV system (T = bone turnover/remodeling, M = mineralization, and V = volume), was developed to standardize the international description of bone histology in CKD patients.

Table 1. Definition of Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD)

A systematic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of the following:

1. Abnormalities of calcium, phosphorus, PTH or vitamin D metabolism
2. Abnormalities of bone turnover, mineralization, volume, linear growth or strength
3. Vascular or other soft tissue calcification

Note: From (34), with permission.

Prior to this new classification system of the bone component of CKD-MBD, the primary emphasis of bone had been on turnover (34), and the majority of the literature on bone in CKD focuses on turnover. High turnover bone disease due to secondary hyperparathyroidism results in abnormal bone ranging from mild resorptive changes to more advanced osteitis fibrosa cystica. Mixed uremic osteodystrophy is seen when there are features of high turnover disease and mineralization defects. Low turnover renal osteodystrophy was formally due to aluminum-induced osteomalacia, but now adynamic bone disease is the predominant cause of low turnover bone disease, characterized by a paucity of both osteoblasts and osteoclasts (35-37). The etiology of this disorder is unclear, but may be due to abnormal differentiation of osteoblasts and osteoclasts. The proportion of dialysis patients with adynamic bone disease has increased over the past 20 years to 37-50%, whereas the proportion of patients with high bone turnover is quite stable at 40-50% (38-40). Aluminum bone disease is now rarely found. In patients not yet on dialysis, bone biopsy series yield different results depending on the GFR level, and the country in which the study was done (36;41-46). However, it is clear from these data that histologic abnormalities of bone begin very early in the course of CKD. Non-invasive tests of bone, including DXA and CT, also demonstrate marked abnormalities across the spectrum of CKD.

However, no studies have demonstrated that any assessment of bone predicts future fractures in CKD patients (11;47-49).

A major component of CKD-MBD is vascular calcification, which is highly prevalent in CKD patients. Calcification can occur in intimal plaques and atheroma and also in the medial layer, especially in elastic-containing arteries (50;51). In patients starting dialysis, 60-70% of patients have significant coronary artery calcification by CT-based imaging (52). Pathologically on autopsy studies, the medial layer of coronary arteries is thicker in CKD patients than in controls, and medial coronary calcification is found in 20% of patients with CKD stages 4 and 5 (50). Calcification is also very common in the peripheral arteries, with 50-80% of dialysis patients demonstrating evidence of calcification by radiographic imaging (53-55) with histology demonstrating both intimal and medial calcification (56). Both coronary artery (23) and peripheral artery calcification (53-55) are associated with increased mortality in patients on dialysis. The risk factors associated with arterial calcification detected by imaging (ultrasound or CT) across multiple series include advanced age, diabetes, obesity, hypertension, dyslipidemia, inflammatory markers, hypoalbuminemia, use of calcium-containing phosphate binders and disordered mineral metabolism (38;54;57-61).

Vascular calcification was previously thought to be a passive process, due to the elevations in calcium and phosphorus observed in patients with advanced CKD. Recent evidence confirms this is an active process due to a series of events (Fig. 1). The initial step is thought to be a transformation or de-differentiation of vascular smooth muscle cells (VSMCs) to osteo/chondrogenic-like cells. These cells can then form matrix vesicles or apoptotic bodies that mineralize on an extracellular matrix, presumably in a manner similar to bone. The existence of abnormal bone remodeling in CKD may accelerate the process by providing excess calcium and phosphate for the matrix vesicles. The overall pathogenesis is regulated by a balance of pro-calcifying factors and inhibitors. Unfortunately in CKD, the pro-calcifying factors including hyperphosphatemia and hyper-parathyroidism are common, and inhibitors such as fetuin-A and matrix gla protein are reduced.

The phenotypic transformation of VSMCs into osteoblast-like cells is demonstrated by the increased expression of Runx2/Cbfa1 and decreased expression of the smooth muscle cell marker Sm22α (62). There is evidence of the presence of Runx2 and bone matrix proteins (*e.g.*, bone sialoprotein, osteopontin, type I collagen) in areas of medial calcification in patients with and without CKD (63;64). Jono *et al*. first demonstrated that VSMCs are induced to express Runx2 and calcify by phosphate *in vitro*, a process dependent on the type III sodium phosphate co-transporter (type III NPC) or Pit-1 (65). Our group further demonstrated that VSMCs also express Runx2/Cbfa1 when incubated with serum from dialysis patients, and this effect was above that induced by phosphate and not inhibited with phosphonoformic acid (63). Therefore, elevated phosphate and the other abnormal factors in CKD work together to initiate and enhance calcification. There are several additional factors in CKD that may play a role, such as parathyroid hormone (66-68), calcitriol (69), and leptin (70;71). However, hyperphosphatemia and secondary hyperparathyroidism are the most consistent factors in animal and human studies.

Neves *et al*. evaluated the role of PTH in the pathogenesis of vascular calcification using nephrectomized rats given a continuous infusion of PTH to achieve supraphysiologic levels. They demonstrated arterial calcification with hyperphosphatemia regardless of PTH, but also arterial calcification with hyperparathyroidism even with low phosphate levels (67). Graciolli and colleagues performed similar experiments, and found that uremic rats with supraphysiologic PTH levels fed a high or low phosphate diet had vascular calcification and increased expression of Runx2 and type I collagen on calcified vessels, while

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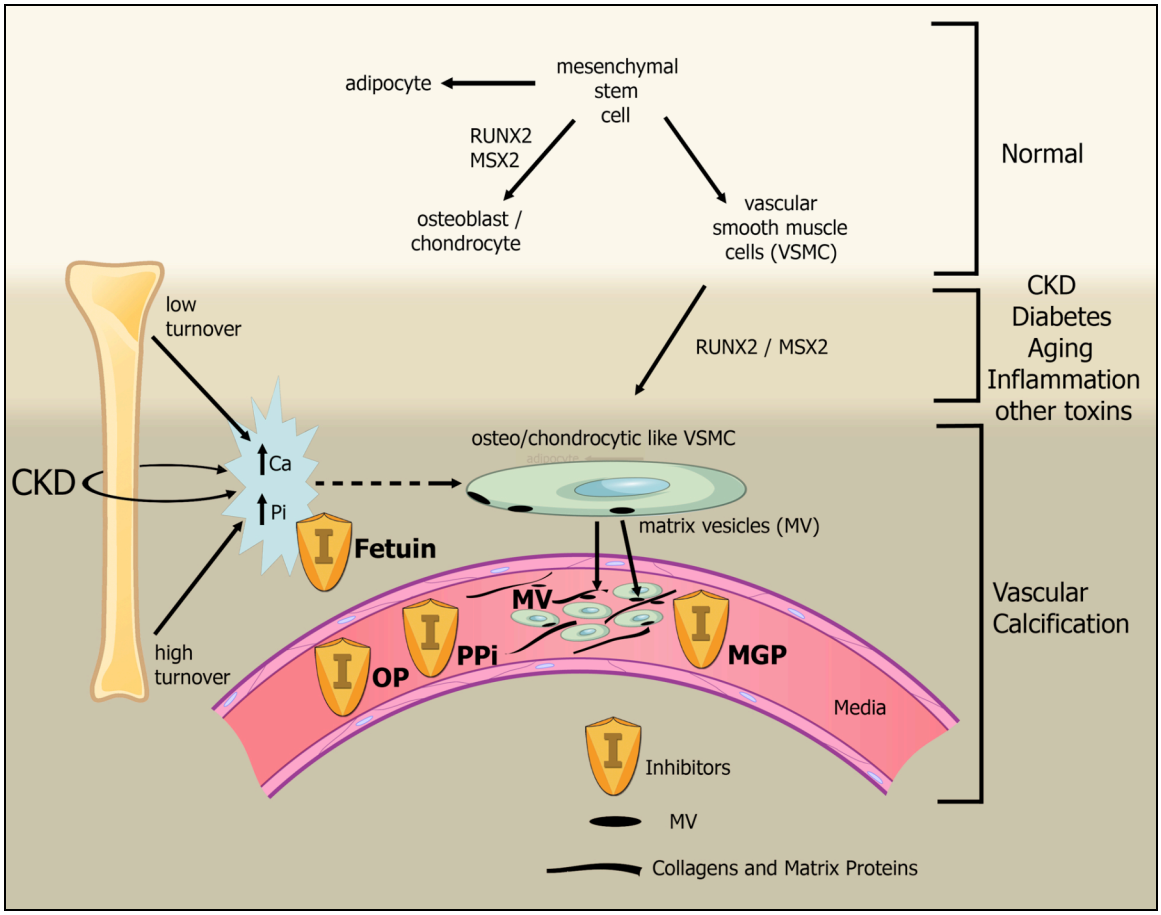

Fig. 1. Hypothesized pathogenesis of vascular calcification. Normally, mesenchymal stem cells differentiate into adipocytes, osteoblasts, chondrocytes, and vascular smooth muscle cells (VSMCs). In the setting of chronic kidney disease (CKD), diabetes, aging, inflammation and multiple other toxins, these VSMCs can de-differentiate or transform into chondrocyte/osteoblast-like cells by upregulation of transcription factors such as Runx2 and Msx2. These transcription factors are critical for normal bone development and thus their upregulation in VSMCs is indicative of a phenotypic switch. These osteo/chondrocytic-like VSMCs then become calcified in a process similar to bone formation. These cells lay down collagen and non-collagenous proteins in the intima or media and incorporate calcium and phosphorus into matrix vesicles to initiate mineralization and further grow the mineral into hydroxyapatite. The overall positive calcium and phosphorus balance of most dialysis patients feeds both the cellular transformation and the generation of matrix vesicles. In addition, the extremes of bone turnover in CKD (low and high or adynamic and hyperparathyroid bone, respectively) will increase the available calcium and phosphorus by altering the bone content of these minerals. Ultimately, whether an artery calcifies or not depends on the strength of the army of inhibitors standing by in the circulation (fetuin-A) and in the arteries. MGP = matrix gla protein; OP = osteopontin; PPi = pyrophosphate. Reprinted with permission from (76).

uremic rats fed a high phosphate diet and having normal PTH levels only expressed Runx2 (72). These data suggest that hyperparathyroidism may have a direct effect on vascular calcification. In addition, PTH may induce high bone turnover by increasing net bone resorption, releasing phosphate and calcium into the bloodstream, thereby increasing extra-skeletal calcification. Supporting this is a study demonstrating that coronary and carotid artery calcification are significantly decreased after subtotal parathyroidectomy (73). Furthermore, low turnover bone disease also increases the risk of coronary artery calcification. Barreto and colleagues performed bone biopsies and CT scans of coronary arteries at baseline and one year later. They found an inverse correlation between bone turnover and progression of

arterial calcification in hemodialysis patients (74). The bones of patients with adynamic bone disease are less able to take up a calcium infusion (75), therefore increasing available calcium substrate for extra-skeletal calcification. Thus, both hyperphosphatemia and secondary hyperparathyroidism are important contributors to vascular calcification in CKD patients, and both extremes of bone turnover (high and low) increase the risk.

Interestingly, some dialysis patients do not have vascular calcification and continue to be calcification-free on follow-up despite similar risk factors (77). This implies the presence of inhibitors of calcification and/or genetic protection. Fetuin-A (α 2-Heremans-Schmid glycoprotein) is a liver-produced protein that circulates in the blood, binding to calcium and phosphate particles. It is a reverse acute-phase reactant, such that levels are low in the presence of inflammation. Fetuin-A null mice have diffuse extra-osseous calcification, albeit the majority is in soft tissue and not in the vasculature (78). Low levels of fetuin-A are associated with mortality and arterial calcification in patients on dialysis (79-81). In animals, fetuin A inhibits, but may not reverse calcification. Westenfield and colleagues (82) induced vascular calcification in fetuin A/apolipoprotein E (ApoE)-deficient mice, comparing them to *ApoE*(-/-) and wild-type mice. Both uremic and non-uremic double knockout mice and *ApoE*(-/-) mice developed intimal atherosclerotic lesions in comparable degree to *ApoE*(-/-) mice but intimal vascular calcification was exhibited more extensively in double knockout mice. *In vitro*, adding fetuin-A back to uremic serum from dialysis patients can prevent calcification of VSMCs (81).

Another important inhibitor is the locally produced matrix gla protein (MGP), as mice null for MGP have severe medial artery calcification (83). Carboxylation of MGP to its active form, via vitamin K-dependent processes, may be abnormal in CKD patients. The levels of undercarboxylated MGP increase with deterioration of renal function. Furthermore, undercarboxylated MGP was found in the medial layer of calcified atherosclerotic carotid arteries. In addition, serum levels were positively correlated with arterial calcification scores in both nondialysis and ESRD patients (84). A third important inhibitor is pyrophosphate. Levels are inversely correlated with the prevalence and progression of arterial calcification in late stages of CKD (85). Thus, the presence or absence of calcification is in part due to the balance of the promoters and inhibitors of calcification. The factors maintaining and disturbing this balance are not fully elucidated.

In conclusion, CKD-MBD is a new term for a common clinical entity observed in patients with CKD that involves abnormal mineral metabolism, bone disease and vascular calcification. These derangements are known to be associated with increased morbidity and mortality, but the precise inter-relationship between these three factors is not yet known. The pathogenesis remains an active area of research. However, it is likely that correction of some of these inciting factors, such as hyperparathyroidism and hyperphosphatemia, may at least stabilize extra-osseous calcification and improve bone although definitive clinical trials are not available. Another approach would be to enhance the amount and/or type of calcification inhibitors, but this has not been tested in humans. It is hoped that the development of the new term CKD-MBD will encourage increased awareness of, and research into, this complex systemic disease.

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References

1. Imai E, Horio M, Watanabe T, Iseki K, Yamagata K, Hara S, Ura N, Kiyohara Y, Moriyama T, Ando Y, Fujimoto S, Konta T, Yokoyama H, Makino H, Hishida A, Matsuo S. Prevalence of chronic kidney disease in the Japanese general population. *Clin Exp Nephrol*. 2009 Dec;13(6):621-30.

2. Ingsathit A, Thakkinstian A, Chaiprasert A, Sangthawan P, Gojaseni P, Kiattisunthorn K, Ongaiyooth L, Vanavanan S, Sirivongs D, Thirakhupt P, Mittal B, Singh AK; Thai-SEEK Group. Prevalence and risk factors of chronic kidney disease in the Thai adult population: Thai SEEK study. *Nephrol Dial Transplant*. 2010 May;25(5):1567-75.

3. 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 Aug;(113):S1-130.

4. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2005 Jun;67(6):2089-100.

5. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: results of the study to evaluate early kidney disease. *Kidney Int*. 2007 Jan;71(1):31-8.

6. Fukagawa M, Kazama JJ. With or without the kidney: the role of FGF23 in CKD. *Nephrol Dial Transplant*. 2005 Jul;20(7):1295-8.

7. Oliveira RB, Cancela AL, Graciolli FG, Dos Reis LM, Draibe SA, Cuppari L, Carvalho AB, Jorgetti V, Canziani ME, Moysés RM. Early control of PTH and FGF23 in normophosphatemic CKD patients: a new target in CKD-MBD therapy? *Clin J Am Soc Nephrol*. 2010 Feb;5(2):286-91.

8. Sprague SM. The role of the bone biopsy in the diagnosis of renal osteodystrophy. *Semin Dial*. 2000 May-Jun;13(3):152-5.

9. Malluche HH, Monier-Faugere MC. Renal osteodystrophy: what's in a name? Presentation of a clinically useful new model to interpret bone histologic findings. *Clin Nephrol*. 2006 Apr;65(4):235-42.

10. Barreto FC, Barreto DV, Moyses RM, Neves CL, Jorgetti V, Draibe SA, Canziani ME, Carvalho AB. Osteoporosis in hemodialysis patients revisited by bone histomorphometry: a new insight into an old problem. *Kidney Int*. 2006 May;69(10):1852-7.

11. Stehman-Breen CO, Sherrard D, Walker A, Sadler R, Alem A, Lindberg J. Racial differences in bone mineral density and bone loss among end-stage renal disease patients. *Am J Kidney Dis*. 1999 May;33(5):941-6.

12. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis*. 1998 Nov;32(5 Suppl 3):S112-9.

13. Alem AM, Sherrard DJ, Gillen DL, Weiss NS, Beresford SA, Heckbert SR, Wong C, Stehman-Breen C. Increased risk of hip fracture among patients with end-stage renal disease. *Kidney Int*. 2000 Jul;58(1):396-9.

14. Coco M, Rush H. Increased incidence of hip fractures in dialysis patients with low serum parathyroid hormone. *Am J Kidney Dis*. 2000 Dec;36(6):1115-21.

15. Atsumi K, Kushida K, Yamazaki K, Shimizu S, Ohmura A, Inoue T. Risk factors for vertebral fractures in renal osteodystrophy. *Am J Kidney Dis*. 1999 Feb;33(2):287-93.

16. Jamal SA, Hayden JA, Beyene J. Low bone mineral density and fractures in long-term hemodialysis patients: a meta-analysis. *Am J Kidney Dis*. 2007 May;49(5):674-81.

17. Dooley AC, Weiss NS, Kestenbaum B. Increased risk of hip fracture among men with CKD. *Am J Kidney Dis*. 2008 Jan;51(1):38-44.

18. Fried LF, Biggs ML, Shlipak MG, Seliger S, Kestenbaum B, Stehman-Breen C, Sarnak M, Siscovick D, Harris T, Cauley J, Newman AB, Robbins J. Association of kidney function with incident hip fracture in older adults. *J Am Soc Nephrol*. 2007 Jan;18(1):282-6.

19. Nickolas TL, McMahon DJ, Shane E. Relationship between moderate to severe kidney disease and hip fracture in the United States. *J Am Soc Nephrol*. 2006 Nov;17(11):3223-32.

20. Ball AM, Gillen DL, Sherrard D, Weiss NS, Emerson SS, Seliger SL, Kestenbaum BR, Stehman-Breen C. Risk of hip fracture among dialysis and renal transplant recipients. *JAMA*. 2002 Dec 18;288(23):3014-8.

21. Mittalhenkle A, Gillen DL, Stehman-Breen CO. Increased risk of mortality associated with hip fracture in the dialysis population. *Am J Kidney Dis*. 2004 Oct;44(4):672-9.

22. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM. Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. *J Am Soc Nephrol*. 2004 Aug;15(8):2208-18.

23. Block GA, Raggi P, Bellasi A, Kooienga L, Spiegel DM. Mortality effect of coronary calcification and phosphate binder choice in incident hemodialysis patients. *Kidney Int*. 2007 Mar;71(5):438-41.

24. Danese MD, Kim J, Doan QV, Dylan M, Griffiths R, Chertow GM. PTH and the risks for hip, vertebral, and pelvic fractures among patients on dialysis. *Am J Kidney Dis*. 2006 Jan;47(1):149-56.

25. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med*. 2004 Sep 23;351(13):1296-305.

26. Young EW, Albert JM, Satayathum S, Goodkin DA, Pisoni RL, Akiba T, Akizawa T, Kurokawa K, Bommer J, Piera L, Port FK. Predictors and consequences of altered mineral metabolism: the Dialysis Outcomes and Practice Patterns Study. *Kidney Int*. 2005 Mar;67(3):1179-87.

27. USRDS. *2003 Annual Data Report: Atlas of End-Stage Renal Disease in the United States*. NIH and NIDDK: Bethesda, MD; 2003.

28. Kalantar-Zadeh K, Kuwae N, Regidor DL, Kovesdy CP, Kilpatrick RD, Shinaberger CS, McAllister CJ, Budoff MJ, Salusky IB, Kopple JD. Survival predictability of time-varying indicators of bone disease in maintenance hemodialysis patients. *Kidney Int*. 2006 Aug;70(4):771-80

29. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol*. 2005 Feb;16(2):520-8.

30. Braun J, Oldendorf M, Moshage W, Heidler R, Zeitler E, Luft FC. Electron beam computed tomography in the evaluation of cardiac calcification in chronic dialysis patients. *Am J Kidney Dis*. 1996 Mar;27(3):394-401.

31. Mehrotra R, Adler S. Coronary artery calcification in nondialyzed patients with chronic kidney diseases. *Am J Kidney Dis*. 2005 May;45(5):963.

32. Mehrotra R, Budoff M, Christenson P, Ipp E, Takasu J, Gupta A, Norris K, Adler S. Determinants of coronary artery calcification in diabetics with and without nephropathy. *Kidney Int*. 2004 Nov;66(5):2022-31.

33. Moe SM, Chen NX. Pathophysiology of vascular calcification in chronic kidney disease. *Circ Res*. 2004 Sep 17;95(6):560-7.

34. Moe S, Drüeke T, Cunningham J, Goodman W, Martin K, Olgaard K, Ott S, Sprague S, Lameire N, Eknoyan G; Kidney Disease: Improving Global Outcomes (KDIGO). Definition, evaluation, and classification of renal osteodystrophy: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). *Kidney Int*. 2006 Jun;69(11):1945-53.

35. Monier-Faugere MC, Malluche HH. Trends in renal osteodystrophy: a survey from 1983 to 1995 in a total of 2248 patients. *Nephrol Dial Transplant*. 1996;11 Suppl 3:111-20.

36. Torres A, Lorenzo V, Hernández D, Rodríguez JC, Concepción MT, Rodríguez AP, Hernández A, de Bonis E, Darias E, González-Posada JM, et al. Bone disease in predialysis, hemodialysis, and CAPD patients: evidence of a better bone response to PTH. *Kidney Int.* 1995 May;47(5):1434-42.

37. Sherrard DJ, Hercz G, Pei Y, Maloney NA, Greenwood C, Manuel A, Saiphoo C, Fenton SS, Segre GV. The spectrum of bone disease in end-stage renal failure--an evolving disorder. *Kidney Int*. 1993 Feb;43(2):436-42.

38. Barreto DV, Barreto FC, Carvalho AB, Cuppari L, Cendoroglo M, Draibe SA, Moyses RM, Neves KR, Jorgetti V, Blair A, Guiberteau R, Fernandes Canziani ME. Coronary calcification in hemodialysis patients: the contribution of traditional and uremia-related risk factors. *Kidney Int*. 2005 Apr;67(4):1576-82.

39. Adragao T, Herberth J, Monier-Faugere MC, Branscum AJ, Ferreira A, Frazao JM, Dias Curto J, Malluche HH. Low bone volume--a risk factor for coronary calcifications in hemodialysis patients. *Clin J Am Soc Nephrol*. 2009 Feb;4(2):450-5.

40. Herberth J, Branscum AJ, Mawad H, Cantor T, Monier-Faugere MC, Malluche HH. Intact PTH combined with the PTH ratio for diagnosis of bone turnover in dialysis patients: a diagnostic test study. *Am J Kidney Dis*. 2010 May;55(5):897-906.

41. Ingham JP, Stewart JH, Posen S. Quantitative skeletal histology in untreated end-stage renal failure. *Br Med J*. 1973 Jun 30;2(5869):745-8.

42. Eastwood JB. Quantitative bone histology in 38 patients with advanced renal failure. *J Clin Pathol*. 1982 Feb;35(2):125-34.

43. Dahl E, Nordal KP, Attramadal A, Halse J, Flatmark A. Renal osteodystrophy in predialysis patients without stainable bone aluminum. A cross-sectional bone-histomorphometric study. *Acta Med Scand*. 1988;224(2):157-64.

44. Hernandez D, Concepcion MT, Lorenzo V, Martinez ME, Rodriguez A, De Bonis E, Gonzalez-Posada JM, Felsenfeld AJ, Rodriguez M, Torres A. Adynamic bone disease with negative aluminium staining in predialysis patients: prevalence and evolution after maintenance dialysis. *Nephrol Dial Transplant*. 1994;9(5):517-23.

45. Coen G, Mazzaferro S, Ballanti P, Sardella D, Chicca S, Manni M, Bonucci E, Taggi F. Renal bone disease in 76 patients with varying degrees of

predialysis chronic renal failure: a cross-sectional study. *Nephrol Dial Transplant*. 1996 May;11(5):813-9.

46. Spasovski GB, Bervoets AR, Behets GJ, Ivanovski N, Sikole A, Dams G, Couttenye MM, De Broe ME, D'Haese PC. Spectrum of renal bone disease in end-stage renal failure patients not yet on dialysis. *Nephrol Dial Transplant*. 2003 Jun;18(6):1159-66.

47. Stehman-Breen C. Bone mineral density measurements in dialysis patients. *Semin Dial*, 14(3): p. 228-9, 2001

48. Moe SM, Yu BO, Sprague SM. Maintenance of bone mass in patients receiving dialytic therapy. *Am J Kidney Dis*. 1993 Aug;22(2):300-7.

49. Nickolas TL, Stein E, Cohen A, Thomas V, Staron RB, McMahon DJ, Leonard MB, Shane E. Bone mass and microarchitecture in CKD patients with fracture. *J Am Soc Nephrol*. 2010 Aug;21(8):1371-80.

50. Schwarz U, Buzello M, Ritz E, Stein G, Raabe G, Wiest G, Mall G, Amann K. Morphology of coronary atherosclerotic lesions in patients with end-stage renal failure. *Nephrol Dial Transplant*. 2000 Feb;15(2):218-23.

51. Nakano T, Ninomiya T, Sumiyoshi S, Fujii H, Doi Y, Hirakata H, Tsuruya K, Iida M, Kiyohara Y, Sueishi K. Association of kidney function with coronary atherosclerosis and calcification in autopsy samples from Japanese elders: the Hisayama study. *Am J Kidney Dis*. 2010 Jan;55(1):21-30.

52. Block GA, Spiegel DM, Ehrlich J, Mehta R, Lindbergh J, Dreisbach A, Raggi P. Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. *Kidney Int*. 2005 Oct;68(4):1815-24.

53. Adragão T, Pires A, Birne R, Curto JD, Lucas C, Gonçalves M, Negrão AP. A plain X-ray vascular calcification score is associated with arterial stiffness and mortality in dialysis patients. *Nephrol Dial Transplant*. 2009 Mar;24(3):997-1002.

54. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*. 2003 Sep;18(9):1731-40.

55. London GM, Marchais SJ, Guérin AP, Métivier F. Arteriosclerosis, vascular calcifications and cardiovascular disease in uremia. *Curr Opin Nephrol Hypertens*. 2005 Nov;14(6):525-31.

56. Moe SM, O'Neill KD, Duan D, Ahmed S, Chen NX, Leapman SB, Fineberg N, Kopecky K. Medial artery calcification in ESRD patients is associated with deposition of bone matrix proteins. *Kidney Int*. 2002 Feb;61(2):638-47.

57. Goodman WG, Goldin J, Kuizon BD, Yoon C, Gales B, Sider D, Wang Y, Chung J, Emerick A, Greaser L, Elashoff RM, Salusky IB. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med*. 2000 May 18;342(20):1478-83.

58. Russo D, Palmiero G, De Blasio AP, Balletta MM, Andreucci VE. Coronary artery calcification in patients with CRF not undergoing dialysis. *Am J Kidney Dis*. 2004 Dec;44(6):1024-30.

59. Tomiyama C, Higa A, Dalboni MA, Cendoroglo M, Draibe SA, Cuppari L, Carvalho AB, Neto EM, Canziani ME. The impact of traditional and non-traditional risk factors on coronary calcification in pre-dialysis patients. *Nephrol Dial Transplant*. 2006 Sep;21(9):2464-71.

60. Kraśniak A, Drozdz M, Pasowicz M, Chmiel G, Michałek M, Szumilak D, Podolec P, Klimeczek P, Konieczyńska M, Wicher-Muniak E, Tracz W, Khoa TN, Souberbielle JC, Drueke TB,

Sulowicz W. Factors involved in vascular calcification and atherosclerosis in maintenance haemodialysis patients. *Nephrol Dial Transplant*. 2007 Feb;22(2):515-21.

61. Kestenbaum BR, Adeney KL, de Boer IH, Ix JH, Shlipak MG, Siscovick DS. Incidence and progression of coronary calcification in chronic kidney disease: the Multi-Ethnic Study of Atherosclerosis. *Kidney Int*. 2009 Nov;76(9):991-8.

62. Steitz SA, Speer MY, Curinga G, Yang HY, Haynes P, Aebersold R, Schinke T, Karsenty G, Giachelli CM. Smooth muscle cell phenotypic transition associated with calcification: upregulation of Cbfa1 and downregulation of smooth muscle lineage markers. *Circ Res*. 2001 Dec 7;89(12):1147-54.

63. Moe SM, O'Neill KD, Duan D, Ahmed S, Chen NX, Leapman SB, Fineberg N, Kopecky K. Medial artery calcification in ESRD patients is associated with deposition of bone matrix proteins. *Kidney Int*. 2002 Feb;61(2):638-47.

64. 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 Mar;63(3):1003-11.

65. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res*. 2000 Sep 29;87(7):E10-7.

66. Sebastian EM, Suva LJ, Friedman PA. Differential effects of intermittent PTH(1-34) and PTH(7-34) on bone microarchitecture and aortic calcification in experimental renal failure. *Bone*. 2008 Dec;43(6):1022-30.

67. Neves KR, Graciolli FG, dos Reis LM, Graciolli RG, Neves CL, Magalhães AO, Custódio MR, Batista DG, Jorgetti V, Moysés RM. Vascular calcification: contribution of parathyroid hormone in renal failure. *Kidney Int*. 2007 Jun;71(12):1262-70.

68. Moe SM, Chen NX, Seifert MF, Sinders RM, Duan D, Chen X, Liang Y, Radcliff JS, White KE, Gattone VH 2nd. A rat model of chronic kidney disease-mineral bone disorder. *Kidney Int*. 2009 Jan;75(2):176-84.

69. Jono S, Nishizawa Y, Shioi A, Morii H. 1,25-Dihydroxyvitamin D3 increases in vitro vascular calcification by modulating secretion of endogenous parathyroid hormone-related peptide. *Circulation*. 1998 Sep 29;98(13):1302-6.

70. Parhami F, Tintut Y, Ballard A, Fogelman AM, Demer LL. Leptin enhances the calcification of vascular cells: artery wall as a target of leptin. *Circ Res*. 2001 May 11;88(9):954-60.

71. Zeadin M, Butcher M, Werstuck G, Khan M, Yee CK, Shaughnessy SG. Effect of leptin on vascular calcification in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol*. 2009 Dec;29(12):2069-75.

72. Graciolli FG, Neves KR, dos Reis LM, Graciolli RG, Noronha IL, Moyses RM, Jorgetti V. Phosphorus overload and PTH induce aortic expression of Runx2 in experimental uraemia. *Nephrol Dial Transplant*. 2009 May;24(5):1416-21.

73. Bleyer AJ, Burkart J, Piazza M, Russell G, Rohr M, Carr JJ. Changes in cardiovascular calcification after parathyroidectomy in patients with ESRD. *Am J Kidney Dis*. 2005 Sep;46(3):464-9.

74. Barreto DV, Barreto FC, Carvalho AB, Cuppari L, Draibe SA, Dalboni MA, Moyses RM, Neves KR, Jorgetti V, Miname M, Santos RD, Canziani ME. Association of changes in bone remodeling and coronary calcification in hemodialysis patients: a prospective study. *Am J Kidney Dis*. 2008 Dec;52(6):1139-50.

75. Kurz P, Monier-Faugere MC, Bognar B, Werner E, Roth P, Vlachojannis J, Malluche HH. Evidence for abnormal calcium homeostasis in patients with adynamic bone disease. *Kidney Int*. 1994 Sep;46(3):855-61.

76. Moe SM, Chen NX. Mechanisms of vascular calcification in chronic kidney disease. *J Am Soc Nephrol*. 2008 Feb;19(2):213-6.

77. Bellasi A, Kooienga L, Block GA, Veledar E, Spiegel DM, Raggi P. How long is the warranty period for nil or low coronary artery calcium in patients new to hemodialysis? *J Nephrol*. 2009 Mar-Apr;22(2):255-62.

78. Schafer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W. The serum protein alpha 2-Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest*. 2003 Aug;112(3):357-66.

79. Ketteler M. Fetuin-A and extraosseous calcification in uremia. *Curr Opin Nephrol Hypertens*. 2005 Jul;14(4):337-42.

80. Ketteler M, Bongartz P, Westenfeld R, Wildberger JE, Mahnken AH, Böhm R, Metzger T, Wanner C, Jahnen-Dechent W, Floege J. Association of low fetuin-A (AHSG) concentrations in serum with cardiovascular mortality in patients on dialysis: a cross-sectional study. *Lancet*. 2003 Mar 8;361(9360):827-33.

81. Moe SM, Reslerova M, Ketteler M, O'Neill K, Duan D, Koczman J, Westenfeld R, Jahnen-Dechent W, Chen NX. Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). *Kidney Int*. 2005 Jun;67(6):2295-304.

82. Westenfeld R, Schäfer C, Krüger T, Haarmann C, Schurgers LJ, Reutelingsperger C, Ivanovski O, Drueke T, Massy ZA, Ketteler M, Floege J, Jahnen-Dechent W. Fetuin-A protects against atherosclerotic calcification in CKD. *J Am Soc Nephrol*. 2009 Jun;20(6):1264-74.

83. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR, Karsenty G. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. *Nature*. 1997 Mar 6; 386(6620):78-81.

84. Schurgers LJ, Barreto DV, Barreto FC, Liabeuf S, Renard C, Magdeleyns EJ, Vermeer C, Choukroun G, Massy ZA. The circulating inactive form of matrix gla protein is a surrogate marker for vascular calcification in chronic kidney disease: a preliminary report. *Clin J Am Soc Nephrol*. 2010 Apr;5(4):568-75.

85. O'Neill WC, Sigrist MK, McIntyre CW. Plasma pyrophosphate and vascular calcification in chronic kidney disease. *Nephrol Dial Transplant*. 2010 Jan;25(1):187-91.