Serum bone markers in ROD patients across the spectrum of decreases in GFR: Activin A increases before all other markers

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Abstract

Introduction: Renal osteodystrophy (ROD) develops early in chronic kidney disease (CKD) and progresses with loss of kidney function. While intact parathyroid hormone (PTH), 1,25-dihydroxyvitamin D3 (1,25D), and fibroblast growth factor-23 (FGF-23) levels are usually considered the primary abnormalities in ROD development, the role of serum activin A elevations in CKD and its relationships to ROD have not been explored. The aims of this study were to evaluate serum activin A at different CKD stages, and to establish the relationships between activin A, bone biomarkers, and bone histomorphometric parameters.

Materials and methods: 104 patients with CKD stages 2 – 5D underwent bone biopsies. We measured in the serum activin A, BSAP, DKK1, FGF-23, α-Klotho, intact PTH, sclerostin, TRAP-5b, and 1,25D. Biochemical results were compared across CKD stages and with 19 age-matched controls with normal kidney function.

Results: Median activin A levels were increased in all stages of CKD compared to controls from 544 pg/mL in CKD 2 (431 – 628) to 1,135 pg/mL in CKD 5D (816 – 1,456), compared to 369 pg/mL in controls (316 – 453, p < 0.01). The increase of activin A in CKD 2 (p = 0.016) occurred before changes in the other measured biomarkers. Activin A correlated with intact PTH and FGF-23 (r = 0.65 and 0.61; p < 0.01) and with histomorphometric parameters of bone turnover (BFR/BS, Acf, ObS/BS and OcS/BS; r = 0.47 – 0.52; p < 0.01). These correlations were comparable to those found with intact PTH and FGF-23.

Conclusion: Serum activin A levels increase starting at CKD 2 before elevations in intact PTH and FGF-23. Activin A correlates with bone turnover similar to intact PTH and FGF-23. These findings suggest a role for activin A in early development of ROD.

Introduction

In the US, there are ~ 30 million patients with chronic kidney disease (CKD). The majority of these patients has chronic kidney disease mineral and bone disorder (CKD-MBD) which represents a pervasive health problem [1]. CKD-MBD presents with dysregulated mineral metabolism, increased risk for bone fracture, cardiovascular calcification, left ventricular hypertrophy, and increased mortality [2, 3, 4, 5, 6]. Renal osteodystrophy (ROD) represents the bone manifestation of CKD-MBD; it starts in patients as early as CKD stage 2 and progresses with further loss of kidney function [7, 8]. Virtually all patients requiring replacement of kidney function by dialysis have evidence of renal osteodystrophy. Progressive loss of kidney function is associated with an increase in intact parathyroid hormone (PTH), serum phosphorus, fibroblast growth factor-23 (FGF-23), and a decrease in 1,25-dihydroxyvitamin D3 (1,25D). These abnormalities are considered to be the main pathologic factors for renal osteodystrophy [7, 9, 10], but they are not sufficient to explain the bone changes that may occur as early as stage 2 [7, 11]. Identification of novel factors, especially in the early stages of CKD, is important for a more complete understanding of the pathogenesis of ROD.

Activin A, a multifunctional cytokine [12], has recently been studied in experimental animals with reduced kidney function [13, 14]. It is the most abundant of the transforming growth factor-beta (TGF-β) family of protein found in bone matrix [15]. Moreover, its expression has been shown to be coupled with bone resorption [16] and inhibition of activin signaling results in stimulation of bone growth [13]. Activin receptor type II A inhibition by the ligand trap RAP011 was shown to inhibit osteoclast formation in vitro and bone remodeling in CKD diabetic mice [13]. These data point to a potentially important role for activin
in bone turnover. Bone turnover abnormalities are an integral part of pathologic features of renal osteodystrophy, and therefore it appears important to study blood levels of activin A in patients across the spectrum of loss of glomerular filtration rate (GFR).

The aims of this study were to: 1) evaluate serum activin A levels in patients at different stages of CKD, 2) compare activin A levels with other known biomarkers of ROD at different stages of CKD, and 3) establish the relationships between histomorphometric parameters of ROD and serum levels of activin A versus the other known ROD bone markers.

Material and methods

Patients

This is a cross-sectional study of 104 CKD patients, stage 2 – 5D (on dialysis) who agreed to undergo bone biopsies for research purposes or workup for bone loss diagnosed by DXA. Before biopsy, for bone labeling, demeclocycline hydrochloride (150 mg b.i.d.) was administered for 2 days and tetracycline hydrochloride (250 mg b.i.d.) for 4 days, each separated from the other by a period of 10 days. Biopsies were performed 3 days after completion of the second label. All patients had blood draw at time of the bone biopsy. Inclusion criteria were age ≥ 18 years with a diagnosis of CKD stages 2 – 5D. Exclusion criteria were history of renal transplantation, history of parathyroidectomy, use of medications known to affect bone metabolism (except for calcitriol or cinacalcet), and life-threatening comorbid conditions such as malignancy, active infection, and hepatic disease. In addition, blood was drawn during the same time period from 19 individuals with normal kidney function who served as controls. These individuals were not receiving any anti-osteoporosis drugs at the time of the biopsy and during 2 years before biopsy. Informed consent was signed by all patients, and the study was reviewed and approved by the Institutional Review Board at the University of Kentucky. The study was conducted according to the Declaration of Helsinki. The patients’ medical records were reviewed to obtain demographic data, medication usage comorbidities, and data about dialysis vintage.

Serum biochemistry

Activin A levels were measured using R&D Systems kits (Indianapolis, ID, USA), sclerostin and DKK1 levels using Biomedica kits (Vienna, Austria), FGF-23 using Kainos kits (Tokyo, Japan), α-Klotho using IBL kits (Fujioka-Shi, Gunma, Japan), BSAP and TRAP-5b using Quidel kits (San Diego, CA, USA). Intact PTH and 1,25D were measured using chemiluminescence analyzers (DiaSorin, Stillwater, MN, USA). Serum creatinine, calcium, and phosphorus levels were measured by automated techniques. All measurements were performed in duplicate. Estimated GFR (eGFR) was determined using the MDRD formula.

Mineralized bone histology and bone histomorphometry

Bone samples were obtained by bone biopsies of the anterior iliac crest under local anesthesia and sedation. They were fixed in ethanol at room temperature, dehydrated, and embedded in methyl methacrylate as described previously [17]. Sections were stained with the modified Masson-Goldner trichrome stain [18], the aurin tricarboxylic acid stain [19], and solochrome azurine stain [20]. Unstained sections were prepared for phase-contrast and fluorescence light microscopy. Bone histomorphometry for static and dynamic parameters of bone structure, formation, and resorption was done at a magnification of 200 × using the OsteoMeasure (OsteoMetrics, Atlanta, GA, USA). All measured histomorphometric parameters are in compliance with the recommendations of the nomenclature committee of the American Society of Bone and Mineral Research [21, 22].

Statistical analyses

Results were reported as means (±SD) or medians (25th – 75th quartiles, IQR) when values were not normally distributed. Categorical variables were expressed as percentages. Comparisons of continuous variables were done using Kruskal-Wallis or Mann-Whitney U-tests as appropriate. Correlations between activin A and other biochemical parameters, and between activin A and bone
histomorphometric parameters were examined using Spearman’s rho (ρ) tests. Cutoff values for determination of low vs. non-low and high vs. non-high bone turnover were obtained by using the Youden’s J statistic. All statistical analyses were performed using SPSS version 24 (IBM Corp., Armonk, NY, USA). Group comparisons with p < 0.05 were considered statistically significant, and p < 0.005 was considered statistically significant for multiple correlations.

Results

There were 104 patients, consisting of 75 females and 29 males with mean age of 59 (± 15) years. 22 patients were in CKD stage 2, 29 patients in CKD stage 3, 19 patients in stages 4 or 5, and 34 patients on maintenance hemodialysis (CKD 5D). Disease etiologies included: 18% diabetes, 11% hypertension, 4% glomerulonephritis, 2% polycystic kidney disease, 30% other etiologies, and 34% unknown. Clinical and biochemical characteristics of the patients are shown in Table 1. Biochemical parameters such as phosphorus, calcium, BSAP, and TRAP-5b were significantly higher in CKD 5D compared to other CKD groups.

### Activin A, sclerostin, and other biochemical parameters across CKD stages

Serum activin A levels increased with declining eGFR (Figure 1) (ρ = 0.580, p < 0.001). Compared to controls, median levels of activin A were significantly elevated in CKD 2 (p = 0.016) (Figure 2). There was a trend to a further increase without significant differences between CKD 2, 3, and 4/5, while in CKD 5D there was a further significant increase in serum activin A.
Activin A and other bone markers in CKD patients

There were no significant differences in activin A levels between diabetics and non-diabetics and patients with or without active vitamin D metabolites among the patients with stages 2 – 5 (p = 0.911 and 0.290, respectively), as well as in CKD 5D patients (p = 0.800 and 0.276, respectively). The results on changes of activin A in patients with CKD versus controls were not altered by exclusion of patients with diabetes or vitamin D treatment.

Compared to controls, median levels of sclerostin and intact PTH became elevated in CKD 3 (p = 0.018 and 0.290, respectively) (Figure 3), while median levels of 1,25D fell and FGF23 levels increased in CKD 4/5 (p = 0.008 and p < 0.001, respectively) (Figure 3).

Across all CKD patients, serum activin A levels correlated (in descending orders) with intact PTH, FGF-23, TRAP-5b, phosphorous, 1,25D, BSAP and sclerostin (Table 2). Serum sclerostin levels also correlated in descending order with 1,25D, phosphorous, FGF-23, intact PTH, and to a lesser extent with TRAP-5b.

**Relationships between activin A, other serum biochemical bone markers, and histomorphometric results (Table 3)**

Across all CKD stages, serum activin A levels correlated with parameters of bone formation and resorption such as activation frequency, bone formation rate, osteoblast surface, osteoclast surface, osteoid thickness, and cortical porosity. These correlations were similar to those found between intact PTH, FGF-23, and histomorphometric bone parameters. The correlations between sclerostin and the histomorphometric bone parameters were less strong and there were no significant correlations with activation frequency and osteoblast surface. DKK1 correlated with osteoid surface only while there was no correlation between histomorphometric bone parameters and α-Klotho. Intact PTH, FGF-23, 1,25D, BSAP, and TRAP-5b showed the expected relationships with histomorphometric parameters of bone formation and resorption.

**Prediction of bone turnover by activin A and other serum biochemical bone markers**

Patients were classified as having low, normal, and high bone turnover based on reference values for activation frequency, bone formation rate, and numbers of osteoclasts and osteoblasts [23, 24, 25]. There were 57 subjects with low bone turnover (55%), 13 with normal (13%), and 34 with high bone turnover (33%). Levels of activin A, sclerostin, intact PTH, FGF-23, 1,25D, BSAP, and TRAP-5b separated by bone turnover are shown in Figures 4 and 5. Serum levels of activin A were significantly different between bone turnover states (ANOVA
The other markers also varied significantly (ANOVA p’s < 0.01) (Figure 5), except for sclerostin. Activin A showed similar AUC results, specificity, and sensitivity in predicting high turnover as intact PTH, BSAP, and FGF-23 (Table 4). Vitamin 1,25D and TRAP–5b showed less sensitivity and specificity for identification of bone turnover.

Discussion

The current data demonstrate the novel findings of a significant increase in activin A blood levels as early as CKD stage 2. Compared to controls, intact PTH and sclerostin increase significantly at CKD stage 3, while FGF-23 does not increase significantly and 1,25D decreases significantly at stage 4/5. The PTH findings are in agreement with sev-
eral prior studies [7, 10]. Sclerostin has been recently shown to increase at CKD stage 3 [11] [26]. In agreement with our results, Gutierrez et al. [4, 9] found FGF-23 to increase in some patients at stage 3 with a significant increase at stage 4, while Isakova et al. [27] studying 3,879 participants found significant increases already at stage 3. Our results on 1,25D changes with CKD are in agreement with Levin et al. [10] who showed in a study of 1,814 CKD patients similar median lev-

els of 1,25D by CKD stages. PTH, FGF-23, and 1,25D are commonly considered to be contributors to the pathogenesis of ROD [28, 29, 30, 31, 32], and sclerostin might play a role in the bone loss of ROD [33]. Our findings ascribe a role to activin A as the earliest documentable serum abnormality in the development of CKD-MBD; they open up a promising new avenue for research addressing the early pathogenesis of, and possible therapeutic approaches to, ROD.

The strong associations between activin A and intact PTH, FGF-23, and 1,25D, factors involved in bone formation, resorption and mineralization, show that activin A appears to be associated with bone turnover. This is corroborated by the observation that activin A showed similar specificity as intact PTH and FGF-23 in the discrimination of high versus non-high turnover. In experimental animals with reduced kidney function [13, 14], activin A expression has been shown to be coupled with bone resorption [16] and inhibition of activin signaling results in stimulation of bone growth [13]. Activin A also has been shown to enhance osteoclast activity, and activin receptor type II A inhibition by the ligand trap, RAP011, was shown to inhibit osteoclast formation in vitro and bone remodeling in diabetic mice with CKD [13].

### Table 3. Correlation coefficients ($\rho$) between serum biochemical results and bone histomorphometric parameters in patients with CKD stages 2 – 5D.

| Spearman's $\rho$ | Activin A | Sclerostin | Intact PTH | FGF-23 | 1,25D | BSAP | TRAP-5b | Phosphorus | Calcium | DKK1 | $\alpha$-Klotho |
|-------------------|-----------|------------|------------|--------|-------|------|--------|------------|---------|------|----------------|
| Bone formation rate/Bone surface | 0.51** | 0.28** | 0.59** | 0.55** | –0.42** | 0.55** | 0.35** | 0.43** | –0.27** | –0.22 | –0.25 |
| Activation frequency | 0.47** | 0.18 | 0.53** | 0.51** | –0.38** | 0.50** | 0.31** | 0.37** | –0.19 | –0.26 | –0.25 |
| Osteoblast surface/Bone surface | 0.49** | 0.16 | 0.58** | 0.54** | –0.45** | 0.39** | 0.23 | 0.42** | –0.17 | –0.24 | –0.10 |
| Osteoclast surface/Bone surface | 0.52** | 0.35** | 0.64** | 0.55** | –0.50** | 0.57** | 0.43** | 0.48** | –0.36** | –0.18 | –0.21 |
| Osteoid surface/Bone surface | 0.52** | 0.32** | 0.64** | 0.54** | –0.41** | 0.46** | 0.30** | 0.40** | –0.32** | –0.36** | –0.21 |
| Mineralization lag time | 0.20 | 0.13 | 0.25 | 0.12 | –0.15 | 0.13 | 0.11 | 0.12 | –0.17 | –0.13 | –0.05 |
| Bone volume/Tissue volume | 0.15 | 0.17 | 0.24 | 0.18 | –0.35** | 0.31** | 0.18 | 0.12 | –0.33** | 0.11 | –0.08 |
| Trabecular thickness | –0.03 | 0.10 | 0.04 | 0.06 | –0.21 | 0.22 | –0.01 | 0.02 | –0.12 | 0.16 | –0.03 |

**$p > 0.01$.**

![Figure 4. Levels of serum activin A in low, normal, and high bone turnover patients with CKD from stage 2 to 5D (group means + 95% confidence intervals). Results sharing the same letters are not significantly different.](image)
In mice with CKD-MBD induced by Alport syndrome, RAP011 decreased elevated osteoclast numbers and bone resorption; importantly, osteoblast numbers were not decreased, and the reduced bone formation rate per osteoblast associated with CKD-MBD was corrected [14]. Taken together, these data point to an important role for activin in bone turnover.

Activin A showed no relationship with Klotho or DKK1 neither of which varied with CKD stages. Thus, activin A appears to be independent of α-Klotho or DKK1 in ROD. The α-Klotho results are in contrast with experimental results in animals and clinical observations in patients with acute kidney injury [34] and CKD [35]. Akimoto et al. [36, 37], using the same assay as employed in our study, found in agreement with our results no significant changes in α-Klotho and DKK1 by CKD stage. The DKK1 results are in agreement with human studies in CKD [33, 38] but in contrast with findings of elevated DKK1 in a mouse model of CKD stage 5 [39]. These discrepancies in α-Klotho and DKK1 results might be related to differences in the employed assays.

Limitations of the study are given by description of correlations that cannot establish causality. However, in mice with CKD-MBD, activin receptor type II A inhibition by the ligand trap RAP011 prevented develop-
ment of renal osteodystrophy, that is, there was correction of high bone turnover and improvement of osteoblastic function [14]. Moreover, use of the natural antagonist of activin A, inhibin, in mice resulted in an increase in bone mineral density (BMD) [40]. Taken together, these data point to an important role for activin in renal osteodystrophy.

Further limitations of the study are related to its cross-sectional nature. Even though the total number of 104 subjects studied including bone biopsies is respectable, when broken down into CKD stages, the number of patients in each group is relatively small and does not allow analysis regarding diagnostic value of serum parameters for low versus high turnover and the role of factors such as diabetes and specific therapies. The present results provide justification for a prospective, multicenter, long-term study with bone biopsies in a larger number of patients. Currently available assays for novel markers such as α-Klotho and DKK1 are still undergoing refinements and standardizations, and could limit interpretation and comparisons of results across publications including ours.

In conclusion, activin A is a novel player observed in ROD in addition to or independent of the known abnormalities in PTH, FGF-23, 1,25D, and sclerostin. Activin A levels increase in blood of patients with CKD starting as early as stage 2 and the relationships with bone turnover abnormalities are as strong as those found with PTH and FGF-23. The present findings open up a new avenue for research in animals and subsequently in humans addressing the early pathogenesis of ROD and potential new therapeutic approaches to this serious abnormality of CKD-MBD.

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Conflict of interest

The authors declare no potential conflict of interest.

References

[1] Saran R, Robinson B, Abbott KC, Agodoa LYC, Bhave N, Bragg-Gresham J, Balkrishnan R, Dietrich X, Eckard A, Eggers PW, Gaipov A, Gillen D, Gipson D, Hailpern SM, Hall YN, Han Y, He K, Herman W, Hwang M, Hirth RA, et al. US Renal Data System 2017 Annual Data Report: Epidemiology of kidney disease in the United States. Am J Kidney Dis. 2018; 71 (3S1): A7. CrossRef PubMed

[2] Black GA, Port FK. Re-evaluation of risks associated with hyperphosphatemia and hyperparathyroidism in dialysis patients: recommendations for a change in management. Am J Kidney Dis. 2000; 35: 1226-1237. CrossRef PubMed

[3] Drücke TB. [The new Kidney Disease: Improving Global Outcomes (KDIGO) guideline for the mineral and bone disorder associated with chronic kidney disease (MBD-CKD)]. Nephrol Ther. 2010; 6: 149-150. PubMed

[4] Gutiérrez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, Sarwar A, Hoffmann U, Coglianese E, Christenson R, Wang TJ, deFilippis C, Wolf M. Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. Circulation. 2009; 119: 2545-2552. CrossRef PubMed

[5] Nickolas TL, Leonard MB, Shane E. Chronic kidney disease and bone fracture: a growing concern. Kidney Int. 2008; 74: 721-731. CrossRef PubMed

[6] Pimentel A, Ureña-Torres P, Zillichens MC, Bover J, Cohen-Solal M. Fractures in patients with CKD-diagnosis, treatment, and prevention: a review by members of the European Calcified Tissue Society and the European Renal Association of Nephrology Dialysis and Transplantation. Kidney Int. 2017; 92: 1343-1355. CrossRef PubMed

[7] Malluche HH, Ritz E, Lange HP, Kutschera L, Hodgson M, Seiffert U, Schoeppe W. Bone histology in incipient and advanced renal failure. Kidney Int. 1976; 9: 355-362. CrossRef PubMed

[8] Moe S, Drücke 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; 69: 1945-1953. CrossRef PubMed

[9] Gutiérrez O, Isakova T, Rhee E, Shah A, Holmes J, Collerone G, Jäppner H, Wolf M. Fibroblast growth factor-23 mitigates hyperphosphatemia but accentuates calcitriol deficiency in chronic kidney disease. J Am Soc Nephrol. 2005; 16: 2205-2215. CrossRef PubMed

[10] Levin A, Le Barbier M, Er L, Andress D, Sigrist MK, Djurdjev O. Incident isolated 1,25(OH)(2) D(3) deficiency is more common than 25(OH)D deficiency in CKD. J Nephrol. 2012; 25: 204-210. CrossRef PubMed

[11] Pelletier S, Dubourg L, Carlier MC, Hadi-Aissa A, Fouque D. The relation between renal function and serum sclerostin in adult patients with CKD. Clin J Am Soc Nephrol. 2013; 8: 819-823. CrossRef PubMed

[12] Macchini A, Nijima Y, Kojima I. Activin A: an autocrine regulator of cell growth and differentiation in renal proximal tubular cells. Kidney Int. 2002; 62: 446-454. CrossRef PubMed

[13] Sugatani T, Agapova OA, Fang Y, Berman AG, Wallace JM, Malluche HH, Faugere MC, Smith W, Song Y, Hruska KA. Ligand trap of the activin receptor type IIA inhibits osteoclast stimulation of bone remodeling in diabetic mice with chronic
kidney disease. Kidney Int. 2017; 91: 86-95. CrossRef PubMed

[14] Ogiwa MJ, Sugatani T, Agapova OA, Fang Y, Gault JP, Faugere MC, Malluche HH, Hruaska KA. The activin receptor is stimulated in the skeleton, vasculature, heart, and kidney during chronic kidney disease. Kidney Int. 2018; 93: 147-158. CrossRef PubMed

[15] Ogawa Y, Schmidt DK, Nathan RM, Armstrong RM, Miller KL, Sawamura SJ, Ziman JM, Erickson KL, de Leon ER, Rosen DM, et al. Bovine bone activin enhances bone morphogenetic protein-4 induced ectopic bone formation. J Biol Chem. 1992; 267: 14233-14237. PubMed

[16] Sakai R, Eto Y, Hirofujii M, Shinoda H. Activin release from bone coupled to bone resorption in organ culture of neonatal mouse calvaria. Bone. 2000; 26: 235-240. CrossRef PubMed

[17] Malluche HH, Faugere MC. Atlas of Mineralized Bone Histology. New York and Basel: S. Karger; 1986.

[18] Goldner J. A modification of the masson trichrome technique for routine laboratory purposes. Am J Pathol. 1938; 14: 237-243. PubMed

[19] Lillie PD, Fullmer HM. Histopathologic Technique and Practical Histchemistry (4th ed). New York: McGraw-Hill; 1976. p. 534.

[20] Denton J, Freemont AJ, Ball J. Detection and distribution of aluminium in bone. J Clin Pathol. 1984; 37: 136-142. CrossRef PubMed

[21] Dempster DW, Compston JE, Drezer MK, Glocious FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Parfitt AM. Standardized nomenclature, symbols, and units for bone histomorphometry: a 2012 update of the report of the ASBMR Histomorphometry Nomenclature Committee. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research. 2013; 28; 2: 1-17.

[22] Parfitt AM, Drezer MK, Glocious FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research. 1987; 2: 595-610.

[23] Heritage J, Brancum 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; 55: 897-906. CrossRef PubMed

[24] Malluche HH, Porter DS, Monier-Faugere MC, Mawad H, Pienkowski D. Differences in bone quality in low- and high-turnover renal osteodystrophy. J Am Soc Nephrol. 2012; 23: 525-532. CrossRef PubMed

[25] Malluche HH, Porter DS, Pienkowski D. Evaluating bone quality in patients with chronic kidney disease. Nat Rev Nephrol. 2013; 9: 671-680. CrossRef PubMed

[26] Enevendel P, D’Haeze P, Brandenburg V. Sclerotin and DKK1: new players in renal bone and vascular disease. Kidney Int. 2015; 88: 235-240. CrossRef PubMed

[27] Isakova T, Wahl P, Vargas GS, Gutierrez OM, Schiaffino S, Nie H, Appleby D, Nessel L, Bellovich K, Chos J, Hann M, Gadebecku C, Horwitz E, Townsend RR, Anderson CA, Lash JP, Hsu CY, Leonard MB, Wolf M. Fibroblast growth factor 23 is elevated before parahyroid hormone and phosphorus in chronic kidney disease. Kidney Int. 2011; 79: 1370-1378. CrossRef PubMed

[28] Dhyat NA, Ackermann D, Pruijn M, Ponte B, Ehret G, Guissous I, Lechtle AB, Paccoud F, Mohaupt M, Fiedler GM, Davayot O, Pechère-Bertschi A, Burnier M, Martin PV, Bochud M, Vogt B, Fossier D. Fibroblast growth factor 23 and markers of mineral metabolism in individuals with preserved renal function. Kidney Int. 2016; 90: 648-657. CrossRef PubMed

[29] Hruaska KA, Sugatani T, Agapova O, Fang Y. The chronic kidney disease - Mineral bone disorder (CKD-MBD): Advances in pathophysiology. Bone. 2017; 100: 80-86. CrossRef PubMed

[30] Lima F, El-Husseini A, Monier-Faugere MC, David V, Mawad H, Quares M, Malluche HH, FGF-23 serum levels and bone histomorphometric results in adult patients with chronic kidney disease on dialysis. Clin Nephrol. 2014; 82: 287-295. CrossRef PubMed

[31] Malluche HH, Davenport DL, Cantor T, Monier-Faugere MC. Bone mineral density and serum biochemical predictors of bone loss in patients with CKD on dialysis. Clin J Am Soc Nephrol. 2014; 9: 1254-1262. CrossRef PubMed

[32] Prist D, Ureña Torres P, Friedlander G. [Fibroblast Growth Factor 23-Klotho: a new axis of phosphate balance control]. Med Sci (Paris). 2009; 25: 489-495. PubMed

[33] Čepka D, Paradura-Rodríguez D, Pichler S, Marzolesca R, Kramer J, Kneissel M, Gross T, Reisinger A, Pahor D, Monier-Faugere MC, Haas M, Malluche HH. Only minor differences in renal osteodystrophy features between wild-type and sclerosin knockout mice with chronic kidney disease. Kidney Int. 2016; 90: 826-834. CrossRef PubMed

[34] Shi M, Flores B, Gillings N, Biao A, Cho HJ, Yan S, Liu Y, Levine B, Moe OW, Hu MC. αKlotho Mitigates Progression of AKI to CKD through Activation of Autophagy. J Am Soc Nephrol. 2015; 27: 2331-2345. CrossRef PubMed

[35] Hu MC, Kuro-o M, Moe OW. Secreted klotho and chronic kidney disease. Adv Exp Med Biol. 2012; 728: 126-157. CrossRef PubMed

[36] Akimoto T, Shizaki K, Sagase T, Watanabe Y, Yoshizawa H, Otani N, Numata A, Takeshima E, Yamazaki T, Miki T, Ito C, Pastor JF, Iwazu Y, Saito O, Muto S, Kuro-o M, Kusano E. The relationship between the soluble Klotho protein and the residual renal function among peritoneal dialysis patients. Clin Exp Nephrol. 2012; 16: 442-447. CrossRef PubMed

[37] Akimoto T, Yoshizawa H, Watanabe Y, Numata A, Yamazaki T, Takeshima E, Iwazu K, Komada T, Otani N, Morishita Y, Ito C, Shizaki K, Ando Y, Muto S, Kuro-o M, Kusano E. Characteristics of urinary and serum soluble Klotho protein in patients with different degrees of chronic kidney disease. BMC Nephrol. 2012; 13: 155. CrossRef PubMed

[38] Behets GJ, Viaene L, Meijers B, Blocki F, Brandenburg VM, Verhulst A, D’Haeze PC, Everepool P. Circulating levels of sclerostin but not DKK1 associate with laboratory parameters of CKD-MBD. PLoS One. 2017; 12: e0176411. CrossRef PubMed

[39] Fang Y, Ginsberg C, Seiffert M, Agapova O, Sugatani T, Register TC, Freedman BI, Monier-Faugere MC, Malluche H, Hruaska KA. CKD-induced wingless/integration 1 inhibitors and phosphorus cause the CKD-mineral and bone disorder. J Am Soc Nephrol. 2014; 25: 1760-1773. CrossRef PubMed

[40] Perrein DS, Akel NS, Edwards PK, Curver AA, Bendre MS, Swain FL, Skinner RA, Hoyward R, Nicks KM, Pierson TM, Suva LJ, Gaudy D. Inhibin A is an endocrine stimulator of bone mass and strength. Endocrinology. 2007; 148: 1654-1665. CrossRef PubMed