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Disorders in the System of Mineral and Bone Metabolism Regulators—FGF-23, Klotho and Sclerostin—in Chronic Kidney Disease: Clinical Significance and Possibilities for Correction

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Abstract

The chapter discusses the current understanding of the system of mineral and bone metabolism regulators—FGF-23, Klotho and sclerostin—disturbances in chronic kidney disease (CKD). In the chapter we presented the date, including our own results, which allow to suggest the change in the ratio of FGF-23-Klotho-sclerostin in CKD as an early biomarker not only for the chronic kidney damage but also for high cardiovascular (CV) risk. Results of studies show that disorders in FGF-23-Klotho-sclerostin ratio correlate with the frequency and severity of hypertension, vascular calcification, cardiac remodelling, anaemia, malnutrition, inflammation and strong aggravate CV risk in CKD. It was found independent from blood pressure (BP) action of increased serum FGF-23 on the myocardium as well as the correlation of serum high-sensitive troponin I with increased serum FGF-23 and low Klotho levels in CKD patients. At the same time, it was shown that renoprotective therapy, including renin-angiotensin blockers, low-protein diet with amino/keto acid supplementation and phosphate binders, erythropoiesis stimulators, vitamin D metabolites used to get the target levels of BP, serum phosphorus, haemoglobin, parathyroid hormone and nutritional status disorders correction can reduce the risk of CV events, as the major cause of death in CKD patients.

Keywords: chronic kidney disease, FGF-23, Klotho, sclerostin, vascular calcification, cardiac remodelling

1. Introduction

Chronic kidney disease (CKD) is a global problem that has not only medical but also great social and economic importance, due to a significant prevalence in the population (10–15%), high mortality rate from cardiovascular complications (CVC), as well as the need for high-cost treatment for the end CKD stage (dialysis, transplantation). As compared with general population, mortality rate due to CVC in patients with chronic renal failure is 10 times higher, and in young people, this risk is higher by 100 or more time. Many patients with CKD die from CVC on pre-dialysis stages, not reaching the end stage of CKD [1, 2].

Among cardiovascular damage in CKD, the progressive both cardiac remodelling and vascular calcification have leading contribution, which together lead to an urgently high cardiovascular mortality in patients with CKD [3, 4]. Understanding of the early mechanisms of arterial calcification as well as left ventricular hypertrophy (LVH) is important for the development of new therapeutic strategies aimed for cardiovascular morbidity reducing, pre-dialysis period prolonging and overall survival of CKD patients improving.

Clarification of CKD progression mechanisms and possible early markers of CVC has led to interest in studying of the identified in recent years factors such as morphogenetic proteins—fibroblast growth factor-23 (FGF-23), Klotho protein and sclerostin glycoprotein—which were estimated initially only as bone-mineral metabolism regulators in CKD [5].

Nowadays, the broader functional role of FGF-23, Klotho and sclerostin in organism has become understandable, including them significance as humoral factors involved in the processes of remodelling and calcification of the heart and vessels in CKD [6, 7]. Furthermore, the accumulated recently data allow to consider these factors as a possible therapeutic option for reducing mortality in CKD patients, but new randomized trials are still needed to clarify the individual mechanisms of their influence on remodelling and calcification of the heart and blood vessels as well as the optimal therapeutic modalities for correction of these disturbances.

The aim of the review was to systematize accumulated information and to establish the significance of the changes in serum levels of morphogenetic proteins (FGF-23, Klotho) and sclerostin glycoprotein, based on available literature data, including the results of our own studies, to assess renal and cardiac prognosis and possibilities for improving of cardio-renoprotective strategy in CKD.

2. FGF-23, Klotho and sclerostin in mineral bone disorders (MBD) in CKD

Disorders of phosphorus-calcium metabolism begin to be detected already on the 3A stage of CKD, when serum phosphorus starts to increase in serum due to glomerular filtration rate (GFR) decreasing [8]. PTH and vitamin D (calcitriol) were considered as main phosphor-regulating hormones for a long time. However, in recent years, it has been established, including our data, that FGF-23 begins to increase in serum in response to phosphorus retention, earlier

than PTH [8, 9], that allows to reconsider the traditional concept of secondary hyperparathyroidism (SGPT) pathogenesis.

FGF-23, produced by osteocytes, is a phosphaturic hormone that maintains a normal serum phosphorus concentration by increasing excretion of phosphorus in the urine and reducing its absorption from the gastrointestinal tract by inhibiting synthesis of 1,25-dihydroxyvitamin D in kidneys. Physiological stimuli for FGF-23 secretion are both a diet with an excess of phosphorus content and an increase in $1,25(OH)_2D_3$ levels in circulation [5, 8]. In the kidneys, FGF-23 suppresses $1,25(OH)_2D_3$ formation by inhibiting 1α-hydroxylase enzyme activity, which converts $25(OH)D_3$ transition to $1,25(OH)_2D_3$, as well as FGF-23 stimulates the formation of 24-hydroxylase, which converts $1,25(OH)_2D_3$ into its inactive metabolites. At the same time, FGF-23 suppresses the expression of the sodium-phosphorus co-transporters of both types (IIa and IIc) located on the apical surface of the epithelial cells of the proximal renal tubules; as a result, renal excretion of phosphorus increases [5, 8, 10].

At the same time, a date on the direct blocking effect of FGF-23 on PTH secretion was obtained [11]. It was found that FGF-23 stimulates mitogen-activated protein kinase (PKA) pathway, and so it inhibits the expression of PTH mRNA and PTH secretion both in vivo in rats and in vitro in parathyroid cell culture [11]. Since PTH is the inducer of $1,25(OH)_2D_3$ gene expression, the suppression of PTH, caused by FGF-23, reduces the serum level of $1,25(OH)_2D_3$, thereby closing the negative feedback of vitamin D homeostasis.

As the renal 1α-hydroxylase is inhibited and secretion of $1,25(OH)_2D_3$ is decreased, hypocalcaemia occurs, which stimulates PTH overproduction [12]. Thus, normal levels of calcium and phosphorus in serum are maintained that is successful at the early CKD stages.

Initially, at the early CKD, the increase in FGF-23 is a compensatory response aimed at normalizing phosphorus serum levels while decreasing the functioning nephron number [8, 12]. The serum FGF-23 level increases in parallel with the progressive decrease in kidney function, and the serum phosphorus does not increase significantly until GFR falls <30 mL/min/1.73 m^2 [5, 8]. When this stage of CKD is reached, the above compensation mechanism becomes ineffective, and constant hyperphosphatemia occurs that stimulates the increasing secretion of FGF-23 and PTH [8]. By the time when patients reach the end stage of CKD, FGF-23 level exceeds its normal range by 100 or more times [8].

To realize its effects in the kidneys, FGF-23 needs a co-receptor which is a transmembrane form of Klotho protein [5, 10]. Klotho was originally identified as an "ageing suppressor" [13]. The Klotho gene encodes a transmembrane protein, which is expressed predominantly in the epithelial cells of distal tubules in the kidneys, in parathyroid glands (PTG) and in the cerebral vascular plexus. Two forms of Klotho protein were found: transmembrane and secreted forms, each of which has different functions. The membrane Klotho form acts as an obligate co-receptor for FGF-23, inducing the excretion of phosphate in the urine. Secreted Klotho form (sKlotho) is detected in human serum and cerebrospinal fluid. It was found that it is formed as a result of Klotho protein cleavage from the cell membrane of the distal tubules of the kidneys [5, 10]. The decrease of Klotho protein expression in the kidneys due to CKD advancement allows to consider it as an early diagnostic marker of kidney damage [5, 14].

Unlike the membrane form, the secreted form of Klotho has systemic effects: it regulates endothelial production of NO [15], maintains the integrity and permeability of the endothelium [16] and calcium homeostasis in the kidneys [17] and suppresses intracellular signals of insulin and insulin-like growth factor-1 as mechanisms evolutionarily associated with life expectancy [18]. The low serum level of sKlotho is associated with an increase of CVC [6] and all causes mortality [19].

In recent years, there is increasing evidence that sKlotho decreasing, as CKD advancement, occurs early (2–3A stage of CKD) and may be also an important reason for the inducing of FGF-23 serum increase. Koh et al. [20], based on the analysis of the kidney biopsy that results in ten patients with a histological nephrosclerosis, found a significant decrease in the expression of Klotho mRNA as well as in sKlotho level and also the role of Klotho deficiency as nephrosclerosis advances, in the development of numerous complications of CKD, including uncontrolled FGF-23 serum increase.

The reduced expression of Klotho transmembrane form on the surface of parathyroid glands (PTGs) cell membranes (Klotho is also a co-receptor for FGF-23 in PTGs) at advanced stages of CKD is attributed to the resistance of PTG receptors to FGF-23, even in FGF-23 maximum concentrations [10, 12, 21].

In recent years, data on the important role of sclerostin glycoprotein in CKD are accumulating [22, 23]. Sclerostin, synthesized by osteocytes, blocks Wnt signalling pathway that leads to suppression of bone formation, as a result of decreased osteoblast differentiation and proliferation and osteocyte apoptosis [24]. The level of sclerostin increases as CKD advances [22, 25].

To date, sclerostin is an established regulator of bone mineralization, while its role in the pathophysiology of vessels in CKD is actively explored [22, 26]. It is important to determine the clinical significance of changes in sclerostin metabolism in CKD, because the relationship between adynamic bone disease (ABD) and calcification of the heart and blood vessels in patients with CKD is considered proven [27]. At the same time, available-to-date publications on the role of sclerostin in ectopic calcification still remain contradictory [22, 23, 25, 26].

It has been shown in experimental studies that in the case of hyperphosphatemia, the function of the skeleton as a phosphorus reservoir is blocked, although the need of bone in phosphorus, on the contrary, is increased, which stimulates a rising of its level in the blood, and soft tissues and vessels become a new reservoir for phosphate deposition [27].

Thus, in patients with end CKD stages, hyperphosphatemia, Klotho's and $1,25(OH)_2D_3$ deficiency and increased PTH and sclerostin occur, despite a very high level of FGF-23. At the same time, the frequency of ABD associated with a relatively low PTH and high sclerostin serum levels increases [12, 24, 27]. These changes, together with a decreased calcium excretion, may be responsible for the development of such complications of CKD as renal osteodystrophy, cardiovascular calcification following CVC and adverse outcomes in CKD [6, 7, 26].

It is suggested that an increase in sclerostin serum levels leads to a slowdown in osteogenesis in CKD. At the same time, there are reasons to believe that this mechanism is blocked in CKD and an increase in sclerostin is directed mainly to the inhibition of the extraosteal calcification [22, 25]. In addition it is believed that an increase in sclerostin expression by osteocytes in CKD causes bone resistance to PTH [24].

According to the results of recent studies, a disorder of the FGF-23-Klotho-sclerostin ratio in CKD is an early biomarker of the degree of chronic renal damage, preceded to the changes in other established markers of CKD advancement such as hyperphosphatemia, hyperparathyroidism and hypovitaminosis D, considering early as emerging cardiovascular risk factors in CKD patients [5, 26]. In addition, in interventional trials, intake of phosphate binders, cinacalcet or active vitamin D did not exert a consistently beneficial effect to reduce in cardiovascular event rate [28].

2.1. The relation of FGF-23, Klotho and sclerostin with cardiovascular remodelling and calcification in CKD

Mineral bone disorders (MBD) in CKD are the main contributor in CVC risk and in general prognosis of this patient cohort (**Figure 1**).

If the increase in FGF-23 serum levels at early CKD stages is adaptive, then the rapid FGF-23 rising from 3B CKD stage acquires a pathological significance. It was shown that an increase in serum FGF-23 level in CKD 3B-5 stages is associated with an endothelium dysfunction, Left ventricular hypertrophy (LVH) and an increase in cardiovascular mortality [7, 29–31].

It is also believed that a markedly increased FGF-23 level in CKD leads to a non-selective binding of it to FGF receptors in the heart, which are usually activated by local growth factors, such as FGF-2 [32]. Thus, an elevated FGF-23 level was directly associated with an increased risk of LVH development, which was detected with prolonged exposure with FGF-2 in experiment. In addition, it was found that an increase in left ventricular mass index (LVMI) accompanied with increased serum FGF-23 was independent from serum phosphorus level [7, 9].

FGF-23 is an earlier, than phosphorus, marker of CVC in patients with CKD, even when a phosphorus serum level is within the normal range [8, 9]. The pathogenetic relationship
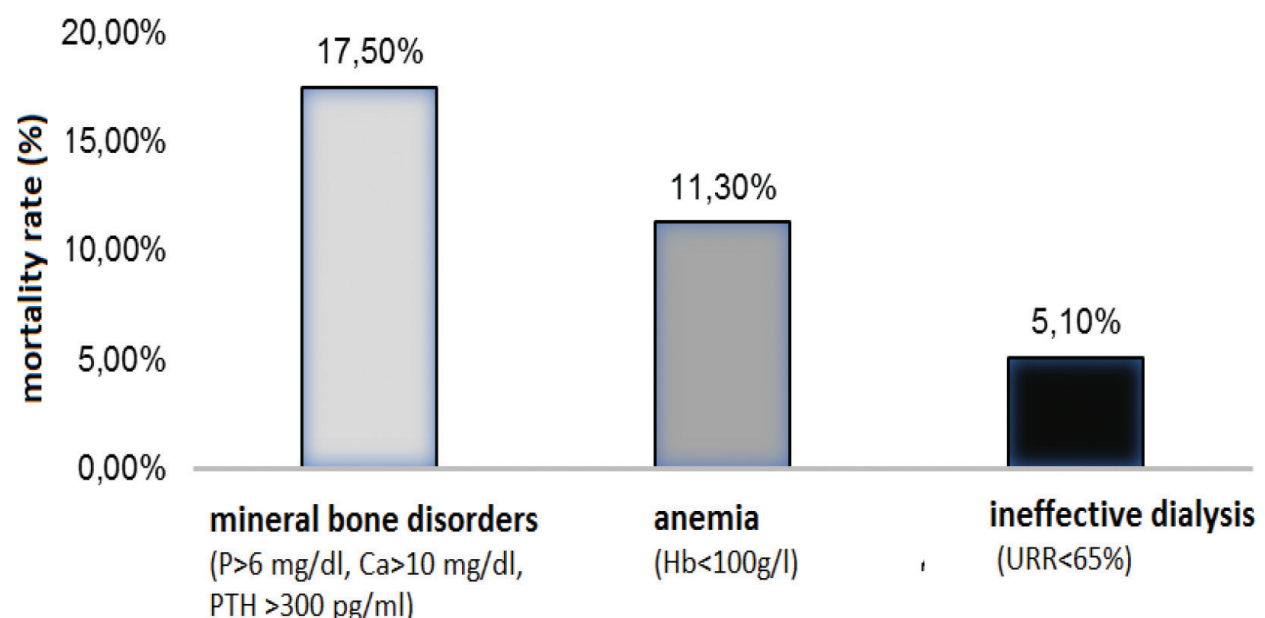
Figure 1. The position of MBD among the main causes of mortality in CKD haemodialysis patients (according to Block GA et al. J. Am. Soc. Nephrol. 2004).

between FGF-23 serum level and LVH was fully confirmed in the fundamental clinical work of Faul and Ansel [7] which showed that an increase in serum FGF-23 levels can directly lead to LVH development in CKD patients. The study consists of several stages; at the first stage, more than 3000 patients with renal insufficiency were examined for serum FGF-23 and echocardiography (EchoCG) at baseline and 1 year later. Each increase in serum FGF-23 on 1 logarithmic unit was associated with an increase in LVMI on 1.5 g/m^2. After 2.9 ± 0.5 years, the researchers re-examined 411 patients who had normal EchoCG parameters at the beginning of the study. In 84 (20%) patients with normal blood pressure (BP) levels, LVH was firstly detected. At the same time, each increase in FGF23 on 1 logarithmic unit led to an increase in the frequency of LVH de novo detection by 4.4 times; and significantly high levels of FGF-23 caused a sevenfold increase in the frequency of LVH detection, independently of the arterial hypertension (AH).

In order to confirm the hypothesis of the direct influence of FGF-23 on cardiomyocytes, Kardami [32] conducted the experimental study in which an effect of exogenous FGF-23 on the cardiomyocytes of newborn rats was evaluated using immunohistochemical and morphometric analysis. As a result, the hypertrophy of cardiomyocytes was revealed, as well as the increase in them, a level of alpha-actinin protein that indicates an increase of sequentially connected sacrometer units in the cardiomyocytes, and an increase in expression of heavy embryonic beta-myosin chains and depression of mature alpha-myosin chains. This switching of heavy chains from mature to embryonic isoforms indicates on the reactivation of embryonic gene programme, which is associated with hypertrophic transformation.

In the work of Di Marco et al. [33], prohypertrophic effect of FGF-23 and FGF-2 on cardiomyocytes was noted, which disappeared after the use of FGF-23 receptor inhibitor, PD173074, that authors consider as evidence of direct FGF-23 action, independently of Klotho protein. It is important to note that the use of PD173074 prevented the development of LVH in rats, despite the presence of hypertension in them.

According to our data [34], an increase in serum FGF-23 levels was associated with a moderately elevated level of troponin I. At the same time, in the patients with increased central BP (>120/80 mm Hg) as well as with normal central BP (90–120/60–79 mm Hg), mean levels of FGF-23 were about the same [629 ± 118 and 489 ± 85, respectively], indicating, an independent from the BP, FGF-23 action on the myocardium. The association of troponins with ischaemic heart disease and their role as predictors of an unfavourable cardiovascular outcome is known that also allows to suggest FGF-23 as an important prognostic cardiomarker in CKD.

In addition, it was established [35] that an increase in serum FGF-23 levels accelerated the development of vascular arteriosclerosis almost by sixfold, with the direct correlation with vascular calcification. However, in multivariate analysis, this relationship was statistically weak, which may indicate a possible indirect effect of FGF-23 on vascular calcification. Further obtained data indicate the effect of FGF-23 on fetuin A level, which is known to be synthesized by osteoblasts and is an inhibitor of vascular smooth muscle cell (VSMS) calcification [35, 36].

In the prospective cohort ArMORR study [37] involving 10,044 patients, it was shown that a high FGF-23 serum level of patients, starting treatment with programmed HD, is an

independent predictor of annual mortality and the patients with high levels of FGF-23 from a higher quartile had a sixfold increase in the risk of mortality compared to similar patients from the lower quartile according to multidimensional corrected model.

In another prospective the mild to moderate kidney disease (MMKD) study [38] involving 227 patients with nondiabetic CKD 1–4 stages, the patients were followed up for 53 months to assess the progression of the nephropathy. Based on the results, an independent direct relationship between increased serum FGF-23 level and CKD progression rate was established. FGF-23 was recognized as an important independent predictor of adverse renal and cardiovascular prognosis, and in addition, in the regression Cox analysis, phosphorus levels lost prognostic value after adjustment to serum FGF-23 level.

Accumulating recent data allow to consider FGF-23 as an earlier and important predictor of mortality than serum phosphorus and PTH levels in patients with CKD. Elevated serum FGF-23 level is currently considered as an independent trigger factor in the pathogenesis of uremic cardiomyopathy and vascular calcification that served as the basis for suggesting FGF-23 *as a new uremic toxin*, earlier than PTH [39].

At the same time, part of the pathological effects of FGF-23 may be due to Klotho deficiency in CKD advancement. It has been shown that kidneys are the main producers of Klotho forms in organism [5, 10, 14], so CKD is a state of Klotho deficiency. Deficit of Klotho causes development of multiple systemic manifestations (i.e. premature ageing syndrome), an integral part of which is severe cardiovascular impairments [6, 13, 15]. According to recent update, it has been proved now that Klotho downregulation is not merely an early biomarker for kidney damage but also plays a pathogenic independent role in the advancing of CKD as well as in principal complications of CKD, important part of which is vascular calcification [6, 9]. As it was recently summarized, Klotho's anti-calcification effect is possibly via at least three mechanisms: a phosphaturic hormone, the preservation of GFR and a direct effect on soft tissues including the vascular smooth muscle [6, 21]. In experiment Klotho overexpression slowed down CKD advancement, improves phosphate metabolism and protects the vasculature from calcification [5, 6, 10].

The role of soluble Klotho form in phosphate homeostasis was recognized as soon as Klotho was discovered, because Klotho-deficient mouse demonstrates severe hyperphosphatemia [10, 13, 18]. This was further confirmed by the fact that there was low serum phosphate in Klotho-overexpressing mice [18]. A patient with homozygous missense mutation (H193R) in Klotho gene had severe calcinosis, dural and carotid artery calcifications, severe hyperphosphatemia, hypercalcemia and high-serum $1,25(OH)_2$ vitamin D and FGF-23. This mutation conceivably destabilizes KL1 domain of Klotho, thereby attenuating production of membrane-bound and sKlotho protein [40]. Therefore, Klotho is now considerable as a novel candidate gene for genetic hyperphosphatemia and calcinosis.

It was established that a decrease in Klotho level is also possible due to the inhibition of its extrarenal production. In this connection, the results of Takeshita et al. [41] study, indicating the presence of Klotho gene expression in sinoatrial node and a high rate of sudden cardiac death due to arrhythmias caused by dysfunction of sinoatrial node in mice with blocked Klotho gene, are interesting.

One of the most important effects of Klotho and sclerostin is its ability to inhibit Wnt signal pathway and through it to slow down vascular calcification [42]. Reduction of serum Klotho levels impairs this protective effect. Besides this, it has been demonstrated that Klotho mitigates the increased cell senescence and apoptosis triggered by oxidative stress in endothelial cells [43] and Klotho also suppresses TNF-β-induced expression of intracellular adhesion molecule-1 and vascular cell adhesion molecule-1, attenuates NF-kappaB activation and reverses the inhibition of eNOS phosphorylation by TNF-α. Thus Klotho protein also protects the vascular endothelium by inhibition of endothelial inflammation [44].

The most definitive study to date of the direct effects of Klotho on the endothelium was conducted by Kusaba et al. [16]. Klotho-deficient mice have increased VEGF-mediated calcium influx, downregulation of vascular endothelial cadherin, increased apoptosis and excessive permeability of vessels. The KL2 domain of Klotho protein binds directly to VEGF receptor 2 and endothelial transient-receptor potential Ca^{2+} channel 1 on the extracellular side and promotes their co-internalization, thereby reducing the Ca^{2+}-activated and caspase-mediated destruction of catenin and vascular endothelial cadherin on the cell surface. Thus, it may be one more effect of soluble Klotho's protein cardioprotection.

In vitro studies have shown that along with the increase in phosphaturia, stabilization of GFR and regulation of vascular endothelial permeability, Klotho suppresses Na-dependent capture of phosphorus by the endothelium and vascular smooth muscle cell (VSMC) and prevents differentiation of VSMC and mineralization induced by hyperphosphatemia [5, 14].

In our study, an association of increased serum FGF-23 and low Klotho levels with the presence of inflammation (as C-reactive protein level increasing) as well as with protein-energy deficiency, and proteinuria, was found [45]. These data are in agreement with the results of other authors [44, 46] who consider CKD as a state of chronic inflammation, based on the consideration of elevated C-reactive protein level as a nonspecific marker of inflammation and endothelial dysfunction in CKD patients. Frequent coexistence triad—malnutrition, inflammation and atherosclerosis (MIA) syndrome—contributes to the risk of CVC in CKD [2, 3, 47]. The obtained data clearly indicate that circulating form of Klotho protein functions as a humoral factor that protects the cardiovascular system from the development of inflammatory endothelial changes and prevents the progression of atherosclerosis and pathological calcification [5, 14–16].

Less understood is the role of sclerostin in CV calcification processes in CKD. Our data go in agreement with the results of authors, who demonstrated a protective effect of sclerostin in calcification in CKD [22, 48, 49]. Its inhibitory effect on osteogenesis and negative association of sclerostin with level of parathyroid hormone as uremic toxin can attest in favour of this date [48, 49].

Viaene et al. [50] showed that in patients on haemodialysis, the serum concentration of sclerostin is higher than in general population. In the future, these data will be repeatedly confirmed by the results of other studies devoted to identify the role of sclerostin in patients on regular haemodialysis [23, 49].

Emerging evidence indicates that Wnt plays a role in vascular biology including vascular calcification, angiogenesis and atherosclerosis [42]. Wnt signalling occurs when the Wnt ligand

binds to co-receptors, Frizzled and low-density lipoprotein receptor-related protein (LRP), which induces β-catenin translocation to the nucleus to regulate the transcription of Wnt target genes. The Wnt pathway is involved in many aspects of biology including cell survival, stem cell development and cell differentiation, including bone and vascular lineages [24, 42].

Register et al. [25] found that high sclerostin was associated with less calcified carotid plaque in diabetic African American men and was not associated with aortic or coronary calcification. Authors' hypothesis to explain this situation is that increased overproduction of sclerostin may be a physiological adaptation to increased calcification.

Sclerostin slows the canonical Wnt signalling pathway and inhibits osteoblast activity and bone formation by sequestering LRP5 and LRP6 [24, 42]. Retarding the Wnt signalling pathway by using a dominant-negative LRP has been depicted to significantly reduce VSMC proliferation. In addition to VSMC proliferation, animal models of intimal thickening have revealed increased β-catenin levels, which suggest the involvement of the Wnt-β-catenin pathway also in VSMC migration. Moreover, Wnt proteins are also known to promote the migration of various cell types, including monocytes and endothelial cells. Furthermore, the Wnt pathway has been described to play an important role in the regulation of endothelial inflammation, vascular calcification and mesenchymal stem cell differentiation. As a result, considering the fact that atherosclerosis and calcification are both an actively regulated and progressive process, we might speculate that high sclerostin levels might be indicative of a sort of defensive mechanism that may attenuate the upregulation of the canonical Wnt pathway and lead to the restoration of quiescent Wnt signalling observed under healthy conditions [24, 49].

Sclerostin has been demonstrated to be upregulated in VSMC, which previously transformed into osteocytic phenotype under calcifying circumstances [22, 24]. Recently, it has been suggested that increased circulating sclerostin levels might protect dialysis patients from cardiovascular calcification and that low bone-specific alkaline phosphatase activity may be the causal pathway [23, 49]. Sclerostin is a potent inhibitor of alkaline phosphatase activity, which inactivates the potent calcification inhibitor, the inorganic pyrophosphate. Accumulating data suggest that Wnt signalling pathway inhibitor overexpression in calcifying vasculature (advanced carotid plaques and calcified aortas) might be vasculoprotective and anti-calcific [25, 49].

PTH increases FGF-23 expression via Wnt and protein kinase (PKA) signalling pathways by blocking the inhibitory effect of sclerostin (**Figure 2**).

According to several authors, the overexpression of sclerostin by osteocytes in patients on haemodialysis is associated with a decrease in overall cause's mortality, including CVC, in dialysis patients [49].

In the same time, more research for confirmation of sclerostin role in FGF-23-Klotho-sclerostin system as a protector of pathogenic transformation of VSMC, triggered by phosphate and FGF-23, is seen as a priority. It is likely that sclerostin confronts effects of low Klotho levels and high levels of FGF-23, allowing for some time to maintain a certain compensatory balance in the system of FGF-23-Klotho-sclerostin. Increasing levels of sclerostin in CKD are likely directed at suppression of processes of calcification, but cannot fully inhibit them, because
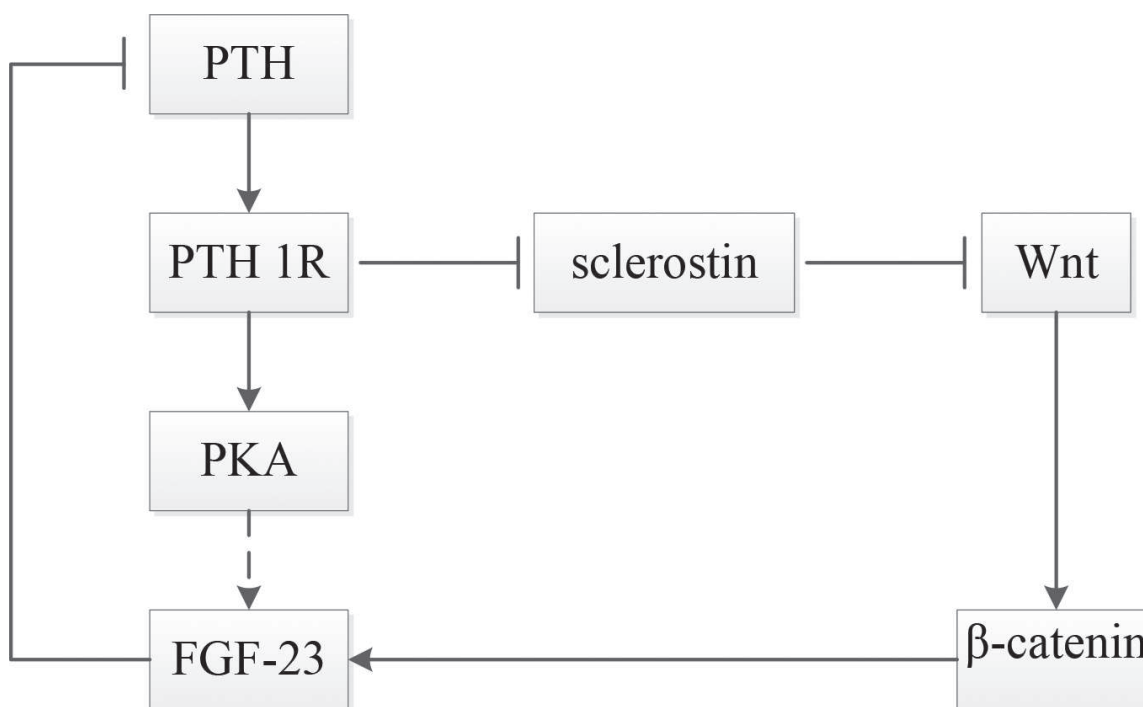
Figure 2. The relationship between PTH, FGF-23 and sclerostin (materials of ISN Nexus Symposium «Bone and the Kidney» September 2012, Copenhagen, Denmark).

reduction of Klotho may be much more potent stimulus for progression of calcification, and increased PTH suppresses sclerostin. Because levels of sclerostin increase as CKD progresses and as levels of Klotho at the same time reduce, some authors may mistakenly interpret the role of sclerostin as a factor, which potentiates calcification. In reality (the results of the multivariate analysis), it is likely that dramatic fall of Klotho levels in the course of CKD outbalances and levels down protective effects of sclerostin.

To sum up, we can consider all three factors (FGF-23, Klotho, sclerostin) as a discrete system of factors influencing cardiovascular risk. Apparently, such high CV risk is determined by joint effect of all of these three factors that appear along with early CKD stages and connect not only between themselves but also with traditional factors, which snowballed quickly following added, potentiate one another as CKD advanced and thereby strongly increase the risk of CV mortality. Influence of each group of these factors may have different impacts depending on the stage of CKD. Importantly, date indeed suggests that the FGF-23-Klotho-sclerostin axis may be a potential novel target in cardio-renal medicine.

3. Possibilities for correction of FGF-23, soluble Klotho and sclerostin serum disorders in CKD by traditional renoprotective therapy

The appearance preliminary results of few clinical trials indicate the possibility of influencing traditional renoprotective therapy such as early correction of arterial hypertension, anaemia,

hyperphosphatasemia and nutritional status disorders on the maintenance of Klotho protein synthesis and FGF-23 overproduction suppression [51–53].

Since the serum FGF-23 level (as more earlier marker of MBD than PTH in CKD) rises before serum phosphorus increases as CKD advances, a preventive decrease in phosphorus diet content in CKD patients with elevated serum FGF-23 levels and the use of phosphate-binding drugs (for the control of serum phosphorus levels below 6.5 mg/dL) in CKD advancing are becoming an important therapeutic task in CKD patients. It can contribute not only in the prevention of SGPT but of CVC in CKD.

In our study [51], in the group of CKD 5D, patients who managed to reach and maintain the target level of serum phosphorus (0.9–1.45 mmol/L), compared to the matched group of patients with uncorrected hyperphosphatemia (>1.45 mmol/L), lower FGF-23 and PTH ($p <$ 0.01 and $p < 0.05$ respectively) in serum were noted, mainly among those patients who used phosphate-binding drugs that did not contain calcium (sevelamer hydrochloride) for correction of hyperphosphatasemia.

Among 17 patients who received low-protein diet (LPD) in combination with phosphate binders for at least 12 months before starting HD and who achieved the target level of serum phosphorus during the first year of treatment with regular HD, the formation of SGPT was noted significantly less ($\chi^2 = 8, 2$; $p < 0.05$) than among those patients who has begun correction of elevated serum phosphorus simultaneously starting with haemodialysis treatment. In these patients, CVC such as worsening of the functional class of angina pectoris, acute coronary syndrome and acute myocardial infarction [51] were also reliably less noted.

In experimental studies, increased Klotho expression was accompanied by a decrease in proteinuria and a significant decrease in angiotensin II in the hypertensive chronic glomerulonephritis mice [54, 55].

Data on the role of the asymmetric dimethylarginine complex 17/transforming growth factor-α/endothelial growth factor (ADAM17/TGF-α/EGFR) induced both due to renin-angiotensin system (RAS) activation and calcitriol deficiency, in the restructuring of the PTGs and in the decrease of Klotho expression in kidneys, allows to suggest the importance of the effective blockade of RAS by renin angiotensin blockers as well as D-hormone deficiency correction for the prevention and treatment of SGPT [55]. Our preliminary results [53, 56] confirm the experimental studies on the ability of AT II blockers to maintain the renal production of sKlotho protein.

It has been reported that angiotensin II and aldosterone, through the initiation of oxidative stress, have the ability to low Klotho expression in rat kidneys, even in minimal concentrations, while exogenous sKlotho infusion contributed to the inversion of renal damage caused by angiotensin II [55].

In our study in patients with CKD stages 1–5D [56] when comparing the patients with hypertension who were receiving antihypertensive monotherapy, the highest serum levels of Klotho protein were observed in those of them whose target BP level was achieved primarily through angiotensin II receptor blockers, compared to those who were administered with another drug group or have not reached the target blood pressure level ($p = 0.008$ and $p = 0.067$ respectively).

On experimental model of mice with CKD and arterial hypertension (AH), it was established that one of the mechanisms of sKlotho cardioprotection is also its ability to block the calcium channels in cardiomyocytes (TRPC6) that contributes to more adequate correction of AH and slower remodelling of the left ventricular myocardium [57].

A number of studies have shown that hypoxia due to anaemia is an independent factor of sKlotho protein production reduction as CKD advances [5, 6, 14]. According to our data, in patients with CKD with anaemia who managed to reach the target haemoglobin with the help of epoetin and iron and maintain it in this range and, as a result, eliminating the hypoxia of vital organs, including the kidneys, the preservation of decreasing of sKlotho protein was noted [58].

According to the results of our study [52], the use of Low Protein Diet (LPD) in combination with keto/amino acids, during not less than 12 months, in patients with 3B–4 stages of CKD, can prevent the development of nutritional status disorders, as well as stimulate sKlotho expression and suppress FGF-23 production. In addition, in these group patients, impairment of vascular damping function (according to the assessment of pulse wave velocity and augmentation indices by «SphygmoCor» device) as well as cardiac (by EchoCG, semiquantitative scale) and aorta calcification (by Kauppila method), and the formation of LVH, was less common.

In addition, according to our data, in patients with CKD 3B–4 stages using of LPD (0.6 g protein per kg body weight/day) supplemented with calcium salts of keto acids, it was possible to achieve and maintain the target level of serum phosphorus and calcium by using lower doses of phosphate-binding drugs, compared with the patients who used LPD, but did not take keto/amino acids [51, 52].

Maintenance of the phosphorus and calcium target serum levels can be a factor that inhibits FGF-23 overproduction and reduces the risk of ectopic mineralization and FGF-23-dependent LVH in CKD 3B–4 stages [51].

It is known that sKlotho paracrine functions include the activation of calcium channel receptors (TRPVs), especially TRPV5 and TRPV6 [14, 59]. TRPV5 are located mainly in the distal renal tubules and participate in the reabsorption of calcium in the kidneys [14]. TRPV6 is expressed in intestinal epithelial cells, where it is involved in the intestinal calcium absorption [14, 59]. In mice with a defect in Klotho gene expression, an increase in the serum level of phosphorus and calcium was revealed [5, 14]. Taking into account the participation of sKlotho in providing the constancy of serum calcium concentration by changing its reabsorption in the kidneys and intestines, it can be assumed that as a result of the intake of calcium salts of keto acids, it is possible to stimulate sKlotho production with its effect on the prevention of transient hypercalcaemia episodes in CKD advancement.

4. Conclusion

Understanding of the role of Klotho, FGF-23 and sclerostin in CKD is important, because it is known that mortality from cardiovascular complications in 20-year-old patients with terminal kidney disease is comparable with such of 80-year-old subjects in total population

[2]. And this very high mortality risk cannot be explained solely by influence of traditional CVD risk factors, which are highly prevalent in patients with CKD as well as by traditional CKD factors such as phosphorus and PTH, correction of which did not result to enough beneficial effects on cardiovascular survival.

Initial disturbances of mineral bone metabolism begin early, already with 3A stage of CKD, with an increase in serum FGF-23 and sclerostin and decrease of Klotho levels. The manifestation of these early changes may be as an increase in phosphorus excretion. From this moment, cardiovascular risk begins to be pawned, although the levels of phosphorus and PTH in serum are usually normal yet.

The accumulated data allow to consider the disturbances in FGF-23-Klotho-sclerostin ratio as one of the early markers of CKD advancement, disorders of mineral metabolism developing and cardiovascular prognosis. Alteration of the FGF-23/sKlotho/sclerostin ratio in serum as CKD advancement is accompanied by the development of vascular calcification, formation of cardiac remodelling and increasing risk of death from cardiovascular events, independently of the serum phosphorus and PTH levels. Changing the ratio of FGF-23, sKlotho and sclerostin can be regarded as independent early marker of cardiovascular and overall prognosis of patients with CKD.

In the reduction of Klotho expression in the kidneys, the role of ischemia, oxidative stress, intracellular elevation of angiotensin II and inflammation was established. These changes require careful correction to maintain Klotho's production as a potent strong cardio-renoprotective factor.

The preliminary results obtained on the positive effects of hypertension and anaemia correction on sKlotho protein maintenance, as well as the possibility of the FGF-23 overproduction suppression by correcting hyperphosphatemia, demonstrate the need for a personalized approach to the choice of cardio-renoprotective therapy from the early stages of CKD based on the degree of morphogenetic proteins and sclerostin system dysfunction as well as open prospects for studying of cardio-nephroprotective strategy in the new aspect.

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