Portofoliu lucrări științifice relevante

Conf. Dr. Diana Tania Luminița Moldovan

Nr.	Articol	Pagina
1.	**Moldovan D,** Rusu CC, Potra AR, Tirinescu D, Ticala M, Maslyennikov Y, Bărar AA, Urs A, Kacso IM. Nutritional Intervention and Musculoskeletal Health in Chronic Kidney Disease. Nutrients. 2025; 17(5):896.https://doi.org/10.3390/nu17050896 https://www.webofscience.com/wos/woscc/full-record/WOS:001442411600001 **ISI, IF=4.8; Q1.** prim autor	2
2.	**Moldovan D,** Rusu C, Potra A, Tirinescu D, Ticala M, Kacso I. Food to Prevent Vascular Calcification in Chronic Kidney Disease. Nutrients. 2024; 16, 617. https://doi.org/10.3390/nu16050617 https://www.webofscience.com/wos/woscc/full-record/WOS:001183043400001 **ISI, IF=4.8; Q1.** prim autor	24
3.	**Moldovan D.** The Severity of Carotid Calcifications, but Not Fibroblast Growth Factor 23, Is Associated with Mortality in Hemodialysis: A Single Center Experience. Diseases. 2025; 13(3):73. https://doi.org/10.3390/diseases13030073 **ISI, IF=2.9; Q2.** prim autor	40
4.	**Moldovan D,** Rusu C, Potra A, Moldovan I, Patiu IM, Gherman-Caprioara M, Kacso IM. Osteoprotegerin and uremic osteoporosis in chronic hemodialysis patients. Int Urol Nephrol. 2017; 49(5):895-901.https://doi.org/10.1007/s11255-017-1529-7 https://www.webofscience.com/wos/woscc/full-record/WOS:000400031800021 **ISI, IF=1.692; Q3.** prim autor	53
5.	**Moldovan D,** Kacso IM, Rusu C, Potra A, Bondor CI, Moldovan I, Patiu IM, Vladutiu D, Caprioara MG. Role of osteoprotegerin in vascular disorders of the end-stage renal disease patients. Biomarkers. 2015; 20(2):116-22. https://doi.org/10.3109/1354750X.2014.1000376 https://www.webofscience.com/wos/woscc/full-record/WOS:000358062100003 **ISI, IF=2.016; Q3.** prim autor	60
6.	**Moldovan D,** Rusu C, Potra A, Bondor C, Ticala M, Tirinescu D, Coman A, Orasan O, Moldovan I, Orasan R, Kacso I. Arterial calcifications and osteoprotegerin in chronic hemodialysis patients: impact on 6-year survival. Int Urol Nephrol 2022; 54:1135-1143. https://doi.org/10.1007/s11255-021-02988-3 https://www.webofscience.com/wos/woscc/full-record/WOS:000695634800001 **ISI, FI =2.26**. prim autor	67
7.	**Moldovan D,** Racasan S, Kacso IM, Rusu C, Potra A, Bondor C, Patiu IM, Gherman-Căprioară M. Survival after parathyroidectomy in chronic hemodialysis patients with severe secondary hyperparathyroidism. Int Urol Nephrol. 2015; 47(11):1871-7. https://doi.org/10.1007/s11255-015-1106-x https://www.webofscience.com/wos/woscc/full-record/WOS:000363940800017 **ISI, FI=1=292; Q3.** prim autor	76
8.	**Moldovan D,** Moldovan I, Rusu C, Kacso I, Patiu I, Gherman-Caprioara. FGF-23, vascular calcification and cardiovascular diseases in chronic hemodialysis patients. Int Urol Nephrol 2014; 46(1):121-8. https://doi.org/10.1007/s11255-013-0422-2 https://www.webofscience.com/wos/woscc/full-record/WOS:000330345300015 **ISI, IF=1.519; Q3.** prim autor	83
9.	Maslyennikov Y, Bărar AA, Rusu CC, Potra AR, Tirinescu D, Ticala M, Urs A, Pralea IE, Iuga CA, **Moldovan DT,** et al. The spectrum of minimal change disease/focal segmental glomerulosclerosis: from pathogenesis to proteomic biomarker research. Int J Mol Sci. 2025; 26(6):2450. https://doi.org/10.3390/ijms26062450 **ISI, FI=4.9; Q1** autor corespondent	91
10.	**Moldovan D,** Moldovan I, Rusu C, et al. Vascular calcifications and renal osteodystrophy in chronic hemodialysis patients: what is the relationship between them? Int Urol Nephrol. 2011; 43:1179-86.https://doi.org/10.1007/s11255-010-9841-5 https://www.webofscience.com/wos/woscc/full-record/WOS:000297477300034 ISI, IF=1.471; Q3. prim autor	115

nutrients

MDPI

Review

Nutritional Intervention and Musculoskeletal Health in Chronic Kidney Disease

Diana Moldovan [1,2,*], Crina Claudia Rusu [1,2], Alina Ramona Potra [1,2], Dacian Tirinescu [1,2], Maria Ticala [1,2], Yuriy Maslyennikov [1], Andrada Alina Bărar [1], Alexandra Urs [1] and Ina Maria Kacso [1,2]

[1] Department of Nephrology, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, 400012 Cluj-Napoca, Romania; alina.potra@umfcluj.ro (A.R.P.); tirinescu.dacian@umfcluj.ro (D.T.); cosa.maria@umfcluj.ro (M.T.); maslyennikov_yuriy@elearn.umfcluj.ro (Y.M.); barar_andrada_alina@elearn.umfcluj.ro (A.A.B.)
[2] Nephrology Clinic, Emergency County Hospital Cluj-Napoca, 400012 Cluj-Napoca, Romania
* Correspondence: diana.moldovan@umfcluj.ro

Abstract: Chronic kidney disease (CKD) is a leading condition in terms of prevalence and overall health impact. With the increased life expectancy of the CKD population and the improvement in medical care, controlling musculoskeletal complications remains a tough challenge. Patients with CKD are prone to falls, fractures and sarcopenia, enhancing the risk of death. A multitude of mechanisms contribute to fractures, and treatment is suboptimal; therefore, prevention must stand out as a key step. This review aims to provide an overview of the most relevant data regarding the impact of nutrition on bone disorders and sarcopenia in CKD. The newest relevant studies emphasize that plant protein intake is associated with a lower production of uremic toxins, lower serum phosphorus levels, and stronger bones. We conclude that patients with CKD should adopt specific diets tailored to the presence of osteoporosis, renal osteodystrophy, and muscle wasting. Low-protein diets or plant-dominant diets containing an adequate amount of protein could be better choices for predialysis patients with CKD in order to protect their bones and muscles, whereas in the dialysis population, a higher protein intake could be essential to prevent osteoporosis and sarcopenia. In all patients with CKD, focusing on antioxidant food intake could provide a strong antiaging benefit through ensuring good musculoskeletal health.

Keywords: chronic kidney disease; mineral and bone disorders; osteoporosis; sarcopenia; lifestyle; nutritional intervention; antioxidants

Academic Editor: Hiroshi Itoh

Received: 15 February 2025
Revised: 21 February 2025
Accepted: 25 February 2025
Published: 4 March 2025

Citation: Moldovan, D.; Rusu, C.C.; Potra, A.R.; Tirinescu, D.; Ticala, M.; Maslyennikov, Y.; Bărar, A.A.; Urs, A.; Kacso, I.M. Nutritional Intervention and Musculoskeletal Health in Chronic Kidney Disease. *Nutrients* **2025**, *17*, 896. https://doi.org/10.3390/nu17050896

1. Introduction

1.1. CKD—Epidemiology

We are living in a time in the history of medicine when non-communicable diseases have the greatest impact on survival, and among these, chronic kidney disease (CKD) is one of the most prevalent [1]. It is currently estimated that over 850 million persons have CKD worldwide [2], and as a result of the population aging, prediction data indicate that CKD will become the fifth global cause of death by 2040 and the second cause of death in countries with a long life expectancy by 2100 [3]. Moreover, the number of patients with end-stage renal disease (ESRD) is rapidly increasing, and these patients are confronting the most severe outcomes. Kidney transplantation, the best option for renal replacement therapy (RRT), is hampered by a shortage of kidney donors; therefore, a high demand for both hemodialysis (HD) and peritoneal dialysis has been registered in ESRD [3]. It is unfortunate that the survival benefit in chronic dialysis compared to

conservative management is overshadowed by the multitude of uncontrolled chronic symptoms and complications.

1.2. Bone and Muscle Health

The increasing life span of the global population has resulted in high interest in physical fitness and the prevention of disabilities. Yet, older adults struggle with frailty rather than enjoying mobility and health. In patients with CKD, musculoskeletal complications are common across all stages; however, the worst situation is observed in patients with ESRD when bone and muscle health suffering become difficult to tolerate [4]. Advancements in treatments for osteoporosis and other bone disorders come as a response to the need for autonomy and for the overall well-being of every person, even in elderly individuals [5]. However, in patients with CKD, none of the available treatments can halt bone suffering, and it is even more problematic that musculoskeletal disorders are associated with prolonged hospitalization and high mortality. One of the main risk factors for these negative outcomes is malnutrition [6].

1.3. Nutrition and CKD

The development of treatments for osteoporosis and sarcopenia is remarkable, but at the same time, we are witnessing increasing awareness regarding the role of lifestyle interventions as preventive measures. The progress has been slow and focused on removing unnecessary restrictions, mainly for food with clear health benefits for the general population. Natural bioactive compounds could be useful in treating CKD and can improve body composition in favor of muscles and healthy bone structure [7]. Studies on the impact of variable nutritional patterns on the musculoskeletal system in patients with CKD have provided conflicting results. Some of them revealed a relationship between different nutrients with better bone mineral density (BMD) or bone biopsy features, while others found a worse significant association [8,9]. The aims of this paper are to review the current knowledge about the interactions of nutritional intervention with bone disorders and sarcopenia in patients with CKD and to conclude what the best food choices are to achieve musculoskeletal strength.

2. Musculoskeletal Involvement in CKD—Pathogeny

2.1. Chronic Kidney Disease—Mineral and Bone Disorders (CKD-MBD)

The term CKD-MBD refers to a systemic syndrome involving mineral abnormalities of calcium (Ca), phosphorus (P), parathyroid hormone (PTH), or vitamin D levels, leading to bone complications vascular calcification due to CKD [10,11]. Renal osteodystrophy is a complex of different bone diseases in CKD, defined according to imagistic and histological abnormalities. There are several types of morphological bone modifications in CKD-MBD depending on turnover, mineralization, and strength. The remodeling process consists of the formation and resorption of bone tissues depending on the balance of osteoblast and osteoclast activities [8]. Renal osteodystrophy includes four entities, namely fibrocystic osteomalacia, adynamic bone disease, and mixed lesions. Fibrocystic osteitis is associated with high bone turnover secondary to hyperparathyroidism, while osteomalacia is the consequence of vitamin D deficiency, and adynamic bone disease occurs due to low bone turnover, which is frequently seen in patients with CKD with low PTH, particularly after parathyroidectomy [12]. The pathogeny of CKD-MBD implies the dysregulation of Ca and P, which depends on the action of parathormone (PTH), of the nutritional and active forms of vitamin D, and of the FGF23-Klotho axis and their impacts on bone. The body systems involved in this regulation process are the parathyroid glands, the small intestines, the bones, and the kidneys. They all interact to maintain normal serum levels of Ca and

P [13]. The clinical impact of this interplay is important in CKD, especially in advanced stages. In chronic HD, increased mortality was reported in patients with high P and Ca levels accompanied by high or low PTH levels [14].

In CKD-MBD, different factors contribute to frailty, such as uremic toxicity [15] or metabolic acidosis [16]. The amount of acid produced in the organism needs to be buffered, but in CKD, there is a decreased ability to generate bicarbonate. Studies have shown that acid loading adversely affects phosphorus homeostasis [16]. Prolonged metabolic acidosis decreases bone strength and favors fracture risk due to increased osteoclastic activity, and due to malnutrition [16].

Other important contributors to CKD-MBD are inflammation, oxidative stress, and mitochondrial dysfunction. CKD-MBD and malnutrition–inflammation complex syndromes are often associated in chronic dialysis patients, leading to a vicious cycle in which each perpetuates the other's progression [17].

2.2. Osteoporosis

Osteoporosis is one of the most common systemic bone diseases in clinical practice and mostly occurs in postmenopausal women [18]. Osteoporosis is a degradation of bone tissue structure with a decrease in the BMD, bone fragility, and high risk of fracture. About 9 million osteoporosis-related fractures are globally reported every year [5,18]. The pathogeny of bone loss and fracture in CKD is complex and multi-factorial, as bone strength is compromised due to the deterioration of both bone quantity and quality [19]. This could be an explanation for the difficulty of reaching a consensus on the optimal diagnostic method of osteoporosis in patients with CKD. As a result, osteoporosis assessment in CKD may include dual-energy x-ray absorptiometry (DXA) or quantitative computed tomography for quantity and imagistic trabecular bone score or bone biopsy for quality [20].

2.3. Sarcopenia

Sarcopenia is a progressive muscle disease characterized by loss of muscle mass, muscle strength and physical performance. Sarcopenia is age-related, has a high prevalence in CKD, up to 19%, and becomes more prominent as CKD progresses [21]. In children with CKD, the muscle–bone axis influences growth, and it is responsible for their muscle mass loss. Mechanisms such as systemic inflammation, anorexia, physical inactivity, vitamin D deficiency, impaired growth hormone/insulin growth factor 1 axis play important roles in physical underdevelopment of ESRD children [22]. The pathophysiology also involves ubiquitin, myostatin, uremic toxicity, reduced energy or nutrient intake, altered contractile activity, reduced myogenesis and reduced protein synthesis. All of these contribute to muscle degradation and impaired regeneration [23]. The theory of the functional muscle–bone unit has a physiological basis, and it is supported by two recent studies showing positive correlations between skeletal and, respectively, psoas muscle mass index with BMD in HD patients [24,25].

The pathogenic pathways leading to musculoskeletal disorders in CKD are numerous and the process is complex and multifactorial (Figure 1).

Intact bones, joints and muscles, good physical performance and the absence of specific symptoms, signs, laboratory and imagistic modifications are central to musculoskeletal health.

Figure 1. Pathogenic pathways associated with musculoskeletal disorders in CKD.

3. Impact of Musculoskeletal Disorders on Outcome in CKD Patients

3.1. Clinical Impact of CKD-MBD

As CKD progresses, more patients develop bone and mineral metabolism disorders. Clinical manifestations of CKD-MBD consist of bone pain, muscle–tendon ruptures, pruritus, increased incidence of fractures, bone deformities and growth arrest in children. The reported global incidence of falls in CKD patients is far higher than the general population. Approximately 30–60% of dialysis patients experienced falls, and of these, 16% resulted in fractures and 4% in death [26]. Fractures create a vicious cycle, reducing physical activity, which consequently causes muscle weakness, fear of falling, further decreased physical activity and, eventually, higher risk of falling [12,27]. Good physical function is a prerequisite for being involved in social and other different daily life activities. One of the main goals in the care of CKD patients in all stages is to prevent disability, because many patients with CKD-MBD experience functional impotence and lower quality of life. An accurate and feasible assessment of the CKD patient must include the evaluation of physical function [28]. CKD-MBD is an important risk factor for mortality and CKD progression leads to risk augmentation [29,30]. Mortality in HD patients with fractures is significantly higher than in those without fractures [31]. Therefore, measures to prevent fractures need to be actively implemented.

3.2. Clinical Impact of Osteoporosis in CKD

A Canadian study on 19,973 individuals analyzed the relationship between frailty and bone health. Frailty consists of at least three of these five symptoms: weakness, slow walking speed, low physical activity, exhaustion, and unintentional weight loss. The study demonstrated that having at least one frailty criterion was associated with a higher risk of fracture and a lower BMD [32]. CKD may be considered a risk factor for fractures, as low BMD in patients with CKD stages 3a–5D has been linked to a 1.5- to 2-fold higher fracture risk compared with BMD-matched patients without CKD [20]. Concomitant loss of BMD, bone strength, muscle mass and strength in CKD may lead to mechanical impairment of the muscle–bone axis with detrimental impact on social roles and, consequently, low quality of life [32].

3.3. Clinical Impact of Sarcopenia in CKD

CKD is responsible for muscle mass loss, with a great influence on physical performance. CKD also interferes in the biomechanical communication between muscles and bones, with an impact on outcomes. A recent registry study on a large number of adult Korean HD patients demonstrated that lean body mass has a significant role in predicting mortality in HD patients across different age groups [33].

4. Management of Musculoskeletal Disorders in CKD

There is a global focus on the goal of extending dialysis-free time and delaying dialysis initiation in CKD populations, with a secondary benefit in preventing some of the musculoskeletal problems [34]. However, specific approaches are needed. Kidney Disease Improving Global Outcomes (KDIGO) 2017 Clinical Practice Guideline for CKD-MBD has recommended thorough treatment decisions for the various typical bone diseases in CKD patients, but also for osteoporosis [10,11,14].

4.1. CKD-MBD Treatment

The current approach for CKD-MBD targets mainly hyperphosphatemia, hypocalcemia and vitamin D deficiency. The control of secondary hyperparathyroidism is the cornerstone for the management of CKD-MBD. Evidence has indicated that hyperphosphatemia is "a toxic factor" at multiple levels, and it has been associated with severe complications and increased mortality [12]. In controlling hyperphosphatemia, phosphorus binders plays a key role in eliminating the gastro-intestinal load. The range of agents extends from calcium-based (Ca carbonate or acetate) to noncalcic phosphate binders (sevelamer, lanthanum, or sucroferric oxyhydroxide). The first category can increase the risk of vascular calcification, and the second can be used only in advanced CKD stages. As for vitamin D use, in all stages of CKD, serum calcidiol should be maintained within the normal range. The use of calcitriol or any other active analogs is not routinely recommended, being reserved for severe hyperparathyroidism cases. For ESRD patients in chronic dialysis programs, calcimimetics are useful treatments for secondary hyperparathyroidism [13]. Adequate dialysis is essential in controlling CKD-MBD in CKD stage 5D, with the potential to eliminate phosphorus and normalize serum Ca levels. In patients with unresponsive or tertiary hyperparathyroidism, surgical removal of the parathyroid glands should be considered. The current recommendation is to perform parathyroidectomy if PTH levels remain above 800 pg/mL despite adequate medical treatment and if it is accompanied by clearly related signs and symptoms [11].

4.2. Osteoporosis Treatment

Some currently prescribed drugs for osteoporosis may not be suitable for CKD patients. The first-line anti-osteoporosis drugs, bisphosphonates, may worsen kidney function or, in ESRD, are relatively contraindicated. Denosumab, a humanized monoclonal RANKL antibody, is an antiresorptive factor not cleared by the kidney and has been used for treatment of osteoporosis, with positive effects in terms of BMD and fracture incidence. An interesting study has proven the beneficial effect of denosumab for muscle and bone tissue in osteoporotic mice and female humans [35]. It can be safely used, but in ESRD, there are concerns about the risk of severe hypocalcemia [20]. Romosozumab, a humanized monoclonal antibody and inhibitor of sclerostin, has been proven to cause a significant change in BMD in all kidney function categories [36]. Teriparatide, an anabolic, bone formation drug, is not recommended is most cases, being of special use in parathyroidectomized patients or those with high suspicion of low bone turnover disease. A recent meta-analysis highlighted the effectiveness of anti-osteoporotic agents (teriparatide, denosumab, romosozumab, ralox-

ifene) in lowering vertebral fracture risk in CKD patients, particularly in stages 1–3, with no benefit in stages 4 and 5 [36]. The absence of reported side effects encourages further research, especially because advanced CKD patients encounter the lowest BMD, and the needs are not yet met.

4.3. The Key Therapeutic Options for Sarcopenia

The key therapeutic options for sarcopenia management include one or a combination of exercise, nutrition, and pharmacological interventions. A growing body of literature has reported specific benefits of these interventions in CKD-associated sarcopenia across the disease spectrum [37]. There is a growing interest in the question of combining nutrition and exercise in CKD, and dialysis can improve the musculoskeletal outcomes [38,39].

5. Impact of Nutrition on Bone Disorders and Sarcopenia in CKD Patients

Defining healthy nutrition is somehow difficult, because it is a continually changing concept. This happens as a consequence of increasing focus on research in the food domain and due to ongoing study of the effects that different nutrients exert on the human body. Nutrition patterns and individual needs should be adjusted every time new and significant evidence points to a positive influence that a specific food group has on general health and on the prevention of non-communicable diseases, including CKD [40]. Conversely, given the potential of some foods to become risk factors for diseases, the "World Health Organization (WHO) Global Action Plan for the Prevention and Control of Noncommunicable Diseases" included strategies against behavioral risk factors, with a focus on unhealthy diets [41]. For the general population, WHO recommended balancing energy intake, increasing consumption of fruits and vegetables, and limiting the intake of saturated fats, sugar and salt [41].

Nutritional habits contribute to 20–40% of the risk of osteoporosis [42]. Chronic kidney disease is a condition associated with higher rates of osteoporosis than the general population. Therefore, dietary intervention to prevent the osseous manifestations of CKD is even more important. At the same time, optimal nutrition is more difficult to accomplish due to numerous risk factors and restrictions. As for CKD-MBD, the current diet recommendation includes low phosphate intake, reduced dietary acid loads, measures to correct Ca and vitamin D levels [11,43]. The latest guidelines for the management of CKD recommend increased consumption of plant-based foods and decreased consumption of animal-based foods and ultraprocessed foods. Education about dietary adaptations regarding P, Ca, sodium, potassium, protein, and even water intake should be an important step in CKD management. All these measures aim to delay progression to ESRD and to prevent chronic complications, particularly CKD-MBD and osteosarcopenia [34]. Lifestyle interventions such as modifying nutritional habits proved to be effective in reducing risk of vascular calcification [44] and frailty in CKD-MBD [45,46]. The efficacy and safety of these interventions in patients on musculoskeletal involvement in people with CKD are still undetermined. Thus, there is an urgent need for relevant and reliable information on whether specific foods are beneficial for all various bone diseases in CKD patients.

5.1. Proteins

A low-protein diet is often recommended to predialysis CKD patients. According to the KDOQI 2020 clinical practice guidelines for nutrition in CKD, daily protein intake recommendation should vary according to CKD stage. In stages 3–5, CKD patients are prescribed low protein intake (0.55 to 0.60 g/kg/day), aiming to postpone dialysis initiation by reducing uremic complications associated with protein catabolism [47]. The alternative

is very low protein intake (0.28–0.43 g/kg/day) with keto-amino acid analogues. With respect to the amount of protein intake, the KDIGO guidelines for CKD management updated to 2024 are less demanding, allowing in CKD stages 3–5 a protein intake of up to 0.8 g/kg/day, including at least 50% of proteins of high biological value [34].

Metabolic acidosis is one of the concerns regarding animal protein intake in CKD, due to its detrimental effects on bones and muscles. Food sources of acid are meat, cheese, and eggs, as all these increase the intake and metabolization of acid in the body. On the other hand, fruit and vegetables are good buffers, correcting metabolic acidosis. Contemporary fast-food diets deliver a high acid load, which has to be neutralized by the bones and muscles. Some studies reported that reducing acidic food by replacing meat with vegetal proteins can protect bones and muscles and slow CKD progression [48]. Several benefits emerge from metabolic acidosis correction in adult CKD patients, such as reduced protein degradation and muscle wasting, better serum albumin levels and nutritional status [16,19]. In a clinical setting, the nutritional status of CKD patients is best assessed using questionnaires, biochemical parameters such as serum albumin, cholesterol, even phosphorus and creatinine in HD patients and anthropometric measures and muscle strength. The follow-up is an important step for assessing adherence [34]. In a small study on subjects with CKD stages 3–4, an omnivorous diet containing 70% protein from plants for 4 weeks was efficacious in lowering urine phosphorus and titratable acid. The effect of such a diet was successful on mineral metabolism and acidosis in CKD. Therefore, it should implicitly be helpful in musculoskeletal health, as phosphorus and acidosis are the main pathogenic agents in CKD-MBD [49] (Table 1).

High-protein diets are essential for healthy bones, being associated with higher BMD. Undoubtedly, building or preserving muscle mass starts from a foundation of proteins. Chronic dialysis treatment leads to loss of proteins and increased muscle catabolism; hence, the KDOQI guidelines for nutrition in CKD recommend that patients treated with dialysis be prescribed high protein intake (1.0–1.2 g/kg/day) [47]. Based on the National Health and Nutrition Examination Survey (NHANES) data, Lee et al. evaluated bone densities of different femoral areas according to different protein diets. In subjects without CKD, higher-protein diets led to higher femoral BMD. High-protein CKD patients did not develop higher femoral BMD and neither did those with low-protein diets reduce their femoral BMD. However, CKD resulted as a risk factor for reduced BMD over the intertrochanteric bone region [9] (Table 1). In patients with ESRD, protein malnutrition is common and is usually caused by low-protein diets, CKD progression, and prolonged dialysis treatment. Hemodialysis is responsible for a loss of 3–8 g of aminoacids per session [50]. In a post hoc analysis of the IHOPE trial, HD patients were randomized for 12 months to placebo, 30 g of whey protein supplementation, or protein plus intradialytic bicycling. The study demonstrated that protein supplementation with or without exercise training is associated with improved hip BMD, preventing the loss of bone mass in elderly individuals [51] (Table 1).

Considering sarcopenia, it is common in ESRD, as these patients are highly sedentary and at risk of malnutrition. A recent Australian single-center cohort of maintenance HD patients reported a sarcopenia prevalence of 18%. In this study, low serum albumin and phosphate resulted as significant risk factors for muscle wasting. These findings confirm that protein malnutrition is an important risk factor for sarcopenia [52] (Table 1).

Specific protein consumption has gained impressive popularity in recent years as a complementary approach to body building. There is an ongoing debate about these protein supplements, especially creatine, one of the most commonly used, in regard to beneficial or harmful effects in CKD patients. Creatine is an essential contributor to cellular energy homeostasis. Recent findings suggest that endogenous creatine production

progressively decreases with increasing stages of CKD. Creatine deficiency in CKD was associated with fatigue, muscle wasting, impaired cognition, and high mortality. Creatine coming from meat and dairy increasingly becomes an essential nutrient and there is a debate about the fact that newer recommendations for plant-based diets may worsen this deficiency [53]. As might be expected, numerous concerns are addressed to the potential of creatine to impair kidney function. However, a clinical trial consisting of 12-week creatine supplementation showed a significant increase in serum creatinine, while cystatin C remained unchanged [54]. Other studies found no change in serum creatinine after creatine supplementation [55]. Consequently, in CKD patients receiving creatine supplementation, creatinine levels become unreliable in evaluating the renal function and cystatin C clearance should be used to assess glomerular filtration rate [53]. A recent small double-blinded study conducted on 40 HD patients explored the effect of creatine supplementation on body composition and malnutrition–inflammation score. At 1 year, creatine supplementation was associated with increased fat-free mass and skeletal muscle mass, but the authors assume that the simultaneous increase in intracellular water may influence results. There was no attenuation of malnutrition–inflammation score [56]. The results indicated that increasing creatine intake (even without resistance training) enhances fat-free mass and skeletal muscle mass in HD patients, indicating a potential role of creatine in the future nutritional management of sarcopenia in CKD (Table 1). In conclusion, healthy proteins from lean poultry, peas, chickpeas, beans, lentils, and fatty fish are possible choices in CKD for the benefits on muscular and osteoarticular functionality. The amount of protein depends on the CKD stage, and creatine may play a role in the future of CKD-MBD nutrition [57].

5.2. Phosphorus, Vitamin D, Calcium and Magnesium

Efforts to control hyperphosphatemia in patients with CKD are essential for preventing secondary hyperparathyroidism and renal osteodystrophy [58]. General recommendations for maintaining serum phosphorus within normal limits include monitoring the dietary intake of phosphorus and taking phosphate binders. Dietary strategies to reduce phosphate levels include abstaining from phosphate-rich foods, as well as paying attention to bioavailability [59]. The dietary phosphorus burden in kidney disease contributes to the pathogeny of CKD-MBD. Dietary phosphate restriction is used commonly to improve outcomes, but the fact that phosphate intake usually parallels protein intake makes the situation more complicated, as CKD patients need to receive an adequate amount of dietary protein to avoid malnutrition [58]. The real restriction should be addressed to phosphate additives. Such inorganic phosphate additives are widely used to produce fast foods, restructured meat, soft drinks, spreadable cheeses, sauces, and frozen bread products [60–62]. Sherman et al. measured the phosphorus, potassium, and protein content in uncooked meat and poultry products, and they discovered that phosphorus and potassium content were two- and three-fold higher, respectively, due to additive content, and this information is often lacking from food labels [60].

An interesting experiment was conducted by Gutierrez et al., assessing the impact of additive intake on bone. After an additive-enhanced diet, healthy individuals, but also mice had modified bone biomarkers, and the BMD decreased in mice [63] (Table 1). There is a very fine line regarding the balance of nutrients in food. Many of the natural foods known to be rich in phosphorus were traditionally disapproved in CKD-MBD. The capacity to absorb phosphate should also be considered while deciding on appropriate nutrition, as the intestinal absorptive rate of additive phosphate is over 80%, while for phosphate contained in plants, it is about 30% [64]. Lately, foods like beans and nuts have received more attention for use by CKD patients with the knowledge that the phosphorus is less

absorbed. Thus, in predialysis patients, beans and nuts may be more acceptable protein sources, because the phytate and fiber content leads to a lower phosphorus absorption rate [65]. A recent study has evaluated adults' phosphorus knowledge and dietary intake of phosphorus, hypothesizing that there would be a negative relationship, in which high phosphorus knowledge scores would contribute to lower consumption of phosphorus-rich foods. The results showed no association with phosphorus knowledge scores and dietary intake of phosphorus, indicating a gap in understanding and a need for tailored nutrition education among adults on dialysis [66].

Vitamin D deficiency is common in patients with CKD and associated with poor outcomes. Vitamin D is a well-known modulator of musculoskeletal health in CKD through its effects on Ca, P, PTH and, consequently, on bones [67]. Oxidative stress in CKD is also impactful on bones and exacerbated by vitamin D deficiency. Nonclassical vitamin D actions are thought to be related to effects on uremic inflammatory status, and on immune dysfunction. It was therefore considered as reasonable to improve 25-dihydroxyvitamin D supply for extrarenal production of 1,25-dihydroxyvitamin D. In HD patients, cholecalciferol therapy led to a significant decrease in inflammatory markers and cytokines [68]. Current clinical practice guidelines recommend supplementation with nutritional vitamin D, as for the general population. The deficit is usually corrected with oral medication, but natural sources of vitamin D such as fatty fish, hazelnuts and mushrooms should be seriously advised [69].

There may be a concern that excess magnesium (Mg) may impede bone mineralization. Sakaguchi et al. conducted a study on a large nation-wide database of patients undergoing HD in Japan with no history of hip fracture. The follow-up lasted 2 years and they identified 2% new hip fractures. The risk of hip fracture was not elevated in HD patients with mild hypermagnesemia, but lower serum Mg levels were significantly associated with an increased risk of hip fracture. The population-attributable fraction of serum Mg level for new hip fractures was 13.7% which was much higher than that of serum Ca, P, and PTH levels [70] (Table 1). A higher dietary amount of Mg can be obtained from cashews, peanuts, almonds or spinach intake. Interestingly, there is a significant interaction between Mg and phosphate in terms of absorption and bone effects [71].

5.3. Plant-Based Diets and Microbiota

5.3.1. Plant-Based Diets and Plant-Dominant Diets (PLADO)

A plant-based diet leads to a 12% lower risk of glomerular filtration rate decline [72]. Even new CKD cases can be prevented by eating plant-based foods. A community-based prospective Korean cohort study demonstrated that a diet rich in vegetables reduces the incidence of CKD [73]. Individual components of such a diet have a beneficial influence on blood pressure, lipid levels, thrombosis and fibrosis risks, oxidative stress, inflammatory responses, and endothelial function in CKD patients. Plant-based diets were also associated with a better quality of life, better management of complications and decreased mortality in adults with CKD [74,75]. Evidence supports the use of plant-based diets for their survival benefit. Important findings come from a registry study which proved the association between a diet with a higher proportion of protein from plant sources and lower mortality in CKD stages 3a–5 [74]. The consumption of fruits and vegetables in patients with stage 3 CKD is seen as an effective method for correcting metabolic acidosis and preserving renal function [76]. The latest KDOQI guidelines recommend an increased dietary intake of fruits and vegetables in adults with CKD 1-4, to decrease net acid production in order to reduce the rate of decline of kidney function and to protect bones from MBD [47]. Acidemia is a major contributor to bone abnormalities in CKD-MBD. Plant foods contain citrate, which is metabolized to bicarbonate and can help reduce metabolic acidosis [77],

leading to improvements in bone metabolism [16]. Among other nutrients, citrate is involved in pathophysiology and the management of bone diseases in CKD. Bone tissue is the main intrinsic source of citrate, being produced by osteoblasts. At the same time, citrate influences osteoblasts' differentiation and functionality. Increasing consumption of oranges, lemons or other types of citrus fruits with high citrate content may be helpful in neutralizing the acid load, and, consequently, in managing CKD-MBD [78]. A new experimental study demonstrated that diosmin, a bioflavonoid contained in citrus, protects against CKD-induced osteopenia in CKD rats, preserving bone mass and strength [79] (Table 1). Thus, we may assume that citrus consumption may have anti-osteoporosis properties in CKD patients.

In a single-center cross-sectional study on autosomal dominant polycystic kidney disease (ADPKD) patients, higher adherence to DASH diet (rich in vegetables, fruits and whole grains) was associated with low risk of reduced handgrip strength, indicating that the DASH dietary pattern may promote the preservation of muscle strength in ADPKD patients [80] (Table 1).

Yet, plant proteins are less anabolic and less digestible than animal proteins. Accordingly, for a similar anabolic effect on muscles and bones, the adequate amount of plant protein should be higher than the animal proteins [81]. Mansouri et al. evaluated the association between pro-vegetarian dietary patterns and the risk of protein-energy wasting and sarcopenia in CKD patients. Their findings indicated that greater adherence to pro-vegetarian diets was negatively associated with the odds of protein-energy wasting, but no association was shown between these diets and the odds of sarcopenia. Therefore, while these results encourage the consumption of plant-based foods, this dietary pattern may not effectively reduce the risk of sarcopenia in CKD patients, particularly if it causes insufficient protein intake [82].

High-protein plant-based food is advised if the potassium content is not too high [11]. Even regarding potassium-related restrictions, we have new surprising data. In a prospective study on HD patients, decreased dietary potassium intake was associated with higher mortality risk. These findings suggest that excessive dietary restriction may be harmful in HD patients [83]. These may indicate the need for a paradigm shift in judging "bad potassium" indiscriminately.

5.3.2. Uremic Toxins and Microbiota

There is a large body of evidence on the accumulation of uremic toxins in CKD patients and its detrimental influence on bone quality and quantity [84]. In most studies, indoxyl sulfate and p-cresyl sulfate, the most important uremic toxins, reduce bone turnover and suppress bone formation. Uremic toxins play a crucial role in the development of low bone turnover disease in CKD through downregulation of PTH receptor expression on osteoblasts and through induction of oxidative stress. Reactive oxygen species inhibit osteoblast functions and stimulate osteoclast proliferation, induce PTH resistance, contributing to the installation and progression of adynamic bone disease in CKD [85]. The results of Barreto et al. indicate a positive association between indoxyl sulfate levels and bone formation rate, osteoblast surface area, osteoid volume, and bone fibrosis volume [86]. Such findings may be explained by the role indoxyl sulfate plays in the pathogeny of bone resistance to PTH and, eventually, of low turnover bone disease [86,87] (Table 1). The impact of a uremic inflammatory environment is not negligible, with CKD inducing decreased muscle protein synthesis and muscle breakdown.

Microbiota plays a specific role in bone metabolism and function. CKD induces dysbiosis, with consequences for various pathological processes. A recent review highlighted important pathogenetic connections between gut microbiota and bone health in CKD

patients, demonstrating how the accumulation of indole and p-cresol in CKD disrupts microbiota and impairs bone formation by inducing PTH resistance in bone cells [88]. The decrease in gastrointestinal absorption and the increase in uremic toxin removal may be important in the treatment of uremic osteoporosis [89]. Probiotics are living microorganisms which restore the balance of intestinal microbiota and improve mucosal integrity. Foods rich in probiotics such as yogurt, kefir, pickles, sauerkraut or kimchi, can mitigate uremic toxicity and protect the musculoskeletal function [90]. Recently, a growing body of evidence has emerged on the benefits of plant-dominant low-protein diet (PLADO) in favoring healthy microbiomes. Adopting PLADO prevents constipation and, as a result, reduces hyperkalemia and relieves uremic toxin burden, with a better preventive effect against metabolic complications in CKD compared to animal protein-dominant intake [91]

A long-standing but untested component of the low-phosphorus diet is the promotion of refined grains over whole grains. Due to the beneficial fiber content of whole grains, this recommended restriction should be thoroughly analyzed. A recent paper reviewed scientific evidence for avoiding whole grains in the dialysis population. Although estimated phosphorus intake was higher, the modification of serum phosphorus was insignificant, concluding that there is no strong evidence in favor of restricting whole grain over refined grain [92].

The attempt to restrict phosphorus intake has led to other inappropriate recommendations, namely, to avoid nuts in CKD. However, less than a third of the dietary organic phosphorus from nuts is absorbed, so it is unlikely that they contribute to dietary phosphorus burden in CKD [93,94]. Various kinds of nuts have high fiber content, with benefits for creating a healthy microbiota. In an experimental model of CKD, a Brazil nut-enriched diet was able to modulate enteric glial cells and gut microbiota [95]. A different study proved the possibility of modulating BMD using a Brazil nut-enriched diet in a CKD experimental model [96].

5.4. Energy-Dense Foods

5.4.1. Energy-Dense Diets

Energy-dense diets are detrimental in regards to CKD, as they promote the main causes of CKD, obesity and diabetes mellitus. The least healthy choice consists of saturated fats. However, reverse epidemiology in advanced CKD links mortality with low body weight, so energy-dense diets may be helpful in preventing weight loss, often an indicator of frailty, which is an important risk factor for falls and fractures. In a group of older patients with advanced CKD, Molinari et al. have demonstrated that frailty is associated with malnutrition–inflammation syndrome, underscoring the importance of addressing malnutrition in order to prevent the onset of frailty in this population [97]. However, returning to its nephrotoxic potential, energy-dense food also has negative consequences on CKD-MBD. It induces decreased absorption and activation of vitamin D, hyperphosphatemia, high levels of FGF23, low levels of Klotho, and, eventually, renal osteodystrophy. Furthermore, obesity is a proinflammatory factor, promoting the progression of CKD and musculoskeletal disease. The implementation of caloric restriction with or without aerobic exercise is feasible and can improve body weight, as well as reduce fat mass and proinflammatory and oxidative stress [98]. We should be aware that dietary recommendations on energy intake should be different according to age, gender and physical activity, targeting an ideal body mass index (BMI)/waist-to-height ratio to be achieved through nutrition [34,47]. The latest KDIGO guideline on CKD management recommends a minimum of 150 min of moderate exercise to supplement the effects of nutrition [34]. Still, not all fat is bad.

5.4.2. Omega-3 Fatty Acids

Omega-3 fatty acids are polyunsaturated fatty acids (PUFAs), the active ingredients of oily fish, with important benefits for general health. An analysis of the NHANES cohort population proved a significant inverse relation between dietary omega-3 intake and all-cause mortality in patients with CKD [99]. Omega-3 fatty acids improve bone quality by promoting bone mineralization and preventing bone decay [100]. Alternative sources can be seafood, sesame, pumpkin chia or flax seeds. Recently, an original study demonstrated the causal beneficial effect of PUFAs on BMD and fracture risk [101]. The influence of omega-3 fatty acids on skeletal muscle protein metabolism and mitochondrial bioenergetics was demonstrated through muscle biopsies [102]. Liu et al. analyzed data from 8889 participants from the NHANES cohort and tested the relationship between the presence of osteopenia or osteoporosis based on BMD scores and dietary omega-3 intake. This study displayed a significant correlation between low dietary omega-3 fatty acid intake and high osteoporosis risk, suggesting that omega-3s play a crucial role in bone health. These findings are exciting, but patients with chronic renal failure were excluded from this study [103].

5.4.3. Olive Oil

Olive oil has been found to improve bone quality, by increasing alkaline phosphatase activity and bone mineralization through its content in hydroxytyrosol. This is a phenolic compound that has antioxidant, anti-inflammatory and antiosteoporotic properties. Olives and olive oil favor maturation of osteoblastic cells and prevent the loss of bone mass induced by inflammation and CKD [43,104].

5.4.4. Nuts

Some specific nuts, such as walnuts, pecan and pistachios, have a high content of omega-3 PUFAs. Nut consumption is another source of healthy fats, with valuable effects on CKD and mortality in the United States [105].

5.5. Antioxidant and Anti-Inflammatory Foods

Oxidative stress and abnormal osteocyte apoptosis are often related to dysregulation of bone turnover, and so vegetables or fruits with high antioxidant potential may play an important role in the prevention of chronic bone loss. An exciting new subfield of nutrition addresses antioxidant and anti-inflammatory foods. These are also called antiaging, senolytic, functional foods or superfoods. Redox signaling and inflammatory pathways play important roles in CKD-associated cachexia, sarcopenia and osteoporosis pathogeny [106]. Antioxidant depletion has been involved in chronic diseases and abnormal bone remodeling, which are signs of osteoporosis [107]. Exercise capacity in skeletal muscle is positively correlated with mitochondrial function, which is mainly controlled by mitochondrial biosynthesis and degradation. Therefore, in natural antioxidants lie great potential to reduce inflammation, oxidative stress and to prevent CKD-MBD [108].

5.5.1. Diets Rich in Berries

Diets rich in berries, especially blueberries or bilberries, which contain a large number of phytochemicals, also provide health benefits. The most prominent of these phytochemicals, termed anthocyanidins, have potent anti-inflammatory and antioxidant effects. Data suggests that blueberries might inhibit osteoclastogenesis in model mice and so could also be useful for the prevention of bone loss and osteoporosis. Delphinidin, one of the major anthocyanidins, prevents bone loss through the inhibition of excessive osteoclastogenesis in osteoporosis model mice [109]. Blueberry juice protects osteocytes and bone precursor cells

against oxidative stress, partly through the activation of SIRT1; reduced SIRT1 expression has been associated with osteoporotic hip fracture [110]. Various berries (blueberry, cranberry, raspberry, and strawberry) could possibly improve the uremic condition by reducing the levels of uremic toxins via modulation of the gut microbiota and, consequently, protect from bone and muscle loss [111].

5.5.2. Resveratrol Is a Polyphenol

Resveratrol is a polyphenol found in grape skins, blueberries, cocoa, and peanuts and has proven efficacy in reducing reactive oxygen and nitrogen species. There is evidence in favor of grape-based food supplementation to prevent the development of CKD [112]. It can contribute to bone restructuring through enhancing osteogenic differentiation and mitochondrial biogenesis from human periosteum-derived mesenchymal stem cells [113]. Murillo-Ortiz et al. conducted a study on 40 HD patients to test the effects of resveratrol and curcumin supplementation on the recovery of bone and muscle mass and protein oxidation, lipid peroxidation and iron overload. Curcumin is a polyphenolic compound and has been reported to have potential benefits for oxidative stress and inflammatory disease; it has the potential to prevent muscle damage and to increase osteoblast survival by downregulating nuclear factor KB. They proved that gain of bone and muscle mass was possible with combined supplementation with curcumin and resveratrol for 12 weeks [114] (Table 1). A recent study evaluated the effect of polyphenols on physical performance and body composition of 40 sarcopenic CKD patients. The authors used functional bars based on fruit, vegetables, extra virgin olive oil, micronized grape pomace, grape seeds and olive leaf powder derived from the recovery of waste from agri-food supply chains combined with adapted physical activity for 12 weeks. These ingredients were selected to evaluate the possible positive effects of foods rich in polyphenols in patients with CKD. This combination was helpful to counteract several CKD-related complications, such as arterial hypertension and uremic sarcopenia, and improve the CKD patients' quality of life [115] (Table 1).

5.5.3. Sulforaphane

Sulforaphane is a bioactive compound present in cruciferous vegetables and several beneficial functions have been observed in CKD. Extensive literature has shown that the main route of action is activation of the transcription nuclear factor erythroid 2 (Nrf2), which has a key role in the antioxidant response. As a result, sulforaphane protects against mitochondrial damage and helps normalize the gut microbiota [116].

5.5.4. Lycopene

Lycopene is one of the most powerful antioxidants in the diet. The Framingham Osteoporosis Study has demonstrated a positive association between the intake of lycopene, a phytonutrient of the carotenoid family, and increased BMD in lumbar vertebrae, in addition to a lower risk of non-vertebral and hip fractures at 4 years [117]. A small study evaluated the effects of lycopene and calcifediol on CKD-MBD through alkaline phosphatase, PTH, as surrogate bone markers and on oxidative stress. Tomato-derived lycopene decreased cholesterol oxidation products and, in association with daily calcifediol leads to normalization of alkaline phosphatase and PTH in elderly CKD patients, suggesting preventive effects on bone disorders [118].

Mounting evidence in support of antioxidant and anti-inflammatory nutrition in CKD leads to knowledge that can provide the foundation for the prevention of musculoskeletal disorders [7,119,120]. In a recent cross-sectional study on 2569 CKD participants from NHANES, dietary inflammatory potential was positively associated with sarcopenia in patients with CKD. The dietary inflammatory potential was calculated by the dietary in-

flammation index score based on dietary recall interviews, and sarcopenia was assessed by low skeletal muscle mass measured by dual-energy X-ray absorptiometry. The prevalence of sarcopenia was 19.11% of patients with CKD [121].

5.5.5. Brazil Nuts

Brazil nuts are an important source of selenium, which is an essential nutrient involved in bone metabolism [95]. The role in osseous remodeling is linked with a decrease in pro-inflammatory cytokine activity and direct action on osteoblasts, as the expression of selenoproteins [122]. A recent meta-analysis demonstrated that selenium intake is associated with higher BMD and a lower risk of osteoporosis and hip fracture [123].

5.5.6. Glutathione

Additionally, including foods naturally high in glutathione, like avocados, spinach, okra, and asparagus, may help decrease oxidative stress. Glutathione is one of the body's potent antioxidants. Several human and animal studies have found that eating sulfur-rich vegetables may reduce oxidative stress by increasing glutathione levels. Sulfur is required for the synthesis of glutathione, and the main sources are animal proteins and cruciferous vegetables like broccoli, Brussels sprouts, cauliflower and kale or allium vegetables like garlic and onions. Selenium is an essential mineral and a glutathione cofactor, the best sources being meats, cottage cheese and Brazil nuts. Nrf2, a transcription factor activated by oxidative stress, plays a crucial role in protecting against the harmful effects of excessive free radicals. Deletion of Nrf2 in osteoblasts results in significantly increased reactive oxygen species production due to lower glutathione levels [85].

Food has the potential to become an elixir for good and functional living in CKD, if used correctly and early in the course of the disease (Figure 2). Nephrologists should individualize dietary intervention to control osteoporosis, sarcopenia and renal osteodystrophy, according to co-morbidities and personal risk factors.

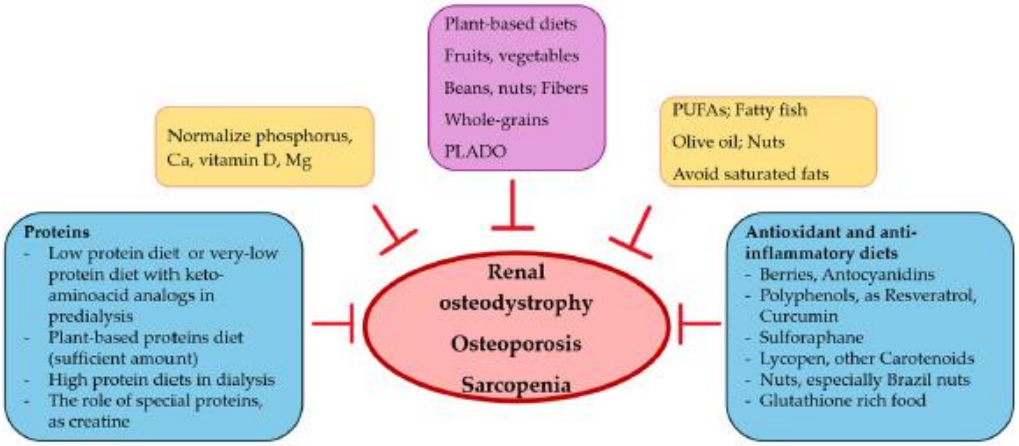

Figure 2. Nutritional intervention, bones and muscles in CKD.

Table 1. Impact of nutrition on musculoskeletal complications in CKD.

Study	Design	Results
Moorthi 2015 [49]	Thirteen subjects with CKD 3–4 received an omnivorous diet containing 70% protein from plants for 4 weeks.	Over the 4-week period, urine phosphorus and titratable acid significantly decreased on the diet. No significant changes in serum FGF23, P or PTH were noted. Hand grip strength and fat-free mass did not change. A 70% plant protein diet is safe and efficacious in lowering urine phosphorus.
Lee 2020 [9]	Based on the NHANES data, BMD of different femoral areas was evaluated according to different protein diets. 12,812 subjects were assigned to: (a) <0.8 g/kg/day, (b) 0.8–1.0 g/kg/day, (c) 1.0–1.2 g/kg/day, and (d) ≥1.2 g/kg/day.	Higher-protein diets led to higher femoral BMD only in subjects without CKD. Those with low-protein diet did not reduce their femoral BMD. CKD was a risk factor for reduced BMD over the intertrochanteric bone region.
Biruete 2016 [51]	A post hoc analysis of the IHOPE trial. 138 HD patients were randomized for 12 months to placebo, protein supplementation, or protein + exercise training.	Patients ≥ 60 years old on protein supplementation maintained hip-BMD. Hip-BMD decreased in placebo group. Similar trend was observed for the femoral neck BMD. There was a lack of effect on patients < 60 years old. There was no effect of protein supplementation on body composition or blood markers of bone metabolism (Ca, P, and PTH) in either age group. In conclusion, the intradialytic protein supplementation attenuated the decrease in hip-BMD, a predictor of fractures, in older HD patients.
Umakanthan 2021 [52]	Australian single-center cohort of 39 maintenance HD patients. Muscle mass, strength and function were evaluated using bioimpedance spectroscopy, hand grip dynamometer and the timed up and go test, respectively.	The prevalence of sarcopenia was 18%. Sarcopenia was associated with low serum albumin and low phosphate levels. Low serum albumin and phosphate, as markers of protein malnutrition, resulted as significant risk factors for muscle wasting.
Marini 2024 [56]	An exploratory 1-year, balanced, double-blind study on 40 HD patients assessed the effect of creatine supplementation on body composition, and malnutrition–inflammation score was evaluated. The follow-up period was 1-year.	Creatine supplementation in HD patients for 1 year increased fat-free mass and skeletal muscle mass, associated with an increase in intracellular water, and it did not attenuate the malnutrition–inflammation score.
Gutierrez 2015 [63]	10 individuals were fed a diet providing 1000 mg of phosphorus daily using low-additive diet for 1 week, followed by a diet containing identical food items but additive-enhanced. Parallel studies were conducted in animals fed low- and high-phosphorus diets for 5 or 15 weeks. The impact of phosphorus-rich additives on bone was tested.	After an additive-enhanced diet, healthy individuals, but also mice had modified bone biomarkers, FGF23, osteopontin, and osteocalcin levels increased and sclerostin decreased. The BMD decreased in mice.
Sakaguchi 2018 [70]	The study was conducted on a nationwide database with 113,683 patients undergoing HD in Japan with no history of hip fracture. The influence of serum magnesium (Mg) on the incidence of hip fractures was evaluated on 2-year follow-up.	2% new hip fractures The incidence rate was higher among patients in the lower quartiles of serum Mg. Lower serum Mg levels were significantly associated with an increased risk of hip fracture. The risk of hip fracture was not elevated in HD patients with mild hypermagnesemia. The population-attributable fraction of serum Mg level for incident hip fractures was 13.7% which was much higher than that of serum Ca, P, and PTH levels.
Sharma 2022 [79]	The osteoprotective effect of diosmin, a citrus-derived bioflavonoid, was tested in CKD rats.	FGF23 and PTH were increased in CKD and diosmin suppressed both. CKD reduced bone mass and deteriorated the microarchitecture of trabecular bones, and diosmin maintained both at control levels. Bone formation and strength were impaired in CKD and diosmin maintained these levels at control levels.
Ryu 2021 [80]	Cross-sectional study, 68 participants with ADPKD. Muscle strength was assessed based on handgrip strength. The relationship between DASH diet and muscle strength was tested.	27.9% had low handgrip strength. Higher adherence to DASH diet was associated with low risk of reduced handgrip strength. The DASH diet can be considered as a nutritional strategy to maintain muscle strength and prevent sarcopenia in ADPKD patients

Table 1. *Cont.*

Study	Design	Results
Barreto 2014 [86]	A post hoc analysis of a study on bone biopsy findings tested the relationship between indoxyl sulfate levels and bone formation rate in a group of 49 predialysis CKD patients.	The study found positive correlation between indoxyl sulfate levels and bone formation rate, osteoblast surface area, osteoid volume, and bone fibrosis volume.
Da Cruz 2024 [96]	Male Wistar rats were assigned to the following groups: sham, Nx, nephrectomized rats, and NxBN, nephrectomized rats and an enriched diet with 5% Brazil nut. Body composition parameters were obtained by DXA.	The NxBN group exhibited a significantly higher total body BMD than the Nx group. Brazil nut-enriched diet modulated BMD in CKD experimental model.
Molinari 2024 [97]	A cross-sectionally study evaluated the associations between frailty, malnutrition–inflammation syndrome and circulating inflammatory cytokines in 115 older individuals with advanced CKD.	Protein energy wasting was associated with frailty, as a manifestation of sarcopenia.
Murillo Ortiz 2019 [114]	A randomized, double-blind, placebo-controlled trial on 40 HD patients with iron overload which received combined supplementation with curcumin and resveratrol for 12 weeks	The treated group recovered bone and muscle mass.
Marrone 2024 [115]	40 CKD patients received functional foods (food bars from grape seed, grape pomace and olive leaf powders) and adapted physical activity training for 12 weeks. The progression of CKD-related comorbidities was evaluated.	This combination can help counteract uremic sarcopenia as well as arterial hypertension, dyslipidemia and improve the CKD patients' quality of life.
Mansouri 2024 [82]	Cross-sectional study evaluated the association between pro-vegetarian dietary pattern and the risk of protein-energy wasting (assessed by low protein intake, low body and muscle mass, low albumin levels) and sarcopenia (low muscle mass, strength and function) in 109 CKD patients.	Greater adherence to pro-vegetarian diets was negatively associated with the odds of protein-energy wasting, but no association was shown between these diets and the odds of sarcopenia.
Carluccio 2016 [118]	In octogenarians, nonagenarians and centenarians with predialysis CKD, vitamin D deficiency and abnormal ALP, PTH blood values, the effects of daily lycopene supplementation on blood oxysterols as markers of oxidative stress were evaluated. The effects of calcifediol administration together with daily lycopene supplementation on PTH and ALP blood concentrations were also investigated.	Tomato-derived lycopene decreased cholesterol oxidation products. Calcifediol and lycopene were associated with normalization of ALP and PTH, suggesting preventive effects on bone disorders.
Huang 2022 [121]	The study was cross-sectional on 2569 CKD participants from NHANES. The dietary inflammatory potential was calculated by the dietary inflammation index score based on dietary recall interviews. Sarcopenia was assessed by low skeletal muscle mass measured by DXA.	The prevalence of sarcopenia was 19.11% of patients with CKD. The dietary inflammatory potential was positively associated with sarcopenia in patients with CKD.

Abbreviations: CKD, chronic kidney disease; FGF23, fibroblast growth factor 23; P, phosphorus; PTH, parathyroid hormone; NHANES, National Health and Nutrition Examination Survey; BMD, bone mineral density; HD, hemodialysis; Ca, calcium; Mg, magnesium; ADPKD, autosomal dominant polycystic kidney disease; DASH, Dietary Approach to Stop Hypertension; ALP, alkaline phosphatase; DXA, dual-energy X-ray absorptiometry.

The publication of the newest controversial conference on CKD-MBD conclusions emphasizes the importance of bone complications and proposes two clinical syndromes: CKD-associated osteoporosis, encompassing increased fracture risk in patients with CKD, and CKD-associated cardiovascular disease, including vascular calcification [124].

6. Conclusions

Musculoskeletal disorders are increasingly more prevalent as CKD progresses to more advanced stages. There is a global recognition of the importance of preventing and mitigating CKD-MBD, osteoporosis and sarcopenia in the CKD population given the goal of extending a life with quality and without fractures, frailty or hospitalization. There is an increasing number of health professionals stating that the management of CKD should emphasize the role of diet. Traditional renal diets became questionable, as new evidence points to the benefits of nutrients that were previously restricted. This review highlights the urgent need for intensive efforts to improve lifestyle quality as a strategy for preventing and diminishing the burden of CKD-MBD, as we aim to have less disabled CKD persons with renal osteodystrophy. In future studies, it should be confirmed whether a specific nutritional intervention may prevent deterioration in bone strength and sarcopenia in the CKD population. Vitality is a goal for people with CKD and it is achievable, as there is evidence that food may act as a geroprotector and may modulate bone and muscle functionality. Using tailored precision nutrition approaches with or without other beneficial strategies may help prevent and treat CKD-MBD and osteosarcopenia.

Author Contributions: Conceptualization, D.M.; literature information gathering, D.M., C.C.R. and I.M.K.; writing—original draft preparation, D.M.; writing—review and editing, D.M., A.R.P., D.T. and M.T.; visualization, Y.M., A.A.B. and A.U.; supervision, D.M. and I.M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Bikbov, B.; Purcell, C.A.; Levey, A.S.; Smith, M.; Abdoli, A.; Abebe, M.; Owolabi, M.O. Global, regional, and national burden of chronic kidney disease, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2020**, *395*, 709–733. [CrossRef]
2. Jager, K.J.; Kovesdy, C.; Langham, R.; Rosenberg, M.; Jha, V.; Zoccali, C. A Single Number for Advocacy and Communication-Worldwide, More Than 850 million Individuals Have Kidney Diseases. *Nephrol. Dial. Transplant.* **2019**, *34*, 1803–1805. [CrossRef] [PubMed]
3. Chesnaye, N.C.; Ortiz, A.; Zoccali, C.; Stel, V.S.; Jager, K.J. The Impact of Population Ageing on The Burden of Chronic Kidney Disease. *Nat. Rev. Nephrol.* **2024**, *20*, 569–585. [CrossRef]
4. Kalantar-Zadeh, K.; Jafar, T.H.; Nitsch, D.; Neuen, B.L.; Perkovic, V. Chronic Kidney Disease. *Lancet* **2021**, *398*, 786–802. [CrossRef]
5. Compston, J.E.; McClung, M.R.; Leslie, W.D. Osteoporosis. *Lancet* **2019**, *393*, 364–376. [CrossRef] [PubMed]
6. Tseng, H.K.; Cheng, Y.J.; Yu, H.K.; Chou, K.T.; Pang, C.Y.; Hu, G.C. Malnutrition and Frailty Are Associated with a Higher Risk of Prolonged Hospitalization and Mortality in Hospitalized Older Adults. *Nutrients* **2025**, *17*, 221. [CrossRef] [PubMed]
7. Mafra, D.; Borges, N.A.; Lindholm, B.; Shiels, P.G.; Evenepoel, P.; Stenvinkel, P. Food as Medicine: Targeting the Uraemic Phenotype in Chronic Kidney Disease. *Nat. Rev. Nephrol.* **2021**, *17*, 153–171. [CrossRef]
8. Malluche, H.H.; Monier-Faugere, M.C.; Blomquist, G.; Davenport, D.L. Two-year cortical and trabecular bone loss in CKD-5D: Biochemical and clinical predictors. *Osteoporos. Int.* **2018**, *29*, 125–134. [CrossRef]

9. Lee, C.L.; Tsai, S.F. The Impact of Protein Diet on Bone Density in People With/Without Chronic Kidney Disease: An Analysis of The National Health and Nutrition Examination Survey Database. *Clin. Nutr.* **2020**, *39*, 3497–3503. [CrossRef]
10. KDIGO Clinical Practice Guideline for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD–MBD). *Kidney Int.* **2009**, *76*, S1–S13.
11. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). *Kidney Int. Suppl.* **2017**, *7*, 1–59. [CrossRef] [PubMed]
12. Lafage-Proust, M.H. Bone and Chronic Kidney Disease. *Semin. Musculoskelet. Radiol.* **2023**, *27*, 463–470. [CrossRef] [PubMed]
13. Cannata-Andía, J.B.; Martín-Carro, B.; Martín-Virgala, J.; Rodríguez-Carrio, J.; Bande-Fernández, J.J.; Alonso-Montes, C.; Carrillo-López, N. Chronic Kidney Disease—Mineral and Bone Disorders: Pathogenesis and Management. *Calcif. Tissue Int.* **2021**, *108*, 410–422. [CrossRef]
14. Ketteler, M.; Block, G.A.; Evenepoel, P.; Fukagawa, M.; Herzog, C.A.; McCann, L.; Moe, S.M.; Shroff, R.; Tonelli, M.A.; Toussaint, N.D.; et al. Executive Summary of the 2017 KDIGO Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) Guideline Update: What's changed and why it matters. *Kidney Int.* **2017**, *92*, 26–36. [CrossRef] [PubMed]
15. Chao, C.-T.; Lin, S.-H. Uremic Toxins and Frailty in Patients with Chronic Kidney Disease: A Molecular Insight. *Int. J. Mol. Sci.* **2021**, *22*, 6270. [CrossRef]
16. Khairallah, P.; Isakova, T.; Asplin, J.; Hamm, L.; Dobre, M.; Rahman, M.; Sharma, K.; Leonard, M.; Miller, E.; Jaar, B.; et al. Acid Load And Phosphorus Homeostasis in CKD. *Am. J. Kidney Dis.* **2017**, *70*, 541–550. [CrossRef]
17. Yamada, S.; Tsuruya, K.; Kitazono, T.; Nakano, T. Emerging Cross-Talks Between Chronic Kidney Disease–Mineral and Bone Disorder (CKD–MBD) and Malnutrition–Inflammation Complex Syndrome (MICS) in Patients Receiving Dialysis. *Clin. Experiment. Nephrol.* **2022**, *26*, 613–629. [CrossRef] [PubMed]
18. Liu, J.; Curtis, E.M.; Cooper, C.; Harvey, N.C. State of the Art in Osteoporosis Risk Assessment and Treatment. *J. Endocrinol. Invest.* **2019**, *42*, 1149–1164. [CrossRef]
19. Khairallah, P.; Nickolas, T.L. Management of Osteoporosis in CKD. *Clin. J. Am. Soc. Nephrol.* **2018**, *13*, 962–969. [CrossRef]
20. Abdalbary, M.; Sobh, M.; Elnagar, S.; Elhadedy, M.; Elshabrawy, N.; Abdelsalam, M.; Asadipooya, K.; Sabry, A.; Halawa, A.; El-Husseini, A. Management of Osteoporosis in Patients with Chronic Kidney Disease. *Osteoporos. Int.* **2022**, *33*, 2259–2274. [CrossRef]
21. Pereira, C.D.; Guimarães, C.; Ribeiro, V.S.; Vaz, D.C.; Martins, M.J. Low-Protein Diets, Malnutrition, and Bone Metabolism in Chronic Kidney Disease. *Nutrients* **2024**, *16*, 3098. [CrossRef] [PubMed]
22. Karava, V.; Dotis, J.; Christoforidis, A.; Kondou, A.; Printza, N. Muscle-Bone Axis in Children with Chronic Kidney Disease: Current Knowledge and Future Perspectives. *Pediatr. Nephrol.* **2021**, *36*, 3813–3827. [CrossRef]
23. Nishi, H.; Takemura, K.; Higashihara, T.; Inagi, R. Uremic Sarcopenia: Clinical Evidence and Basic Experimental Approach. *Nutrients* **2020**, *12*, 1814. [CrossRef]
24. Ito, K.; Ookawara, S.; Hibino, Y.; Imai, S.; Fueki, M.; Bandai, Y.; Morishita, Y. Skeletal Muscle Mass Index is Positively Associated with Bone Mineral Density in Hemodialysis Patients. *Front. Med.* **2020**, *7*, 187. [CrossRef]
25. Ito, K.; Ookawara, S.; Sanayama, H.; Kakuda, H.; Kanai, C.; Iguchi, K.; Shindo, M.; Tanno, K.; Ishibashi, S.; Kakei, M.; et al. Association Between Psoas Muscle Mass Index and Bone Mineral Density in Patients Undergoing Hemodialysis. *Sci. Rep.* **2025**, *15*, 544. [CrossRef]
26. Cook, W.L.; Tomlinson, G.; Donaldson, M.; Markowitz, S.N.; Naglie, G.; Sobolev, B.; Jassal, S.V. Falls and fall-related injuries in older dialysis patients. *Clin. J. Am. Soc. Nephrol.* **2006**, *1*, 1197–1204. [CrossRef]
27. Shirai, N.; Inoue, T.; Ogawa, M.; Okamura, M.; Morishita, S.; Suguru, Y.; Tsubaki, A. Relationship Between Nutrition-Related Problems and Falls in Hemodialysis Patients: A Narrative Review. *Nutrients* **2022**, *14*, 3225. [CrossRef] [PubMed]
28. Koufaki, P. Assessment of Function Limitations in People with Chronic Kidney Disease for Implementation in Clinical Practice. *Kidney Dial.* **2022**, *2*, 234–244. [CrossRef]
29. Moldovan, D.; Rusu, C.; Potra, A.; Bondor, C.; Ticala, M.; Tirinescu, D.; Coman, A.; Orasan, O.; Moldovan, I.; Orasan, R.; et al. Arterial Calcifications and Osteoprotegerin in Chronic Hemodialysis Patients: Impact On 6-Year Survival. *Int. Urol. Nephrol.* **2022**, *54*, 1135–1143. [CrossRef]
30. Morishita, S.; Tsubaki, A.; Shirai, N. Physical Function Was Related to Mortality in Patients with Chronic Kidney Disease and Dialysis. *Hemodial. Int.* **2017**, *21*, 483–489. [CrossRef]
31. Wakasugi, M.; Kazama, J.J.; Wada, A.; Hamano, T.; Masakane, I.; Narita, I. Long-term excess mortality after hip fracture in hemodialysis patients: A nationwide cohort study in Japan. *J. Bone Miner. Metab.* **2020**, *38*, 718–729. [CrossRef] [PubMed]
32. Dufour, A.; Kurtz, K.A.; Vachey, C.; Mac-Way, F. Association Between Frailty and Bone Health in Early-Stage Chronic Kidney Disease: A Study from the Population-Based CARTaGENE Cohort. *Clin. Kidney J.* **2025**, *18*, sfaf015. [CrossRef]
33. Kim, J.E.; Yi, J.; Kim, J.H.; Kim, K.; Song, J.H.; Lee, S.W.; Hwang, S.D. The Role of Lean Body Mass in Predicting Mortality in Hemodialysis Patients Across Different Age Groups. *Sci. Rep.* **2025**, *15*, 2150. [CrossRef]

34. Kidney Disease: Improving Global Outcomes, C.K.D.W.G. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int.* **2024**, *105*, S117–S314. Available online: https://kdigo.org/guidelines/ckd-evaluation-and-management/ (accessed on 10 February 2025).
35. Bonnet, N.; Bourgoin, L.; Biver, E.; Douni, E.; Ferrari, S. RANKL Inhibition Improves Muscle Strength and Insulin Sensitivity and Restores Bone Mass. *J. Clin. Investig.* **2019**, *129*, 3214–3223. [CrossRef]
36. Sabaghian, T.; Delkash, P.; Rahmannia, M.; Shahidi Bonjar, A.H.; Centis, R.; D'Ambrosio, L.; Sotgiu, G.; Nasiri, M.J.; Migliori, G.B. Efficacy and Safety of Anti-Osteoporotic Agents across CKD Stages: A Meta-Analysis of Randomized Clinical Trials. *Kidney Blood Press. Res.* **2024**, *49*, 581–587. [CrossRef] [PubMed]
37. Noor, H.; Reid, J.; Slee, A. Resistance Exercise and Nutritional Interventions for Augmenting Sarcopenia Outcomes in Chronic Kidney Disease: A Narrative Review. *J. Cachexia Sarcopenia Muscle* **2021**, *12*, 1621–1640. [CrossRef]
38. Meade, A.; McLaren, C.; Bennett, P.N. Combining Exercise and Nutrition in Chronic Kidney Disease and Dialysis: Can We Learn from the Performance Nutrition of Athletes? *Semin. Dial.* **2024**, *37*, 3–9. [CrossRef] [PubMed]
39. Notaras, S.; Lambert, K.; Perz, J.; Makris, A. Diet in the Management of Non-Dialysis Dependent Chronic Kidney Disease: Perceptions and Practices of Health Professionals. *BMC Nephrol.* **2022**, *23*, 158. [CrossRef]
40. Cena, H.; Calder, P.C. Defining a Healthy Diet: Evidence for the Role of Contemporary Dietary Patterns in Health and Disease. *Nutrients* **2020**, *12*, 334. [CrossRef]
41. World Health Organization. *Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013–2020*; World Health Organ: Geneva, Switzerland, 2013.
42. Reid, I.R. A broader strategy for osteoporosis interventions. *Nat. Rev. Endocrinol.* **2020**, *16*, 333–339. [CrossRef]
43. Bargagli, M.; Arena, M.; Naticchia, A.; Gambaro, G.; Mazzaferro, S.; Fuster, D.; Ferraro, P.M. The Role of Diet in Bone and Mineral Metabolism and Secondary Hyperparathyroidism. *Nutrients* **2021**, *13*, 2328. [CrossRef] [PubMed]
44. Moldovan, D.; Rusu, C.; Potra, A.; Tirinescu, D.; Ticala, M.; Kacso, I. Food to Prevent Vascular Calcification in Chronic Kidney Disease. *Nutrients* **2024**, *16*, 617. [CrossRef] [PubMed]
45. Mayes, J.; Young, H.M.L.; Blacklock, R.M.; Lightfoot, C.J.; Chilcot, J.; Nixon, A.C. Targeted Non-Pharmacological Interventions for People Living with Frailty and Chronic Kidney Disease. *Kidney Dial.* **2022**, *2*, 245–261. [CrossRef]
46. Agarwal, S.K. Lifestyles and Their Effect on Chronic Kidney Diseases. *East Afr. Sch. J. Med. Sci.* **2021**, *4*, 183–193. [CrossRef]
47. Ikizler, T.A.; Burrowes, J.D.; Byham-Gray, L.D.; Campbell, K.L.; Carrero, J.-J.; Chan, W.; Fouque, D.; Friedman, A.N.; Ghaddar, S.; Goldstein-Fuchs, D.J.; et al. KDOQI Clinical Practice Guideline for Nutrition in CKD: 2020 Update. *Am. J. Kidney Dis.* **2020**, *76*, S1–S107. [CrossRef]
48. Angéloco, L.R.N.; de Souza, G.C.A.; Romão, E.A.; Chiarello, P.G. Alkaline Diet and Metabolic Acidosis: Practical Approaches to the Nutritional Management of Chronic Kidney Disease. *J. Ren. Nutr.* **2018**, *28*, 215–220. [CrossRef] [PubMed]
49. Moorthi, R.N.; Armstrong, C.L.; Janda, K.; Ponsler-Sipes, K.; Asplin, J.R.; Moe, S.M. The Effect of a Diet Containing 70% Protein from Plants on Mineral Metabolism and Musculoskeletal Health in Chronic Kidney Disease. *Am. J. Nephrol.* **2014**, *40*, 582–591. [CrossRef]
50. Sahathevan, S.; Khor, B.H.; Ng, H.M.; Gafor, A.H.A.; Daud, Z.A.M.; Mafra, D.; Karupaiah, T. Understanding Development of Malnutrition in Hemodialysis Patients: A Narrative Review. *Nutrients* **2020**, *12*, 3147. [CrossRef]
51. Biruete, A.; Fitschen, P.; Jeong, J.; Wu, P.-T.; Tomayko, E.; Wilund, K. TH-OR067 Intradialytic Protein Supplementation Increases Protein Intake in Older, but Not Younger, Hemodialysis Patients and is Associated with Improved Hip Bone Mineral Density. *J. Am. Soc. Nephrol.* **2016**, *27*, 17A.
52. Umakanthan, M.; Li, J.W.; Sud, K.; Duque, G.; Guilfoyle, D.; Cho, K.; Brown, C.; Boersma, D.; Gangadharan Komala, M. Prevalence and Factors Associated with Sarcopenia in Patients on Maintenance Dialysis in Australia—A Single Centre, Cross-Sectional Study. *Nutrients* **2021**, *13*, 3284. [CrossRef] [PubMed]
53. Post, A.; Tsikas, D.; Bakker, S.J.L. Creatine is a Conditionally Essential Nutrient in Chronic Kidney Disease: A Hypothesis and Narrative Literature Review. *Nutrients* **2019**, *11*, 1044. [CrossRef] [PubMed]
54. Gualano, B.; Ugrinowitsch, C.; Novaes, R.B.; Artioli, G.G.; Shimizu, M.H.; Seguro, A.C.; Harris, R.C.; Lancha, A.H. Effects of Creatine Supplementation on Renal Function: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. *Eur. J. Appl. Physiol.* **2008**, *103*, 33–40. [CrossRef] [PubMed]
55. Lugaresi, R.; Leme, M.; de Salles Painelli, V.; Murai, I.H.; Roschel, H.; Sapienza, M.T.; Lancha, A.H., Jr.; Gualano, B. Does long-term creatine supplementation impair kidney function in resistance-trained individuals consuming a high-protein diet? *J. Int. Soc. Sports Nutr.* **2013**, *10*, 26. [CrossRef]
56. Marini, A.C.B.; Schincaglia, R.M.; Candow, D.G.; Pimentel, G.D. Effect of Creatine Supplementation on Body Composition and Malnutrition-Inflammation Score in Hemodialysis Patients: An Exploratory 1-Year, Balanced, Double-Blind Design. *Nutrients* **2024**, *16*, 615. [CrossRef]
57. Kim, J.W.; Yang, S.J. Dietary Patterns, Kidney Function, and Sarcopenia in Chronic Kidney Disease. *Nutrients* **2025**, *17*, 404. [CrossRef]

58. St-Jules, D.E.; Rozga, M.R.; Handu, D.; Carrero, J.J. Effect of Phosphate-Specific Diet Therapy on Phosphate Levels in Adults Undergoing Maintenance Hemodialysis. A Systematic Review and Meta-Analysis. *Clin. J. Am. Soc. Nephrol.* **2021**, *16*, 107–120. [CrossRef]
59. Shah, A. Phosphorus and other aspects of CKD-MBD in the conservative management of chronic kidney disease. *Panminerva Med.* **2017**, *59*, 124–132. [CrossRef]
60. Asan, N.B.; Kun, D.W.W.; Ooi, Y.B.H.; Khor, B.H. A Survey on nutrition labeling for sodium, potassium, and phosphorus of packaged food and beverages. *J. Ren. Nutr.* **2025**, *35*, 229–233. [CrossRef]
61. Sherman, R.A.; Mehta, O. Phosphorus and Potassium Content of Enhanced Meat and Poultry Products: Implications for Patients Who Receive Dialysis. *Clin. J. Am. Soc. Nephrol.* **2009**, *4*, 1370–1373. [CrossRef]
62. Salera, D.; Bellasi, A.; Del Vecchio, L.; Locatelli, F. Update on current and emerging treatment paradigms for hyperphosphatemia in chronic kidney disease. *Expert Opin. Pharmacother.* **2025**, *26*, 85–100. [CrossRef] [PubMed]
63. Gutiérrez, O.M.; Luzuriaga-McPherson, A.; Lin, Y.; Gilbert, L.C.; Ha, S.W.; Beck, G.R., Jr. Impact of Phosphorus-Based Food Additives on Bone and Mineral Metabolism. *J. Clin. Endocrinol. Metab.* **2015**, *100*, 4264–4271. [CrossRef]
64. Rysz, J.; Franczyk, B.; Rokicki, R.; Gluba-Brzózka, A. The Influence of Dietary Interventions on Chronic Kidney Disease–Mineral and Bone Disorder (CKD-MBD). *Nutrients* **2021**, *13*, 2065. [CrossRef] [PubMed]
65. Elder, G.J.; Malik, A.; Lambert, K. Role of Dietary Phosphate Restriction in Chronic Kidney Disease. *Nephrology* **2018**, *23*, 1107–1115. [CrossRef] [PubMed]
66. Schneider, S.T.; Klug, A.; Andrade, J.M. Phosphorus Knowledge and Dietary Intake of Phosphorus of US Adults Undergoing Dialysis. *Nutrients* **2024**, *16*, 2034. [CrossRef]
67. Molina, P.; Chauveau, P.; Mazzaferro, S.; Torres, P.U.; Bover, J.; Carrero, J.J. Vitamin D, a Modulator of Musculoskeletal Health in Chronic Kidney Disease. *J. Cachexia Sarcopenia Muscle* **2017**, *8*, 686–701. [CrossRef]
68. Matias, P.J.; Jorge, C.; Ferreira, C.; Borges, M.; Aires, I.; Amaral, T.; Gil, C.; Cortez, J.; Ferreira, A. Cholecalciferol Supplementation in Hemodialysis Patients: Effects on Mineral Metabolism, Inflammation, and Cardiac Dimension Parameters. *Clin. J. Am. Soc. Nephrol.* **2010**, *5*, 905–911. [CrossRef] [PubMed]
69. Jørgensen, H.S.; Vervloet, M.; Cavalier, E.; Bacchetta, J.; de Borst, M.H.; Bover, J.; Evenepoel, P.; Cozzolino, M.; Ferreira, A.C.; Hansen, D.; et al. The Role of Nutritional Vitamin D in CKD-MBD in Children and Adults with CKD, on Dialysis and After Kidney Transplantation—A European Consensus Statement. *Nephrol. Dial. Transplant.* **2025**, gfae293. [CrossRef]
70. Sakaguchi, Y.; Hamano, T.; Wada, A.; Hoshino, J.; Masakane, I. Magnesium and Risk of Hip Fracture among Patients Undergoing Hemodialysis. *J. Am. Soc. Nephrol.* **2018**, *29*, 991–999. [CrossRef]
71. Sakaguchi, Y. The emerging role of magnesium in CKD. *Clin. Experiment. Nephrol.* **2022**, *26*, 379–384. [CrossRef]
72. Palmer, S.C.; Maggo, J.K.; Campbell, K.L.; Craig, J.C.; Johnson, D.W.; Sutanto, B.; Ruospo, M.; Tong, A.; Strippoli, G.F. Dietary interventions for adults with chronic kidney disease. *Cochrane Database Syst. Rev.* **2017**, *2017*, CD011998. [CrossRef] [PubMed]
73. Jhee, J.H.; Kee, Y.K.; Park, J.T.; Chang, T.I.; Kang, E.W.; Yoo, T.H.; Kang, S.W.; Han, S.H. A Diet Rich in Vegetables and Fruit and Incident CKD: A Community-Based Prospective Cohort Study. *Am. J. Kidney Dis.* **2019**, *74*, 491–500. [CrossRef] [PubMed]
74. Chen, X.; Wei, G.; Jalili, T.; Metos, J.; Giri, A.; Cho, M.E.; Boucher, R.; Greene, T.; Beddhu, S. The Associations of Plant Protein Intake with All-Cause Mortality in CKD. *Am. J. Kidney Dis.* **2016**, *67*, 423–430. [CrossRef]
75. Carrero, J.J.; González-Ortiz, A.; Avesani, C.M.; Bakker, S.J.L.; Bellizzi, V.; Chauveau, P.; Clase, C.M.; Cupisti, A.; Espinosa-Cuevas, A.; Molina, P.; et al. Plant-based Diets to Manage the Risks and Complications of Chronic Kidney Disease. *Nat. Rev. Nephrol.* **2020**, *16*, 525–542. [CrossRef] [PubMed]
76. Goraya, N.; Simoni, J.; Jo, C.H.; Wesson, D.E. Treatment of Metabolic Acidosis in Patients with Stage 3 Chronic Kidney Disease with Fruits and Vegetables or Oral Bicarbonate Reduces Urine Angiotensinogen and Preserves Glomerular Filtration Rate. *Kidney Int.* **2014**, *86*, 1031–1038. [CrossRef]
77. Chen, Y.; Wu, J.; Yu, D.; Liu, M. Plant or Animal-based or PLADO diets: Which Should Chronic Kidney Disease Patients Choose? *J. Ren. Nutr.* **2022**, *33*, 228–235. [CrossRef]
78. Granchi, D.; Baldini, N.; Ulivieri, F.M.; Caudarella, R. Role of Citrate in Pathophysiology and Medical Management of Bone Diseases. *Nutrients* **2019**, *11*, 2576. [CrossRef]
79. Sharma, S.; Porwal, K.; Kulkarni, C.; Pal, S.; Sihota, P.; Kumar, S.; Chattopadhyay, N.; Tiwari, M.C.; Katekar, R.; Kumar, A.; et al. Diosmin, a citrus fruit-derived phlebotonic bioflavonoid protects rats from chronic kidney disease-induced loss of bone mass and strength without deteriorating the renal function. *Food Funct.* **2022**, *13*, 2184–2199. [CrossRef]
80. Ryu, H.; Yang, Y.J.; Kang, E.; Ahn, C.; Yang, S.J.; Oh, K.H. Greater Adherence to the Dietary Approaches to Stop Hypertension Dietary Pattern is Associated with Preserved Muscle Strength in Patients with Autosomal Dominant Polycystic Kidney Disease: A Single-Center Cross-Sectional Study. *Nutr. Res.* **2021**, *93*, 99–110. [CrossRef]
81. Massini, G.; Caldiroli, L.; Molinari, P.; Carminati, F.M.L.; Castellano, G.; Vettoretti, S. Nutritional Strategies to Prevent Muscle Loss and Sarcopenia in Chronic Kidney Disease: What Do We Currently Know? *Nutrients* **2023**, *15*, 3107. [CrossRef]

82. Mansouri, F.; Shateri, Z.; Jahromi, S.E.; Mahmudi-Zadeh, M.; Nouri, M.; Babajafari, S. Association Between Pro-Vegetarian Dietary Pattern and the Risk of Protein-Energy Wasting and Sarcopenia in Patients with Chronic Kidney Disease. *J. Health Popul. Nutr.* **2024**, *43*, 110. [CrossRef] [PubMed]
83. Narasaki, Y.; Okuda, Y.; Kalantar, S.S.; You, A.S.; Novoa, A.; Nguyen, T.; Streja, E.; Nakata, T.; Colman, S.; Kalantar-Zadeh, K.; et al. Dietary Potassium Intake and Mortality in a Prospective Hemodialysis Cohort. *J. Ren. Nutr.* **2021**, *31*, 411–420. [CrossRef]
84. Hung, K.-C.; Yao, W.-C.; Liu, Y.-L.; Yang, H.-J.; Liao, M.-T.; Chong, K.; Peng, C.-H.; Lu, K.-C. The Potential Influence of Uremic Toxins on the Homeostasis of Bones and Muscles in Chronic Kidney Disease. *Biomedicines* **2023**, *11*, 2076. [CrossRef] [PubMed]
85. Yiang, G.T.; Su, W.L.; Zheng, C.M.; Liao, M.T.; Cheng, T.H.; Lu, C.L.; Lu, K.C. The Influence of Uremic Toxins on Low Bone Turnover Disease in Chronic Kidney Disease. *Tzu Chi Med. J.* **2024**, *36*, 38–45. [CrossRef] [PubMed]
86. Barreto, F.C.; Barreto, D.V.; Canziani, M.E.; Tomiyama, C.; Higa, A.; Mozar, A.; Glorieux, G.; Vanholder, R.; Massy, Z.; de Carvalho, A.B. Association Between Indoxyl Sulfate and Bone Histomorphometry in Pre-Dialysis Chronic Kidney Disease Patients. *J. Bras. Nefrol.* **2014**, *36*, 289–296. [CrossRef]
87. Leong, S.C.; Sirich, T.L. Indoxyl Sulfate—Review of Toxicity and Therapeutic Strategies. *Toxins* **2016**, *8*, 358. [CrossRef] [PubMed]
88. Rodrigues, F.G.; Ormanji, M.S.; Heilberg, I.P.; Bakker, S.J.L.; de Borst, M.H. Interplay between gut microbiota, bone health and vascular calcification in chronic kidney disease. *Eur. J. Clin. Investig.* **2021**, *51*, e13588. [CrossRef]
89. Lobel, L.; Cao, Y.G.; Fenn, K.; Glickman, J.N.; Garrett, W.S. Diet Posttranslationally Modifies the Mouse Gut Microbial Proteome to Modulate Renal Function. *Science* **2020**, *369*, 1518–1524. [CrossRef]
90. Meijers, B.; Evenepoel, P.; Anders, H.J. Intestinal Microbiome and Fitness in Kidney Disease. *Nat. Rev. Nephrol.* **2019**, *15*, 531–545. [CrossRef]
91. Du, J.; Zhao, X.; Ding, X.; Han, Q.; Duan, Y.; Ren, Q.; Zhu, H.; Wang, H.; Song, C.; Wang, X.; et al. The Role of the Gut Microbiota in Complications among Hemodialysis Patients. *Microorganisms* **2024**, *12*, 1878. [CrossRef]
92. Winkelman, D.; Gallant, K.H.; Moe, S.; St-Jules, D.E. Seeing the Whole Picture: Evaluating the Contribution of Whole Grains to Phosphorus Exposure in People with Kidney Failure Undergoing Dialysis Treatment. *Semin. Dial.* **2024**, *37*, 326–333. [CrossRef] [PubMed]
93. Kalantar-Zadeh, K.; Gutekunst, L.; Mehrotra, R.; Kovesdy, C.P.; Bross, R.; Shinaberger, C.S.; Noori, N.; Hirschberg, R.; Benner, D.; Nissenson, A.R.; et al. Understanding Sources of Dietary Phosphorus in the Treatment of Patients with Chronic Kidney Disease. *Clin. J. Am. Soc. Nephrol.* **2010**, *5*, 519–530. [CrossRef]
94. Kalantar-Zadeh, K.; Joshi, S.; Schlueter, R.; Cooke, J.; Brown-Tortorici, A.; Donnelly, M.; Schulman, S.; Lau, W.-L.; Rhee, C.M.; Streja, E.; et al. Plant-Dominant Low-Protein Diet for Conservative Management of Chronic Kidney Disease. *Nutrients* **2020**, *12*, 1931. [CrossRef] [PubMed]
95. Almeida, P.P.; Da Cruz, B.O.; Thomasi, B.; Menezes, A.C.; Brito, M.L.; da Costa, N.S.; Almeida Ito, R.V.; Degani, V.A.N.; Daleprane, J.B.; D'Angelo, C.M.; et al. Brazil Nut-Enriched Diet Modulates Enteric Glial Cells and Gut Microbiota in an Experimental Model of Chronic Kidney Disease. *J. Am. Nutr. Assoc.* **2023**, *43*, 201–212. [CrossRef] [PubMed]
96. Da Cruz, B.O.; Almeida, P.P.; Silva-Costa, N.; Brito, M.L.; Degani, V.A.N.; da Silva, E.M.; Magliano, D.C.; Mebarek, S.; Brizuela, L.; Cardozo, L.F.; et al. Brazil Nut–Enriched Diet Modulates Bone Mineral Density and Body Composition in An Experimental Model of Chronic Kidney Disease. *Nutrition* **2024**, *125*, 112482. [CrossRef]
97. Molinari, P.; Caldiroli, L.; Abinti, M.; Nardelli, L.; Armelloni, S.; Cesari, M.; Castellano, G.; Vettoretti, S. Frailty Is Associated with Malnutrition–Inflammation Syndrome in Older CKD Patients. *Nutrients* **2024**, *16*, 2626. [CrossRef]
98. Ikizler, T.A.; Robinson-Cohen, C.; Ellis, C.; Headley, S.A.E.; Tuttle, K.; Wood, R.J.; Evans, E.E.; Milch, C.M.; Moody, K.A.; Germain, M.; et al. Metabolic Effects of Diet and Exercise in Patients with Moderate to Severe CKD: A Randomized Clinical Trial. *J. Am. Soc. Nephrol.* **2018**, *29*, 250–259. [CrossRef]
99. Li, W.L.; Zhang, N.H.; Ge, S.W.; Xu, G. Dietary Omega-3 Fatty Acid Intake and Mortality in CKD Population: A 1999–2014 NHANES Analysis. *Am. J. Nephrol.* **2022**, *52*, 909–918. [CrossRef]
100. Sharma, T.; Mandal, C.C. Omega-3 Fatty Acids in Pathological Calcification and Bone Health. *J. Food Biochem.* **2020**, *44*, e13333. [CrossRef]
101. Tao, S.S.; Wang, P.; Wang, X.Y.; Yin, K.J.; Yang, X.K.; Wang, Z.X.; Wang, D.-G.; Pan, H.F. Causal Effect of Polyunsaturated Fatty Acids on Bone Mineral Density and Fracture. *Front. Nutr.* **2022**, *9*, 1014847. [CrossRef]
102. Lalia, A.Z.; Dasari, S.; Robinson, M.M.; Abid, H.; Morse, D.M.; Klaus, K.A.; Lanza, I.R. Influence of Omega-3 Fatty Acids on Skeletal Muscle Protein Metabolism and Mitochondrial Bioenergetics in Older Adults. *Aging* **2017**, *9*, 1096–1129. [CrossRef] [PubMed]
103. Liu, Z.; Cai, S.; Chen, Y.; Peng, Z.; Jian, H.; Zhang, Z.; Huang, H. The Association Between Dietary Omega-3 Intake and Osteoporosis: A NHANES Cross-Sectional Study. *Front. Nutr.* **2025**, *11*, 1467559. [CrossRef] [PubMed]
104. Hagiwara, K.; Goto, T.; Araki, M.; Miyazaki, H.; Hagiwara, H. Olive Polyphenol Hydroxytyrosol Prevents Bone Loss. *Eur. J. Pharmacol.* **2011**, *662*, 78–84. [CrossRef] [PubMed]

105. Wang, K.; Qian, D.; Hu, Y.; Cheng, Y.; Ge, S.; Yao, Y. Nut Consumption and Effects on Chronic Kidney Disease and Mortality in the United States. *Am. J. Nephrol.* **2022**, *53*, 503–512. [CrossRef]
106. Simões, E.; Silva, A.C.; Cheung, W.W.; Oliveira, E.A.; Mak, R.H. Redox Signaling in Chronic Kidney Disease-Associated Cachexia. *Antioxidants* **2023**, *12*, 945. [CrossRef]
107. Basu, S.; Michaelsson, K.; Olofsson, H.; Johansson, S.; Melhus, H. Association Between Oxidative Stress and Bone Mineral Density. Biochem. *Biophys Res.Commun.* **2001**, *288*, 275–279. [CrossRef]
108. Lee, O.Y.A.; Wong, A.N.N.; Ho, C.Y.; Tse, K.W.; Chan, A.Z.; Leung, G.P.-H.; Kwan, Y.W.; Yeung, M.H.Y. Potentials of Natural Antioxidants in Reducing Inflammation and Oxidative Stress in Chronic Kidney Disease. *Antioxidants* **2024**, *13*, 751. [CrossRef]
109. Moriwaki, S.; Suzuki, K.; Muramatsu, M.; Nomura, A.; Inoue, F.; Into, T.; Yoshiko, Y.; Niida, S. Delphinidin, One of The Major Anthocyanidins, Prevents Bone Loss Through the Inhibition of Excessive Osteoclastogenesis in Osteoporosis Model Mice. *PLoS ONE* **2014**, *9*, e97177. [CrossRef]
110. Domazetovic, V.; Marcucci, G.; Pierucci, F.; Bruno, G.; Di Cesare, M.L.; Ghelardini, C.; Brandi, M.L.; Iantomasi, T.; Meacci, E.; Vincenzini, M.T. Blueberry Juice Protects Osteocytes and Bone Precursor Cells Against Oxidative Stress partly through SIRT1. *FEBS Open Bio* **2019**, *9*, 1082–1096. [CrossRef]
111. Coutinho-Wolino, K.S.; Melo, M.F.; Mota, J.C.; Mafra, D.; Guimarães, J.T.; Stockler-Pinto, M.B. Blueberry, cranberry, raspberry, and strawberry as modulators of the gut microbiota: Target for treatment of gut dysbiosis in chronic kidney disease? From current evidence to future possibilities. *Nutr. Rev.* **2024**, *82*, 248–261. [CrossRef]
112. Zhu, J.; Du, C. Could Grape-Based Food Supplements Prevent the Development of Chronic Kidney Disease? *Crit. Rev. Food Sci. Nutr.* **2019**, *60*, 3054–3062. [CrossRef] [PubMed]
113. Moon, D.K.; Kim, B.G.; Lee, A.R.; Choe, Y.L; Khan, I.; Moon, K.M.; Jeon, R.H.; Byun, J.H.; Hwang, S.C.; Woo, D.K. Resveratrol Can Enhance Osteogenic Differentiation And Mitochondrial Biogenesis From Human Periosteum-Derived Mesenchymal Stem Cells. *J. Orthop. Surg. Res.* **2020**, *15*, 203. [CrossRef]
114. Murillo Ortiz, B.O.; Fuentes Preciado, A.R.; Ramírez Emiliano, J.; Martínez Garza, S.; Ramos Rodríguez, E.; de Alba Macías, L.A. Recovery of Bone and Muscle Mass in Patients with Chronic Kidney Disease and Iron Overload on Hemodialysis and Taking Combined Supplementation with Curcumin and Resveratrol. *Clin. Interv. Aging* **2019**, 2055–2062. [CrossRef] [PubMed]
115. Marrone, G.; Murri, A.; Urciuoli, S.; Di Lauro, M.; Grazioli, E.; Vignolini, P.; Noce, A. Functional Foods and Adapted Physical Activity as New Adjuvant Therapy for Chronic Kidney Disease Patients. *Nutrients* **2024**, *16*, 2325. [CrossRef]
116. Cardozo, L.F.; Alvarenga, L.A.; Ribeiro, M.; Dai, L.; Shiels, P.G.; Stenvinkel, P.; Lindholm, B.; Mafra, D. Cruciferous Vegetables: Rationale for Exploring Potential Salutary Effects of Sulforaphane-Rich Foods in Patients with Chronic Kidney Disease. *Nutr. Rev.* **2021**, *79*, 1204–1224. [CrossRef] [PubMed]
117. Sahni, S.; Hannan, M.T.; Blumberg, J.; Cupples, L.A.; Kiel, D.P.; Tucker, L.K. Inverse Association of Carotenoid Intakes With 4-Y Change in Bone Mineral Density in Elderly Men and Women: The Framingham Osteoporosis Study. *Am. J. Clin. Nutr.* **2009**, *89*, 416–424. [CrossRef]
118. Carluccio, F.; Lenucci, M.; Piro, G.; Siems, W.; Luño, J. Integrated Medicine with Vegetable Derived Antioxidant and Vitamin D: Effects on Oxidative Stress and Bone Mineral Metabolism of Aged Patients with Renal Disease. *Funct. Foods Health Dis.* **2016**, *6*, 379–387. [CrossRef]
119. Supriyadi, R.; Koswara, M.I.A.; Soelaeman, M.A.; Huang, I. The Effect of Antioxidants Supplementation on Oxidative Stress and Proinflammatory Biomarkers in Patients with Chronic Kidney Disease: A Systematic Review and Meta-Analysis. *Eur. Rev. Med. Pharmacol. Sci.* **2023**, *27*, 1413–1426. [CrossRef]
120. Rapa, S.F.; Marzocco, S.; Di Iorio, B.R.; Heidland, A.; Campiglia, P. Inflammation and Oxidative Stress in Chronic Kidney Disease—Potential Therapeutic Role of Minerals, Vitamins and Plant-Derived Metabolites. *Int. J. Mol. Sci.* **2019**, *21*, 263. [CrossRef]
121. Huang, Y.; Zeng, M.; Zhang, L.; Shi, J.; Yang, Y.; Liu, F.; Sun, L.; Xiao, L. Dietary Inflammatory Potential Is Associated with Sarcopenia Among Chronic Kidney Disease Population. *Front. Nutr.* **2022**, *9*, 856726. [CrossRef]
122. Qi, Z.; Duan, A.; Ng, K. Selenoproteins in Health. *Molecules* **2024**, *29*, 136. [CrossRef] [PubMed]
123. Xie, H.; Wang, N.; He, H.; Yang, Z.; Wu, J.; Yang, T.; Wang, Y. The Association Between Selenium and Bone Health: A Meta-Analysis. *Bone Joint Res.* **2023**, *12*, 423–432. [CrossRef] [PubMed]
124. Ketteler, M.; Evenepoel, P.; Holden, R.M.; Isakova, T.; Jørgensen, H.S.; Komaba, H.; Wong, J.; Nickolas, T.L.; Sinha, S.; Vervloet, M.G.; et al. Chronic kidney disease–mineral and bone disorder: Conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int.* **2025**, *107*, 405–423. [CrossRef] [PubMed]

nutrients

MDPI

Review

Food to Prevent Vascular Calcification in Chronic Kidney Disease

Diana Moldovan [1,2,*], Crina Rusu [1,2], Alina Potra [1,2], Dacian Tirinescu [1,2], Maria Ticala [1,2] and Ina Kacso [1,2]

[1] Department of Nephrology, "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, 400347 Cluj-Napoca, Romania; claudia.rusu@umfcluj.ro (C.R.); alina.potra@umfcluj.ro (A.P.); tirinescu.dacian@umfcluj.ro (D.T.); cosa.maria@umfcluj.ro (M.T.); maria.kacso@umfcluj.ro (I.K.)

[2] Nephrology Clinic, Emergency County Hospital Cluj-Napoca, 400347 Cluj-Napoca, Romania

* Correspondence: diana.moldovan@umfcluj.ro

Abstract: Vascular calcification (VC) is a consequence of chronic kidney disease (CKD) which is of paramount importance regarding the survival of CKD patients. VC is far from being controlled with actual medication; as a result, in recent years, diet modulation has become more compelling. The concept of medical nutritional therapy points out the idea that food may prevent or treat diseases. The aim of this review was to evaluate the influence of food habits and nutritional intervention in the occurrence and progression of VC in CKD. Evidence reports the harmfulness of ultra-processed food, food additives, and animal-based proteins due to the increased intake of high absorbable phosphorus, the scarcity of fibers, and the increased production of uremic toxins. Available data are more supportive of a plant-dominant diet, especially for the impact on gut microbiota composition, which varies significantly depending on VC presence. Magnesium has been shown to prevent VC but only in experimental and small clinical studies. Vitamin K has drawn considerable attention due to its activation of VC inhibitors. There are positive studies; unfortunately, recent trials failed to prove its efficacy in preventing VC. Future research is needed and should aim to transform food into a medical intervention to eliminate VC danger in CKD.

Keywords: food; nutrition; lifestyle; vascular calcification; chronic kidney disease

Citation: Moldovan, D.; Rusu, C.; Potra, A.; Tirinescu, D.; Ticala, M.; Kacso, I. Food to Prevent Vascular Calcification in Chronic Kidney Disease. *Nutrients* **2024**, *16*, 617. https://doi.org/10.3390/nu16050617

Academic Editor: Kelly Lambert

Received: 30 January 2024
Revised: 14 February 2024
Accepted: 16 February 2024
Published: 23 February 2024

1. Introduction

Chronic kidney disease (CKD) is an emerging public health priority associated with high mortality rates and demanding complex management, including lifestyle changes, medications, and, sometimes, renal replacement therapy. Due to its very high prevalence in over 10% of the general population, CKD has a heavy social and financial burdens [1]. Above all other effects, CKD produces a significant negative impact on patients' lives, leading to complications that affect their quality and becoming life-threatening over time. One of the most important, yet unsolved, complications is chronic kidney disease—mineral and bone disorder (CKD-MBD) [2–4]. As CKD progresses, cardiovascular and osteo-articular complications occur and may have different impacts on patients' quality of life. CKD-MBD leads to a multitude of symptoms, including decreased function and social roles, depression, and a shorter life span [4,5]. Vascular calcification (VC) is the main abnormality from the complex CKD-MBD in terms of associated cardiovascular morbidity and mortality [2,3,6]. The goals of management in different stages of CKD are to slow the progression of kidney disease, to postpone the need for replacement therapy, and to control complications, such as hyperkalemia, metabolic acidosis, inflammation, protein malnutrition, anemia, high blood pressure, and mineral and bone disorders, which are particularly important for chronic management [1].

Food and drug intake may sometimes have a poisonous effect on the kidneys; therefore, a large number of patients with kidney failure have reached a point of no return due to their everyday life choices. Lifestyle impacts epigenetics, body composition, and function,

so people will eventually develop CKD if their habits are harmful. In the most prominent diseases of this century, including diabetes mellitus, hypertension, cardiovascular diseases, and cancer, studies have been carried out and have proven that different environmental factors contribute to each of these diseases' pathogeny [7,8]. Type 2 diabetes mellitus is associated with a diet that is rich in sweets, soft drinks, snacks, nuggets, and other ultra-processed foods [7]; hypertension has a strong connection with salt intake [9]; and cardiovascular diseases have strong connections with fatty foods. And, as is well known, the above-mentioned illnesses are the main causes of CKD. The ageing process itself is associated with common "burden of lifestyle" diseases, which include CKD. Interestingly, aging and CKD share important features; CKD is a condition which leads to an increased biological age [10], and CKD-MBD raises its invalidity rate and death toll [11]. We live in times of increasing awareness of the potential of meals to damage health. Consequently, a useful idea that has captured public attention is that food may become a tool to prevent or treat diseases and might be considered as medicine. This idea was conceptualized as medical nutritional therapy [12] or food as medicine [13,14]. When properly used, food may heal and may slow down and alleviate disease. Besides all of these well-known factors, different dietary patterns may be important influencers for chronic diseases, including CKD and its main chronic complication, CKD-MBD. Energy and action define everyone's way of living; therefore, smart choices must be made regarding food as energy supply based on valid scientific data. There is hope that lifestyle changes will slow or stop CKD-MBD features and prevent or even lead to regression in VC [15–17]. Clear knowledge about what to eat, how much to eat, and in what combination is necessary for patients with CKD-MBD [15]. Medical nutrition therapy (MNT) is an evidence-based process aiming to treat or manage a disease through nutrition. Its components are comprehensive and include the evaluation of nutritional status, intervention in diets, and nutrition therapies [12].

In this study, we aim to provide a comprehensive review of the effects of diet on VC as a part of the CKD-MBD spectrum that is known to be associated with severe clinical outcomes in patients with CKD. We will present the current state of this research field by reviewing the key publications from recent years, and we will highlight controversial and diverging hypotheses regarding this approach.

2. Vascular Calcification in CKD

CKD is characterized by features of accelerated ageing, such as increased levels of cellular senescence, and epigenetic modifications, such as telomere attrition, arterial calcification, osteoporosis, sarcopenia, frailty, and depression [11]. According to the KDIGO guidelines, the term "chronic kidney disease—mineral and bone disorder (CKD–MBD)" is a clinical syndrome which comprises mineral, bone, and calcific cardiovascular abnormalities in CKD. It includes modifications of calcium, phosphorus, parathyroid hormone (PTH), vitamin D, bone metabolism, and vascular or other soft-tissue calcification [2,3]. CKD-MBD is a consequence of CKD that has led to extended research and the development of a wide variety of treatments; despite specialists' implications, it continues to produce a multitude of symptoms and deleterious effects for the people who have it, including the following:

- Vascular calcification (VC) is a phenomenon involving the deposition of calcium and phosphorus within the layers of the arteries. Medial calcification, which presents as rail-train deposits along the vasculature, is particularly prevalent in patients suffering from CKD, but it is associated with aging and diabetes mellitus, too. It mainly affects the aorta and peripheral arteries. The deposition of mineral content within the media is preceded by phenotypic changes in vascular smooth muscle cells (VSMCs) and leads to arterial stiffness, significantly contributing to heart failure and increased cardiovascular morbidity. The accumulation of uremic toxins, the imbalance of calcium and phosphate, and a lack of calcification inhibitors have been implicated in the pathogenesis of calcification.
- Intimal calcification displays a patchy distribution pattern and preferentially affects the coronary and carotid arteries. It is part of the atherosclerosis process. In patients

with dyslipidemia and hypertension and smokers, atherosclerotic plaques occur as a consequence of inflammation and endothelial damage. It is common to find both types of calcifications in CKD patients. Accumulation of mineral content in atherosclerotic plaques may increase the risk of ischemic events such as stroke, ischemic coronary syndromes, or ischemic arteriopathy of the lower limbs [18,19].

- Other ectopic extraskeletal calcifications may occur. Valvular calcification is highly prevalent in CKD patients, contributing to chronic heart failure; calcifications in the joints can cause pain and functional impotence, and calcifications in the subcutaneous tissue can lead to resistant pruritus.

3. Food for CKD Patients

According to Global Burden of Disease Study, dietary risk factors are major contributors to millions of deaths, leading to higher mortality rates than well-known risk factors such as smoking [20]. Lifestyle interventions, such as healthy nutritional habits, proved to be effective in reducing cardiovascular risk factors in the general population [21]. High intake of sodium and sugar and a low intake of whole grains, vegetables, and fruits can cause type 2 diabetes mellitus, hypertension, cardiovascular disease, cancer, and CKD [22]. A study conducted in the Netherlands on over 78,000 people with a follow-up of 3.6 years revealed new evidence that ultra-processed food consumption leads to kidney function decline [23]. An observational study from Brazil demonstrated that elderly patients on hemodialysis (HD) have a worse dietary quality and higher consumption of ultra-processed food than elderly without CKD [24]. Some diets, as the DASH (Dietary Approaches to Stop Hypertension) diet and Mediterranean diet, provided important evidence regarding efficacy in promoting health [25]; these diets especially involve the reduction in salt, fat, and processed food intake. Tyson et al. demonstrated in CKD patients that the reduced-sodium DASH diet is efficient in reducing blood pressure [26].

CKD people are constantly exposed to conditions that alter epigenetic regulation such as toxins and shifts in dietary patterns. CKD-MBD leads to changes in DNA or histones, which are heritable from one cell to its descendants [27]. Neytchev et al. compared dialysis patients with transplant patients and controls and demonstrated that the uremic milieu drives genome-wide methylation changes that are partially reversed with kidney failure replacement therapy [28]. Studies have shown that different life variables, including food choices, may lead to epigenomic reprogramming [27,29]. Recent research of these nutritional interventions in CKD patients with VC gives rise to the hope of finding solutions.

4. Phosphorus, Vitamin D, and Calcium and Vascular Calcification in CKD

In patients with CKD, mineral disorders are associated with hyperparathyroidism, renal osteodystrophy, arterial calcification, and cardiovascular mortality [30,31]. CKD-MBD is marked by high serum phosphate levels, low serum active vitamin D, and low serum calcium levels.

Increased phosphate levels lead to VC and high cardiovascular death. In their experimental study, Turner et al. discovered that the arteries acutely deposit large amounts of amorphous phosphate to control the elevation in the bloodstream, thereby altering the systemic disposition of phosphate; therefore, they identified the arteries as a participatory mineral homeostatic organ [32]. Nephrologists encounter serious difficulties in controlling phosphate levels, and phosphate impacts the CKD-MBD patients' prognosis, even when receiving specific medication. Yet, the benefits of phosphate-lowering medication on VC, arterial stiffness, and clinical outcomes in predialysis CKD stages remain uncertain [33,34]. Nevertheless, there is evidence in favor of phosphate lowering; a recent Japanese study has proven that consistently strict phosphate control may slow the progression of coronary and valvular calcifications in incident patients undergoing HD (Table 1) [35].

Table 1. The effects of food on vascular calcification in patients with CKD.

Article	Design	Results
Shimizu 2023 [35]	Japanese study 64 incident HD patients Phosphate levels CAC by CT scans	Consistently strict phosphate control may slow the progression of coronary and valvular calcifications
Machado 2018 [36]	PROGREDIR study 373 non-dialysis CKD patients Food questionnaire Coronary artery calcification (CAC) by CT scans	Increased intake of food rich in phosphorus, calcium, and magnesium is associated with CAC
Ter Braake 2019 [37]	Klotho-deficient mice High dietary Mg	Mg prevents VC in Klotho deficiency
Talari 2019 [38]	RCT 54 HD patients with diabetes Mg oxide or placebo	Decrease in intima–media thickness after Mg supplementation
Sakaguchi 2019 [39]	RCT of 96 non-dialysis CKD patients Mg oxide versus carbon adsorbent CAC by CT scans Follow-up 2 years	CAC score was significantly smaller in the Mg oxide group
Bressendorf 2023 [40]	Magical-CKD study 150 CKD patients Supplementation with Mg for 12 months CAC by CT scans	No effect on CAC
Zhang 2023 [41]	170 CKD patients and 62 healthy controls Blood zinc levels CAC by CT scans	Low zinc with moderate–severe CAC and CDV events
McCabe 2013 [42]	Rats with adenine-induced chronic renal failure and warfarin-induced VC	High dietary vitamin K1 increased vitamin K tissue concentrations and blunted the development of VC
El Shinnawy 2022 [43]	RCT on 120 HD patients given supplements of vitamin K2, vitamin K1, and placebo Matrix Gla protein levels	Matrix Gla protein levels showed a significant increase in the vitamin K2 group compared with vitamin K1 and placebo groups
Li 2017 [44]	100 HD patients Used vitamin-K-enriched dialysate	Decreased VC scores as the effect of vitamin K
Haroon 2023 [45]	Trevasc-HDK RCT on 138 HD patients; CAC scores Vitamin K2 supplementation	No effect on VC
Levy-Schousboe 2021 [46]	RenaKvit RCT on 48 dialysis patients Vitamin K or placebo for 2 years Abdominal aortic calcification	No difference in VC
Holden 2022 [47]	iPACK-HD RCT on 86 HD patients Vitamin K1 for 12 months Coronary artery calcium score	No difference in progression of coronary artery calcification
Kanai 2011 [48]	Warfarin-induced medial arterial calcification in a rat model	Decreased medial arterial calcification after omega-3 fatty acid supplementation
Nakamura 2017 [49]	Eicosapentaenoic acid in Klotho mutant mice	Eicosapentaenoic acid limit VC
Son 2012 [50]	Cross-sectional study 31 HD patients Erythrocyte membrane content of monounsaturated fatty acids Plain radiographs for VC	Monounsaturated fatty acid erythrocyte content is significantly higher in HD patients with arterial medial calcification of the feet than in those without calcifications
Lan 2022 [51]	Cell culture Animal studies Ketone body β-hydroxybutyrate and VC in CKD model	Ketogenic diet through β-hydroxybutyrate suppresses VC in CKD through downregulation of HDAC9
Merino-Ribas 2022 [52]	Cross-sectional study 44 CKD patients on peritoneal dialysis (PD) Gut and blood microbiomes VC on plain radiographs	Differences in microbiota between PD patients with and without VC
Wei 2023 [53]	CKD Rats with 1,25-dihydroxyvitamin D3 induced VC. Lactobacillus rhamnosus	Lactobacillus rhamnosus GG supplements worsened the VC in CKD

Table 1. *Cont.*

Article	Design	Results
Sanchis 2016 [54]	69 non-dialysis CKD patients Food questionnaire evaluated the phytate (Myo-inositol hexaphosphate) intake. VC on plain radiographs	Increased phytate intake was associated with less abdominal aorta calcification
Raggi 2020 [55]	RCT 274 HD patients Myo-inositol hexaphosphate Cardiovascular calcification on CT scan 52 weeks	Slowed progression of cardiovascular calcification with myo-inositol hexaphosphate
Li 2023 [56]	Data from NHANES 1862 participants Information on 35 dietary components VC on plain radiographs	Low contents of proteins, fiber and vitamin A and high contents of lipids and caffeine were associated with abdominal aorta calcification. High adherence to the plant-based pattern was associated with a lower risk of VC
Zhang 2016 [57]	Resol	Resveratrol is a scavenger for many free radicals and ameliorates VC in CKD
Chang 2017 [58]	Rats with adenin-induced chronic renal failure. Quercetin	Quercetin exerted a protective effect on VC

Abbreviations: CKD, chronic kidney disease; CAC, coronary artery calcification; HD, hemodialysis; Mg, magnesium; VC, vascular calcification; RCT, randomized control trial; HDAC9, histone deacetylase 9; NHANES, National Health and Nutrition Examination Survey.

In PROGREDIR study on non-dialysis CKD patients, Machado et al. demonstrated that an increased intake of phosphorus- and calcium-rich food is associated with coronary artery calcification (Table 1) [36].

Sources of phosphorus include meats, fish, poultry, dairy products, nuts, beans, and food additives. Animal foods and inorganic phosphorus from food additives and preservatives have higher phosphorus absorption than plant foods; the industrial use of phosphate in additives used for ultra-processed food is strongly linked to cardiorenal disease risk [59,60]. An increasing number of specialists recommend a more plant-based diet to control phosphate. Phosphate bioavailability is lower with a vegetarian diet compared to a diet based on animal protein or processed foods and beverages. Many foods that have traditionally been labeled high in phosphate (such as beans and nuts) may actually be acceptable because phosphate from these sources is only partially and slowly absorbed. The plant-derived phosphate found in unprocessed foods is in the form of phosphorus phytate, and the human intestine does not secrete phytase, the enzyme required for absorption [60,61]. In addition, such a diet, rich in legumes, nuts, and whole grains, may also result in higher fiber intake while offering wider food choices and preventing constipation with better digestive phosphorus elimination [62,63]. These data highlight the importance of phosphate bioavailability in different foods in CKD patients as a mediator of cardiovascular risk.

In cases of severe and progressive secondary hyperparathyroidism, the 2017 KDIGO guidelines recommend the use of calcitriol and vitamin D analogs [3]. We have to be aware of the potential double-edged sword effect of vitamin D, since both deficiency and excess may be related to VC. While deficiency produces hyperparathyroidism and VC, treatment with calcitriol and vitamin D analogs, even if reducing the PTH level, can lead to the development of VC by increasing the intestinal absorption of calcium and phosphate [64]. The intake of food rich in vitamin D, such as ergocalciferol and cholecalciferol, can decrease the required dose of active vitamin D, thus mitigating the VC risk associated with the latter. Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) are recognized as fat-soluble prohormones, having different sources. Part of vitamin D as a nutrient is synthesized by the body through the action of sunlight, and some foods are fortified with the vitamin. Yet, there are foods naturally rich in vitamin D, including salmon, herring, mackerel, sardines, mushrooms, cashews, and hazelnuts [22,65]. Well controlled studies are needed to determine whether nutritional vitamin D slows the rate of progression of VC.

5. Magnesium and Vascular Calcification in CKD

The capacity of magnesium to inhibit calcium phosphate crystallization has been well documented in the context of VC. Magnesium effectively suppresses phosphate-induced calcification of VSMCs, as proved in different experiments [66]. Magnesium is known to suppress the maturation of calciprotein particles, which may play a pivotal role in the pathogenesis of VC. A high-magnesium diet prevented aortic calcification in animal models of CKD, such as Klotho knockout mice [37] (Table 1).

Patients with diabetes and CKD on HD showed reduced carotid intima–media thickness after magnesium supplementation, emphasizing a preventive role against VC [38]. Sakaguchi conducted a randomized trial comparing magnesium oxide and oral carbon adsorbent in predialysis CKD patients with coronary artery calcifications. The study proved the efficacy of magnesium to prevent coronary artery calcification progression [39] (Table 1).

A systematic review analyzed prospective clinical trials testing interventions to attenuate VC in people with CKD. It concluded that, in general, data are insufficient or conflicting, yet magnesium appears to be one of the few promising therapies [67].

The more recent Magical-CKD trial failed to demonstrate an improvement of coronary artery calcification progression after magnesium supplementation [40] (Table 1). Nevertheless, magnesium is one of the few nutritional elements with supportive data in terms of VC protection. Hypomagnesemia is not rare in patients with CKD, and several causes can be identified; the dietary restriction of potassium limits the intake of magnesium, and diuretics are known to enhance its urinary excretion. Almonds, peanuts, cashew, and spinach are foods rich in magnesium; these can be a good source especially in patients with low serum magnesium levels.

Zinc is considered an essential nutrient, having numerous benefits for health. A recent study demonstrated associations of low blood zinc levels with coronary artery calcification and future cardiovascular events in CKD patients. Good sources of zinc include seafood, meat, nuts, whole grains, and dairy products, which are recommended to avoid a zinc deficit [41].

6. Vitamin K and Vascular Calcification in CKD

There is a close relationship between vitamin K and biomineralization. Vitamin K enables normal calcification processes in bones and soft tissues. This role is associated with vitamin-K-dependent proteins, including osteocalcin, matrix γ-carboxyglutamic acid (Gla) protein, and growth arrest specific 6 (Gas6). Matrix Gla protein, a vitamin K-dependent protein produced by VSMCs, is a powerful inhibitor of VC in culture cells with medial and intimal calcification. In view of the key role played by vitamin K, it is not surprising that patients with vitamin K deficiency and those who are using long-term anticoagulant therapy with vitamin K antagonists are prone to develop VC. There are two types of vitamin K. Vitamin K1 (phylloquinone) is found primarily in foods, especially plant-based oils, green vegetables (e.g., broccoli, spinach, and cabbage), and cow's milk. The forms of vitamin K2 (menaquinones) are produced by bacteria, being found in meat, dairy products, and fermented foods, and are also synthesized in the intestine by colonic bacteria [68] (Figure 1).

McCabe et al. studied rats with adenine-induced chronic renal failure and showed that administration of a high dose of vitamin K protected against the development of warfarin-induced calcification [42] (Table 1).

In a study on HD patients, the levels of matrix Gla proteins displayed a significant increase in patients receiving vitamin K2 compared with vitamin K1 and placebo groups [43].

A study on HD patients from China demonstrated that the VC scores decreased as an effect of a vitamin-K-enriched dialysate [44].

Figure 1. Food and vascular calcification (VC) in chronic kidney disease (CKD). Green arrows refer to a protective effect of the nutritional components against VC and red arrows indicate favorable VC development.

Recently, a few randomized controlled trials were designed to test the anticalcification properties of vitamin K. Trevasc-HDK failed to prove that vitamin K2 can reduce progression of coronary artery calcification in HD patients [45]. The conclusion of RenaKvit, a double-blind, randomized, placebo-controlled trial, was that vitamin K supplementation does not modify the progression of arterial calcification in dialysis [46]. Similar results were reported by the iPACK-HD trial; there was improvement in vitamin K levels but no significant modification of VC progression [47] (Table 1).

In conclusion, vitamin K had no consistent benefit in VC reduction in CKD patients. Vitamin K1 showed better efficacy in correcting vitamin K status, and it had very positive results in experimental studies as a protector against VC. Further clinical studies are needed to shed light on the effect of vitamin K supplementation on arterial health, mostly because there is hope from experimental studies.

Regarding the effects of other vitamins, vitamin E has proven anti-atherogenic and antioxidant attributes, which have been correlated with improved cardiovascular outcomes. Wheat germ oil, sunflower seeds, and avocado have an increased content of vitamin E [69]. A recent study on non-dialysis CKD patients suggested that a higher intake of vitamin B5 (pantothenic acid) may have a small protective effect on coronary calcification [36].

7. Lipids and Vascular Calcification in CKD

Dyslipidemia plays a pivotal role in arterial intima calcification. Among the risk factors for atherosclerosis, cholesterol and lipid deposition are strongly associated with plaque formation and calcification. Clinical trials involving HMG-CoA reductase inhibitors showed good efficacy in reducing lipid levels and cardiovascular risk yet could not consistently demonstrate attenuation of VC [67]. KDOQI guidelines for nutrition in CKD highlight the importance of food choices and suggest that prescribing a Mediterranean diet may improve lipid profiles in adults with CKD 1–5 not on dialysis, having dyslipidemia or not. Prescribing increased fruit, legume, and vegetable intake may decrease body weight and blood pressure in CKD 1–4 patients [70].

Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid and docosahexaenoic acid, are part of a class of lipids with various biological functions. They reduce inflammation and atherogenesis, and, as a result, they can decrease the cardiovascular mortality [71]. Omega-3 PUFAs are used as medication for hypertriglyceridemia in patients with CKD. They are also a component of food, being present in fish oil; in a variety of microorganisms, including bacteria and marine microalgae; and in plant sources, such as flaxseeds, chia, and walnuts [72] (Figure 1). When it comes to mortality and cardiovascular disease, the current KDOQI guidelines in nutrition do not routinely recommend PUFA supplementation, even if it is acknowledged that lipid profile will be improved [69]. Several studies have investigated PUFAs' effects in CKD; PUFAs increase the membrane potential and ATP levels in mitochondria with a protective effect on the kidneys and arteries. A recent study demonstrated that maslinic acid can reduce renal interstitial fibrosis and can prevent CKD progression and complications [73]. Interestingly, randomized controlled trials comparing the effect of omega-3 PUFA supplementation with placebo have shown significant relief of uremic pruritus, which is associated with CKD-MBD [74].

Experimental studies documented a preventive role of omega-3 fatty acids in pathological calcification, leading to decreased warfarin-induced medial arterial calcification in a rat model [64] (Table 1). It has been reported that patients with CKD have low serum expression of Klotho, which has been proved to be an arterial calcification inhibitor [75]. Nakamura et al. has shown that eicosapentaenoic acid can limit arterial calcification in Klotho mutant mice [49] (Table 1). A recent large study demonstrated an association of higher plasma levels of omega-3 PUFAs with an increased arterial elasticity [76].

Monounsaturated fatty acids are known to have deleterious effects on health (Figure 1). As opposed to PUFAs' protective attributes, Son et al. demonstrated that the erythrocyte membrane content of monounsaturated fatty acids is significantly higher in HD patients with arterial medial calcification of the feet than in the patients without calcifications [50].

The ketogenic diet, which consists of a limited intake of carbohydrates and a liberal intake of fats, has recently attracted considerable interest. It is proven as an efficient intervention in controlling type 2 diabetes mellitus [77] and in slowing renal cyst growth [78]; therefore, patients with diabetic kidney disease and autosomal-dominant polycystic kidney disease may derive benefits from this diet, including a delay in progression and, eventually, in the complications rate [79]. It may cause a rise in cholesterol levels, so, when adopted, it should be accompanied by close monitoring and treatment for dyslipidemia [77]. Dietary modulation can increase and maintain circulating ketone bodies, especially β-hydroxybutyrate (β-HB), which is one of the most abundant ketone bodies in human circulation [80]. A very important finding was reported by Lan et al.; the ketone body β-hydroxybutyrate (BHB) produced in the ketogenic diet has been demonstrated to suppress VC in CKD through downregulation of HDAC9 [51] (Table 1).

Intermittent fasting, as a model of caloric restriction, has broad-spectrum benefits for many health conditions, such as atherosclerosis, cardiovascular disease, and obesity, as preclinical studies and clinical trials have shown [81]; as far as the impact on VC, it remains a topic for future studies.

8. Uremic Toxins, Microbiota, Fibers, and Vascular Calcification in CKD

Accumulation of various uremic toxins, including inorganic phosphate (Pi), interleukins (IL-1β, IL-6), tumor necrosis factor alpha (TNFα), and indoxyl-sulfate, have been linked to VC. Pi induces the upregulation of several osteoblast-like transition molecules like BMP2 (bone morphogenetic protein 2), RUNX2 (Runt-related transcription factor 2), and osteopontin that initiate the pro-calcifying trans-differentiation of VSMCs. Indoxyl-sulfate stimulates transforming growth factor beta (TGFβ) expression and medial layer hyperplasia. Uremic toxins act on endothelial cells to induce vasoconstriction, upregulation of extracellular matrix degradation molecules such as matrix metalloproteinases 2 and 9, and oxidative stress. Calcium and Pi deposition in the form of hydroxyapatite crystals induces medial VC [82].

The load of uremic toxins can be reduced through dialysis, yet their production is influenced by dietary habits, mostly important in pre-dialysis stages. Some foods are sources for protein-bound uremic toxins (e.g., indoxyl sulphate). These are by-products of aromatic amino acids (phenylalanine, tryptophan, and tyrosine) from protein disintegration by gut microbiota [83]. Colonic bacteria transform tryptophan to indol, which, through oxidation and sulfation in the liver, will lead to indoxyl sulphate formation. Rodrigues et al. have similarly explained the pathophysiology of the interplay between gut microbiota, bone health, and VC in CKD [84]. Interesting results come from studies investigating the influence of different dietary habits on the uremic gut microbiota. Merino-Ribas et al. found differences in the type of microbiota of CKD on peritoneal dialysis with or without VC, namely Coprobacter, Coprococcus 3, Lactobacillus, and the Eubacterium eligens group in the gut and Cutibacterium, Pajaroellobacter, Devosia, Hyphomicrobium, and Pelomonas in the blood. These results may indicate a link between microbiota and VC in CKD patients on peritoneal dialysis [52] (Table 1). Such results from similar studies led to the hypothesis that inflammation and gut dysbiosis are important drivers of CKD–MBD [85]. An association between dietary inflammatory index and cardiovascular disease and mortality was recently proven [86]. A study identified an association of the proatherogenic metabolite trimethylamine N-oxide (TMAO), which increases due to gut dysbiosis, with cardiovascular outcomes in HD patients [87].

Lactobacillus rhamnosus GG is a probiotic with great promise in bone formation, but an experimental study recently proved an association with worsening of VC in CKD [53] (Table 1).

Fiber intake is an important health promoter in the general population. In their recent study, conducted on over 3800 Korean patients with CKD, Kwon et al. reported an inverse association between dietary fiber intake and all-cause mortality at 10 years in CKD patients [88]. Higher fiber intake was associated with less inflammation, less myocardial hypertrophy, and lower risk of cardiovascular events in dialysis patients [89]. Fibers are needed for the effective absorption of nutrients. Fibers demonstrated salutary benefits, including improved glycemic and lipid control, blood pressure, gastrointestinal motility, and gut microbiota composition [90]. In a study published on HD patients, increased dietary fiber intake led to the reduction in indoxyl sulphate levels by 29% [91].

Adequate consumption of phytate (containing myo-inositol hexaphosphate) can prevent abdominal aortic calcification in patients with CKD [54]. The phytate comes from whole-grain cereals, bran, and lentils. Nuts are also a good source of antioxidants and dietary fiber (Figure 1). The Calipso trial demonstrated the effect of myo-inositol hexaphosphate in slowing progression of cardiovascular calcification in patients on HD [55] as additional evidence for the usefulness of this component of fibers (Table 1).

Finding the balance in gut microbiota and regulating microbiota-derived metabolites by dietary intervention and probiotics are new targets for the improvement of the gut–kidney–arteries axis, which indicates innovative interventions of VC in CKD [16,92].

9. Protein Intake and Vascular Calcification in CKD

The foundation of nutrition intervention in CKD was laid for decades on a low-protein diet to slow progression and on restriction of plant foods, such as vegetables and fruits, to prevent hyperkalemia. Lately, this paradigm has changed, and the plant-dominant low-protein (PLADO) diets seem to have become a better choice for patients with CKD [21]. In a sub-analysis of the NHANES III study on 14,000 participants patients with a glomerular filtration rate < 60 mL/min, a diet with a higher proportion of protein from plant sources was associated with lower mortality, probably due to lower production of uremic toxins and lower serum phosphorus levels [30]. The vegetarian diet or a reduced intake of red meat has been associated with a reduction in the generation of uremic toxins [93–96]. Such a diet is based on fruits, vegetables, seeds, nuts, tea, cocoa, and whole-grain cereals [97]. Among plant-based foods, Brazil nuts seem to have important benefits in CKD, even in

end-stage kidney disease patients, due to their contents of proteins, selenium, omega-3 fatty acids, and fibers [98,99] (Figure 1).

The DIET-HD study demonstrated for a large number on HD patients that the highest intake of fruit and vegetables had the lowest risk for all-cause and cardiovascular mortality [100,101]. On the contrary, the CRIC study did not find a significant association between higher diet scores and reduced risk for atherosclerosis or mortality [102] (Figure 1).

As for the risk of hyperkalemia, CKD patients have been advised for a long time to reduce their intake of fruits, vegetables, and nuts. Nevertheless, we must be aware that meat and ultra-processed food have a high content in potassium, with a high absorption rate [103–109].

Nuts have high content of phosphorus, which is one of the traditional nutrients restricted in advanced CKD to avoid hyperphosphatemia [108], but the latest studies demonstrated that non-animal protein does not lead to hyperphosphatemia, as previously believed [105,110].

To identify dietary components associated with abdominal aorta calcification, data from NHANES were employed in a cross-sectional study. Low contents of proteins, fiber and vitamin A, and high contents of lipids and caffeine exhibited an association with abdominal aorta calcification. High adherence to the plant-based pattern was associated with a lower risk of VC, as a new and valuable result in favor of PLADO [109].

10. Bioactive and Senolytic Food and Vascular Calcification in CKD

Bioactive and senolytic food has antioxidant and anti-inflammatory effects. Resveratrol, quercetin, curcumin, anthocyanins, and cruciferous and cocoa powder are part of this category [56] (Table 1). Anthocyanins, present in purple fruits and vegetables, exert their beneficial effects through improvements in oxidative stress, inflammation, gut microbiota, and modulation of neuropeptides. Their health benefits in humans include protection of the cardiovascular system and kidneys, among others [111].

Resveratrol, a dietary polyphenol compound, has anti-inflammatory and antioxidative properties [112]. Recently, studies also showed that resveratrol is a scavenger for many free radicals and ameliorates VC in CKD [113].

Cocoa contains fatty acids and polyphenolic bioactives, with proanthocyanidins being the most abundant and methylxanthine alkaloids. Dark chocolate led to a reduction in TNFα and no change in potassium and phosphorus plasma levels. These are the results of a clinical trial of 2 months on HD patients [57] (Table 1).

Blueberry, cranberry, raspberry, and strawberry are modulators of the gut microbiota and a target for treatment of gut dysbiosis in CKD [114] (Figure 1).

Dietary senolytics, such as quercetin (found in apples), fisetin (in strawberries), and organosulphur compounds and flavonoids (aged garlic) may be alternative approaches to reduce cardiovascular risk in CKD [13,115]. Quercetin exerted a protective effect on VC in adenine-induced chronic renal failure rats, possibly through the modulation of oxidative stress [58] (Table 1).

Iron supplementation is highly recommended to improve cardiovascular function in CKD, but it remains controversial when it comes to VC. Recent studies demonstrated that iron targets some pathways of VC dependent on phosphorus-induced osteoblastic transformation of VSMCs to calciproteins, apoptosis, and inflammation, since it is effective both in prevention and when calcification is already established [116].

Selenium works as an antioxidant in the body by preventing vascular cell damage. In a recent study, a higher dietary selenium intake was negatively associated with severe abdominal aorta calcification incidence in CKD patients [117]. The selenium content of foods can vary considerably depending on the geographic area; nuts, oats, seeds, mushrooms, beans, and eggs can be good sources.

As for all the benefits discovered in mentioned studies, the concept of food as medicine for protecting the kidneys and heart and avoiding VC in CKD patients seems to have moved closer to reality [118].

11. Conclusions

The search for eternal youth, as an emblem for health, is as old as mankind. But in the case of the patients with CKD and VC, it is more of a struggle for life, a fight against many deadly factors, because VC is strongly associated with cardiovascular mortality. Most of the efforts are made to fix problems with a focus on the other end of the spectrum of CKD, and yet medication failed to show consistent efficacy in preventing VC.

Food is essential for life; thus, prevention of VC in CKD through nutrition seems to be the logical approach. High phosphorus absorption, high production of uremic toxins, and gut dysbiosis are consequences of the increased intake of animal-based proteins, processed food, salt, and sugar. Available research links all of the above with the presence of VC. A diet involving vitamin K, magnesium, plant-based diets, fibers, omega 3 fatty acids, or bioactive food appears to be the most promising in protecting against VC. These results are based on experimental and relatively small clinical studies but still are not negligible. Even though clinical trials on magnesium and vitamin K were not able to prove the efficacy of the nutritional interventions in CKD patients with VC, the effects exist, and more research needs to be conducted. Finding the best variants of meals may lead to reduced VC incidence and progression and may allow eating to be transformed into a scientific act and medical intervention with effective outcomes. Food is supplied for life, and data are available to be discovered on the best nutrient choices to disrupt the vicious cycle of the gut–kidney–arteries axis and prevent cardiovascular calcification in the CKD population.

Author Contributions: Conceptualization, D.M. and I.K.; gathering research literature, D.M., C.R. and I.K.; writing—original draft preparation, D.M.; writing—review and editing, A.P., D.T. and M.T; visualization, D.M., A.P., D.T. and M.T.; supervision, D.M. and I.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Kalantar-Zadeh, K.; Jafar, T.H.; Nitsch, D.; Neuen, B.L.; Perkovic, V. Chronic kidney disease. *Lancet* **2021**, *398*, 786–802. [CrossRef] [PubMed]
2. Eckardt, K.-U.; Kasiske, B.L. KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int.* **2009**, *76*, S1–S130. [CrossRef] [PubMed]
3. Kidney Disease: Improving Global Outcomes (KDIGO). CKD-MBD Update Work Group. KDIGO 2017 Clinical Practice Guideline Update for the Diagnosis, Evaluation, Prevention, and Treatment of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). *Kidney Int.* **2017**, *7*, 1–59. [CrossRef] [PubMed]
4. Cannata-Andía, J.B.; Martín-Carro, B.; Martín-Virgala, J.; Rodríguez-Carrio, J.; Bande-Fernández, J.J.; Alonso-Montes, C.; Carrillo-López, N. Chronic kidney disease—Mineral and bone disorders: Pathogenesis and management. *Calcif. Tissue Int.* **2021**, *108*, 410–422. [CrossRef] [PubMed]
5. Bikbov, B.; Purcell, C.A.; Levey, A.S.; Smith, M.; Abdoli, A.; Abebe, M.; Owolabi, M.O. Global, regional, and national burden of chronic kidney disease, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2020**, *395*, 709–733. [CrossRef] [PubMed]
6. Moldovan, D.; Rusu, C.; Potra, A.; Bondor, C.; Ticala, M.; Tirinescu, D.; Coman, A.; Orasan, O.; Moldovan, I.; Orasan, R.; et al. Arterial calcifications and osteoprotegerin in chronic hemodialysis patients: Impact on 6-year survival. *Int. Urol. Nephrol.* **2022**, *54*, 1135–1143. [CrossRef] [PubMed]
7. Srour, B.; Fezeu, L.K.; Kesse-Guyot, E.; Allès, B.; Debras, C.; Druesne-Pecollo, N.; Chazelas, E.; Deschasaux, M.; Hercberg, S.; Galan, P.; et al. Ultraprocessed food consumption and risk of type 2 diabetes among participants of the NutriNet-Santé prospective cohort. *JAMA Intern. Med.* **2019**, *180*, 283–291. [CrossRef] [PubMed]
8. Liu, X.; Wu, S.; Song, Q.; Wang, X. Reversion from pre–diabetes mellitus to normoglycemia and risk of cardiovascular disease and all-cause mortality in a chinese population: A prospective cohort study. *J. Am. Heart Assoc.* **2021**, *10*, e019045. [CrossRef]
9. Kawarazaki, W.; Fujita, T. Kidney and epigenetic mechanisms of salt-sensitive hypertension. *Nat. Rev. Nephrol.* **2021**, *17*, 350–363. [CrossRef]
10. Wen, L.; Yang, H.L.; Lin, L.; Ma, L.; Fu, P. The Emerging role of epigenetic methylation in kidney disease. *Curr. Med. Chem.* **2022**, *29*, 3732–3747. [CrossRef]

11. Chou, Y.H.; Chen, Y.M. Aging and renal disease: Old questions for new challenges. *Aging Dis.* **2021**, *12*, 515–528. [CrossRef]
12. Kalantar-Zadeh, K.; Mattix-Kramer, H.J.; Moore, L.W. Culinary medicine as a core component of the medical nutrition therapy for kidney health and disease. *J. Ren. Nutr.* **2021**, *31*, 1–4. [CrossRef]
13. Mafra, D.; Borges, N.A.; Lindholm, B.; Shiels, P.G.; Evenepoel, P.; Stenvinkel, P. Food as medicine: Targeting the uraemic phenotype in chronic kidney disease. *Nat. Rev. Nephrol.* **2021**, *17*, 153–171. [CrossRef]
14. Alvarenga, L.; Reis, D.C.; Kemp, J.A.; Teixeira, K.T.R.; Fouque, D.; Mafra, D. Using the concept of food as medicine to mitigate inflammation in patients undergoing peritoneal dialysis. In *Therapeutic Apheresis and Dialysis*; Wiley: Hoboken, NJ, USA, 2024. [CrossRef]
15. Hu, E.A.; Steffen, L.M.; Grams, M.E.; Crews, D.C.; Coresh, J.; Appel, L.J.; Rebholz, C.M. Dietary patterns and risk of incident chronic kidney disease: The atherosclerosis risk in communities study. *Am. J. Clin. Nutr.* **2019**, *110*, 713–721. [CrossRef] [PubMed]
16. Yin, L.; Li, X.; Ghosh, S.; Xie, C.; Chen, J.; Huang, H. Role of gut microbiota-derived metabolites on vascular calcification in CKD. *J. Cell. Mol. Med.* **2021**, *25*, 1332–1341. [CrossRef] [PubMed]
17. Cretoiu, D.; Ionescu, R.F.; Enache, R.M.; Cretoiu, S.M.; Voinea, S.C. Gut microbiome, functional food, atherosclerosis, and vascular calcifications—Is there a missing link? *Microorganisms* **2021**, *9*, 1913. [CrossRef] [PubMed]
18. Ebert, T.; Neytchev, O.; Witasp, A.; Kublickiene, K.; Stenvinkel, P.; Shiels, P.G. Inflammation and oxidative stress in chronic kidney disease and dialysis patients. *Antioxid. Redox Signal.* **2021**, *35*, 1426–1448. [CrossRef]
19. Hutcheson, J.D. Cardiovascular calcification heterogeneity in chronic kidney disease. *Circ. Res.* **2023**, *132*, 993–1012. [CrossRef] [PubMed]
20. Fraser, S.D.S.; Roderick, P.J. Kidney disease in the Global Burden of Disease Study 2017. *Nat. Rev. Nephrol.* **2019**, *15*, 193–194. [CrossRef] [PubMed]
21. Chen, Y.; Wu, J.; Yu, D.; Liu, M. Plant or animal-based or PLADO diets: Which should chronic kidney disease patients choose? *J. Ren. Nutr.* **2023**, *33*, 228–235. [CrossRef]
22. Afshin, A.; Sur, P.J.; Fay, K.A.; Cornaby, L.; Ferrara, G.; Salama, J.S.; Murray, C.J. Health effects of dietary risks in 195 countries, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2019**, *393*, 1958–1972. [CrossRef] [PubMed]
23. Cai, Q.; Duan, M.J.; Dekker, L.H.; Carrero, J.J.; Avesani, C.M.; Bakker, S.J.L.; de Borst, M.H.; Navis, G.J. Ultraprocessed food consumption and kidney function decline in a population-based cohort in the Netherlands. *Am. J. Clin. Nutr.* **2022**, *116*, 263–273. [CrossRef] [PubMed]
24. Martins, A.M.; Moreira, A.S.B.; Canella, D.S.; Rodrigues, J.; Santin, F.; Wanderley, B.; Lourenço, R.A.; Avesani, C.M. Elderly patients on hemodialysis have worse dietary quality and higher consumption of ultraprocessed food than elderly without chronic kidney disease. *Nutrition* **2017**, *41*, 73–79. [CrossRef] [PubMed]
25. Appel, L.J.; Moore, T.J.; Obarzanek, E.; Vollmer, W.M.; Svetkey, L.P.; Sacks, F.M.; Bray, G.A.; Vogt, T.M.; Cutler, J.A.; Windhauser, M.M.; et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N. Engl. J. Med.* **1997**, *336*, 1117–1124. [CrossRef] [PubMed]
26. Tyson, C.C.; Lin, P.-H.; Corsino, L.; Batch, B.C.; Allen, J.; Sapp, S.; Barnhart, H.; Nwankwo, C.; Burroughs, J.; Svetkey, L.P. Short-term effects of the DASH diet in adults with moderate chronic kidney disease: A pilot feeding study. *Clin. Kidney J.* **2016**, *9*, 592–598. [CrossRef] [PubMed]
27. Vineis, P.; Chatziioannou, A.; Cunliffe, V.T.; Flanagan, J.M.; Hanson, M.; Kyrtopoulos, S.; Kirsch-Volders, M. Epigenetic memory in response to environmental stressors. *FASEB J.* **2017**, *31*, 2241. [CrossRef]
28. Neytchev, O.; Witasp, A.; Nordfors, L.; Qureshi, A.R.T.; Wennberg, L.; Erlandsson, H.; Stenvinkel, P. FC 123 Renal transplantation mitigates increased biological (epigenetic) age in chronic kidney disease. *Nephrol. Dial. Transplant.* **2021**, *36* (Suppl. S1), gfab147-002. [CrossRef]
29. Witasp, A.; Luttropp, K.; Qureshi, A.R.; Barany, P.; Heimbürger, O.; Wennberg, L.; Ekström, T.J.; Shiels, P.G.; Stenvinkel, P.; Nordfors, L. Longitudinal genome-wide DNA methylation changes in response to kidney failure replacement therapy. *Sci. Rep.* **2022**, *12*, 470. [CrossRef]
30. Chang, A.R.; Lazo, M.; Appel, L.J.; Gutiérrez, O.M.; Grams, M.E. High dietary phosphorus intake is associated with all-cause mortality: Results from NHANES III. *Am. J. Clin. Nutr.* **2014**, *99*, 320–327. [CrossRef] [PubMed]
31. Moldovan, D.; Rusu, C.; Potra, A.; Moldovan, L.; Patiu, I.M.; Gherman-Caprioara, M.; Kacso, I.M. Osteoprotegerin and uremic osteoporosis in chronic hemodialysis patients. *Int. Urol. Nephrol.* **2017**, *49*, 895–901. [CrossRef] [PubMed]
32. Turner, M.E.; Rowsell, T.S.; Lansing, A.P.; Jeronimo, P.S.; Lee, L.H.; Svajger, B.A.; Adams, M.A. Vascular calcification maladaptively participates in acute phosphate homeostasis. *Cardiovasc. Res.* **2023**, *119*, 1077–1091. [CrossRef] [PubMed]
33. Lioufas, N.M.; Pascoe, E.M.; Hawley, C.M.; Elder, G.J.; Badve, S.V.; Block, G.A.; Johnson, D.W.; Toussaint, N.D. Systematic review and meta-analyses of the effects of phosphate-lowering agents in nondialysis CKD. *J. Am. Soc. Nephrol.* **2022**, *33*, 59–76. [CrossRef] [PubMed]
34. Toussaint, N.D.; Pedagogos, E.; Lioufas, N.M.; Elder, G.J.; Pascoe, E.M.; Badve, S.V.; IMPROVE-CKD Trial Investigators. A randomized trial on the effect of phosphate reduction on vascular end points in CKD (IMPROVE-CKD). *J. Am. Soc. Nephrol.* **2020**, *31*, 2653–2666. [CrossRef] [PubMed]
35. Shimizu, M.; Fujii, H.; Kono, K.; Goto, S.; Watanabe, K.; Sakamoto, K.; Nishi, S. Clinical implication of consistently strict phosphate control for coronary and valvular calcification in incident patients undergoing hemodialysis. *J. Atheroscler. Thromb.* **2023**, *30*, 64159. [CrossRef]

36. Machado, A.D.; Gómez, L.M.; Marchioni, D.M.L.; Dos Anjos, F.S.N.; Molina, M.D.C.B.; Lotufo, P.A.; Benseñor, I.J.M.; Titan, S.M.O. Association between dietary intake and coronary artery calcification in non-dialysis chronic kidney disease: The PROGREDIR Study. *Nutrients* **2018**, *10*, 372. [CrossRef]
37. Ter Braake, A.D.; Smit, A.E.; Bos, C.; van Herwaarden, A.E.; Alkema, W.; van Essen, H.W.; Bravenboer, N.; Vervloet, M.G.; Hoenderop, J.G.J.; de Baaij, J.H.F. Magnesium prevents vascular calcification in Klotho deficiency. *Kidney Int.* **2020**, *97*, 487–501. [CrossRef]
38. Talari, H.R.; Zakizade, M.; Soleimani, A.; Bahmani, F.; Ghaderi, A.; Mirhosseini, N.; Eslahi, M.; Babadi, M.; Mansournia, M.A.; Asemi, Z. Effects of magnesium supplementation on carotid intima-media thickness and metabolic profiles in diabetic haemodialysis patients: A randomised, double-blind, placebo-controlled trial. *Br. J. Nutr.* **2019**, *121*, 809–817. [CrossRef]
39. Sakaguchi, Y.; Hamano, T.; Obi, Y.; Monden, C.; Oka, T.; Yamaguchi, S.; Matsui, I.; Hashimoto, N.; Matsumoto, A.; Shimada, K.; et al. A randomized trial of magnesium oxide and oral carbon adsorbent for coronary artery calcification in predialysis CKD. *J. Am. Soc. Nephrol.* **2019**, *30*, 1073–1085. [CrossRef]
40. Bressendorff, I.; Hansen, D.; Schou, M.; Kragelund, C.; Svensson, M.; Hashemi, B.; Brandi, L. The effect of magnesium supplementation on vascular calcification in CKD: A randomized clinical trial (MAGiCAL-CKD). *J. Am. Soc. Nephrol.* **2023**, *34*, 886–894. [CrossRef]
41. Zhang, D.; Zhu, Y.; Li, H.; Wang, Y.; Niu, Z.; Zhou, W.; Wang, D. Associations of whole blood zinc levels with coronary artery calcification and future cardiovascular events in CKD patients. *Biol. Trace Elem. Res.* **2024**, *202*, 46–55. [CrossRef] [PubMed]
42. McCabe, K.M.; Booth, S.L.; Fu, X.; Shobeiri, N.; Pang, J.J.; Adams, M.A.; Holden, R.M. Dietary vitamin K and therapeutic warfarin alter the susceptibility to vascular calcification in experimental chronic kidney disease. *Kidney Int.* **2013**, *83*, 835–844. [CrossRef] [PubMed]
43. El Shinnawy, H.; Elsaid, T.; Farid, S.; Shamseldin, A.; Ibrahim, S.; Elsharabasy, R. The effect of oral vitamin K2 versus K1 on vascular calcification in hemodialysis patients: A randomized controlled trial. *Nephrol. Dial. Transplant.* **2022**, *37* (Suppl. S3), gfac080.026. [CrossRef]
44. Li, Y.; Xie, Z.; Xu, D. Inhibition of maintenance hemodialysis related vascular calcification by vitamin K in chronic kidney disease. *Int. J. Clin. Exp. Med.* **2017**, *10*, 15309–15315.
45. Haroon, S.; Davenport, A.; Ling, L.H.; Tai, B.C.; Schurgers, L.; Chen, Z.; Wong, W.K. Randomized controlled clinical trial of the effect of treatment with vitamin K2 on vascular calcification in hemodialysis patients (Trevasc-HDK). *Kidney Int. Rep.* **2023**, *8*, 1741–1751. [CrossRef] [PubMed]
46. Levy-Schousboe, K.; Frimodt-Møller, M.; Hansen, D.; Peters, C.D.; Kjærgaard, K.D.; Jensen, J.D.; Strandhave, C.; Elming, H.; Larsen, C.T.; Sandstrøm, H.; et al. Vitamin K supplementation and arterial calcification in dialysis: Results of the double-blind, randomized, placebo-controlled RenaKvit trial. *Clin. Kidney J.* **2021**, *14*, 2114–2123. [CrossRef] [PubMed]
47. Holden, R.M.; Booth, S.L.; Zimmerman, D.; Moist, L.; Norman, P.; Day, A.G.; Heyland, D.K. Inhibit progression of coronary artery calcification with vitamin k in hemodialysis patients (the iPACK-HD study): A randomized, placebo-controlled multi-centre, pilot trial. *Nephrol. Dial. Transplant.* **2022**, *38*, 746–756. [CrossRef] [PubMed]
48. Kanai, S.; Uto, K.; Honda, K.; Hagiwara, N.; Oda, H. Eicosapentaenoic acid reduces warfarin-induced arterial calcification in rats. *Atherosclerosis* **2011**, *215*, 43–51. [CrossRef]
49. Nakamura, K.; Miura, D.; Saito, Y.; Yunoki, K.; Koyama, Y.; Satoh, M.; Ito, H. Eicosapentaenoic acid prevents arterial calcification in klotho mutant mice. *PLoS ONE* **2017**, *12*, e0181009. [CrossRef]
50. Son, Y.K.; Lee, S.M.; Kim, S.E.; Kim, K.H.; Lee, S.Y.; Bae, H.R.; Han, J.Y.; Park, Y.; An, W.S. Association between vascular calcification scores on plain radiographs and fatty acid contents of erythrocyte membrane in hemodialysis patients. *J. Ren. Nutr.* **2012**, *22*, 58–66. [CrossRef]
51. Lan, Z.; Chen, A.; Li, L.; Ye, Y.; Liang, Q.; Dong, Q.; Yan, J. Downregulation of HDAC9 by the ketone metabolite β-hydroxybutyrate suppresses vascular calcification. *J. Pathol.* **2022**, *258*, 213–226. [CrossRef]
52. Merino-Ribas, A.; Araujo, R.; Pereira, L.; Campos, J.; Barreiros, L.; Segundo, M.A.; Silva, N.; Costa, C.F.F.A.; Quelhas-Santos, J.; Trindade, F.; et al. Vascular calcification and the gut and blood microbiome in chronic kidney disease patients on peritoneal dialysis: A pilot study. *Biomolecules* **2022**, *12*, 867. [CrossRef] [PubMed]
53. Wei, J.; Li, Z.; Fan, Y.; Feng, L.; Zhong, X.; Li, W.; Guo, T.; Ning, X.; Li, Z.; Ou, C. Lactobacillus rhamnosus GG aggravates vascular calcification in chronic kidney disease: A potential role for extracellular vesicles. *Life Sci.* **2023**, *331*, 122001. [CrossRef] [PubMed]
54. Sanchis, P.; Buades, J.M.; Berga, F.; Gelabert, M.M.; Molina, M.; Íñigo, M.V.; Grases, F. Protective effect of myo-inositol hexaphosphate (phytate) on abdominal aortic calcification in patients with chronic kidney disease. *J. Ren. Nutr.* **2016**, *26*, 226–236. [CrossRef] [PubMed]
55. Raggi, P.; Bellasi, A.; Bushinsky, D.; Bover, J.; Rodriguez, M.; Ketteler, M.; Chertow, G.M. Slowing progression of cardiovascular calcification with SNF472 in patients on hemodialysis: Results of a randomized phase 2b study. *Circulation* **2020**, *141*, 728–739. [CrossRef] [PubMed]
56. Tchkonia, T.; Kirkland, J.L. Aging, cell senescence, and chronic disease: Emerging therapeutic strategies. *JAMA* **2018**, *320*, 1319–1320. [CrossRef]
57. Ribeiro, M.; Fanton, S.; Paiva, B.R.; Baptista, B.G.; Alvarenga, L.; Ribeiro-Alves, M.; Cardozo, L.F.; Mafra, D. Dark chocolate (70% cocoa) attenuates the inflammatory marker TNF-a in patients on hemodialysis. *Clin. Nutr. ESPEN* **2023**, *53*, 189–195. [CrossRef]

58. Chang, X.Y.; Cui, L.; Wang, X.Z.; Zhang, L.; Zhu, D.; Zhou, X.R.; Hao, L.R. Quercetin attenuates vascular calcification through suppressed oxidative stress in adenine-induced chronic renal failure rats. *BioMed Res. Int.* **2017**, *2017*, 5716204. [CrossRef]
59. Calvo, M.S.; Dunford, E.K.; Uribarri, J. Industrial use of phosphate food additives: A mechanism linking ultra-processed food intake to cardiorenal disease risk? *Nutrients* **2023**, *15*, 3510. [CrossRef]
60. Su, G.; Saglimbene, V.; Wong, G.; Bernier-Jean, A.; Carrero, J.J.; Natale, P.; Strippoli, G.F. Dietary phosphorus, its sources, and mortality in adults on haemodialysis: The DIET-HD Study. *Nutrients* **2022**, *14*, 4064. [CrossRef] [PubMed]
61. Miyamoto, K.I.; Oh, J.; Razzaque, M.S. Common Dietary Sources of Natural and Artificial Phosphate in Food. In *Phosphate Metabolism*; Springer: Cham, Switzerland, 2022; pp. 99–105.
62. Byrne, F.N.; Gillman, B.A.; Kiely, M.; Palmer, B.; Shiely, F.; Kearney, P.M.; Earlie, J.; Bowles, M.B.; Keohane, F.M.; Connolly, P.P.; et al. Pilot randomized controlled trial of a standard versus a modified low-phosphorus diet in hemodialysis patients. *Kidney Int. Rep.* **2020**, *5*, 1945–1955. [CrossRef] [PubMed]
63. Moorthi, R.N.; Armstrong, C.L.; Janda, K.; Ponsler-Sipes, K.; Asplin, J.R.; Moe, S.M. The effect of a diet containing 70% protein from plants on mineral metabolism and musculoskeletal health in chronic kidney disease. *Am. J. Nephrol.* **2014**, *40*, 582. [CrossRef]
64. Henley, C.; Colloton, M.; Cattley, R.C.; Shatzen, E.; Towler, D.A.; Lacey, D.; Martin, D. 1,25-Dihydroxyvitamin D3 but not cinacalcet HCl (Sensipar/Mimpara) treatment mediates aortic calcification in a rat model of secondary hyperparathyroidism. *Nephrol. Dial. Transplant.* **2005**, *20*, 1370–1377. [CrossRef]
65. Lee, S.M.; An, W.S. Supplementary nutrients for prevention of vascular calcification in patients with chronic kidney disease. *Korean J. Intern. Med.* **2019**, *34*, 459–469. [CrossRef]
66. Ter Braake, A.D.; Shanahan, C.M.; de Baaij, J.H.F. Magnesium counteracts vascular calcification: Passive interference or active modulation? *Arterioscler. Thromb. Vasc. Biol.* **2017**, *37*, 1431–1445. [CrossRef] [PubMed]
67. Xu, C.; Smith, E.R.; Tiong, M.K.; Ruderman, I.; Toussaint, N.D. Interventions to attenuate vascular calcification progression in chronic kidney disease: A systematic review of clinical trials. *J. Am. Soc. Nephrol.* **2022**, *33*, 1011–1032. [CrossRef] [PubMed]
68. Cozzolino, M.; Fusaro, M.; Ciceri, P.; Gasperoni, L.; Cianciolo, G. The role of vitamin K in vascular calcification. *Adv. Chronic Kidney Dis.* **2019**, *26*, 437–444. [CrossRef] [PubMed]
69. Kumar, M.; Deshmukh, P.; Bhatt, A.; Sinha, A.H.; Chawla, P. Vitamin E Supplementation and Cardiovascular Health: A Comprehensive Review. *Cureus* **2023**, *15*, e48142. [CrossRef]
70. Ikizler, T.A.; Burrowes, J.D.; Byham-Gray, L.D.; Campbell, K.L.; Carrero, J.-J.; Chan, W.; Fouque, D.; Friedman, A.N.; Ghaddar, S.; Goldstein-Fuchs, D.J.; et al. KDOQI Clinical Practice Guideline for Nutrition in CKD: 2020 Update. *Am. J. Kidney Dis.* **2020**, *76*, S1–S107. [CrossRef] [PubMed]
71. Endo, J.; Arita, M. Cardioprotective mechanism of omega-3 polyunsaturated fatty acids. *J. Cardiol.* **2016**, *67*, 22–27. [CrossRef] [PubMed]
72. Saini, R.K.; Prasad, P.; Sreedhar, R.V.; Akhilender Naidu, K.; Shang, X.; Keum, Y.-S. Omega−3 polyunsaturated fatty acids (PUFAs): Emerging plant and microbial sources, oxidative stability, bioavailability, and health benefits—A review. *Antioxidants* **2021**, *10*, 1627. [CrossRef]
73. Sun, W.; Byon, C.H.; Kim, D.H.; Choi, H.I.; Park, J.S.; Joo, S.Y.; Kim, S.W. Renoprotective effects of maslinic acid on experimental renal fibrosis in unilateral ureteral obstruction model via targeting MyD88. *Front. Pharmacol.* **2021**, *12*, 708575. [CrossRef]
74. Panahi, Y.; Dashti-Khavidaki, S.; Farnood, F.; Noshad, H.; Lotfi, M.; Gharekhani, A. Therapeutic effects of omega-3 fatty acids on chronic kidney disease-associated pruritus: A literature review. *Adv. Pharm. Bull.* **2016**, *6*, 509–514. [CrossRef]
75. Kitagawa, M.; Sugiyama, H.; Morinaga, H.; Inoue, T.; Takiue, K.; Ogawa, A.; Makino, H. A decreased level of serum soluble Klotho is an independent biomarker associated with arterial stiffness in patients with chronic kidney disease. *PLoS ONE* **2013**, *8*, e56695. [CrossRef]
76. Garg, P.K.; Guan, W.; Nomura, S.; Weir, N.L.; Karger, A.B.; Duprez, D.; Tsai, M.Y. Associations of plasma omega-3 and omega-6 PUFA levels with arterial elasticity: The multi-ethnic study of atherosclerosis. *Eur. J. Clin. Nutr.* **2022**, *76*, 1770–1775. [CrossRef]
77. O'Neill, B.; Raggi, P. The ketogenic diet pros and cons. *Atherosclerosis* **2019**, *292*, 119–126. [CrossRef]
78. Carriazo, S.; Perez-Gomez, M.V.; Cordido, A.; García-González, M.A.; Sanz, A.B.; Ortiz, A.; Sanchez-Niño, M.D. Dietary care for ADPKD patients: Current status and future directions. *Nutrients* **2019**, *11*, 1576. [CrossRef] [PubMed]
79. Torres, J.A.; Kruger, S.L.; Broderick, C.; Amarlkhagva, T.; Agrawal, S.; Dodam, J.R.; Mrug, M.; Lyons, L.A.; Weimbs, T. Ketosis ameliorates renal cyst growth in polycystic kidney disease. *Cell Metab.* **2019**, *30*, 1007–1023. [CrossRef] [PubMed]
80. Han, Y.M.; Ramprasath, T.; Zou, M.H. β-hydroxybutyrate and its metabolic effects on age-associated pathology. *Exp. Mol. Med.* **2020**, *52*, 548–555. [CrossRef] [PubMed]
81. De Cabo, R.; Matsson, M.P. Effects of intermittent fasting on health, aging and disease. *N. Engl. J. Med.* **2019**, *381*, 2541–2551. [CrossRef]
82. Dube, P.; DeRiso, A.; Patel, M.; Battepati, D.; Khatib-Shahidi, B.; Sharma, H.; Gupta, R.; Malhotra, D.; Dworkin, L.; Haller, S.; et al. Vascular calcification in chronic kidney disease: Diversity in the vessel wall. *Biomedicines* **2021**, *9*, 404. [CrossRef] [PubMed]
83. Ravid, J.D.; Kamel, M.H.; Chitalia, V.C. Uraemic solutes as therapeutic targets in CKD-associated cardiovascular disease. *Nat. Rev. Nephrol.* **2021**, *17*, 402–416. [CrossRef] [PubMed]
84. Rodrigues, F.G.; Ormanji, M.S.; Heilberg, I.P.; Bakker, S.J.L.; de Borst, M.H. Interplay between gut microbiota, bone health and vascular calcification in chronic kidney disease. *Eur. J. Clin. Investig.* **2021**, *51*, e13588. [CrossRef] [PubMed]

85. Evenepoel, P.; Stenvinkel, P.; Shanahan, C.; Pacifici, R. Inflammation and gut dysbiosis as drivers of CKD–MBD. *Nat. Rev. Nephrol.* **2023**, *19*, 646–657. [CrossRef] [PubMed]
86. Sun, S.N.; Ni, S.H.; Li, Y.; Liu, X.; Deng, J.P.; Ouyang, X.L.; Kuang, X.Y. Association between dietary inflammatory index with all-cause and cardiovascular disease mortality among older US adults: A longitudinal cohort study among a nationally representative sample. *Arch. Gerontol. Geriatr.* **2024**, *118*, 105279. [CrossRef] [PubMed]
87. Shafi, T.; Powe, N.R.; Meyer, T.W.; Hwang, S.; Hai, X.; Melamed, M.L.; Banerjee, T.; Coresh, J.; Hostetter, T.H. Trimethylamine N-oxide and cardiovascular events in hemodialysis patients. *J. Am. Soc. Nephrol.* **2017**, *28*, 321–331. [CrossRef]
88. Kwon, Y.J.; Lee, H.S.; Park, G.E.; Lee, J.W. Association between dietary fiber intake and all-cause and cardiovascular mortality in middle aged and elderly adults with chronic kidney disease. *Front. Nutr.* **2022**, *9*, 863391. [CrossRef]
89. Wang, A.Y.-M.; Sea, M.M.-M.; Ng, K.; Wang, M.; Chan, I.H.-S.; Lam, C.W.-K.; Sanderson, J.E.; Woo, J. Dietary fiber intake, myocardial injury, and major adverse cardiovascular events among end-stage kidney disease patients: A prospective cohort study. *Kidney Int. Rep.* **2019**, *4*, 814–823. [CrossRef]
90. Wang, K.; Qian, D.; Hu, Y.; Cheng, Y.; Ge, S.; Yao, Y. Nut consumption and effects on chronic kidney disease and mortality in the United States. *Am. J. Nephrol.* **2022**, *53*, 503–512. [CrossRef]
91. Sirich, T.L.; Plummer, N.S.; Gardner, C.D.; Hostetter, T.H.; Meyer, T.W. Effect of increasing dietary fiber on plasma levels of colon-derived solutes in hemodialysis patients. *Clin. J. Am. Soc. Nephrol.* **2014**, *9*, 1603–1610. [CrossRef]
92. Filipska, I.; Winiarska, A.; Knysak, M.; Stompór, T. Contribution of gut microbiota-derived uremic toxins to the cardiovascular system mineralization. *Toxins* **2021**, *13*, 274. [CrossRef] [PubMed]
93. Black, A.P.; Anjos, J.S.; Cardozo, L.; Carmo, F.L.; Dolenga, C.J.; Nakao, L.S.; de Carvalho Ferreira, D.; Rosado, A.; Eduardo, J.C.C.; Mafra, D.; et al. Does low-protein diet influence the uremic toxin serum levels from the gut microbiota in nondialysis chronic kidney disease patients? *J. Ren. Nutr.* **2018**, *28*, 208–214. [CrossRef] [PubMed]
94. Kandouz, S.; Shendi, A.M.; Zheng, Y.; Sandeman, S.R.; Davenport, A. Reduced protein bound uraemic toxins in vegetarian kidney failure patients treated by haemodiafiltration. *Hemodial. Int.* **2016**, *20*, 610–617. [CrossRef] [PubMed]
95. Gluba-Brzózka, A.; Franczyk, B.; Rysz, J. Vegetarian diet in chronic kidney disease—A friend or foe. *Nutrients* **2017**, *9*, 374. [CrossRef] [PubMed]
96. Kalantar-Zadeh, K.; Joshi, S.; Schlueter, R.; Cooke, J.; Brown-Tortorici, A.; Donnelly, M.; Schulman, S.; Lau, W.-L.; Rhee, C.M.; Streja, E.; et al. Plant-dominant low-protein diet for conservative management of chronic kidney disease. *Nutrients* **2020**, *12*, 1931. [CrossRef] [PubMed]
97. Carrero, J.J.; González-Ortiz, A.; Avesani, C.M.; Bakker, S.J.L.; Bellizzi, V.; Chauveau, P.; Clase, C.M.; Cupisti, A.; Espinosa-Cuevas, A.; Molina, P.; et al. Plant-based diets to manage the risks and complications of chronic kidney disease. *Nat. Rev. Nephrol.* **2020**, *16*, 525–542. [CrossRef]
98. Stockler-Pinto, M.B.; Mafra, D.; Moraes, C.; Lobo, J.; Boaventura, G.T.; Farage, N.E.; Silva, W.S.; Cozzolino, S.F.; Malm, O. Brazil nut (Bertholletia excelsa, H.B.K.) improves oxidative stress and inflammation biomarkers in hemodialysis patients. *Biol. Trace Elem. Res.* **2014**, *158*, 105–112. [CrossRef]
99. Narasaki, Y.; Rhee, C.M.; Kalantar-Zadeh, K. Going nuts to protect kidneys and to live longer with kidney disease. *Am. J. Nephrol.* **2022**, *53*, 423–426. [CrossRef]
100. Saglimbene, V.M.; Wong, G.; Craig, J.C.; Ruospo, M.; Palmer, S.C.; Campbell, K.; Garcia-Larsen, V.; Natale, P.; Teixeira-Pinto, A.; Carrero, J.-J.; et al. The association of Mediterranean and DASH diets with mortality in adults on hemodialysis: The DIET-HD multinational cohort study. *J. Am. Soc. Nephrol.* **2018**, *29*, 1741–1751. [CrossRef]
101. Su, G.; Saglimbene, V.; Wong, G.; Natale, P.; Ruospo, M.; Craig, J.C.; Strippoli, G.F. Healthy lifestyle and mortality among adults receiving hemodialysis: The DIET-HD study. *Am. J. Kidney Dis.* **2022**, *79*, 688–698. [CrossRef]
102. Ricardo, A.C.; Anderson, C.A.; Yang, W.; Zhang, X.; Fischer, M.J.; Dember, L.M.; Fink, J.C.; Frydrych, A.; Jensvold, N.G.; Lustigova, E.; et al. Healthy lifestyle and risk of kidney disease progression, atherosclerotic events, and death in CKD: Findings from the Chronic Renal Insufficiency Cohort (CRIC) Study. *Am. J. Kidney Dis.* **2015**, *65*, 412–424. [CrossRef] [PubMed]
103. Sherman, R.A.; Mehta, O. Phosphorus and potassium content of enhanced meat and poultry products: Implications for patients who receive dialysis. *Clin. J. Am. Soc. Nephrol.* **2009**, *4*, 1370–1373. [CrossRef] [PubMed]
104. Clegg, D.J.; Headley, S.A.; Germain, M.J. Impact of dietary potassium restrictions in CKD on clinical outcomes: Benefits of a plant-based diet. *Kidney Med.* **2020**, *2*, 476–487. [CrossRef] [PubMed]
105. Sakaguchi, Y.; Kaimori, J.Y.; Isaka, Y. Plant-dominant low protein diet: A potential alternative dietary practice for patients with chronic kidney disease. *Nutrients* **2023**, *15*, 1002. [CrossRef] [PubMed]
106. Cases, A.; Cigarrán-Guldrís, S.; Mas, S.; Gonzalez-Parra, E. Vegetable-based diets for chronic kidney disease? It is time to reconsider. *Nutrients* **2019**, *11*, 1263. [CrossRef] [PubMed]
107. Narasaki, Y.; Rhee, C.M. Dietary Therapy for Managing Hyperphosphatemia. *Clin. J. Am. Soc. Nephrol.* **2020**, *16*, 9–11. [CrossRef]
108. Narasaki, Y.; Okuda, Y.; Kalantar, S.S.; You, A.S.; Novoa, A.; Nguyen, T.; Streja, E.; Nakata, T.; Colman, S.; Kalantar-Zadeh, K.; et al. Dietary potassium intake and mortality in a prospective hemodialysis cohort. *J. Ren. Nutr.* **2021**, *31*, 411–420. [CrossRef]
109. Joshi, S.; McMacken, M.; Kalantar-Zadeh, K. Plant-based diets for kidney disease: A guide for clinicians. *Am. J. Kidney Dis.* **2021**, *77*, 287–296. [CrossRef]
110. Li, W.; Huang, G.; Tang, N.; Lu, P.; Jiang, L.; Lv, J.; Lei, D. Identification of dietary components in association with abdominal aortic calcification. *Food Funct.* **2023**, *14*, 8383–8395. [CrossRef]

111. Panchal, S.K.; John, O.D.; Mathai, M.L.; Brown, L. Anthocyanins in chronic diseases: The power of purple. *Nutrients* **2022**, *14*, 2161. [CrossRef]
112. Marx, W.; Kelly, J.; Marshall, S.; Nakos, S.; Campbell, K.; Itsiopoulos, C. The effect of Polyphenol-rich interventions on cardiovascular risk factors in haemodialysis: A systematic review and meta-analysis. *Nutrients* **2017**, *9*, 1345. [CrossRef]
113. Zhang, P.; Li, Y.; Du, Y.; Li, G.; Wang, L.; Zhou, F. Resveratrol ameliorated vascular calcification by regulating Sirt-1 and Nrf2. *Transplant. Proc.* **2016**, *48*, 3378–3386. [CrossRef]
114. Coutinho-Wolino, K.S.; Melo, M.F.; Mota, J.C.; Mafra, D.; Guimarães, J.T.; Stockler-Pinto, M.B. Blueberry, cranberry, raspberry, and strawberry as modulators of the gut microbiota: Target for treatment of gut dysbiosis in chronic kidney disease? From current evidence to future possibilities. *Nutr. Rev.* **2024**, *82*, 248–261. [CrossRef] [PubMed]
115. Gurău, F.; Baldoni, S.; Prattichizzo, F.; Espinosa, E.; Amenta, F.; Procopio, A.D.; Albertini, M.C.; Bonafè, M.; Olivieri, F. Anti-senescence compounds: A potential nutraceutical approach to healthy aging. *Ageing Res. Rev.* **2018**, *46*, 14–31. [CrossRef] [PubMed]
116. Ciceri, P.; Cozzolino, M. The emerging role of iron in heart failure and vascular calcification in CKD. *Clin. Kidney J.* **2021**, *14*, 739–745. [CrossRef] [PubMed]
117. Dong, W.; Liu, X.; Ma, L.; Yang, Z.; Ma, C. Association between dietary selenium intake and severe abdominal aortic calcification in the United States: A cross-sectional study. *Food Funct.* **2024**, *15*, 1575–1582. [CrossRef]
118. Cardozo, L.F.; Borges, N.A.; Ribeiro, M.; Wang, A.Y.M.; Mafra, D. Protect the kidneys and save the heart using the concept of food as medicine. *J. Ren. Nutr.* **2023**, *33*, S110–S117. [CrossRef]

diseases

MDPI

Article

The Severity of Carotid Calcifications, but Not Fibroblast Growth Factor 23, Is Associated with Mortality in Hemodialysis: A Single Center Experience

Diana Moldovan

Department of Nephrology, "Iuliu Hatieganu" University of Medicine and Pharmacy, 400012 Cluj-Napoca, Romania; diana.moldovan@umfcluj.ro

Abstract: Background. The study goal was to assess the mortality effect of carotid vascular calcifications (VC), of fibroblast growth factor 23 (FGF-23), mineral markers, and comorbidities in hemodialysis (HD) patients. Methods. The influence of carotid VC severity, FGF-23, laboratory markers, clinical features, and comorbidities on mortality was analyzed in a cohort of 88 HD patients. The follow-up period lasted 8 years. The cut-off value for carotid VC was 4 for all-cause and cardiovascular mortality. Results. Carotid VC, diabetes, low serum albumin, high serum C-reactive protein (CRP), and the presence of cardiovascular diseases are associated with all-cause and cardiovascular mortality. Carotid VC score over 4 was an independent predictor of all-cause and cardiovascular mortality, along with diabetes, low albumin, and high CRP. FGF-23 was not found to be predictable for the study outcomes. Conclusions. The study documented in a cohort of patients prevalent in chronic HD that carotid VC predicts all-cause and cardiovascular mortality at 8 years and improves risk stratification, but FGF-23 is not associated with mortality. Other risk factors for all-cause and cardiovascular mortality were diabetes, inflammation, and malnutrition. However, future efforts are needed to assess whether a risk-based approach, including VC screening, improves survival.

Keywords: carotid vascular calcification; fibroblast growth factor 23; hemodialysis; all-cause mortality; cardiovascular mortality

Received: 5 February 2025
Revised: 21 February 2025
Accepted: 25 February 2025
Published: 28 February 2025

Citation: Moldovan, D. The Severity of Carotid Calcifications, but Not Fibroblast Growth Factor 23, Is Associated with Mortality in Hemodialysis: A Single Center Experience. *Diseases* **2025**, *13*, 73. https://doi.org/10.3390/diseases13030073

1. Introduction

One can argue that people having end-stage renal disease (ESRD) are part of a privileged category compared with those with other vital organ failure, thanks to renal replacement therapies, which substantially prolong life. Nevertheless, it is estimated that chronic kidney disease accounts globally for 5 to 10 million deaths annually, mainly due to cardiovascular diseases [1]. However, there is a great need for improvement regarding the lifespan of these people. In 2016, chronic kidney disease was the sixteenth leading cause of years of life lost worldwide, and it is expected to be the fifth leading cause of years of life lost worldwide by 2040 [1]. Cardiovascular events account for the primary cause of mortality in hemodialysis (HD) patients.

Vascular disease can have an important mortality effect on patients with ESRD, and the risk for cardiovascular death is enhanced by the presence of atherosclerosis and vascular calcification (VC) [2]. Atherosclerosis is a complex and multifactorial process, characterized by early asymptomatic formation of plaque in the arterial walls, silent plaque progression and calcification that poses flow limitation, and risk of sudden thrombotic occlusion associated with a high rate of fatal evolution [2,3]. The early detection and grading of VC,

or even better, of atherosclerotic plaque, and assessing the predictive value are critically important to prevent clinical consequences [3].

Vascular calcification (VC) is an active process analogous to bone formation and results not only from a passive deposition of calcium phosphate in the vessel walls but also from the activation of osteogenesis [2,4]. The differentiation of vascular smooth muscle cells into osteoblast-like cells [4] results in the synthesis of a bone structure in the arteries. Active inducers of VC in chronic kidney disease include hypercalcemia, hyperphosphatemia, abnormal iPTH levels, FGF-23, uremic toxicity, and inflammation [5]. The loss of mineralization inhibitors such as Klotho, pyrophosphates, and matrix gla protein leads to VC. Noteworthy are the systems of the osteoprotegerin (OPG)/RANK/RANK ligand complex and Wnt signaling pathway inhibitors such as sclerostin, which control bone matrix formation, including in the case of VC [4]. Besides classical risk factors for cardiovascular events, such as diabetes, dyslipidemia, and hypertension, kidney disease-specific risk factors, such as anemia, fluid overload, and chronic kidney disease—mineral bone disorders (CKD-MBD) contribute to this high mortality risk [5,6].

Extraskeletal calcifications represent the mineralization of the extracellular matrix. It is a condition frequently observed in the HD patient population, the main site for abnormal calcium salt deposition being the arteries [4,7]. The mechanisms underlying the pathogenesis of VC are complex, involving factors that facilitate or impede the development of calcification. Ongoing research has detected VC to be a cell-mediated process and a result of the combination of pro-calcifying stimuli and impairment of inhibiting mechanisms [7]. Intimal calcification complicating atherosclerosis and medial calcification are both possible consequences of ESRD that can occur simultaneously [8]. Endothelial cells exposed to various pro-calcification factors are injured, and they easily become prone to VC. Extended research brought evidence in favor of the pathogenetic pathway of VC, explained by the fact that endothelial cells are not simple bystanders, being able to undergo a phenotypic switch and become bone progenitor cells to promote calcification [9]. The crosstalk between vessels and bone is a continuum in VC occurrence, with all the above-mentioned factors acting on arteries and bones consecutively [10]. Arterial calcification and elevated FGF-23 levels may be significantly involved in the development of heart conditions such as left ventricular hypertrophy, coronary ischemic disease, and consequent congestive heart failure in chronic kidney disease, predisposing factors for early cardiovascular death [11].

The role of calcification inhibitors and regulators in the calcification process, as well as their effect on vascular dysfunction and mortality in HD patients, is not completely understood. Fibroblast growth factor 23 (FGF-23) is an important regulator of mineral homeostasis [12] and is a circulating hormone primarily released by bone, produced in osteocytes. Circulating FGF-23 stimulates urinary phosphate excretion and inhibits the production of active 1,25-dihydroxy vitamin D [13]. Serum FGF-23 levels increase early during chronic kidney disease progression before changes in other parameters of bone and mineral metabolism. Several studies have reported that a higher level of FGF-23 is associated with an increased risk of death in patients with ESRD [14] or with other adverse outcomes such as the progression of diabetic nephropathy [15]. No consensus has been obtained yet regarding the role of FGF-23 in the evolution of HD patients.

There are some theories that outcomes for ESRD patients are different according to findings related to CKD-MBD, such as serum levels of FGF-23 and the presence or severity of vascular calcifications; however, it is generally hypothesized that the benefits observed with early interventions depend on how early risk is detected and addressed [11]. More importantly, there is currently no efficient treatment for VC, so understanding the impact on outcome and prevention is crucial.

The primary aim of this study was to investigate the association between carotidal atheroma VC, serum FGF-23 levels, and all-cause mortality in chronic HD patients. The secondary goals were to investigate the causes of death and the impact of different demographical, clinical, and laboratory factors on all-cause and cardiovascular mortality.

2. Materials and Methods

This is a prospective, longitudinal, and analytical study. The study population consisted of a group of 88 patients treated with chronic hemodialysis. Eligibility criteria: adult patients over 18 years old, prevalent in dialysis, who accepted the study protocol. Exclusion criteria: life expectancy less than 6 months, previous parathyroidectomy, previous renal transplant, active infections. Demographic and clinical data (age, gender, HD vintage, HD prescription, treatments with Ca-based phosphates (P) binders, sevelamer, and vitamin D, presence of diabetes and hypertension, cardiovascular diseases). Laboratory evaluation included serum calcium (Ca), inorganic phosphorus (inorganic P), alkaline phosphatase (ALP), intact parathyroid hormone (iPTH), bicarbonate, creatinine, hemoglobin (Hb), ferritin, albumin, and C-reactive protein (CRP); fibroblast growth factor 23 (Human FGF-23; ELISA). Hemodialysis adequacy was presented using spKt/V and urea reduction ratio (URR).

Ultrasound examinations have been performed on three segments of the carotid arteries (bilateral): common carotid artery, bifurcation, and internal carotid artery. Real-time and color Doppler sonography through longitudinal and transversal sections were undertaken. Two exams assured the intra-observer reproducibility of this assessment; in selected cases, a third examination was performed. Carotid vascular calcifications (VC) were defined as patchy hyperechoic images with posterior shadows in the arterial walls, representing calcified atheroma plaques. A VC score was counted ranging from 0 (no calcification) to 6 (calcification of all artery sites examined from both sides); each examined site received 1 point in this score (Figure 1).

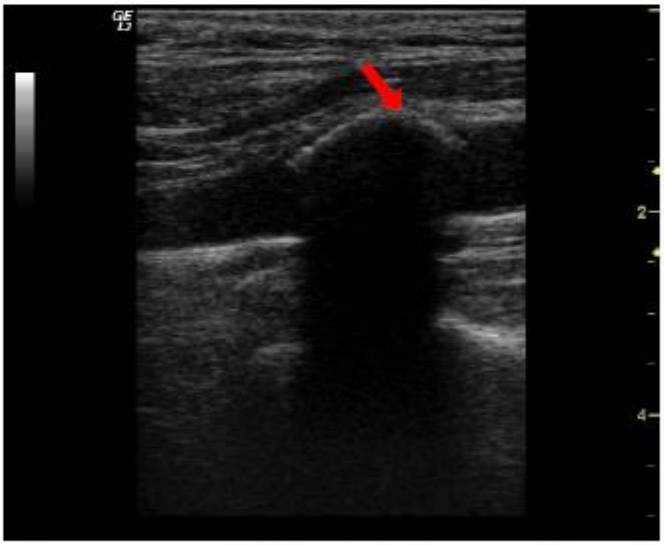

Figure 1. Ultrasound examination. Common carotid artery calcification is the patchy hyperechoic image with a posterior shadow in the arterial wall (red arrow).

Cardiovascular diseases were registered and included ischemic heart disease, heart failure, stroke, arrhythmia, aortic aneurysm, and peripheral artery disease.

All 88 included HD patients had a standard HD schedule of 3 sessions of 4 h/week and achieved a mean spKt/V of 1.53 ± 0.28. They had a mean age of 59.68 ± 14.49 years, and a mean HD vintage of 59.61 ± 52.39 months. Forty-five were males (51.13%), 63 patients (71.6%) had hypertension, 20 patients (22.72%) had diabetes mellitus, 54 patients (61.4%) had carotidal VC, and 25 patients (28.4%) had cardiovascular diseases. The median FGF-23 was 43.5, ranging from 7.6 to 290.8 pg/mL. The patients' characteristics are described in detail in our previous cross-sectional study [16].

The follow-up period lasted 8 years. This study recorded the mortality causes and survival time. Mortality data were obtained from the dialysis center register. The cardiovascular causes of death included myocardial infarction, heart failure, arrhythmia, pulmonary edema, stroke, aortic aneurysm, and peripheral artery disease. Factors with impact on all-cause and cardiovascular death were analyzed.

Statistical Analysis

Normally distributed data are presented as mean and standard deviation (SD) and skewed variables as median and interquartile range. Normality was assessed using Kolmogorov–Smirnov and Shapiro–Wilk tests. Categorical variables are shown as total numbers and percentages. Comparison between groups was performed by the Chi-square test, Fisher exact test, independent samples *t*-test, and Mann-Whitney test as appropriate. Univariate and multivariate Cox proportional hazard models were used to estimate the association between independent predictors and all-cause and cardiovascular mortality in all patients. The multivariable model included potential confounders influencing mortality, which became significant in univariate analysis. Cox proportional hazard models were used to estimate hazard ratios (HR) for the outcome of interest described above. The receiver-operating characteristic (ROC) curve identified the cut-off value of carotidal VC that best-predicted all-cause and cardiovascular deaths. To visualize significant associations between VC score (<4 vs. ≥4) and mortality, Kaplan–Meier curves were generated; these were compared by the log-rank test.

Statistical analyses were performed using SPSS version 25.0 (IBM, Armonk, NY, USA). For all tests, a two-sided p-value < 0.05 was regarded as statistically significant.

3. Results

Death of all causes was recorded and analyzed. In our study group, forty-nine patients died (55.7%) for the entire follow-up period. Among all patients included in the study, 34 patients died due to cardiovascular causes (38.6%). Cardiovascular mortality was the consequence of myocardial infarction in 8 patients, heart failure in 7 patients, arrhythmia in 5 patients, pulmonary edema in 4 patients, stroke in 5 patients, and peripheral artery disease in 5 patients. The other patients died due to infections (7 patients), neoplasia (2 patients), sudden death (3 patients), or unknown cause (3 patients).

The patients were included in 2 categories, deceased and survivors, and compared them. It resulted in a significant association between all-cause mortality and older age, male gender, more severe carotidal VC, increased CRP, low albumin, presence of diabetes, and cardiovascular diseases (Table 1).

Table 1. Comparison between the deceased and survivor groups.

	Deceased (49 Patients)	Survivors (39 Patients)	*p*
Age (years)	63 (59–72)	56 (46–64)	**0.003**
HD vintage (months)	47 (29–64)	49 (29.5–68)	0.628
Gender, males no. (%)	27 (55.1)	18 (46.2)	0.404
Diabetes, no. (%)	17 (34.7)	3 (7.7)	**0.003**
HTN, no. (%)	38 (77.6)	25 (64.1)	0.165
FGF-23 (pg/mL)	42.30 (23.10–72.70)	41.70 (20.95–70.90)	0.592
VC score	4 (1–6)	0 (0–2)	**<0.001**
URR	72.32 (69.57–79.45)	77.33 (69.63–80.65)	0.183
spKt/V	1.46(1.39–1.63)	1.58 (1.40–1.66)	0.183
Bicarbonate (mEq/L)	24 (22.7–25.6)	22.9 (20.5–24.6)	0.084
K (mEq/L)	4.48 ± 0.64	4.72 ± 0.55	0.067
Ca (mg/dL)	9.09 ± 0.65	9.07 ± 0.67	0.898
P (mg/dL)	4.34 ± 1.10	4.57 ± 1.54	0.427
ALP (U/L)	78.68 (64.13–99.56)	67.75 (45.22–103.84)	0.957
iPTH (pg/mL)	212.25 (144.75–547.65)	273.4 (160.95–536)	0.332
Hb (g/dL)	11.3 (10.6–12)	11.4 (10.5–12.35)	0.072
Ferritin (ng/mL)	498.51 (303.45–791.35)	586.22 (402.46–816.45)	0.784
CRP (mg/dL)	0.80 (0.35–2.02)	0.47 (0.28–1.04)	**0.035**
Albumin (g/dL)	3.75 (3.57–3.94)	3.96 (3.80–4.08)	**0.005**
Creatinine (mg/dL)	8.20 ± 1.98	8.73 ± 2.53	0.265
Ca in HD solution	1.5 (1.25–1.5)	1.5 (1.25–1.5)	0.854
Treatment with Ca salts, no. (%)	30 (61.2)	17 (43.6)	0.099
Sevelamer, no. (%)	10 (20.4)	10 (25.6)	0.561
Vitamin D Treatment, no. (%)	10 (20.4)	10 (25.6)	0.561
Cardiovascular diseases, no. (%)	23 (46.9)	4 (10.3)	**<0.001**

Legend: FGF-23, fibroblast growth factor 23; HD, hemodialysis; HTN, arterial hypertension; VC, vascular calcification; URR, urea reduction ratio; spKt/V, dialysis adequacy; K, kalium; Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; iPTH, intact parathyroid hormone; Hb, hemoglobin; CRP, C-reactive protein. Data are expressed as mean ± standard deviation and as median (25th–75th percentiles) or percentages. Statistical significance is marked with bold characters.

Univariate Cox proportional hazards regression analysis for all-cause and cardiovascular mortality in HD patients is detailed in Table 2. The results of univariate regression demonstrated that advanced age ($p = 0.002$), diabetes ($p = 0.001$), carotidal VC ($p < 0.001$), increased CRP ($p < 0.001$), decreased albumin ($p = 0.002$) and presence of cardiovascular diseases ($p < 0.001$) are significantly associated with high risk of all-cause mortality. Serum FGF-23 levels had no significant impact on all-cause ($p = 0.67$) or cardiovascular mortality ($p = 0.84$) (Table 2). The analysis of cardiovascular mortality in univariate regression demonstrated a significant association with increased age ($p = 0.019$), increased carotidal VC score ($p = 0.003$), low URR and spKt/V ($p = 0.02$), high CRP ($p < 0.001$), presence of diabetes ($p = 0.01$) and cardiovascular diseases ($p = 0.013$) (Table 2).

Table 2. Results of univariate Cox regression analysis for all-cause and cardiovascular mortality.

	All-Cause Mortality		Cardiovascular Mortality	
	HR (95% CI)	***p***	**HR (95% CI)**	***p***
Age (years)	1.04 (1.01–1.06)	**0.002**	1.03 (1.00–1.06)	**0.019**
HD vintage (months)	0.99 (0.99–1.00)	0.577	0.99 (0.99–1.00)	0.785
Gender (males)	0.77 (0.43–1.35)	0.361	0.67 (0.34–1.33)	0.254
Diabetes	2.74 (1.50–5.01)	**0.001**	2.56 (1.25–5.24)	**0.01**
HTN	1.41 (0.72–2.77)	0.307	1.11 (0.52–2.39)	0.779
FGF-23 (pg/mL)	1.00 (0.99–1.00)	0.676	1.00 (0.99–1.00)	0.841
Carotid VC score	1.24 (1.11–1.39)	**<0.001**	1.22 (1.07–1.40)	**0.003**
URR	0.98 (0.96–1.00)	0.119	0.97 (0.95–0.99)	**0.026**
spKt/V	0.48 (0.19–1.20)	0.119	0.33 (0.12–0.87)	**0.026**
Bicarbonate (mEq/L)	1.07 (0.98–1.16)	0.092	1.05 (0.95–1.16)	0.342
K (mEq/L)	0.65 (0.39–1.06)	0.085	1.03 (0.58–1.81)	0.917
Ca (mg/dL)	1.01 (0.64–1.60)	0.948	1.27 (0.73–2.23)	0.395
P (mg/dL)	0.88 (0.71–1.09)	0.263	0.89 (0.69–1.16)	0.411
ALP (U/L)	1.00 (0.99–1.00)	0.945	1.00 (0.99–1.00)	0.590
iPTH (pg/mL)	1.00 (0.99–1.00)	0.210	1.00 (0.99–1.00)	0.399
Hb (g/dL)	0.85 (0.70–1.04)	0.117	0.89 (0.71–1.13)	0.375
Ferritin (ng/mL)	1.00 (0.99–1.00)	0.788	1.00 (1.00–1.00)	0.514
CRP (mg/dL)	1.24 (1.10–1.38)	**<0.001**	1.27 (1.13–1.44)	**<0.001**
Albumin (g/dL)	0.41 (0.23–0.71)	**0.002**	0.58 (0.27–1.24)	0.162
Creatinine (mg/dL)	0.95 (0.84–1.06)	0.371	1.03 (0.89–1.19)	0.660
Ca in HD solution	1.03 (0.67–1.58)	0.890	0.79 (0.45–1.30)	0.330
Treatment with Ca salts—patients (%)	1.50 (0.84–2.68)	0.164	1.17 (0.59–2.31)	0.644
Sevelamer—patients (%)	0.69 (0.34–1.39)	0.303	0.71 (0.31–1.64)	0.430
Vitamin D Treatment—patients (%)	0.75 (0.37–1.50)	0.419	0.76 (0.33–1.75)	0.521
Cardiovascular diseases	2.84 (1.61–5.01)	**<0.001**	2.38 (1.20–4.72)	**0.013**

Legend: HR, hazard ratio; CI, confidence interval; FGF-23, fibroblast growth factor 23; HD, hemodialysis; HTN, arterial hypertension; VC, vascular calcification; URR, urea reduction ratio; spKt/V, dialysis adequacy; K, kalium; Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; iPTH, intact parathyroid hormone; Hb, hemoglobin; CRP, C-reactive protein. $p < 0.05$ was statistically significant and was marked with bold.

The study evaluated how the severity of carotidal VC can predict mortality. The cut-off value for the VC score to predict all-cause mortality was 4. The area under the ROC curve was 0.718 ($p < 0.001$; 95% CI = 0.609–0.826). The sensitivity was 55.1% and the specificity was 85%.

The cut-off value for VC score which predicts cardiovascular mortality was also 4. The area under the ROC curve was 0.657 ($p = 0.014$; 95% CI = 0.539–0.775). The sensitivity was 55.9% and the specificity was 74.1%.

The group with a VC score < 4 consisted of 55 patients and 22 deaths (mortality rate = 40%), and the group with a VC score ≥ 4 consisted of 33 patients (37.5%) with 27 deaths (mortality rate = 81.8%) (Figure 2).

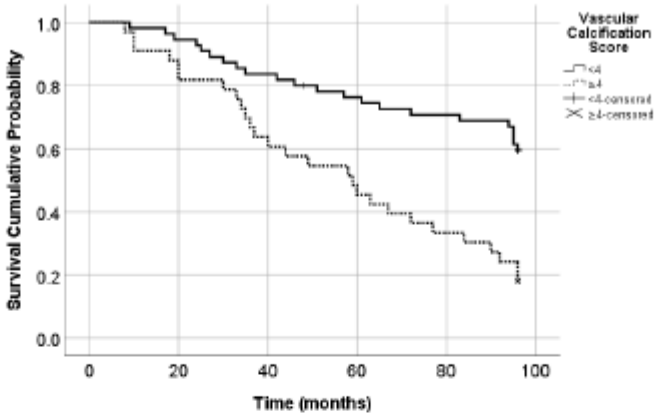

Figure 2. All-cause mortality according to the VC score (Log Rank Mantel Cox; $p < 0.001$). Survival probability at 96 months was 60% for patients with VC score < 4 and 19.2% for patients with VC score < 4.

In 6 patients from the survivors' group (15.4%) and in 27 patients from the deceased group (55.1%), the VC score was ≥4 ($p < 0.001$). The Kaplan-Meyer analysis showed that from the group of 55 patients with VC score < 4, 27.3% died due to cardiovascular causes, and from the group of 33 patients with VC score ≥ 4, 57.6% died due to cardiovascular causes ($p = 0.005$) (Figure 3).

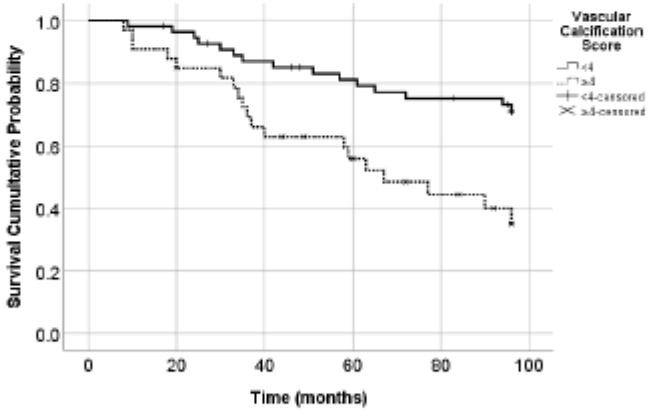

Figure 3. Cardiovascular mortality according to the VC score (Log Rank Mantel Cox; $p = 0.005$). Cardiovascular events produced death in 27.3% of patients with VC score < 4 and in 57.6% of patients with VC score ≥ 4.

Multivariate Cox regression analyzed the influence of carotid VC on mortality. All-cause mortality was associated with carotid VC score, diabetes, and CRP; covariates were all significant factors in univariate analysis and factors relevant to patients' outcomes. No significant relation was found between FGF-23 and all-cause mortality (Table 3).

Table 3. Multivariate Cox regression analysis for all-cause mortality.

	All-Cause Mortality	
	HR (95% CI)	***p***
Age (years)	1.02 (0.99–1.05)	0.185
FGF-23 (pg/mL)	1.00 (0.99–1.00)	0.090
Diabetes	2.35 (1.19–4.64)	**0.014**
VC score	1.19 (1.01–1.39)	**0.031**
K	0.94 (0.51–1.73)	0.860
Bicarbonate	1.09 (0.97–1.23)	0.122
Albumin	0.66 (0.31–1.43)	0.297
CRP	1.28 (1.12–1.46)	**<0.001**
Cardiovascular diseases	1.34 (0.67–2.68)	0.400

Legend: HR, hazard ratio; CI, confidence interval; FGF-23, fibroblast growth factor 23; VC, vascular calcification; K, kalium; CRP, C-reactive protein. A value of $p < 0.05$ marked statistical significance and it was highlighted with bold.

Cardiovascular mortality was also associated with carotid VC score, diabetes, and CRP, but also with HD adequacy assessed by URR; covariates were significant factors in univariate analysis (Table 4).

Table 4. Multivariate Cox regression analysis for cardiovascular mortality.

	Cardiovascular Mortality	
	HR (95% CI)	***p***
Age (years)	1.02 (0.98–1.06)	0.221
FGF-23 (pg/mL)	1.00 (0.99–1.00)	0.432
Diabetes	2.16 (1.03–4.55)	**0.041**
VC score	1.22 (1.02–1.47)	**0.028**
CRP	1.36 (1.18–1.56)	**<0.001**
URR	0.95 (0.93–0.99)	**0.009**
Cardiovascular diseases	1.12 (0.51–2.44)	0.762

Legend: HR, hazard ratio; CI, confidence interval; FGF-23, fibroblast growth factor 23; VC, vascular calcification; CRP, C-reactive protein; URR, urea reduction ratio. A value of $p < 0.05$ marked statistical significance and it was highlighted with bold.

4. Discussion

A high prevalence of VC of the carotid arteries on ultrasound examinations was identified. The arguments in favor of vascular ultrasound evaluations include easy access to devices, a high rate of use in most medical facilities, a large number of physicians trained to perform echography, its non-invasive nature, and safety with no risk added. Numerous studies validated the ability of arterial ultrasound examinations to properly assess the presence and extent of calcifications and similar results were reported by other authors as well [3,16,17]. A recent study demonstrated that the ultrasound-based method correlates with standard computer tomography (CT)–based methods for femoral artery evaluation. Ultrasound-based calcification scores were increased in patients with diabetes, renal failure, and the presence of chronic limb ischemia similar to CT-based femoral calcification [17]. Due to the reliability of the method, in recent years, a higher interest extending even to intravascular ultrasound in specialized centers is acknowledged. The aim of the latest is to detect vascular wall microcalcification because the mechanical stresses are augmented, and the

clinical risk occurs from the early stages of intimal calcium formation [18,19]. Nevertheless, it is generally accepted that the use of common ultrasound by skilled clinicians improves vascular assessment with reasonable accuracy, and by now, this is widely available.

The present study identified a strong, significant association of carotid calcified atheroma plaques with all-cause and cardiovascular mortality. Such correlations between VC and mortality were reported by numerous studies. Coronary artery calcification detected on CT exams increased mortality in patients undergoing hemodialysis [20]. In peritoneal dialysis patients, it was reported that discrete modifications of arteries, such as carotid intima-media thickness, are associated with cardiovascular mortality [21]. The INDEPENDENT study checked for the power of death prediction in incident HD patients of VC assessed with coronary artery calcification Agatston score and with abdominal aorta calcification Kauppila score. Each type of VC predicted all-cause mortality in this study [3]. Cardiovascular risk can also be stratified by assessing the atheroma through ultrasound of arteries [22].

Chang et al. evaluated the aortic arch calcifications of HD patients on chest X-rays using a three-grade scoring of severity. The patients with coexisting moderate-to-severe aortic arch calcification and high alkaline phosphatase had a higher risk of major cardiovascular events, and cardiovascular and all-cause mortality compared to those with non-to-mild aortic arch calcification/low alkaline phosphatase even after adjustments for significant clinical variables. Authors concluded that moderate-severe aortic arch calcification and high serum alkaline phosphatase co-modify the risk of cardiovascular events and mortality among chronic HD patients at 3 years of follow-up [23].

Regarding cardiovascular diseases in the present study, they were associated with all-cause and cardiovascular mortality in univariate analysis; this effect was lost after multivariate analysis. Further larger studies are needed to clarify this relationship.

Low and high PTH and hyperphosphatemia as well facilitate VC. The FGF 23-Klotho axis has also been implicated in VC. FGF-23, a bone-derived hormone maintaining phosphate balance, has emerged as a key player in CKD-MBD pathophysiology. This study did not find any significant relationship between serum FGF-23 levels and all-cause mortality or cardiovascular mortality. A previous report about FGF-23 effects in HD patients demonstrated an association between elevated FGF-23 levels and death [24]. FGF-23 was also associated with death in non-dialysis chronic kidney disease stages 3–5 patients [25]. In a recent report on the EVOLVE trial, FGF-23 was a risk factor for cardiovascular calcification, events, and mortality in HD patients [26,27]. Conversely, our prior cross-sectional study demonstrated that low FGF-23 is associated with the presence of cardiovascular diseases [16]. No relationship between FGF-23 and atherosclerosis, arterial stiffness, and peripheral vascular complications was reported by other studies [28]. In the PREVEND study, on a prospective population-based cohort, high FGF-23 levels were associated with an increased risk of new-onset chronic kidney disease and all-cause mortality, independent of established chronic kidney disease risk factors [29]. However, Olauson et al. [30] and Mizuiri [20] reported no association between high serum FGF-23 and mortality in HD patients, which is consistent with our findings. PACE study reached the same conclusion that FGF-23 is not associated with death [31]. Bouma de Krijger et al. tested the change in FGF-23 concentration over time and its association with all-cause mortality in patients treated with HD or hemodiafiltration. The results of their study, named CONTRAST, was that there is not any association between a single value of FGF-23 and all-cause mortality, but increasing FGF-23 concentrations did identify patients at risk for mortality. No association was found between baseline FGF-23 concentrations and all-cause mortality among prevalent dialysis patients, and this is also a similarity with my study results. CONTRAST study also confirmed that hemodiafiltration is capable of substantially reducing plasma

FGF-23 concentrations. However, since lowering FGF-23 did not improve outcomes, this study found no argument for therapeutically lowering FGF-23 [32]. The CONTRAST study results may help us find an explanation for the lack of correlation between serum FGF-23 levels and mortality. The possibility that high-flux filters used to dialyze our patients could have contributed to FGF-23 clearance, influencing the serum levels, and consequently, the study's results [32]. Further studies may bring clarity regarding this hypothesis.

Carotid intima-media thickness, FGF-23, and mineral bone disorder were analyzed in a cross-sectional study on 42 children aged 2–18 years old with chronic kidney disease stages 2 to 5D. The study has shown that FGF-23 levels increase with chronic kidney disease progression, but there were no significant correlations between carotid intima-media thickness and factors, including mineral markers and FGF-23 levels [33]. The discrepancies between the results of these studies might be at least partially explained by age, lifestyle, and racial differences and also by the HD prescription, efficiency, and even ultrafiltration [34,35]. In conclusion, in prevalent HD patients, baseline FGF-23 value is not associated with all-cause mortality, suggesting that the association between FGF-23 and long-term outcome may disappear with dialysis duration. Furthermore, low FGF-23 concentrations are not associated with a lower cardiovascular risk [16]. This observation argues against the benefit of interventions that lower FGF-23 in prevalent patients with HD.

Diabetes mellitus proved to be a risk factor for all-cause and cardiovascular mortality in ESRD patients. This is a traditional risk factor acknowledged by different studies [36]. Low serum albumin levels were linked to all-cause and also to cardiovascular mortality, emphasizing the role of malnutrition and inflammation in the outcome of HD patients [37]. There is an ongoing interest in prediction models for mortality. A new construction proved that a C-reactive protein–albumin–lymphocyte could be used as a prediction model for all-cause mortality in patients on maintenance hemodialysis, emphasizing the role of serum albumin and CRP in the enhanced risk of death [38]. In our study, serum CRP levels were identified as a strong predictor for all-cause and cardiovascular mortality. Inflammation was outlined in ongoing research as a risk factor for negative outcomes in HD patients [8,39,40], including death [37,41]. A recent study conducted on a cohort of 3262 participants from the US National Health and Nutrition Examination Survey (NHANES) database proved that the systemic inflammatory response is associated with all-cause mortality and cardiovascular mortality in a population with chronic kidney disease. The systemic inflammatory response independently posed a risk for both all-cause and cardiovascular mortality in chronic kidney disease patients [42]. In our study, cardiovascular mortality was also influenced by HD adequacy, low URR increased the death risk by cardiovascular causes.

As a validation of previous studies, the recent KDIGO guideline for the management of chronic kidney disease and the conclusions of the newest controversial conference on CKD-MBD recognize diabetes, malnutrition inflammation, and VC as risk factors for premature death in chronic kidney disease [43].

The primary strength of this study was the long follow-up period with reliable and detailed information. However, there are several potential limitations. The relatively small sample size is the first and probably the most relevant one. Secondly, variables related to cardiovascular risks, such as cardiac echogram parameters, smoking habits, and physical exercise, were not included in this study. Finally, these results were obtained from a single center, and the patients were all Caucasians, so the results might not be applied to all hemodialysis people. The ultrasound-based method shows promise as a simple method for quantifying the extent of carotid artery calcification in patients with ESRD. The correlation with mortality shows that it could be useful for predicting outcomes for HD patients, extending access to VC screening.

5. Conclusions

The study documented in a cohort of patients prevalent in chronic HD that carotid VC predicts all-cause and cardiovascular mortality at 8 years of follow-up and significantly improves risk stratification. FGF-23 was not associated with outcomes. Other significant risk factors for all-cause and cardiovascular mortality were diabetes, high CRP, and low albumin. HD adequacy influenced cardiovascular death risk. In conclusion, our findings emphasize that the adverse prognosis associated with carotid VC in ESRD is exacerbated by the severity and extent of calcifications, encompassing age, diabetes, or inflammation. Conversely, normal serum albumin and an adequate HD may offer protective effects on carotid VC-related poor outcomes, potentially through mechanisms involving nutrition, elimination of uremic toxins, or anti-inflammatory pathways. Therefore, this study illustrates the concept of multiple pathogenic hits contributing to the high risk of death in HD patients. Consequently, effective therapeutic strategies aimed at improving outcomes in HD patients should focus on mitigating the harmful risk factors, including chronic inflammation, malnutrition, and normalizing mineral metabolism and vascular bone remodeling. However, future efforts are needed to assess whether a risk-based approach, including VC screening to guide the management of chronic HD patients improves survival.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee for Scientific Research of the University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania with number 27020/38/15.11.2011, the approval date: 15 November 2011.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The author would like to express her gratitude to the patients involved in the study.

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Foreman, K.J.; Marquez, N.; Dolgert, A.; Fukutaki, K.; Fullman, N.; McGaughey, M.; Pletcher, M.A.; Smith, A.E.; Tang, K.; Yuan, C.W.; et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: Reference and alternative scenarios for 2016–40 for 195 countries and territories. *Lancet* **2018**, *392*, 2052–2090. [CrossRef]
2. Düsing, P.; Zietzer, A.; Goody, P.R.; Hosen, M.R.; Kurts, C.; Nickenig, G.; Jansen, F. Vascular pathologies in chronic kidney disease: Pathophysiological mechanisms and novel therapeutic approaches. *J. Mol. Med.* **2021**, *99*, 335–348. [CrossRef] [PubMed]
3. Bellasi, A.; Di Lullo, L.; Russo, D.; Ciarcia, R.; Magnocavalo, M.; Lavalle, C.; Di Iorio, B.R. Predictive value of measures of vascular calcification burden and progression for risk of death in incident to dialysis patients. *J. Clin. Med.* **2021**, *10*, 376. [CrossRef]
4. Singh, A.; Tandon, S.; Tandon, C. An update on vascular calcification and potential therapeutics. *Mol. Biol. Rep.* **2021**, *48*, 887–896. [CrossRef] [PubMed]
5. Prié, D. FGF23 and cardiovascular risk. *Ann. Endocrinol.* **2021**, *82*, 141–143. [CrossRef] [PubMed]
6. Wanner, C.; Schuchhardt, J.; Bauer, C.; Brinker, M.; Kleinjung, F.; Vaitsiakhovich, T. Risk prediction modeling for cardiorenal clinical outcomes in patients with non-diabetic CKD using US nationwide real-world data. *BMC Nephrol.* **2025**, *26*, 8. [CrossRef] [PubMed]
7. Zhang, Y.X.; Tang, R.N.; Wang, L.T.; Liu, B.C. Role of crosstalk between endothelial cells and smooth muscle cells in vascular calcification in chronic kidney disease. *Cell Prolif.* **2021**, *54*, e12980. [CrossRef] [PubMed]
8. Viegas, C.; Araujo, N.; Marreiros, C.; Simes, D. The interplay between mineral metabolism, vascular calcification and inflammation in chronic kidney disease (CKD): Challenging old concepts with new facts. *Aging* **2019**, *11*, 4274–4299. [CrossRef] [PubMed]
9. Hortells, L.; Sur, S.; St. Hilaire, C. Cell phenotype transitions in cardiovascular calcification. *Front. Cardiovasc. Med.* **2018**, *5*, 27. [CrossRef]

10. Moldovan, D.; Moldovan, I.; Rusu, C.; Racasan, S.; Patiu, I.M.; Brumboiu, A.; Bondor, C.; Parvu, L.; Kacso, I.; Orasan, R.; et al. Vascular calcifications and renal osteodystrophy in chronic hemodialysis patients: What is the relationship between them? *Int. Urol. Nephrol.* **2011**, *43*, 1179–1186. [CrossRef]
11. Ketteler, M.; Evenepoel, P.; Holden, R.M.; Isakova, T.; Jørgensen, H.S.; Komaba, H.; Wong, J. Chronic kidney disease–mineral and bone disorder: Conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int.* **2025**, *107*, 405–423. [CrossRef]
12. Hannemann, A.; Nauck, M.; Völzke, H.; Weidner, H.; Platzbecker, U.; Hofbauer, L.C.; Baschant, U. Interactions of anemia, FGF-23, and bone in healthy adults—Results from the Study of Health in Pomerania (SHIP). *J. Clin. Endocrinol. Metab.* **2021**, *106*, e288–e299. [CrossRef] [PubMed]
13. Lang, E.; Leibrock, C.; Pandyra, A.A.; Stournaras, C.; Wagner, A.; Föller, M. Phosphate Homeostasis, Inflammation and the Regulation of FGF-23. *Kidney Blood Press. Res.* **2018**, *43*, 1742–1748. [CrossRef]
14. Mendoza, J.M.; Isakova, T.; Cai, X.; Bayes, L.Y.; Faul, C.; Scialla, J.J.; Townsend, R.R. Inflammation and elevated levels of fibroblast growth factor 23 are independent risk factors for death in chronic kidney disease. *Kidney Int.* **2017**, *91*, 711–719. [CrossRef] [PubMed]
15. Titan, S.M.; Zatz, R.; Graciolli, F.G.; Dos Reis, L.M.; Barros, R.T.; Jorgetti, V.; Moysés, R.M. FGF-23 as a predictor of renal outcome in diabetic nephropathy. *Clin. J. Am. Soc. Nephrol.* **2011**, *6*, 241–247. [CrossRef] [PubMed]
16. Moldovan, D.; Moldovan, I.; Rusu, C.; Kacso, I.; Patiu, I.M.; Gherman-Caprioara, M. FGF-23, vascular calcification, and cardiovascular diseases in chronic hemodialysis patients. *Int Urol. Nephrol.* **2014**, *46*, 121–128. [CrossRef] [PubMed]
17. Maahs, E.; Schwartz, A.; Berezowitz, A.; Davis, S.; Guzman, R.J. An ultrasound-based femoral artery calcification score. *J. Vasc. Surg. Cases Innov. Tech.* **2024**, *10*, 101381. [CrossRef] [PubMed]
18. Valero, C.; Macías, J.G. Atherosclerosis, vascular calcification and osteoporosis. *Med. Clínica* **2025**, *164*, e13–e20. [CrossRef] [PubMed]
19. Wang, X.; Li, W.; Song, E.; Wang, L.; Fu, Q.; Cao, S.; Lu, Z. Carotid atherosclerosis detected by ultrasonography: A national Cross-Sectional study. *J. Am. Heart Assoc.* **2018**, *7*, e008701. [CrossRef] [PubMed]
20. Mizuiri, S.; Nishizawa, Y.; Doi, T.; Yamashita, K.; Shigemoto, K.; Usui, K.; Masaki, T. Iron, coronary artery calcification, and mortality in patients undergoing hemodialysis. *Ren. Fail.* **2021**, *43*, 371–380. [CrossRef]
21. Asicioglu, E.; Velioglu, A.; Arikan, H.; Koc, M.; Tuglular, S.; Ozener, C. Baseline carotid intima media thickness is associated with cardiovascular morbidity and mortality in peritoneal dialysis patients. *Ther. Apher. Dial.* **2021**, *25*, 962–969. [CrossRef] [PubMed]
22. Kusic Milicevic, J.; Vidakovic, R.; Markovic, R.; Andjelkovic Apostolovic, M.; Korac, M.; Trbojevic Stankovic, J.; Dragovic, G. Cardiovascular risk assessment and coronary artery calcification burden in asymptomatic patients in the initial years of haemodialysis. *Ther. Apher. Dial.* **2022**, *6*, 64–70. [CrossRef] [PubMed]
23. Chang, C.H.; Liou, H.H.; Wu, C.K. Moderate-severe aortic arch calcification and high serum alkaline phosphatase co-modify the risk of cardiovascular events and mortality among chronic hemodialysis patients. *Ren. Fail.* **2025**, *47*, 2449572. [CrossRef] [PubMed]
24. Gutierrez, O.M.; Mannstadt, M.; Isakova, T.; Rauh-Hain, J.A.; Tamez, H.; Shah, A.; Smith, K.; Lee, H.; Thadhani, R.; Jüppner, H.; et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N. Engl. J. Med.* **2008**, *359*, 584–592. [CrossRef]
25. Alderson, H.V.; Ritchie, J.P.; Middleton, R.; Larsson, A.; Larsson, T.E.; Kalra, P.A. FGF-23 and Osteoprotegerin but not Fetuin-A are associated with death and enhance risk prediction in non-dialysis chronic kidney disease stages 3–5. *Nephrology* **2016**, *21*, 566–573. [CrossRef]
26. Salam, S.; Gallagher, O.; Gossiel, F.; Paggiosi, M.; Eastell, R.; Khwaja, A. Vascular calcification relationship to vascular biomarkers and bone metabolism in advanced chronic kidney disease. *Bone* **2021**, *143*, 115699. [CrossRef] [PubMed]
27. Block, G.A.; Chertow, G.M.; Cooper, K.; Xing, S.; Fouqueray, B.; Halperin, M.; Danese, M.D. Fibroblast growth factor 23 as a risk factor for cardiovascular events and mortality in patients in the EVOLVE trial. *Hemodial. Int.* **2021**, *25*, 78–85. [CrossRef] [PubMed]
28. Coban, M.; Yilmaz, U.; Suleyman, D.; Asilturk, E.; Sozer, Y.; Bekir, E.R.; Ellidag, H.Y. Intact Fibroblast Growth Factor 23 and peripheral vascular complications in patients on hemodialysis. *Dicle Tip Dergisi* **2020**, *47*, 66–73. [CrossRef]
29. De Jong, M.A.; Eisenga, M.F.; van Ballegooijen, A.J.; Beulens, J.W.; Vervloet, M.G.; Navis, G.; De Borst, M.H. Fibroblast growth factor 23 and new-onset chronic kidney disease in the general population: The Prevention of Renal and Vascular Endstage Disease (PREVEND) study. *Nephrol. Dial. Transplant.* **2021**, *36*, 121–128. [CrossRef]
30. Olauson, H.; Qureshi, A.R.; Miyamoto, T.; Barany, P.; Heimburger, O.; Lindholm, B.; Stenvinkel, P.; Larsson, T.E. Relation between serum fibroblast growth factor-23 level and mortality in incident dialysis patients: Are gender and cardiovascular disease confounding the relationship? *Nephrol. Dial. Transplant.* **2010**, *25*, 3033–3038. [CrossRef] [PubMed]
31. Fitzpatrick, J.; Kim, E.D.; Sozio, S.M.; Jaar, B.G.; Estrella, M.M.; Monroy-Trujillo, J.M.; Parekh, R.S. Calcification biomarkers, subclinical vascular disease, and mortality among multiethnic dialysis patients. *Kidney Int. Rep.* **2020**, *5*, 1729–1737. [CrossRef]

32. Bouma-de Krijger, A.; de Roij van Zuijdewijn, C.L.; Nubé, M.J.; Grooteman, M.P.; Vervloet, M.G.; CONTRAST Study Group. Change in FGF23 concentration over time and its association with all-cause mortality in patients treated with haemodialysis or haemodiafiltration. *Clin. Kidney J.* **2021**, *14*, 891–897. [CrossRef]
33. Palupi-Baroto, R.; Hermawan, K.; Mumi, I.K.; Nurlita, T.; Prihastuti, Y.; Puspitawati, I.; Tandri, C.C.; Ambarsari, C.G. Carotid intima-media thickness, fibroblast growth factor 23, and mineral bone disorder in children with chronic kidney disease. *BMC Nephrol.* **2024**, *25*, 369. [CrossRef] [PubMed]
34. Nishizawa, Y.; Hosoda, Y.; Horimoto, A.; Omae, K.; Ito, K.; Higuci, C.; Sakura, H.; Nitta, K.; Ogawa, T. Fibroblast growth factor 23 (FGF23) level is associated with ultrafiltration rate in patients on hemodialysis. *Heart Vessel.* **2020**, *36*, 414–423. [CrossRef] [PubMed]
35. Edmonston, D.; Grabner, A.; Wolf, M. FGF23 and klotho at the intersection of kidney and cardiovascular disease. *Nat. Rev. Cardiol.* **2024**, *21*, 11–24. [CrossRef]
36. Kacso, I.M.; Potra, A.R.; Bondor, C.I.; Moldovan, D.; Rusu, C.; Patiu, I.M.; Kacso, G. Adiponectin predicts cardiovascular events in diabetes dialysis patients. *Clin. Biochem.* **2015**, *48*, 860–865. [CrossRef] [PubMed]
37. Kanda, E.; Lopes, M.B.; Tsuruya, K.; Hirakata, H.; Iseki, K.; Karaboyas, A.; Bieber, b.; Jacobson, S.J.; Dasgupta, I.; Robinson, B.M. The combination of malnutrition-inflammation and functional status limitations is associated with mortality in hemodialysis patients. *Sci. Rep.* **2021**, *11*, 1582. [CrossRef]
38. Huang, J.; Hao, J.; Luo, H.; Chen, L.; Luo, H.; Liu, H.; Wang, P. Construction of a C-reactive protein-albumin-lymphocyte index-based prediction model for all-cause mortality in patients on maintenance hemodialysis. *Ren. Fail.* **2025**, *47*, 2444396. [CrossRef]
39. Sánchez-Duffhues, G.; García de Vinuesa, A.; van de Pol, V.; Geerts, M.E.; de Vries, M.R.; Janson, S.G.; van Dam, H.; Lindeman, J.H.; Goumans, M.J.; Ten Dijke, P. Inflammation induces endothelial-to-mesenchymal transition and promotes vascular calcification through downregulation of BMPR2. *J. Pathol.* **2019**, *247*, 333–346. [CrossRef] [PubMed]
40. Donate-Correa, J.; Martín-Núñez, E.; Hernández-Carballo, C.; González-Luis, A.; Mora-Fernández, C.; Martín-Olivera, A.; Navarro-González, J.F. FGF23 as a potential pathophysiological factor in peripheral arterial disease associated with chronic kidney disease. *Int. J. Mol. Sci.* **2024**, *25*, 5457. [CrossRef] [PubMed]
41. Izzo, C.; Secondulfo, C.; Bilancio, G.; Visco, V.; Virtuoso, N.; Migliarino, S.; Vecchione, C. Chronic kidney disease with mineral bone disorder and vascular calcification: An overview. *Life* **2024**, *14*, 418. [CrossRef] [PubMed]
42. Gu, L.; Xia, Z.; Qing, B.; Wang, W.; Chen, H.; Wang, J.; Yuan, Y. Systemic Inflammatory Response Index (SIRI) is associated with all-cause mortality and cardiovascular mortality in population with chronic kidney disease: Evidence from NHANES (2001–2018). *Front. Immunol.* **2024**, *15*, 1338025. [CrossRef] [PubMed]
43. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2024 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int.* **2024**, *105*, S117–S314. Available online: https://kdigo.org/guidelines/ckd-evaluation-and-management/ (accessed on 22 January 2025). [CrossRef] [PubMed]

Int Urol Nephrol (2017) 49:895–901
DOI 10.1007/s11255-017-1529-7

NEPHROLOGY - ORIGINAL PAPER

Osteoprotegerin and uremic osteoporosis in chronic hemodialysis patients

Diana Moldovan[1,4] · Crina Rusu[1] · Alina Potra[1] · Ioan Moldovan[2] · Ioan Mihai Patiu[3] · Mirela Gherman-Caprioara[1] · Ina Maria Kacso[1]

Received: 1 December 2016 / Accepted: 25 January 2017 / Published online: 4 February 2017

Abstract
Introduction Osteoprotegerin (OPG) is a powerful inhibitor of osteoclast activity, and it plays an important role in bone metabolism. In hemodialysis (HD) patients, the relationship between OPG and bone mineral density (BMD) is important, but remains unclear yet. The study objective was to assess the OPG role related to uremic osteoporosis in HD patients.
Methods This cross-sectional study has been realized on a cohort of 63 chronic HD patients. Inclusion criteria: elderly prevalent HD patients with an age over 55 years old. Exclusion criteria: previous bone disease or previous renal transplant; neoplasia; parathyroidectomy, hormone replacement therapy. The data regarding demographical and clinical characteristics, including treatments for mineral and cardiovascular complications, were recorded. Serum OPG and mineral markers levels were measured. BMD was assessed by calcaneus quantitative ultrasound; it measured broadband ultrasound attenuation, speed of sound (SOS) and stiffness index (STI).
Results The high OPG levels were associated with higher bone mineral density (OPG–SOS $P = 0.003$; $R = 0.37$; OPG–STI $P = 0.03$; $R = 0.28$). Malnutrition, anemia and advanced age correlated with bone demineralization. Males had higher bone density parameters than females. In patients treated with vitamin D ($P = 0.005$), the BMD was increased comparing to patients without these treatments.
Conclusions OPG levels had directly correlated with bone mineral density parameters. Our study further confirms the critical role of OPG in the pathogenesis of uremic osteoporosis in ESRD. Whether the increased circulant OPG protect against bone loss in patients undergoing HD remains to be established.

Keywords Osteoprotegerin · Osteoporosis · Hemodialysis

Introduction

Bone damage in patients with chronic kidney disease (CKD), in the spectrum of chronic kidney disease–mineral and bone disorders (CKD–MBD), represents a daily challenge for nephrologists. The impact of CKD on bone health may be immediate regarding biological equilibrium or delayed as fractures and vascular calcifications. Renal osteodystrophy (ROD) occurs in patients with advanced CKD, including osteitis fibrosa cystica, adynamic bone disease, osteomalacia and mixed uremic osteodystrophy [1]. At the present time, diagnosis of bone disease in CKD is based on clinical signs, laboratory findings and bone radiographs. Histomorphometry remains the gold standard to evaluate bone health, but it is rarely performed in clinical practice.

Patients with CKD may have also osteoporosis, either before or after developing kidney disease. Osteoporosis is a common disease in elderly general population that is characterized by low bone mass with microarchitectural disruption and skeletal fragility, resulting in an increased risk of fracture. In the general adult population, the clinical

✉ Diana Moldovan
dnmoldovan@yahoo.com

1 Department of Nephrology, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

2 Military Hospital Cluj-Napoca, Cluj-Napoca, Romania

3 Nefromed Dialysis Center Cluj-Napoca, Cluj-Napoca, Romania

4 Nephrology and Dialysis Clinic, Emergency County Hospital, „Mihai Manasia", 3-5 Clinicilor Street, Cluj-Napoca, Romania

diagnosis of osteoporosis is made in one of two ways: the presence of a low trauma fracture independent of the prevailing bone mineral density (BMD), or in the absence of a preexisting fracture, a certain level of BMD defined in standard deviation score terms, the T-score. Osteoporosis means T-score below −2.5 SD, and osteopenia is T-score between −1 and −2.5 SD [2]. The criteria of osteoporosis refer only to postmenopausal elderly women and are based on measurement of BMD by dual energy X-ray absorptiometry (DXA) examination [3]. In 2008, a World Health Organization (WHO) task force introduced a Fracture Risk Assessment Tool (FRAX), which estimates the 10-year probability of osteoporotic fractures, which does not include any adjustment of risk according to glomerular filtration rate [4]. In the setting of CKD, the diagnosis of osteoporosis is not stated precisely.

Independent of the bone damage type, BMD measurement is important for mortality risk assessment and risk of fractures prediction [5]. Although its predictive value in dialysis is not yet confirmed, many authors recommend DXA to identify fracture risk in end-stage renal disease (ESRD) patients [6]. The technique has certain limits [7], and it is not used in current clinical practice [1].

The target for secondary osteoporosis diagnosis is to identify cases with low bone strength [8]. Bone strength is characterized by BMD, but also by the quality of the bone. The quality of the bone cannot be assessed only using DXA. In addition to the BMD, quantitative ultrasound osteodensitometry (QUS) provides information on the bone elasticity and structure, being complementary investigations. QUS had been accepted as a good predictor of osteoporotic fracture risk [9]. In addition to predicting fracture risk, other studies have found that QUS is at least as good, and possibly better than clinical risk factors for predicting women at risk for osteoporosis [10]. The power to predict the global and hip risk of fractures on fragile bone, especially in elderly women, is equally strong in QUS and DXA [11]; it can be used in conjunction with clinical risk factors to identify patients at high risk of osteoporotic fractures which require initiation of specific therapy [12]. QUS is an acceptable, cheap, non-radiative and easy-to-use method for assessing bone health. In addition, in dialysis patients who are difficult to mobilize, QUS can be realized in the dialysis center, quality that should not be neglected.

Osteoprotegerin (OPG) is a powerful inhibitor of osteoclast activity, and it plays an important role in bone metabolism. It is widely recognized that biomarkers are of main importance in detecting the complications of chronic kidney disease from early stages [13]. In experimental studies, deficit in OPG led to osteoporosis and the excess of OPG resulted in osteopetrosis [14]. Also, OPG administration can produce osteoporosis regression [14]. Some clinical studies demonstrated that increased OPG serum levels are associated with low BMD [15]. Genetically engineered recombinant OPG and anti-RANKL antibodies are a current indication for osteoporosis in elderly patients [16, 17]. Further studies are absolutely and urgently needed in order to determine the effects of OPG on bones in hemodialysis (HD) patients, because OPG-RANKL system could become an essential therapeutic target. Correlations between OPG and BMD have attracted the interest of many researchers both in the general population and in renal patients. In dialysis patients, study results were contradictory, detecting either positive or negative association or even a lack of association between serum OPG levels and BMD. In HD patients, the relationship between OPG and BMD is important, but remains unclear yet.

The study goal was to assess the osteoprotegerin role related to uremic osteoporosis in HD patients and to identify factors which favor the osseous demineralization in elderly HD patients. This research is aimed to bring new elements in understanding the pathogenic mechanisms that favor CKD–MBD in relation to OPG and to evaluate the OPG influence on chronic HD patients' morbidity. The study objectives are: to evaluate the relationship between OPG and bone mineral density in elderly HD patients; to establish the link between biochemical markers of CKD–MBD and bone demineralization from ROD in elderly HD patients; to establish the link between demographic characteristics, nutrition parameters and current treatments and bone demineralization in elderly HD patients.

Methods

This cross-sectional, analytical study has been realized on a cohort of ESRD patients, randomly selected. All were on conventional HD therapy in Nefromed Dialysis Center Cluj-Napoca. *Inclusion criteria*: elderly prevalent HD patients with an age over 55 years old, who also agreed to participate to this research. *Exclusion criteria*: previous bone disease or previous renal transplant; neoplasia; parathyroidectomy, women on hormone replacement therapy. Among all 131 patients on conventional HD therapy in Nefromed Dialysis Center Cluj-Napoca, 63 met the eligibility criteria.

All patients were receiving conventional 4–5 h HD, three times weekly, with synthetic (polysulphone) dialyzers, bicarbonate dialysate and heparin as standard anticoagulants. Dialysis was prescript in order to achieve adequacy ($spKt/V \geq 1.2$).

The following data were recorded: age, gender, presence of diabetes, HD vintage, dialysate calcium, HD prescription and treatments for mineral metabolism complications.

Body mass index (BMI = weight/height^2) was calculated. Serum levels of calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), intact parathyroid hormone (iPTH—Roche second-generation assay), urea, creatinine, albumin, cholesterol, triglycerides, C-reactive protein (CRP) and OPG (human-OPG ELISA, Biomedica, Wien, Austria) were measured. Biochemical evaluation was performed in a central laboratory. Blood samples were drawn prior to the HD session in the same week of the QUS study. Hemodialysis (HD) adequacy was evaluated using the clearance of urea (spKt/V = 2.4X (1 − urea post-HD/urea pre-HD) − 0.276).

Bone mineral density was assessed by calcaneus (heel) quantitative ultrasound. QUS device was OsteoMed PEGASUS Prestige. QUS measures the transmission of ultrasound through accessible limb bones or the reflectance of the ultrasound waves from the bone surface. Pegasus apparatus was used and the following parameters were determined: broadband ultrasound attenuation (BUA) (dB/MHz), speed of sounds (SOS) (m/s), T-score, Z-score and stiffness index (STI). SOS is a measure of BMD and bone elasticity, BUA measures BMD and bone structure, STI is a composite parameter resulting from SOS and BUA.

Statistics

Mean ± standard deviation expressed continuous variables when normal distribution and the median (inter-quartile range) had expressed them when the distribution was not normal. Qualitative variables were expressed as frequencies. The Kolmogorov–Smirnov test was employed for the continuous variables to compare the observed cumulative distribution function with the normal distribution. The statistical comparison was performed using t test for variables with normal distribution or the Mann–Whitney rank sum test for the others. Chi-square or Fisher exact test were used to test the relationship between qualitative variables. Parametric (Pearson) and nonparametric (Spearman) correlations were determined to test the relationship between QUS testing and other parameters. Independent variables associated with bone demineralization were identified using linear regression, stepwise method. Statistically, significance was considered when P value was < 0.05. All statistical analyses were performed using SPSS 16.0 statistics packages.

Ethical Issues

All patients signed an informed consent prior to the study entry. The study protocol conformed to the ethical guidelines. IRB/Ethics Committee approval has been obtained (IRB approval number 178/2014).

Results

Mean age was 68.74 ± 7.92 years old; mean HD vintage was 47.53 ± 48.30 months. All patients were caucasians. In the studied cohort, there were 29 females (46%), 19 diabetes patients (30.15%) and 9 were smokers (14.28%). Fifty-two patients were treated with calcium salts (82.53%); 22 patients were treated with vitamin D (calcitriol) (34.92%); and 15 patients were treated with sevelamer (23.8%) (Table 1). None received calcimimetics or lanthanum. Twenty-nine patients (46%) had 1.25% dialysate calcium and patients (54%) had 1.5% dialysate calcium. Distribution according to T-score was as follows: 40 patients had the T-score ≤ −2.5 (63.5%); 14 patients had the T-score > −2.5 and ≤ −1(22.2%); and 9 patients had the T-score > −1(14.3%).

The following correlations were obtained applying linear regression: OPG–SOS ($P = 0.003$, R = 0.37); OPG–STI ($P = 0.03$, $R = 0.28$); OPG–BUA ($P = 0.37$); and OPG–T-score ($P = 0.85$) (Figs. 1, 2). OPG correlated also with age ($P = 0.03$, $R = 0.27$), BMI ($P = 0.04$, $R = -0.26$), URR ($P = 0.02$, $R = 0.29$) and iPTH ($P = 0.01$, $R = -0.35$).

In linear regression, stepwise method, all quantitative variables were entered into the equation; only BMI ($P = 0.01$; $b = 0.33$ 95%CI = 0.14–0.52) remained a predictor for BUA. Only Hb ($P < 0.01$, $b = 10.26$; 95%CI = 5.65–14.88) and Ca salts ($P < 0.01$, $b = 21.69$; 95%CI = 10.27–33.12) remained predictors for SOS.

The statistically significant correlations between BMD and other parameters are reproduced in Table 2. Serum albumin, iPTH, ALP and CRP were not correlated with indices of QUS measurement.

Comparing BMD parameters according to gender, BUA was significantly increased in males versus females ($P = 0.048$).

The group of patients was divided into two subgroups, according to the treatment. Those who received treatment with vitamin D derivates had significantly increased BUA and STI versus those without vitamin D ($P = 0.005$, respectively, $P = 0.01$). Higher Ca in dialysate was associated with higher SOS ($P = 0.03$). Treatments with calcium salts or sevelamer did not influence bone mineral density.

Discussion

In our studied HD patients, QUS was able to detect bone demineralization. Osteoporosis and osteopenia are medical terms validated for the general population; they are somewhat not characteristic for secondary bone demineralization, as in ESRD [18]. There is uncertainty related to the applicability of the established WHO classification of BMD

Table 1 Characteristics of the studied cohort

Characteristics	Media ± DS/median (25th–75th percentile)	Minim	Maxim
Age (years)	68.74 ± 7.93	56	89
HD vintage (months)	33 (14–60)	2	272
BMI (kg/m^2)	28.37 ± 5.82	17.93	43.82
OPG (pmol/ml)	5.9 (4.20–8.20)	2	19.6
Ca (mg/dl)	8.37 ± 0.48	7.36	9.96
P (mg/dl)	5.09 ± 1.63	1.87	8.83
CaxP (mg^2/dl^2)	42.90 ± 14.61	16	75
iPTH (pg/ml)	219.50 (104.27–420.87)	28	1297
ALP (U/l)	66 (54–84.5)	41	378
URR	73.21 ± 9.82	53.7	100
Kt/V	1.48 ± 0.23	1	2.1
Bicarbonate (U/l)	17.46 ± 3.45	9.1	25.3
Hb (g/dl)	11.61 ± 1.22	8.8	15.2
Cholesterol (mg/dl)	164.5 (140.5–202.5)	58	362
Trygliceride (mg/dl)	133.5 (95.75–189.25)	40	556
Creatinine (mg/dl)	7.96 (6.76–9.4)	2.52	13.7
Albumin (g/dl)	4.10 (3.96–4.32)	3.38	4.81
CRP (mg/dl)	1.15 (0.49–2.70)	0.04	15.44
BUA (dB/MHz)	51.71 (49.42–55.87)	46.11	65.02
SOS (m/s)	1592.36 ± 24.69	1546	1594.1
T-score	−3.04 (−3.42 to −2.3)	−3.77	−0.36
Z-score	−1.60 ± 0.74	−2.64	0.54
STI	98.23 ± 7.87	82.4	114.8

SD standard deviation, *HD* hemodialysis, *OPG* osteoprotegerin, *BMI* body mass index, *Ca* calcium, *P* phosphorus, *iPTH* intact parathyroide hormone, *ALP* alkaline phosphatase, *URR* urea reduction ratio, *Kt/V* clearance of urea, *CRP* C-reactive protein, *BUA* broadband ultrasound attenuation, *SOS* speed of sound, *STI* stiffness index

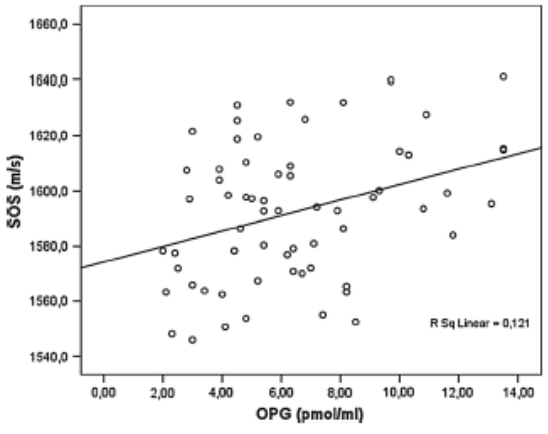

Fig. 1 Correlation between OPG and SOS ($P = 0.003$; $R = 0.348$)

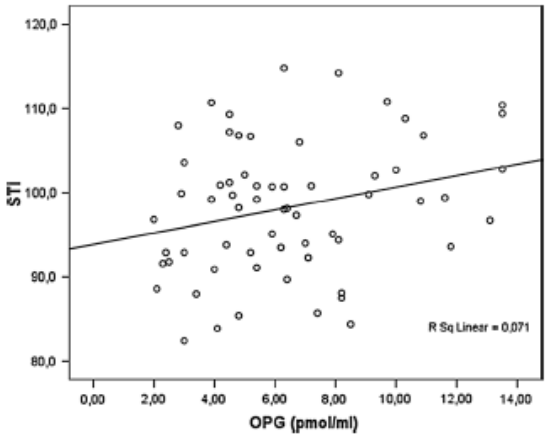

Fig. 2 Correlation between OPG and STI ($P = 0.03$; $R = 0.27$)

according to T-score DXA thresholds. McCloskey's meta-analysis confirmed that quantitative ultrasound is a valuable tool, because it is an independent predictor of fracture for men and women particularly at low QUS values [19].

Similar to other studies [20, 21] our study demonstrated that bone demineralization is prevalent in elderly HD patients. Some studies revealed an acceptable concordance

Table 2 Correlations between BMD and other parameters

	BUA (dB/MHz)	SOS (m/s)	T-score	Z-score	STI
Age (years)	**$P = 0.02$; $R = -0.30$**	**$P = 0.03$; $R = -0.28$**	**$P = 0.04$; $R = -0.26$**	$P = 0.44$; $R = -0.10$	**$P = 0.005$; $R = -0.35$**
HD vintage (months)	$P = 0.14$; $R = -0.19$	**$P = 0.08$; $R = 0.22$**	**$P = 0.02$; $R = -0.28$**	**$P = 0.006$; $R = -0.34$**	$P = 0.36$; $R = 0.12$
BMI (kg/m^2)	**$P = 0.001$; $R = 0.41$**	$P = 0.84$; $R = -0.03$	**$P < 0.001$; $R = 0.52$**	**$P < 0.001$; $R = 0.53$**	$P = 0.64$; $R = 0.06$
Cholesterol (mg/dl)	**$P = 0.05$; $R = 0.27$**	$P = 0.08$; $R = 0.24$	**$P = 0.008$; $R = 0.36$**	**$P = 0.004$; $R = 0.39$**	**$P = 0.03$; $R = 0.29$**
Tryglycerides (mg/dl)	$P = 0.07$; $R = 0.25$	$P = 0.56$; $R = -0.32$	**$P = 0.02$; $R = 0.32$**	**$P = 0.02$; $R = 0.31$**	$P = 0.92$; $R = -0.01$
Creatinine (mg/dl)	**$P = 0.03$; $R = 0.28$**	$P = 0.65$; $R = 0.06$	$P = 0.20$; $R = 0.16$	$P = 0.86$; $R = 0.02$	$P = 0.27$; $R = 0.14$
Hb (g/dl)	$P = 0.09$; $R = 0.22$	$P = 0.001$; $R = 0.42$	$P = 0.23$; $R = 0.15$	$P = 0.76$; $R = 0.04$	**$P = 0 < 0.001$; $R = 0.47$**
URR	**$P = 0.03$; $R = -0.27$**	$P = 0.54$; $R = 0.08$	$P = 0.37$; $R = -0.11$	$P = 0.10$; $R = -0.21$	$P = 0.47$; $R = -0.09$
CRP (mg/dl)	$P = 0.06$; $R = 0.20$	$P = 0.07$; $R = -0.19$	$P = 0.11$; $R = 0.21$	$P = 0.21$; $R = 0.16$	$P = 0.14$; $R = -0.19$
Ca (mg/dl)	$P = 0.57$; $R = 0.07$	**$P = 0.003$; $R = 0.36$**	$P = 0.75$; $R = 0.04$	$P = 0.60$; $R = 0.07$	**$P = 0.005$; $R = 0.35$**
P (mg/dl)	**$P = 0.03$; $R = 0.28$**	$P = 0.10$; $R = 0.21$	**$P = 0.01$; $R = 0.33$**	**$P = 0.05$; $R = 0.25$**	**$p = 0.04$; $R = 0.26$**
Bicarbonate (mmol/l)	$P = 0.46$; $R = 0.10$	$P = 0.63$; $R = 0.06$	$P = 0.19$; $R = 0.17$	$P = 0.14$; $R = 0.19$	$P = 0.40$; $R = 0.11$

Bold values indicate significant correlations

SD standard deviation, *HD* hemodialysis, *OPG* osteoprotegerin, *BMI* body mass index, *Ca* calcium, *P* phosphorus, *URR* urea reduction ratio, *CRP* C-reactive protein, *BUA* broadband ultrasound attenuation, *SOS* speed of sound, *STI* stiffness index

between QUS and DXA in chronic HD patients [22]. However, even in the general population, QUS and DXA compliance is not high enough; the explanation is that they measure different parameters and different skeletal areas [23]. In the general population, QUS plays an important role in assessing bone health [24]. A prospective 10-year follow-up study established that QUS and DXA have the same fraction risk prediction power [25]. The utility of the former was proved in HD patients also, predicting the risk of fractures [26–28].

In our group of HD patients, OPG levels were high comparing with reference values. This result is concordant with Demir's recent study [29]. It has been reported that circulating OPG is increased in experimental animals fed with high-fat diet [30], but in our study OPG was inversely correlated with BMI.

The higher OPG levels were correlated directly with SOS and STI, reflecting an increased bone mineralization. Thus, OPG might act to prevent bone loss in HD patients. In healthy persons, it was shown that OPG serum levels are positively correlated with bone metabolism markers and are negatively correlated with BMD. In chronic HD patients, Nakashima et al. [31] had demonstrated that BMD is positively correlated with OPG and negatively correlated with HD vintage and iPTH levels. Avila et al. [32] had showed there is no association between osteopenia and OPG in women on dialysis. The precise role of OPG in ROD pathogenesis remains unknown, and further studies are needed to elucidate it. OPG/sRANKL system is an independent determinant of bone volume and turnover [20]. A study on postmenopausal osteoporosis HD women observed that serum OPG levels are higher in HD patients with osteoporosis compared to same age women not on HD; it also demonstrated that increased OPG is associated with low BMD in postmenopausal HD patients [33]. It had suggested that it is a consequence of imbalances in kinetics of bones that occurs in CKD. Our study results are in contrast with the data available for the healthy population, but are consistent with the findings of Nakashima [31], which also showed a positive correlation between OPG and BMD. It is well established that elevated serum OPG levels are associated with vascular damage and increased risk of cardiovascular events [34] including in HD patients [35]. Also, vascular calcification and renal osteodystrophy have a pathogenetic link in HD patients [36]. Nascimento et al. [37] reported in a 3-year follow-up study that increased OPG levels were independently associated with increased risk of death in HD patients.

As the post hoc analysis of the FREEDOM trial showed us, at the present time, we have available a new therapeutic tool [38]. Denosumab was effective in reducing fracture risk, improving bone mineral density and was not associated with an increase in adverse events, including changes in estimated glomerular filtration rate, among women with impaired kidney function [38–40]. Currently there are no clinical studies to prove the benefits of antiosteoporotic treatment in reducing the fracture risk in patients selected by QUS measurements. However, the International Society of Clinical Densitometry Official Position is that pharmacological treatment can be initiated in case the fracture probability is sufficiently high even central DXA cannot be done. In this case, fracture probability should be assessed by heel QUS using device-specific thresholds and in conjunction with clinical risk factors [23, 41].

In our study, iPTH was not associated with bone demineralization in HD patients. The relationship between BMD and iPTH is not constant in trials, but it was shown that BMI has a positive influence on BMD [20, 42].

Nutrition (BMI, cholesterol, tryglycerides, creatinine and Hb) was an important determinant of BMD in our study. Some studies have shown that older age, low weight, low albumin and increased ALP are important risk factors for low BMD [33]. Moreover, it has been reported that circulating OPG is increased in experimental animals fed with high-fat diet [30].

In the present study, there was a significant difference between the QUS-measured parameters according to the gender of patients, consistent with the literature indicating that demineralization in women would be more important in patients with ESRD; increased HD vintage was associated with lower Z-score, consistent with other studies [43].

Bone mineralization was better in patients receiving treatment with high calcium dialysate or vitamin D; it was not influenced by the treatment with sevelamer. These results correspond to data available for the general population; the patients deficient in vitamin D with or without associated hypocalcemia develop bone complications. These data can be explained by a potential deficit of Ca and vitamin D, whose correction is beneficial for the bone.

Limitations of the study consist in a relatively reduced number of patients, which restrain us to generalize the results. Its cross-sectional nature does not permit causative associations. QUS examination validity in HD patients is not certain, as it has not been yet compared with the gold standard.

Some challenges remain in the modern management of secondary osteoporosis: development of better diagnostic tools for the quality of bone, the evaluation of fracture risk and the most appropriate selection of patients for therapy [44–46].

Conclusions

Elevated OPG correlated directly with ultrasonographic parameters of good bone mineralization, suggesting that OPG may protect bone against bone loss in HD patients. Advanced age, absence of treatment with vitamin D and malnutrition correlated with bone demineralization. These results justify the statement that OPG is an important piece in CKD–MBD, but its exact role in HD patients remains to be established in future research.

Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest exists.

References

1. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group (2009) KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease–mineral and bone disorder (CKD–MBD). Kidney Int Suppl 113:S1–S130
2. National Institutes of Health (2001) Consensus development panel on osteoporosis prevention D, therapy. Osteoporosis prevention, diagnosis, and therapy. JAMA 285:785–795
3. National Osteoporosis Foundation. Clinician's Guide to Prevention and Treatment of Osteoporosis (2014). http://nof.org/files/nof/public/content/file/2791/upload/919.pdf (Accessed on 16 Oct 2016)
4. Kanis JA on behalf of the World Health Organization Scientific Group (2008) Assessment of osteoporosis at the primary healthcare. In: Technical Report. UK: WHO Collaborative Centre, University of Sheffield
5. West SL, Patel P, Jamal SA (2015) How to predict and treat increased fracture risk in chronic kidney disease. J Intern Med 278(1):19–28
6. Yenchek RH, Ix JH, Shlipak MG, Bauer DC, Rianon NJ, Kritchevsky SB, Fried LF (2012) Bone mineral density and fracture risk in older individuals with CKD. Clin J Am Soc Nephrol 7(7):1130–1136
7. Ureña P, Bernard-Poenaru O, Ostertag A et al (2003) Bone mineral density, biochemical markers and skeletal fractures in haemodialysis patients. Nephrol Dial Transplant 18:2325–2331
8. Iimori S, Mori Y, Akita W, Kuyama T, Takada S, Asai T, Kuwahara M, Sasaki S, Tsukamoto Y (2011) Diagnostic usefulness of bone mineral density and biochemical markers of bone turnover in predicting fracture in CKD stage 5D patients—a single-center cohort study. Nephrol Dial Transplant 27:345–351
9. Khaw KT, Reeve J, Luben R et al (2004) Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus: EPIC-Norfolk prospective population study. Lancet 363:197
10. Hodson J, Marsh J (2003) Quantitative ultrasound and risk factor enquiry as predictors of postmenopausal osteoporosis: comparative study in primary care. BMJ 326:1250
11. Glüer CC, Barkmann R, Blenk T, Stewart A, Kolta S, Finigan J, Graeff C, Gablentz J, Eastell R, Reid DM, Roux C, Felsenberg D (2007) Quantitative ultrasound predicts incident vertebral and hip fractures at least as strongly as DXA: the OPUS study. J Bone Miner Res 22(Suppl 1):1075
12. Hans D, Durosier C, Kans JA, Johansson H, Schott-Pethelaz AM, Krieg MA (2008) Assessment of the 10- year probability of osteoporotic hip fracture combining clinical risk factors and heel bone ultrasound: the EPISEM prospective cohort of 12,958 elderly women. J Bone Miner Res 23(7):1045–1051
13. Bondor C, Kacso Potra A, Moldovan D et al (2015) Relationship of adiponectin to markers of oxidative stress in type 2 diabetic patients: influence of incipient diabetes-associated kidney disease. Int Urol Nephrol 47(7):1173–1180
14. Min H, Morony S, Sarosi I et al (2000) Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. J Exp Med 192:463–474
15. Doumouchtsis KK, Kostakis AI, Doumouchtsis SK et al (2008) Associations between osteoprotegerin and femoral neck BMD in hemodialysis patients. J Bone Miner Metab 26:66–72
16. Lewiecki EM (2009) Denosumab in postmenopausal osteoporosis: what the clinician needs to know. Ther Adv Musculoskelet Dis 1(1):13–26

17. Cummings SR, San Martin J, McClung MR et al (2009) Denosumab for prevention of fractures in postmenopausal women with osteoporosis. N Engl J Med 361:756
18. Ersoy FF (2007) Osteoporosis in the elderly with chronic kidney disease. Int Urol Nephrol 39(1):321–331
19. McCloskey EV, Kanis JA, Odén A, Harvey NC, Bauer D, González-Macias J, Johansson H (2015) Predictive ability of heel quantitative ultrasound for incident fractures: an individual-level meta-analysis. Osteoporos Int 26(7):1979–1987
20. Barreto FC, Barreto DV, Moyses RM et al (2006) Osteoporosis in hemodialysis patients revisited by bone histomorphometry: a new insight into an old problem. Kidney Int 69(10):1852–1857
21. Kuo CW, Ho SY, Chang TH et al (2010) Quantitative ultrasound of the calcaneus in hemodialysis patients. Ultrasound Med Biol 36(4):589–594
22. Arici M, Erturk H, Altun B et al (2000) Bone mineral density in haemodialysis patients: a comparative study of dual-energy X-ray absorptiometry and quantitative ultrasound. Nephrol Dial Transplant 15:1847–1851
23. Krieg MA, Barkmann R, Gonnelli S, Stewart A, Bauer DC, Barquero LR, Kaufman JJ, Lorenc R, Miller PD, Olszynski WP, Poiana C, Scott AM, Lewiecki EM, Hans D (2008) Quantitative ultrasound in the management of osteoporosis: the 2007 ISCD Official Positions. J Clin Densitom 11(1):163–187
24. Guglielmi G, Adams J, Link TM (2009) Quantitative ultrasound in the assessment of skeletal status. Eur Radiol 19(8):1837–1848
25. Moayyeri A, Kaptoge S, Dalzell N et al (2009) Is QUS or DXA better for predicting the 10-year absolute risk of fracture? J Bone Miner Res 24(7):1319–1325
26. West SL, Lok CE, Langsetmo L, Cheung AM, Szabo E, Pearce D, Jamal SA (2015) Bone mineral density predicts fractures in chronic kidney disease. J Bone Miner Res 30(5):913–919
27. Bauer DC, Ewing SK, Cauley JA, Ensrud KE, Cummings SR, Orwoll ES (2007) osteoporotic fractures in men (MrOS) research group. Quantitative ultrasound predicts hip and non-spine fracture in men: the MrOS study. Osteoporos Int 18(6):771–777
28. Bucur RC, Panjwani DD, Turner L, Rader T, West SL, Jamal SA (2015) Low bone mineral density and fractures in stages 3–5 CKD: an updated systematic review and meta-analysis. Osteoporos Int 26:449–458
29. Demir P, Erdenen F, Aral H et al (2016) Serum osteoprotegerin levels related with cardiovascular risk factors in chronic kidney disease. J Clin Lab Anal 30(6):811–817
30. Bernardi S, Fabris B, Thomas M et al (2014) Osteoprotegerin increases in metabolic syndrome and promotes adipose tissue proinflammatory changes. Mol Cell Endocrinol 394(1):13–20
31. Nakashima A, Yorioka N, Doi S et al (2006) Osteoprotegerin and bone mineral density in hemodialysis patients. Osteoporosis Int 17(6):841–846
32. Avila-Diaz M, Prado C, Ventura M et al (2010) Vitamin D receptor gene, biochemical bone markers and bone mineral density in Mexican women on dialysis. Nephrol Dial Transplant 25(7):2259–2265
33. Wu WT, Lee RP, Wang CH et al (2010) The association of serum osteoprotegerin and osteoporosis in postmenopausal hemodialysis patients: a pilot study. J Women's Health 19(4):785–790
34. Yilmaz MI, Siriopol D, Saglam M, et al (2016) Osteoprotegerin in chronic kidney disease: associations with vascular damage and cardiovascular events. Calcif Tissue Int 99(2):121–130
35. Lee JE, Kim HJ, Moon SJ et al (2013) Serum osteoprotegerin is associated with vascular stiffness and the onset of new cardiovascular events in hemodialysis patients. Korean J Intern Med 28(6):668–677
36. Moldovan D, Moldovan I, Rusu C et al (2011) Vascular calcifications and renal osteodystrophy in chronic hemodialysis patients: what is the relationship between them? Internat Urol Nephrol 43(4):1179–1186
37. Nascimento MM, Hayashi SY, Riella MC, Lindholm B (2014) Elevated levels of plasma osteoprotegerin are associated with all-cause mortality risk and atherosclerosis in patients with stages 3 to 5 chronic kidney disease. Braz J Med Biol Res 47(11):995–1002
38. Jamal SA, Ljunggren O, Stehman-Breen C et al (2011) Effects of denosumab on fracture and bone mineral density by level of kidney function. J Bone Miner Res 26(8):1829–1835
39. Block GA, Bone HG, Fang L et al (2012) A single-dose study of denosumab in patients with various degrees of renal impairment. J Bone Miner Res 27(7):1471–1479
40. Schipper LG, Fleuren HW, van den Bergh JP, Meinardi JR, Veldman BA, Kramers C (2015) Treatment of osteoporosis in renal insufficiency. Clin Rheumatol 34(8):1341–1345
41. Hayashi T, Joki N, Tanaka Y, Iwasaki M, Kubo S, Asakawa T, Hase H (2015) The FRAX as a predictor of mortality in Japanese incident hemodialysis patients: an observational, follow-up study. J Bone Mineral Metab 33(6):674–683
42. Castillo RF, de la Rosa RJ (2009) Relation between body mass index and bone mineral density among haemodialysis patients with chronic kidney disease. J Ren Care 35(Suppl 1):57–64
43. Pluskiewicz W, Zwiec J, Gumprecht J et al (2007) Quantitative ultrasound of phalanges of adults with end-stage renal disease or who have undergone renal transplantation. Ultrasound Med Biol 33(9):1353–1361
44. Jamal SA, Nickolas TL (2015) Bone imaging and fracture risk assessment in kidney disease. Curr Osteoporos Rep 3(3):166–172
45. Kazama JJ, Matsuo K, Iwasaki Y, Fukagawa M (2015) Chronic kidney disease and bone metabolism. J Bone Miner Metab 33(3):245–252
46. Pérez-Sáez MJ, Prieto-Alhambra D, Barrios C, Crespo M, Redondo D, Nogués X, Pascual J (2015) Increased hip fracture and mortality in chronic kidney disease individuals: the importance of competing risks. Bone 73:154–159

http://informahealthcare.com/bmk
ISSN: 1354-750X (print), 1366-5804 (electronic)

Biomarkers

Biomarkers, Early Online: 1–7
© 2015 Informa UK Ltd. DOI: 10.3109/1354750X.2014.1000376

informa
healthcare

RESEARCH ARTICLE

Role of osteoprotegerin in vascular disorders of the end-stage renal disease patients

Diana Moldovan[1], Ina Maria Kacso[1], Crina Rusu[1], Alina Potra[1], Cosmina Ioana Bondor[2]#, Ioan Moldovan[3], Ioan Mihai Patiu[4], Dan Vladutiu[1], and Mirela Gherman Caprioara[1]

[1]Department of Nephrology and [2]Department of Statistics, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania, [3]Emergency Military Hospital, Cluj-Napoca, Romania, and [4]Nefromed Dialysis Center, Cluj-Napoca, Romania

Abstract

Aim: To assess the osteoprotegerin (OPG) relationship with cardiovascular complications in hemodialysis (HD) patients.
Methods: The study included 87 HD patients. Clinical characteristics, ankle-arm index (AAI), OPG and mineral markers levels were recorded. Arterial intimal calcification (AIC) and arterial medial calcification (AMC) were registered.
Results: OPG levels were increased in HD patients. Patients with AIC ($p = 0.006$)/ AMC ($p = 0.01$) had higher OPG levels. OPG did not have any relation with cardiovascular diseases. OPG correlated positively with age, increased HD vintage and inversely with albumin and AAI. OPG has not been a risk factor for VC or cardiovascular disease.
Conclusion: OPG rising could be a reaction in defense to vascular aggression, because OPG was associated with VC, but not with vascular disease.

Keywords

Cardiovascular disease, immunotoxicity, renal disease

History

Received 6 November 2014
Accepted 16 December 2014
Published online 14 January 2015

Introduction

Nowadays in nephrology, the most challenging complication in patients with end-stage renal disease (ESRD) treated with dialysis is chronic kidney disease (CKD) – mineral and bone disorders (CKD-MBD). This is a relatively new concept including mineral laboratory abnormalities, renal osteodystrophy and, very important, vascular calcifications (VC; KDIGO, 2009).

Cardiovascular (CDV) calcifications have a high prevalence in patients with CKD and on chronic dialysis treatment (Massy et al., 2013). CKD patients can develop two different types of VC. Arterial intimal calcifications (AIC) appear on atherosclerosis (ATS) plaques and produce occlusion-ischemia phenomenons. Arterial medial calcifications (AMC), also called as arteriosclerosis, can lead to decreased elasticity of large vessels, systolic hypertension, cardiac failure, or sudden death (Schlieper, 2014). It has been demonstrated that all-cause and CDV survival are influenced in a different manner by the two types of VC (Abou-Hassan et al., 2015). Vascular ultrasound is a reliable instrument for detecting VC and can differentiate between calcified atherosclerotic plaques (AIC) and AMC (Lanzer et al., 2014; Moldovan et al., 2014). Pulse pressure (PP) and ankle-arm index (AAI) appreciate stiffness or compliance and provide the vascular functions assessment (Covic et al., 2010).

The risk factors for CDV calcification in CKD patients are considered to be classical factors, uremia-related procalcification factors like uremic serum, calcium (Ca), phosphorus (P), parathyroid hormone (PTH), but, also, a deficit of anti-calcification factors like fetuin-A, osteoprotegerin (OPG), osteopontin, and others. The process of VC is active and it consists in ossification, in which the balance favor of procalcific factors over inhibitors (Scialla et al., 2014).

OPG or osteclast inhibitory factor is a dimeric glycoprotein of the tumor necrosis factor (TNF) receptor family which inhibits receptor activator of NF-kB (RANK) stimulation of osteoclast formation as a soluble decoy receptor. Osteoblasts and stromal cells produce OPG, which binds to and thereby inactivates receptor activator of nuclear factor kappa-B ligand (RANKL). In the absence of OPG, RANKL activates its receptor RANK, found on osteoclasts and preosteoclast precursors (Candido, 2014). OPG's role identified in experimental studies was in bone turn-over regulation and in extraskeletal calcification processes. Animal knock-out models and human single gene defects have confirmed the role of OPG/RANK/RANKL in regulating VC (Lacey et al., 2012). In vascular smooth muscle cells, the OPG expression is increased in uremic serum compared to normal serum (Moe et al., 2005) and OPG inhibits VC (Zhou et al., 2013). Recombinant OPG administration results in stopping the progression of vascular lesions (Lacey et al., 2012; Yavropoulou et al., 2014). Role of OPG in CKD and its

#Cosmina Ioana Bondor is responsible for statistical design/analysis. E-mail: cosmina_ioana@yahoo.com.

Address for correspondence: Dr. Diana Moldovan, Department of Nephrology, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania. E-mail: dnmoldovan@yahoo.com

influence of vessels were assessed also in clinical studies. Serum OPG levels were associated with ATS development and severity (Vik et al., 2010) and with increased aortic stiffness in HD patients (Kim et al., 2013b). OPG was associated with increased CDV mortality (Morena et al., 2006) and with the presence and extent of coronary diseases (Ford et al., 2012) in CKD patients. But, there are studies like Di Bartolo et al.'s (2011) which represent arguments that OPG is not a deleterious factor and that OPG act as a VC inhibitor. In other studies, OPG–VC relationship could not be demonstrated (Jean et al., 2009). However, unfortunately the existing data are as yet sparse. The ongoing dilemmas related to OPG's role in CDV disorders of CKD patients (Liabeuf et al., 2014) represented stimuli for us to bring suplimentary data related to this issue.

The research goal was to assess the OPG role related to VC and CDV complications in HD patients. Objectives were to assess the presence and prevalence of VC, AIC and AMC using ultrasound techniques, to assess the relationship between OPG and ATS (AIC and AAI), the relationship between OPG and medial complications (AMC and PP), the relationship between OPG and the presence of CVD, the relationship between OPG and mineral metabolism markers (Ca, P, CaxP, iPTH, ALP) and other clinical and laboratory markers in HD patients.

Patients and methods

This was a cross-sectional, prospective, analytical study carried on a cohort of ESRD patients, randomly selected. Among all 142 patients on conventional HD therapy in Nefromed Dialysis Center Cluj-Napoca, 87 met the eligibility criteria and, also agreed to participate to this research. All patients were receiving conventional 4–5 h HD, three times weekly, with synthetic (polysulphone) dialyzers, bicarbonate dialysate and heparin as standard anti-coagulants. Dialysis prescription was guided by a goal of achieving a value of spKt/V $\geq$1.2 (KDOQI guidelines, 2006).

Inclusion criteria: prevalent HD patients, age > 18 years.

Exclusion criteria: neoplasia, severe infections; parathyroidectomy, previous renal transplant.

The data regarding demographical and clinical characteristics (age, gender, HD vintage, presence of diabetes, medical history, dialysate calcium, HD prescription), including treatments for mineral and CDV complications, were recorded. The presence of CDV disease was recorded.

Blood pressure (BP) was measured with an upper arm mercury sphygmomanometer according to the recommendations of the American Heart Association. We measured arm systolic (sBP) and diastolic (dBP) BP and ankle sBP in clinostatism after 10 min of repaus, before HD session. We calculated body mass index (BMI = weight/height^2), mean blood pressure (MBP = dBP + (sBP–dBP)/3), PP = sBP–dBP), AAI = ankle sBP/ arm sBP.

Serum levels of calcium (Ca), inorganic phosphorus (P), alkaline phosphatase (ALP), intact parathyroid hormone (iPTH – Roche second-generation assay), urea, albumin and C-reactive protein (CRP), cholesterol, triglycerides andOPG (human-OPG ELISA, Biomedica, Wien, Austria) were measured. The reported normal OPG levels are 3.1 $\pm$ 2.1 pmol/l (62 pg/ml).

Blood samples for the biochemical evaluation were drawn prior to the HD session in the same week of the ultrasound study. Laboratory tests were performed in a central laboratory. The clearance of urea (spKt/V = 2.4 $\times$ (1–urea post-HD/urea pre-HD)–0.276) assessed hemodialysis (HD) adequacy.

Vascular ultrasound was performed to detect VC in carotid and femoral arteries. A Doppler ultrasound machine with a 5–10 MHz linear transducer was used. The ultrasound examiner was blinded to other patient's data. We examined common carotid artery (1 cm proximal to the carotid bifurcation), bifurcation (1–2 cm) and internal carotid artery (1 cm distal to the bifurcation); common and superficial femoral arteries were examined on a distance of 1 cm proximal, respective distal to bifurcation. Real-time B mode and Doppler functions, transversal and longitudinal sections, were used to assess the arteries walls. In order to avoid biases, operator was trained and unaware of patient's clinical and biochemical data. The intra-observer reproducibility of this assessment was assured using two consecutive ultrasound exams; in case of discordance, a third examination was performed in order to settle the right result. The AIC was considered to be calcified atheroma plaques (focal areas of intima thickening, with protrusion in the vascular lumen and hyperechoicaspect with posterior shadows in the arterial walls). The AMC were defined as multiple punctiform hyperechoic images inclavated in the vascular wall, which does not protrude in the lumen and does not affect the blood flow, as they are described in the literature (Lanzer et al., 2014).

The cohort was divided in subgroups: with and without AIC; with and without AMC; with and without CVD. The CVD diseases were defined by ischemic heart disease (acute myocardial infarction, angor pectoris), heart failure, stroke, arrhythmia, aortic aneurysm and peripheral artery disease.

Statistics

Continuous variables were expressed as mean $\pm$ standard deviation if variables had normal distribution and as median (inter-quartile range) if the distribution was not normal. Qualitative variables were expressed as frequencies. The statistical comparison was performed using *t*-test for variables with normal distribution or the Mann–Whitney Rank Sum Test for the others. Chi-square or Fisher exact test were used to test the relationship between qualitative variables. Parametric (Pearson) and non-parametric (Spearman) correlations were determined to test the relationship between OPG, AAI, PP and the other parameters of CDV risk. As age was significantly associated with OPG, partial correlation controlled for age were computed. Independent variables associated with AIC, AMC were identified using binary logistic regression (ENTER method) with age, statine treatment and Ca treatment as covariate. Thereafter, a receiver-operating characteristic (ROC) curve was designed to identify a cut-off value of OPG that best predicted the presence of AIC and AMC, according to the maximum of the Youden Index. Statistically significance was considered when p value was <0.05. All statistical analyses were performed using SPSS 13.0 statistics packages.

Ethical issues

All patients signed an informed consent prior to the study entry. Their privacy was respected. The study protocol conformed to the ethical guidelines and it was approved by the University Ethics Committee.

Results

Demographical and clinical characteristics of the studied cohort are as follows. Mean age for our 87 ESRD patients was 62.74 ± 12.95 years, between 20 and 89 years. The HD vintage ranged between 2 months and 272 months with a mean of 47.96 ± 49.36 months. Regarding vascular access, 62 had an arterio–venous fistula and 25 patients had a central venous catheter (Table 1). A minimum spKt/V of 0.9, maximum of 2.1 and medium of 1.46 ± 0.22 was obtained. A number of 40 patients were females (46.13%), 11 patients were smokers (12.6%), one patient had B-virus infection (1.1%) and five patients had C-virus infection (5.7%). The etiology of ESRD was chronic glomerulonephrites in 14 patients (16.09%), diabetic nephropathy in 21 patients (24.13%), vascular nephropathy in 10 patients (11.49%), tubulo-interstitial diseases in 11 patients (12.64%), polycystic kidney disease in 4 patients (4.59%), others and unknown in 27 patients (31.03%).

Mean albumin was 4.17 ± 0.32 g/dl; mean CRP was 1.67 ± 2.35 mg/dl; mean cholesterol was 171.35 ± 54.35 mg/dl; mean triglyceride was 166.00 ± 117.86 mg/dl; mean HDL-cholesterol was 47.89 ± 12.89 mg/dl; mean VLDL was 33.22 ± 23.56 mg/dl. According to K/DOQI targets for mineral markers: 44.8% had normal Ca levels and most of them (54%) had low Ca levels; 40.2% normal P, 19.5% low P and 17.2% had high P; 39.1% normal; 26.4% low iPTH; 34.5% high PTH. 54% had increased OPG levels. Medium serum OPG levels were increased in our chronic HD patients, 5.97 ± 3.24, ranging from 1.4 to 19.6 pmol/ml (Table 3).

Regarding the VC's prevalence and distribution in HD patients, 82% had VC and the anatomic localization was: 55 patients with carotid AIC; 38 patients with femoral AIC; 21 patients with carotid AMC; 52 patients with femoral AMC. 14% had only AIC, 14% had only AMC and 54% had both types. Thirty-two (36.8%) patients had CVD.

Comparing the subgroups of patients with and without AIC regarding all features recorded and measured, OPG levels ($p = 0.006$), age ($p < 0.0001$) and HD vintage ($p = 0.03$) were significantly higher in AIC subgroup (Table 2). In the AIC subgroup, 76.27% of patients received calcium-based treatments compared with 96.42% in subgroup without AIC ($p = 0.03$). A total of 44% of patients with AIC received CDV treatments compared with 17.85% in the non-AIC subgroup ($p = 0.03$; Table 2). AAI was decreased in 79.66% of AIC patients and 53.57% in non-AIC patients ($p = 0.02$).

Patients with AMC had higher OPG levels ($p = 0.01$), age ($p < 0.001$) and lower creatinine levels ($p = 0.02$) compared with patients without AMC. In the subgroup with AMC, 33.89% had diabetes, only 3.57% from subgroup without AMC had diabetes ($p = 0.004$). In AMC subgroup, 79.7% had decreased AAI and 53% in the subgroup without AMC ($p = 0.02$; Table 3).

Increased OPG was correlated with advanced age ($p = 0.003$; $r = 0.32$) and HD vintage ($p < 0.001$; $r = 0.35$). Inverse significant correlation between OPG levels and AAI ($p = 0.009$; $r = -0.28$) and between OPG levels and serum albumin ($p < 0.001$). After controlling for age, OPG was inverse correlated with BMI($r = -0.25$, $p = 0.03$) and directly correlated with HD vintage ($r = 0.24$, $p = 0.04$). The relation between OPG and PP ($p = 0.75$) or between OPG and CVD ($p = 0.37$) was not significant.

ROC curves detected that cut-off value for OPG was 6.05 pmol/ml to predict AIC and 7.05 pmol/ml to predict AMC. Plasma OPG level had a good prediction of AIC, with an area under ROC curve of 0.68. The cut-off value best predicting AIC was 6.05 pg/ml (sensitivity = 49.15%; specificity = 82.15%). In the group without AIC, 5 patients (17.8%) have OPG over 6.05 and in the group with AIC, 29 patients (49.5%) had OPG over the cut-off value ($p = 0.005$).

Plasma OPG level had a good prediction of AMC, with an area under ROC curve of 0.66. The cut-off value best predicting AMC was 7.05 pg/ml (sensitivity = 37.28%; specificity = 92.85%). Six patients (21.42%) had OPG over the cut-off value in group without AMC, and 28 patients (47.45%) in the group with AMC ($p = 0.02$).

After controlling for age, statins and Ca treatment in a multivariate binary logistic regression the identified risk factor for AIC was albumin (OR = 13.08, 95% CI 1.99–85.71, $p = 0.01$), but not OPG (OR = 1.19, 95% CI 0.95–1.51, $p = 0.13$). Risk factor for AMC with HD vintage, statins and Ca treatment as covariates was DM (OR = 11.29, 95% CI 1.32–96.55, $p = 0.03$), without OPG (OR = 1.14, 95% CI 0.93–1.40, $p = 0.21$).

Table 1. Demographical and clinical cantitative characteristics ($n = 87$).

Feature	Minim	Maxim	Media	Standard deviation
Age (yrs)	20.00	89.00	62.74	12.95
HD vintage (ms)	2.00	272.00	47.96	49.36
W (kg)	44.00	140.00	74.40	17.64
H (m)	1.45	1.83	1.6	0.08
BMI (kg/m^2)	17.93	43.82	27.85	5.82
sBP (mmHg)	90.00	190.00	133.90	19.31
dBP (mmHg)	40.00	100.00	76.89	10.29
PP (mmHg)	30.00	120.00	57.01	14.21
Medium BP (mmHg)	70.00	127.00	95.91	12.31
AAI	0.00	1.25	0.59	0.38

HD, hemodialysis; W, weight; H, height; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; PP, pulse pressure; AAI, ankle arm index.

Discussion

The prevalence of CVD and VC in our studied ESRD patients was increased. Our finding is a new confirmation that CKD is a risk factor for CVD (Jha et al., 2013). In renal population, ATS is more common than in the general population and the level of plaque calcifications is more frequent and more extensive (McCullough et al., 2008). The present research has revealed an increased prevalence of calcification in the atherosclerotic plaque and, also, in the media of the arteries. Most patients had a combination of both types of VC KDIGO guidelines in CKD-MBD recommend that affordable and widely available techniques should be evaluated and used in

Table 2. Comparison of clinical and biochemical profiles of hemodialysis patients with and without arterial intimal calcification (AIC).

Feature	Without AIC ($n = 28$)	With AIC ($n = 59$)	p
OPG (pmol/ml)	**4.4 (2.82–5.87)**	**5.9 (4.5–8.2)**	**0.006**
Age (yrs)	**54.85 ± 15.33**	**66.49 ± 9.74**	**<0.001**
HD vintage (months)	**19 (11–45.75)**	**36 (18–68)**	**0.03**
Blood flow (ml/min)	350 (300–380)	350 (300–380)	0.80
BMI (kg/m^2)	26.29 (23.26–31.31)	27.11 (24.14–31.24)	0.70
sBP (mmHg)	140 (120–140)	130 (120–150)	0.67
dBP (mmHg)	80 (70–80)	80 (70–80)	0.62
PP (mmHg)	55 (50–60)	60 (50–60)	0.61
mBP (mmHg)	94.32 ± 12.92	96.67 ± 12.05	0.40
Hb (g/dl)	11.3 ± 1.43	11.6 ± 1.17	0.20
Ca (mg/dl)	8.34 ± 0.45	8.34 ± 0.51	0.94
P (mg/dl)	5.42 ± 1.71	4.99 ± 1.65	0.26
Creatinine (mg/dl)	7.89 (6.60–10.01)	8.17 (7.26–9.84)	0.23
spKt/V	1.42 (1.34–1.56)	1.47 (1.29–1.57)	0.73
Albumin (g/dl)	**4.07 (3.93–4.32)**	**4.20 (4.04–4.44)**	**0.04**
CRP (mg/dl)	0.87 (0.36–3.1)	0.92 (0.38–1.64)	0.38
iPTH (mg/dl)	222.3 (177.9–409.3)	220.9 (126.85–447)	0.88
ALP (U/l)	62.5 (48.7–83.2)	68.5 (53.2–92.5)	0.35
Cholesterol (mg/dl)	170.5 (136.25–205)	159 (143–202)	0.78
DM, no (%)	5 (17.85)	16 (27.11)	0.49
AVF, no (%)	18 (64.28)	45 (76.27)	0.82
Smokers, no (%)	3 (10.71)	8 (13.55)	1
Calcium, no (%)	25 (89.3)	47 (79.7)	0.36
Vitamin D, no (%)	8 (28.57)	23 (38.98)	0.47
Sevelamer, no (%)	4 (14.28)	17 (28.81)	0.22
ACEI/sartans, no (%)	7 (25)	22 (37.28)	0.37
Betablokers, no (%)	15 (53.57)	44 (74.57)	0.08
Statins, no (%)	5 (17.9)	7 (11.9)	0.51

Bold values represent a statistically significant difference between the two groups.
OPG, osteoprotegerin; HD, hemodyalisis; W, weight; H, height; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; PP, pulse pressure; AAI, ankle arm index; Hb, hemoglobin; Ca, calcium; P, phosphorus; CRP, C-reactive protein; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; DM, diabetes mellitus; AVF, arterio–venous fistula; ACEI, angiotensin converting enzyme inhibitors.

detecting the presence and monitoring the progression of arterial calcification in routine clinical practice (KDIGO, 2009). In this research, ultrasound proved to be efficient in evaluating AIC and AMC as different entities, as in Lanzer's review (2014). AIC and AMC are known to have different consequences on CDV system (Yamada et al., 2014). In the present study, CVD diseases were not favored by a certain type of VC.

Clinical studies have shown that in patients with CKD and on chronic dialysis, serum concentrations of OPG are elevated (Montañez-Barragán et al., 2014). This fact was demonstrated, also, in our cohort of chronic HD patients. Serum OPG was positively correlated with age and duration of HD, suggesting that age-related factors may intervene in the regulation of serum levels of OPG in HD patients. Lewis et al. described this correlation between OPG, renal function and aging (Lewis et al., 2014), but age is not necessarily a confounder in all studies (Ciccone et al., 2013).

Associations between high OPG values with the occurrence and severity of CV were reported by some authors (Wei et al., 2009), but these results could not be confirmed by other studies (Jean et al., 2009). Our result suggest that OPG could be involved in the development of ATS; its serum levels were significantly higher in patients with AIC compared with those without AIC. OPG was negatively correlated with AAI, suggesting that OPG may be a marker for increased risk of atherosclerotic peripheral arterial disease as a complication. Dialysis research offers an interpretation of the fact that OPG could promote the progression and instability of atherosclerotic plaque (Ozkok et al., 2012; Pateinakis et al., 2013). Studies carried out on non-renal diabetic patients have shown that there is an association between OPG and the formation and growth of plaque (Augoulea et al., 2013). Experimental studies instead, noted that OPG deficiency increase CV risk (Callegari et al., 2014). OPG is an independent predictor for coronary VC progression in HD patients (Ozkok et al., 2012). In peritoneal patients, an association between OPG and aortic VC has been recently described (Huang et al., 2014).

The subgroup with AIC had increased age and HD vintage, which fit with the pattern of age – ATS link. The use of less Ca-based phosphate binders can have the significance of lower control of mineral metabolism abnormalities (Ortiz et al., 2013).

In our study, it had been revealed that patients with AMC had significantly higher OPG and that were older. PP was not different between the two subgroups with and without AIC, AMC or both. In studies on HD children, OPG was associated with arterial stiffness and VC (Shroff et al., 2008). It was also demonstrated that arterial stiffness assessed by PWV is related to OPG (Kim et al., 2013a), but in our study, assessing arterial stiffness by PP we could not reproduce this relationship, because PP was not significantly correlated with OPG.AMC included more patients with diabetes, being a well-known association (Aoki et al., 2013).

Table 3. Comparison of clinical and biochemical profiles of hemodialysis patients with and without arterial media calcification (AMC).

Feature	Without AMC ($n = 28$)	With AMC ($n = 59$)	p
OPG (pmol/ml)	**4.72 ± 2.19**	**6.56 ± 3.49**	**0.01**
Age (years)	**55.14 ± 15.81**	**66.35 ± 9.54**	**0.001**
HD vintage (months)	34 (14–66)	30 (12–60)	0.41
Blood flow (ml/min)	350 (300–380)	350 (300–380)	0.63
BMI (kg/m^2)	25.52 (23.26–28.13)	28 (24.24–31.65)	0.06
sBP (mmHg)	130 (120–140)	130 (120–145)	0.52
dBP (mmHg)	75 (70–80)	80 (70–80)	0.27
PP (mmHg)	57.5 (50–60)	55 (50–65)	0.90
mBP (mmHg)	94.11 ± 12.25	96.75 ± 12.26	0.34
Hb (g/dl)	11.49 ± 1.48	11.58 ± 1.16	0.76
Ca (mg/dl)	8.42 ± 0.5	8.31 ± 0.49	0.30
P (mg/dl)	5.19 ± 1.94	5.10 ± 1.56	0.82
Creatinine (mg/dl)	7.7 (6.6–9.7)	8.4 (7.2–9.9)	0.03
spKt/V	1.46 (1.34–1.63)	1.45 (1.29–1.55)	0.36
Albumin (g/dl)	4.16 (3.96–4.4)	4.18 (4.04–4.39)	0.63
CRP (mg/dl)	0.89 (0.34–2.92)	0.91 (0.38–1.64)	0.78
iPTH (mg/dl)	218.6 (115.6–538.6)	223.7 (128.27–424.2)	0.76
ALP (U/l)	68 (53–84)	65 (50.5–91.5)	0.99
Cholesterol (mg/dl)	155 (134–182)	167.5 (147.7–205.2)	0.16
Gender M/F, no (%)	12/16 (42.85/57.15)	35/24 (59.32/40.67)	0.36
DM, no (%)	**1 (3.57)**	**20 (33.89)**	**0.004**
AVF, no (%)	23 (82.14)	40 (67.79)	0.25
Smokers, no (%)	6 (21.42)	5 (84.74)	0.16
Calcium treatments, no (%)	26 (92.85)	46 (77.96)	0.12
Vitamin D, no (%)	9 (32.14)	22 (37.28)	0.81
Sevelamer, no (%)	8 (28.57)	13 (22.03)	0.69
ACEI/sartans, no (%)	7 (25)	22 (37.28)	0.37
Betablokers, no (%)	19 (67.85)	40 (67.79)	0.81
Statins, no (%)	3 (10.7)	9 (15.3)	0.74

Bold values represent a statistically significant difference between the two groups.
HD, hemodyalisis; W, weight; H, height; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; PP, pulse pressure; AAI, ankle arm index; Hb, hemoglobin; Ca, calcium; P, phosphorus; CRP, C-reactive protein; iPTH, intact parathyroid hormone; ALP, alkaline phosphatase; DM, diabetes mellitus; AVF, arterio–venous fistula; ACEI, angiotensin converting enzyme inhibitors.

In addition, studies on non-renal patients have represented subjects for debate about OPG' role. Some authors concluded that there is no doubt about the fact that OPG is a VC inhibitor and marker (Callegari et al., 2014), while others are vehement in saying that OPG is a uremic toxin that increases skeletal resistance iPTH (Vik et al., 2010). In our study, OPG did not correlate with the laboratory markers of mineral metabolism (Ca, P, iPTH, ALP), but it registered an inverse correlation with serum albumin, suggesting that protein malnutrition influence serum OPG levels. Koo et al. (2011) demonstrated the association between malnutrition and increased OPG levels in their study.

A lot of studies have drawn attention on the association between increased serum OPG and CDV mortality and events (Morena et al., 2009; Venuraju et al., 2010). The research of Yokoyama et al. (2008) is singular in reporting an association between low levels of OPG an poor prognosis in Japanese diabetic HD patients. Our study revealed no association between OPG and the presence of CDV diseases in our cohort.

This research outlines the two assumptions: one is that OPG may have dual role, protective or harmful in the development and progression of AIC and AMC and the other that OPG may be increased as a defense mechanism to counteract excessive CV. Although in univariable analysis, higher OPG levels associated with AIC or AMC in multivariable analysis, OPG has not been a risk factor for VC or CDV disease. In light of these results, we consider that OPG has a value as marker of reaction in defense to vascular aggression and not as a pathogenic factor. We interpret the OPG rising to be a reaction in defense to vascular aggression and not a pathogenic factor, because higher OPG levels were associated with VC, but not with vascular disease.

There are some limitations of the present study that should be considered. It results from a relatively reduced number of patients, which restrain us to generalize the results; it is cross-sectional, which precludes validation of causative associations. Ultrasound exam cannot detect details about the components of plaques, as more accurate techniques, such as magnetic resonance imaging (Fayad et al., 2011; van den Bouwhuijsen et al., 2012). Widely available ultrasound machines are not able to measure the inter-adventitia common carotid diameter. (Baldassarreet al., 2012). Comparing their results could be the subject of a future research.

In conclusion, the prevalence of VC and the high serum OPG levels in studied HD patients was increased. Higher OPG levels were associated with VC, but not with vascular disease. This is the reason why we consider the OPG rising to be a reaction in defense to vascular aggression, rather than the aggressor itself. The precise role of OPG in the vascular wall has yet to be determined. Thus, further prospective studies are needed to establish whether increased OPG levels in CKD patients can in fact predict later development of vascular complications.

Acknowledgements

We are indebted to all patients who participated in this study.

Declaration of interest

The authors report no conflicts of interest. This paper was published under the frame of European Social Found, Human Resources Development Operational Program 2007–2013, project no POSDRU/159/1.5/138776.

References

Abou-Hassan N, Tantisattamo E, D'Orsi ET, O'Neill WC. (2015). The clinical significance of medial arterial calcification in end-stage renal disease in women. Kidney Int 87:195–9.

Aoki A, Murata M, Asano T, et al. (2013). Association of serum osteoprotegerin with vascular calcification in patients with type 2 diabetes. Cardiovasc Diabetol 12:11. doi:10.1186/1475-2840-12-11.

Augoulea A, Vrachnis N, Lambrinoudaki I, et al. (2013). Osteoprotegerin as a marker of atherosclerosis in diabetic patients. Int J Endocrinol 2013: Article ID 182060, 6 pages. doi:10.1155/2013/182060.

Baldassarre D, Hamsten A, Veglia F, et al. On Behalf Of The Improve Study Group. (2012). Measurements of carotid intima-media thickness and of interadventitia common carotid diameter improve prediction of cardiovascular events: results of the IMPROVE (Carotid Intima Media Thickness [IMT] and IMT-Progression as Predictors of Vascular Events in a High Risk European Population) study. J Am Coll Cardiol 60:1489–99.

Callegari A, Coons ML, Ricks JL, et al. (2014). Increased calcification in osteoprotegerin-deficient smooth muscle cells: dependence on receptor activator of NF-κB ligand and interleukin 6. J Vasc Res 51:118–31.

Candido R. (2014). The osteoprotegerin/tumor necrosis factor related apoptosis-inducing ligand axis in the kidney. Curr Opin Nephrol Hypertens 23:69–74.

Ciccone MM, Scicchitano P, Gesualdo M, et al. (2013). Serum osteoprotegerin and carotid intima-media thickness in acute/chronic coronary artery diseases. J Cardiovasc Med (Hagerstown) 14:43–8.

Covic A, Kanbay M, Voroneanu L, et al. (2010). Vascular calcification in chronic kidney disease. Clin Sci (Lond) 28;119:111–21.

Di Bartolo BA, Schoppet M, Mattar MZ. (2011). Calcium and osteoprotegerin regulate IGF1R expression to inhibit vascular calcification. Cardiovasc Res 91:537–45.

Fayad ZA, Mani V, Fuster V. (2011). The time has come for clinical cardiovascular trials with plaque characterization as an endpoint. Eur Heart J 33:160–1.

Ford ML, Smith ER, Tomlinson LA, et al. (2012). FGF-23 and osteoprotegerin are independently associated with myocardial damage in chronic kidney disease stages 3 and 4. Another link between chronic kidney disease–mineral bone disorder and the heart. Nephrol Dial Transplant 27:727–33.

Huang J-W, Lien YC, Yang CY, et al. (2014). Osteoprotegerin, inflammation and dyslipidemia are associated with abdominal aortic calcification in non-diabetic patients on peritoneal dialysis. Nutrition Metab Cardiovasc Dis 24:236–42.

Jean G, Bresson E, Terrat JC, et al. (2009). Peripheral vascular calcification in long-haemodialysis patients: associated factors and survival consequences. Nephrol Dial Transplant 24:948–55.

Jha V, Garcia-Garcia G, Iseki K, et al. (2013). Chronic kidney disease: global dimension and perspectives. Lancet 382:260–72.

KDOQI Clinical Practice Guidelines for hemodialysis adequacy 2006 Updates. Available from: http://www.kidney.org/sites/default/files/docs/12-50-0210_jag_dcp_guidelines-hd_oct06_sectiona_ofc.pdf [last accessed 6 Dec 2014].

Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group. (2009). KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease–mineral and bone disorder (CKD–MBD). Kidney Int Suppl 113:S1–130.

Kim HR, Kim HK, Oh DJ. (2013a). Serum osteoprotegerin level is associated with degree of arteriovenous fistula stenosis in patients with hemodialysis. Clin Nephrol 80:322–7.

Kim HR, Kim SH, Han MJ, et al. (2013b). The ratio of osteoprotegerin to fetuin-a is independently associated with vascular stiffness in hemodialysis patients. Nephron Clin Pract 123:165–72.

Koo HM, Do HM, Kim EJ, et al. (2011). Elevated osteoprotegerin is associated with inflammation, malnutrition and new onset cardiovascular events in peritoneal dialysis patients. Atherosclerosis 219:925–30.

Lacey DL, Boyle WJ, Simonet WS, et al. (2012). Bench to bedside: elucidation of the OPG–RANK–RANKL pathway and the development of denosumab. Nat Rev Drug Discov 11:401–19.

Lanzer P, Boehm M, Sorribas V, et al. Medial vascular calcification revisited: review and perspectives. European Heart J 35:1515–25.

Lewis JR, Lim WH, Zhu K, et al. (2014). Elevated osteoprotegerin predicts declining renal function in elderly women: a 10-year prospective cohort study. Am J Nephrol 39:66–74.

Liabeuf S, Okazaki H, Desjardins L, et al. (2014). Vascular calcification in chronic kidney disease: are biomarkers useful for probing the pathobiology and the health risks of this process in the clinical scenario? Nephrol Dial Transplant 29:1275–84.

Massy ZA, Drüeke TB. (2013). Vascular calcification. Curr Opin Nephrol Hypertens 22:405–12.

McCullough PA, Agrawal V, Danielewicz E, Abela GS. (2008). Accelerated atherosclerotic calcification and monckeberg's sclerosis: a continuum of advanced vascular pathology in chronic kidney disease. Clin J Am Soc Nephrol 3:1585–98.

Moe SM, Reslerova M, Ketteler M, et al. (2005). Role of calcification inhibitors in the pathogenesis of vascular calcification in chronic kidney disease (CKD). Kidney Int 67:2295–304.

Moldovan D, Moldovan I, Rusu C, et al. (2014). FGF-23, vascular calcification and cardiovascular diseases in chronic hemodialysis patients. Int Urol Nephrol 46:121–28.

Montañez-Barragán A, Gómez-Barrera I, Sanchez-Niño M, et al. (2014). Osteoprotegerin and kidney disease. J Nephrol 27:607–17.

Morena M, Dupuy AM, Jaussent I, et al. (2009). A cut-off value of plasma osteoprotegerin level may predict the presence of coronary artery calcifications in chronic kidney disease patients. Nephrol Dial Transplant 24:3389–97.

Morena M, Terrier N, Jaussent I, et al. (2006). Plasma osteoprotegerin is associated with mortality in hemodialysis patients. J Am Soc Nephrol 17:262–70.

Ortiz A, Sanchez-Niño MD. (2013). The demise of calcium-based phosphate binders. Lancet 382:1232–34.

Ozkok A, Caliskan Y, Sakaci T, et al. (2012). Osteoprotegerin/RANKL axis and progression of coronary artery calcification in hemodialysis patients. Clin J Am Soc Nephrol 7:965–73.

Pateinakis P, Papagianni A, Douma S, et al. (2013). Associations of fetuin-A and osteoprotegerin with arterial stiffness and early atherosclerosis in chronic hemodialysis patients. BMC Nephrol 14:122.

Schlieper G. (2014). Vascular calcification in chronic kidney disease: not all arteries are created equal. Kidney Int 85:501–3.

Scialla JJ, Kao WH, Crainiceanu C, et al. (2014). Biomarkers of vascular calcification and mortality in patients with ESRD. Clin J Am Soc Nephrol 9:745–55.

Shroff RC, Shah V, Hiorns MP, et al. (2008). The circulating calcification inhibitors, fetuin-A and osteoprotegerin, but not Matrix Gla protein, are associated with vascular stiffness and calcification in children on dialysis. Nephrol Dial Transplant 23:3263–71.

Van Den Bouwhuijsen QJ, Vernooij MW, Hofman A, et al. (2012). Determinants of magnetic resonance imaging detected carotid plaque components: the Rotterdam Study. European Heart J 33:221–9.

Venuraju SM, Yerramasu A, Corder R, Lahiri A. (2010). Osteoprotegerin as a predictor of coronary artery disease and cardiovascular mortality and morbidity. J Am Coll Cardiol 55:2049–61.

Vik A, Mathiesen EB, Johnsen SH, et al. (2010). Serum osteoprotegerin, sRANKL and carotid plaque formation and growth in a general population – the Tromsø study. J Thromb Haemost 8:898–905.

Wei T, Wang M, Wang M, et al. (2009). Relationship of sRANKL level and vascular calcification score to cardiovascular events in maintenance hemodialysis patients. Blood Purif 28:342–45.

Yamada S, Oshima M, Watanabe Y, Miyake H. (2014). Arterial location-specific calcification at the carotid artery and aortic arch for chronic kidney disease, diabetes mellitus, hypertension, and dyslipidemia. Calcified Tissue Int 95:267–74.

Yavropoulou MP, Pikilidou M, Yovos JG. (2014). Anti-osteoporotic drugs and vascular calcification: the bidirectional calcium traffic. J Res 51:37–49.

Yokoyama K, Shigematsu T, Miyaki K, et al. (2008). Low blood osteoprotegerin levels are a predictor to poor prognosis in Japanese patients on hemodialysis due to diabetic nephropathy. Ther Apher Dial b12:259–60.

Zhou S, Fang X, Xin H, et al. (2013). Osteoprotegerin inhibits calcification of vascular smooth muscle cell via down regulation of the Notch1-RBP-Jκ/Msx2 signaling pathway. PLoS One 8:e68987.

International Urology and Nephrology
https://doi.org/10.1007/s11255-021-02988-3

NEPHROLOGY - ORIGINAL PAPER

Arterial calcifications and osteoprotegerin in chronic hemodialysis patients: impact on 6-year survival

Diana Moldovan[1] · Crina Rusu[1] · Alina Potra[1] · Cosmina Bondor[2] · Maria Ticala[1] · Dacian Tirinescu[1] · Anca Coman[1] · Olga Orasan[3] · Ioan Moldovan[4] · Remus Orasan[5] · Ina Kacso[1]

Received: 20 June 2021 / Accepted: 21 August 2021

Abstract

Aim The association between end-stage renal disease and cardiovascular mortality may be influenced through vascular alterations, in particular atherosclerosis and vascular calcification. The study *goal* was to assess the impact of each type of arterial intimal calcifications (AIC) and arterial medial calcifications (AMC), of osteoprotegerin (OPG), mineral metabolism markers and other features on all-cause and cardiovascular mortality in chronic hemodialysis patients.

Methods Ultrasound was performed in 87 patients on the carotid and femoral arteries, and the severity of AIC and AMC was assessed calculating a score according to the extension of calcification. We analyzed the link between AIC, AMC, OPG, mineral markers and mortality after 6 years of follow-up.

Results The cutoff value for OPG determined using ROC was 4.9 pmol/l for all-cause and cardiovascular mortality. Patients with higher serum OPG levels presented higher mortality rates. Our study revealed that AIC, high OPG, low ankle-arm index, presence of diabetes, smoking status, and lack of arteriovenous fistula are associated with all-cause and cardiovascular mortality in univariate regression analysis. Multivariate analysis identified AIC scoring based on the segmentation method as an independent predictor of all-cause and cardiovascular mortality, along with increased OPG levels. AMC scoring was not a predictor of mortality.

Conclusions Identifying and scoring AIC on ultrasound and measuring OPG levels, as a basis of the HD patient assessment may become valuable tools in clinical work, as these have an impact on death toll.

Keywords Arterial calcification · Osteoprotegerin · Hemodialysis patients · Survival

Introduction

End-stage renal disease (ESRD) is the image of a successful story of survival for an end-stage organ damage. No other internal medicine derived specialty can report such long-life span when the function of a vital organ is irreversible lost. The death rate of our end-stage renal disease patients remains still high, when comparing to general population, even in young adults [1]. Uremic syndrome does not lead to death anymore, but cardiovascular complications are major risk factors. In our effort to keep our patients alive, we are interested in factors that impede a good evolution.

Mineral metabolism is commonly disturbed due to progression of chronic kidney disease and loss of kidney functions. This can lead to cardiovascular diseases, especially related to the high rate of vascular calcifications. Vascular calcification is a consequence of calcium phosphate deposition into arteries, either in their intima or media layers. But vascular calcification is not just a simple mechanical process, it is a multifactorial phenomenon which induces a phenotype switch of vascular smooth muscle cells to osteoblast-like cells; this represents a subject of great interest for medical research [2]. A disequilibrium between pro-calcification and

✉ Diana Moldovan
dnmoldovan@yahoo.com

1 Department of Nephrology, University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania

2 Department of Statistics, University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania

3 4th Medical Department, University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania

4 Emergency Military Hospital, Cluj-Napoca, Romania

5 Nefromed Dialysis Center, Cluj-Napoca, Romania

Published online: 09 September 2021

anti-calcification factors contribute highly to this process [3].

One of the most important anti-calcification factors is osteoprotegerin (OPG). OPG is produced by osteoblasts and can inhibit osteoclast activation. It is a soluble receptor of receptor activator of nuclear factor-κB ligand (RANKL). OPG raised the interest of many research groups, but there is no consensus yet regarding its impact on hemodialysis (HD) patients' outcome. Although OPG is an anti-calcification factor, the results of clinical studies indicated poor outcomes. Some papers linked OPG with the pathogenesis of vascular calcification and atherosclerosis. Patients with coronary artery disease or heart failure and high OPG had an increased morbidity and mortality [4]. An association between OPG levels and mortality is present in the general population was the conclusion of a relatively recent meta-analysis [5]. There is no consensus about the relationship between OPG and cardiovascular morbidity and mortality in HD patients, still raising questions regarding its role [3, 6].

The association between ESRD and cardiovascular mortality seems to be influenced through vascular alterations, in particular atherosclerosis and vascular calcification [7].

We *aimed* to study the influence of both types of vascular calcifications (medial and intimal), of OPG serum levels and other specific factors on the survival time to all-cause and cardiovascular death in chronic HD patients.

Patients and methods

The study was designed as longitudinal, prospective, and analytical. It was carried on a randomly selected population of chronic prevalent HD patients. The patients were dialyzed in three sessions of 4–5 h every week, with synthetic high-flux dialyzers. The dialysate respected the standards, bicarbonate was the buffer, and heparin was the anticoagulant agent. We included all prevalent, adult HD patients, who agreed to participate to this study, and we excluded the patients with severe infections, acute illness, neoplasia, parathyroidectomy, previous renal transplant. Evaluation at baseline comprised clinical data, as well as laboratory assessment. We recorded data regarding patients' characteristics, as age, gender, HD vintage, presence of diabetes, medical history, dialysate calcium, HD prescription and medication. Treatment prescriptions were made according to guidelines. HD adequacy was assessed through spKt/V and urea reduction ratio (URR). We calculated body mass index (BMI), pulse pressure (PP) and ankle–arm index (AAI). Osteoprotegerin (OPG) (human-OPG ELISA, Biomedica, Wien, Austria) serum levels were measured. Testing of serum calcium (Ca), inorganic phosphorus (P), alkaline phosphatase (ALP), intact parathyroid hormone (iPTH), pre- and post-HD urea, creatinine, albumin, C-reactive protein (CRP), bicarbonate, hemoglobin (Hb), ferritin, cholesterol, HDL-cholesterol, and triglycerides levels, was performed. Blood samples were drawn before the HD session.

Vascular calcifications were detected in carotid and femoral arteries. The arteries walls were examined using a 5–10 MHz linear transducer and real-time B mode and Doppler functions were used. The operator was unaware of patient's clinical and biochemical data, to avoid biases. Arterial intima calcifications (AIC) referred to calcified atheroma plaques recognized as areas of focal intima thickening, with hyperechoic protrusion in the vascular lumen and posterior shadows. Multiple punctiform hyperechoic images in the vascular wall, not protruding in the lumen represented the aspect of the arterial media calcifications (AMC). Examination was done bilateral on common carotid artery, bifurcation, internal carotid artery, common and superficial femoral arteries, and calcification scores were calculated summarizing the presence of the typical image on each examined site, so the AIC and AMC scores ranged from 0 to 10.

Eighty-seven patients, with 47 males (53.87%) were included. Eleven patients were smokers (12.6%) and 21 patients (24.13%) had diabetes. Arteriovenous fistula (AVF) was the vascular access for 62 patients and 25 patients had a central venous catheter. Mean age was 62.74 ± 12.95 years, mean HD vintage was 47.96 ± 49.36 months and a mean spKt/V of 1.46 ± 0.22 was obtained. Vascular calcifications were identified in 71 patients, 68% had AIC, 68% had AMC and 54% had both. More about the baseline characteristics and descriptive statistics are depicted in our previous cross-sectional study [8].

Evolution, fatal events, death date and cause were registered. Patients were prospectively followed up for 6 years (72 months). All-cause and cardiovascular mortality were analyzed. Cardiovascular mortality was defined as death due to pulmonary edema, heart failure, arrhythmia, ischemic heart disease, peripheral artery disease and stroke. Their frequencies were calculated. Impact of AIC, AMC, OPG and different other factors on mortality was analyzed.

The statistical analysis was realized in IBM SPSS Statistics 25.0 program. Data were expressed as mean ± standard deviation (SD), percentage, or median (25th–75th percentile) for the follow-up period. To compare the means of independent characteristics of two groups, we used Student's *t* test or the Mann–Whitney according to the variable distribution. For comparison of categorical variables, Chi square test or Fisher's exact test was applied. Multiple regression was applied between all-cause and cardiovascular mortality and the potentially associated factors. Significant variables in univariate analysis were entered into multivariate analysis. Survival analysis was performed with Cox regression. The hazard ratios and their 95% confidence intervals for all-cause and CDV deaths were calculated. When the parameter was found significant by Cox's hazard model, Kaplan–Meier

analysis was applied to compare two groups stratified by a cut-off. According to the maximum of the Youden Index, a cutoff value of OPG, AIC and AMC that best predicted the all-cause and cardiovascular deaths was identified using a receiver-operating characteristic (ROC) curve. Survival analysis was performed with log-rank test, survival curves were represented with Kaplan–Meier curve. Statistical significance threshold was considered $p < 0.05$.

Results

All-cause mortality was analyzed in our research group. Among the 87 patients, 43 patients died (49.4%) due to different causes, as cardiovascular events, or other causes, which indicate to 8.23% annual all-cause mortality. Eight patients died due to infections, 3 due to cancer, 1 due to hemorrhage and 5 of unknown cause. Among all patients included in the study, 26 patients died due to cardiovascular causes (29.88%). Cardiovascular death was produced by pulmonary edema and heart failure in 8 patients, ischemic heart disease and myocardial infarction in 7 patients, arrhythmia in 5 patients, stroke in 4 patients and peripheral artery disease in 2 patients.

Comparison between the deceased and survivor groups is presented in Table 1. We found positive significant association between all-cause mortality and age, male gender, current smoking, coexistence of DM, presence of AIC and AMC and a negative association with the treatment with Ca salts (Table 1).

The results of univariate Cox proportional hazards regression analysis for all-cause and cardiovascular mortality in all study patients are shown in Table 2. Univariate regression analysis showed that old age ($p < 0.0001$), male gender ($p = 0.03$), diabetes ($p = 0.008$), smoking ($p = 0.03$), AIC ($p = 0.002$), decreased AAI < 1 ($p = 0.01$), and low usage of treatment with Ca salts ($p = 0.01$) were associated with increased all-cause mortality; there was a trend of association between catheters as vascular access due to lack of AVF and death ($p = 0.05$). The relation between OPG levels and survival time until all-cause death was tested, and no significant association was found ($p = 0.07$) (Table 2). Instead, elevated OPG levels were associated with the decreased survival time in patients who died due to cardiovascular events ($p = 0.03$). In univariate analysis, cardiovascular mortality was also associated with older age ($p < 0.001$), with diabetes ($p = 0.03$), increased AIC score ($p = 0.001$), lack of AVF as vascular access ($p = 0.03$), low AAI ($p = 0.04$) and low prescription of treatments with Ca salts ($p = 0.02$). Cardiovascular related deaths were associated with high AIC score, but there were no significant relationships with AMC score and serum Ca, P, ALP and iPTH levels (Table 2).

The cutoff value of OPG that best predicted the all-cause and cardiovascular deaths was 4.9 pmol/ml with an area under the ROC curve of 0.642 and sensitivity 69.76% and specificity 59.1% (95% CI = 0.525–0.759, $p = 0.022$). The group with OPG < 4.9 pmol/ml consisted in 39 patients and 13 deaths (survival rate = 66.7%) and the group with OPG ≥ 4.9 pmol/ml consisted in 47 patients (54.7%) with 30 deaths (survival 37.5%) (Fig. 1). In 17 patients from survivors' group (39.5%) and in 30 patients from deceased group (69.8%), the OPG levels were ≥ 4.9 pmol/l ($p = 0.005$). The Kaplan–Meier analysis showed that, from the group of 39 patients with OPG < 4.9 pmol/ml, 25.4% died due to cardiovascular causes and from the group of 48 patients with OPG ≥ 4.9 pmol/ml, 41.7% died due to cardiovascular causes ($p = 0.004$) (Fig. 2).

Regarding associations between AIC and AMC with outcomes, we considered three categories of arterial calcifications for each type, according to the number of sites affected. A score was calculated, and patients were included in one group with no AIC or AMC, the second with a score from 1 to 4 and the third with a score from 5 to 10. There were no significant differences at AMC analysis; from survivors' group, 22 patients (51.2%) had no calcification, 5 patients (11.6%) had an AMC score of 1–4 and 16 patients (37.2%) had an AMC score ≥ 5; from the deceased group, 19 patients (44.2%) had no calcification, 13 patients (30.2%) had an AMC score of 1–4 and 11 patients (25.6%) had an AMC score ≥ 5 ($p = 0.095$). Regarding AIC, there were highly significant differences; from survivors: 21 patients (48.8%) had no calcification, 13 patients (30.2%) had an AIC score of 1–4 and 9 patients (20.9%) had an AIC score ≥ 5; from deceased: 6 patients (14%) had no calcification, 9 patients (20.9%) had an AIC score of 1–4 and 28 patients (65.1%) had an AMC score ≥ 5 ($p < 0.0001$).

In Kaplan–Meier analysis, survival was 78.6% in no AIC group (6 deaths in 28 patients), 59.1% in the group with AIC score of 1–4 (9 deaths in 22 patients) and 24.3% in the group with AIC ≥ 5 (28 deaths from all 37 patients) ($p < 0.0001$) (Fig. 3). Kaplan–Meier analysis demonstrated a significant association between AIC and cardiovascular mortality ($p = 0.001$). Cardiovascular mortality was 10.7% in the group with no AIC, 22.7% in the group with an AIC score of 1–4 and 48.6% in those with AIC score 5–10 (Fig. 4).

Multivariate Cox regression tested the impact of OPG and arterial calcifications on mortality. Covariates were DM, smoking, the vascular access, AAI < 1 and treatments with Ca salts. It demonstrated a significant association between all-cause mortality and increased age, male gender, smoking, presence of diabetes, lack of AVF, increased AIC, AAI < 1, low use of treatment with Ca salts. As the age and gender are non-modifiable factors, they were removed from the model; afterwards, OPG and AIC, but not AMC, entered in the model as risk factor

Table 1 Comparison between the deceased and survivor groups

	HD deceased (43 patients)	HD survivors (44 patients)	*p*
OPG (pmol/ml)	5.9 (4.60–8.00)	4.5 (3.15–6.50)	0.103
Age (years)	68 (62–74)	57 (52.50–68.50)	**< 0.0001**
HD vintage (months)	44.26 ± 39.06	51.58 ± 58.62	0.497
Gender 46 males (53.5%)	28 (65.1%)	18 (41.9%)	**0.031**
Smoker 10 pts (11.6%)	8 (18.6%)	2 (4.7%)	**0.044**
DM 21 pts (24.4%)	16 (37.2%)	5 (11.6%)	**0.006**
AVF 61 pts (70.9%)	27 (62.8%)	34 (79.1%)	0.096
BMI (kg/m^2)	27.12 (24.43–29.94)	26.67 (26.79–31.65)	0.855
PP (mmHg)	60 (50–65)	50 (50–60)	0.970
AAI	0.54 (0.38–0.74)	0.75 (0.18–1)	0.375
AIC	6 (3.5–9)	1 (0–4)	**< 0.0001**
AMC	4 (1–6)	4 (0–4)	**0.037**
Ca (mg/dl)	8.38 ± 0.44	8.31 ± 0.55	0.527
P (mg/dl)	5.07 ± 1.67	5.18 ± 1.72	0.762
ALP (U/l)	66 (53–85)	65 (52–90)	0.653
iPTH (pg/ml)	231.85 (147.90–421.70)	203.80 (107.80–353.70)	0.472
Bicarbonate	17.56 ± 3.67	17.07 ± 3.00	0.499
Hb (g/dl)	11.58 ± 1.11	11.53 ± 1.43	0.847
Ferritin (ng/ml)	772.05 (477–897.80)	772.50 (673.30–937.95)	0.339
CRP (mg/dl)	0.90 (0.39–2.27)	0.88 (0.40–1.72)	0.839
Albumin (g/dl)	4.20 (4.04–4.42)	4.11 (3.97–4.32)	0.578
Cholesterol (mg/dl)	159 (144.50–203.50)	161 (143–189)	0.276
HDL-cholesterol (mg/dl)	45.81 ± 12.06	49.91 ± 13.50	0.170
Triglyceride (mg/dl)	139 (107.50–211.50)	133 (84–173)	0.260
Urea (mg/dl)	133.01 ± 32.08	136.74 ± 30.42	0.582
Creatinine (mg/dl)	8.60 ± 2.22	8.43 ± 2.38	0.740
URR	70.57 (64.03–76.11)	73.12 (68.59–77.19)	0.379
spKt/V	1.42 (1.26–1.55)	1.48 (1.37–1.58)	0.379
Ca in HD solution	1.51 ± 0.14	1.53 ± 0.13	0.432
Treatment with Ca salts—71 patients (82.6%)	32 (74.4%)	39 (90.7%)	**0.047**
Treatment with vitamin D (calcitriol)—30 patients (34.9%)	16 (37.2%)	14 (32.6%)	0.651
Sevelamer—21 patients (24.4%)	11 (25.6%)	10 (23.3%)	0.802
ACEI—29 patients (33.7%)	16 (37.2%)	13 (30.2%)	0.494
Betablockers–59 patients (68.6%)	32 (74.4%)	27 (62.8%)	0.245
CCB—39 patients (45.3%)	19 (44.2%)	20 (46.5%)	0.829
Statins—11 patients (12.8%)	4 (9.3%)	7 (16.3%)	0.333

Legend: *OPG* osteoprotegerin; *HD* hemodialysis; *DM* diabetes mellitus; *AVF* arteriovenous fistula; *BMI* body mass index; *PP* pulse pressure; *AAI* ankle-arm index; *AIC* arterial intima calcification; *AMC* arterial media calcification; Ca calcium; P phosphorus; ALP alkaline phosphatase; iPTH intact parathyroid hormone; Hb hemoglobin; CRP C-reactive protein; *URR* urea reduction ratio; spKt/V = dialysis adequacy; *ACEI* angiotensin-converting enzyme inhibitors, *CCB* Calcium channel blockers. Data are expressed as mean ± standard deviation and as median (25th–75th percentiles) or percentages. Statistically significance is marked with bold characters

for all-cause mortality. The factors influencing cardiovascular mortality were also analyzed in multivariate Cox regression multivariate analysis. After removal of age and gender, OPG and AIC remained in the model, as their high levels are significant risk factors for cardiovascular mortality (Table 3).

Discussion

The quality of the arteries is a very important prognostic factor for chronic HD patients; it has a great impact on their health, disease, and survival. An increasing interest

Table 2 Results of univariate Cox regression analysis for all-cause and cardiovascular mortality

	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	*p*	HR (95% CI)	*p*
OPG (pmol/ml)	1.07 (0.99–1.16)	0.098	1.10 (1.01–1.21)	**0.033**
OPG ≥ 4.9 (pmol/ml)	2.45 (1.28–4.71)	**0.005**	3.53 (1.42–8.81)	**0.007**
Age (years)	1.07 (1.03–1.10)	**< 0.0001**	1.12 (1.06–1.17)	**< 0.001**
HD vintage (months)	1.00 (0.99–1.00)	0.588	1.00 (0.99–1.01)	0.54
Gender (males)	1.99 (1.06–3.73)	**0.03**	1.24 (0.57–2.68)	0.58
Smoker	2.27 (1.05–4.90)	**0.03**	1.79 (0.62–5.21)	0.285
DM	2.26 (1.21–4.20)	**0.008**	2.34 (1.06–5.18)	**0.03**
AVF	0.54 (0.29–1.00)	**0.05**	0.44 (0.20–0.95)	**0.03**
BMI	0.99 (0.94–1.04)	0.757	1.00 (0.94–1.07)	0.937
PP	1.00 (0.98–1.02)	0.546	1.00 (0.97–1.03)	0.926
AAI < 1	0.35 (0.16–0.79)	**0.01**	0.33 (0.11–0.96)	**0.04**
AIC score	1.18 (1.09–1.27)	**0.002**	1.20 (1.08–1.34)	**0.001**
AMC score	1.13 (1.03–1.24)	**0.013**	1.13 (1.00–1.28)	0.056
Ca (mg/dl)	1.18 (0.67–2.09)	0.570	0.85 (0.41–1.80)	0.679
P (mg/dl)	0.95 (0.79–1.13)	0.546	1.02 (0.81–1.28)	0.888
ALP (U/l)	1.00 (0.99–1.01)	0.614	1.00 (0.99–1.01)	0.496
iPTH (pg/ml)	1.00 (1.00–1.00)	0.642	1.00 (0.999–1.002)	0.346
Bicarbonate (mmol/l)	1.04 (0.94–1.15)	0.421	1.07 (0.94–1.21)	0.421
Hb (g/dl)	1.03 (0.82–1.28)	0.823	1.04 (0.78–1.38)	0.803
CRP (mg/dl)	1.00 (0.999–1.001)	0.618	1.00 (0.998–1.001)	0.773
Albumin (g/dl)	1.03 (0.91–1.17)	0.688	1.03 (0.86–1.22)	0.671
Cholesterol (mg/dl)	1.22 (0.46–3.28)	0.168	0.77 (0.23–2.60)	0.240
HDL-cholesterol (mg/dl)	1.00 (1.00–1.01)	0.183	1.01 (1.00–1.01)	0.523
Triglyceride (mg/dl)	1.00 (0.999–1.004)	0.176	1.00 (1.00–1.01)	0.688
Urea	1.00 (0.99–1.01)	0.407	0.745 (0.586–1.144)	0.255
Creatinine (mg/dl)	1.01 (0.89–1.15)	0.891	1.09 (0.92–1.28)	0.325
URR	1.00 (0.96–1.02)	0.472	0.97 (0.93–1.01)	0.189
spKt/V	0.61 (0.16–2.37)	0.472	0.30 (0.05–1.80)	0.189
Ca in HD solution	2.78 (0.31–25.11)	0.364	5.73 (0.34–96.91)	0.226
Treatment with Ca salts	0.40 (0.20–0.80)	**0.01**	0.38 (0.16–0.91)	**0.029**
Treatment with vitamin D (calcitriol)	1.18 (0.64–2.20)	0.593	1.47 (0.67–3.20)	0.333
Sevelamer	1.10 (0.56–2.19)	0.779	0.96 (0.39–2.39)	0.929
ACEI	1.18 (0.64–2.19)	0.600	0.88 (0.38–2.03)	0.765
Betablockers	1.53 (0.77–3.04)	0.223	1.19 (0.52–2.74)	0.681
CCB	0.91 (0.50–1.66)	0.757	0.84 (0.39–1.84)	0.668
Statins	0.65 (0.23–1.83)	0.416	0.84 (0.25–2.80)	0.775

OPG osteoprotegerin; *HD* hemodialysis; *DM* diabetes mellitus; *AVF* arteriovenous fistula; *BMI* body mass index; *PP* pulse pressure; *AAI* ankle–arm index; *AIC* arterial intima calcification; *AMC* arterial media calcification; Ca = calcium; P = phosphorus; ALP = alkaline phosphatase; *iPTH* intact parathyroid hormone; Hb = hemoglobin; *CRP* C-reactive protein; URR = urea reduction ratio; spKt/V = dialysis adequacy; *ACEI* angiotensin-converting enzyme inhibitors, *CCB* Calcium channel blockers. Statistically significance is marked with bold characters

on arterial structure and functions' assessment can be observed in everyday practice as a consequence of positive research results. The main strength of our study is the prospective data of HD patients over a follow-up period of 72 months, in a group known to be at high risk of death.

In our study, the presence of arterial calcification was identified as belonging to the atheroma plaques (AIC) or being situated in the media of the arteries (AMC). These are two different categories of vascular calcifications with different consequences on arterial functions, broadly speaking, vessel occlusion after AIC and stiffness in case of AMC [9]. We demonstrated that severity of AIC, stratified on three categories, was associated with all-cause and cardiovascular mortality; AMC had no impact on outcome. The AIC were

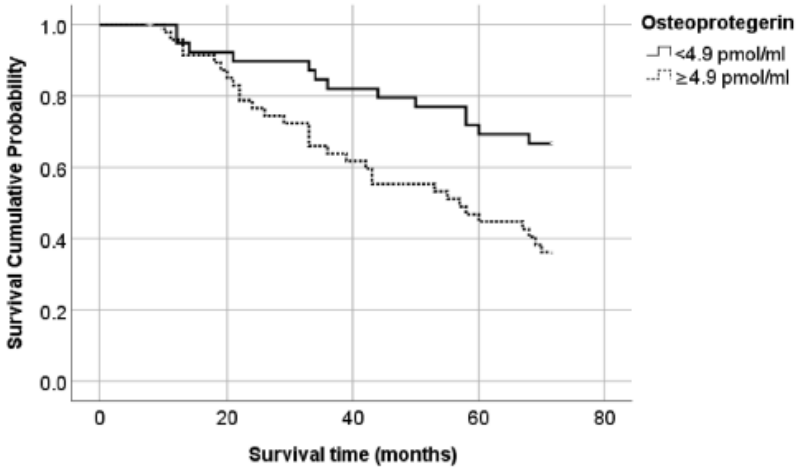

Fig. 1 All-cause mortality comparison according to OPG. Kaplan–Meier curve illustrates 72-month survival. Survival rate was 66.7% in the group with OPG < 4.9 pmol/ml and 37.5% in the group with OPG ≥ 4.9 pmol/ml ($p = 0.005$)

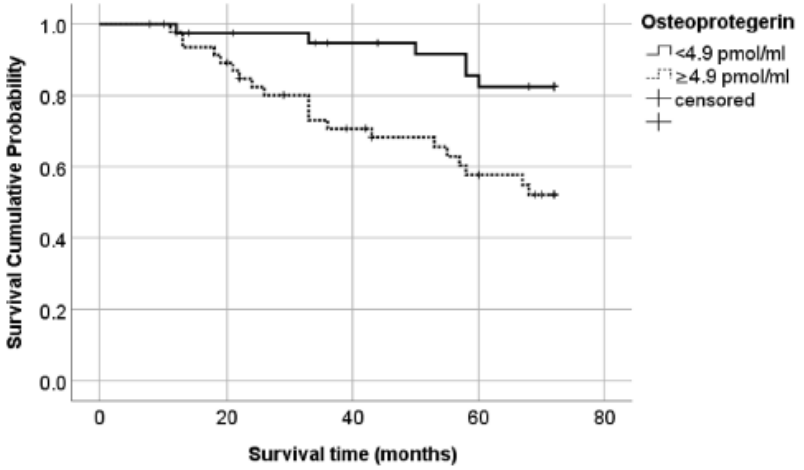

Fig. 2 Kaplan–Meier curve illustrates 72-month survival according to OPG. Cardiovascular mortality was 25.4% in the group with OPG < 4.9 pmol/ml and 41.7% in the group with OPG ≥ 4.9 pmol/ml ($p = 0.004$)

significant important factors influencing the outcome in our HD patients. Other recent studies evaluated this relation. Some authors used CT and radiograph to examine of the arteries. The presence and extension of vascular calcifications, detected on coronary arteries and abdominal aorta, predicted risk of all-cause death in patients starting hemodialysis [10]. The aortic arch calcification was measured on chest radiographs and its progression was associated with mortality in HD patients [11]. Ultrasound, as one of the most valuable and also available tools, can easily detect morphological abnormalities of the vascular walls. Discrete modifications of carotid intima media thickness increase cardiovascular mortality in peritoneal dialysis patients [12]. Different scoring of AMC realized on ultrasound exam of the lower limbs was associated with chronic complications of diabetes mellitus, especially the nephropathy [13]. There is a constant concern related to possible intervention strategies to prevent and regress the vascular calcification in dialysis patients [14, 15].

Biomarkers may be essential elements influencing the cardiovascular outcome of ESRD patients [16, 17]. We analyzed, also, other factors impacting the mortality in HD patients. One important biomarker in patients with chronic kidney disease is OPG. Increased levels are present in ESRD patients and indicate a crosstalk between the bone and vessels in chronic HD patients [16]. In one of our previous studies, we demonstrated that increased circulating OPG levels are associated with vascular calcifications [8]. The present research identified a significant relationship between high OPG serum levels and all-cause mortality in chronic HD

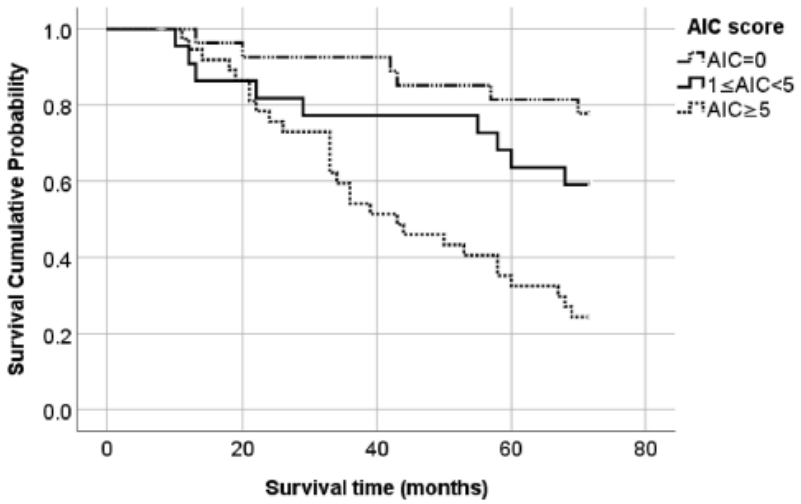

Fig. 3 All-cause mortality comparison between groups according to AIC score. Survival in no AIC group was 78.6%, in AIC = 1–4 group was 59.1% and in AIC = 5–10 was 24.3% ($p < 0.0001$)

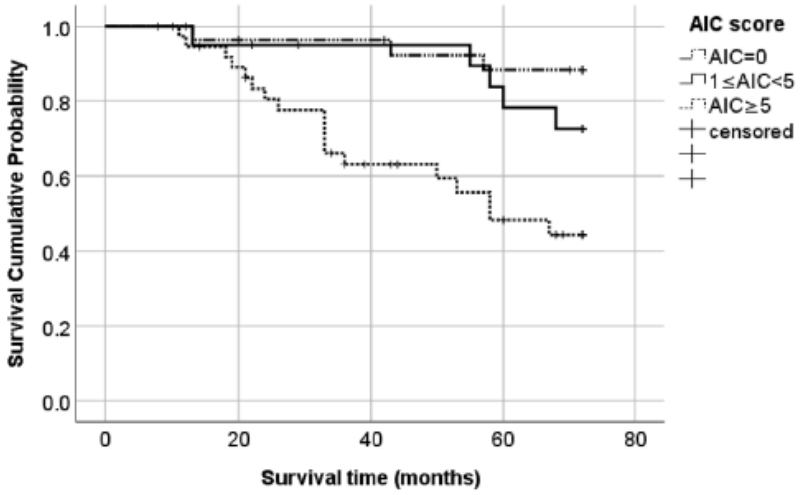

Fig. 4 Kaplan–Meier curve illustrates 72-month survival according to AIC score. Cardiovascular mortality rate was 10.7% in the group with no AIC, 22.7% in the group with AIC = 1–4 and 48.6% in the group with AIC = 5–10 ($p = 0.01$)

Table 3 Multivariate Cox regression analysis for all-cause and for cardiovascular mortality

	All-cause mortality		Cardiovascular mortality	
	HR (95% CI)	*p*	HR (95% CI)	*p*
OPG ≥ 4.9 (pmol/ml)	2.976 (1.380–6.418)	**0.005**	5.838 (1.943–17.542)	**0.002**
AIC score	1.797 (1.148–2.813)	**0.01**	2.078 (1.145–3.771)	**0.016**
AMC score	1.331 (0.878–2.019)	0.178	1.476 (0.881–2.474)	0.139

OPG osteoprotegerin, *AIC* arterial intima calcification; *AMC* arterial media calcification. Covariates were DM, smoking, vascular access, AAI, treatments with Ca salts. Statistically significance is marked with bold characters

patients, after 6 years of follow-up. Cardiovascular mortality was also significantly associated with increased OPG levels. A literature-based meta-analysis involving a high number of participants analyzed possible links between OPG and cardiovascular outcomes in the general population; it concluded that high OPG is associated with an increased risk of incident cardiovascular disease [5]. In predialysis chronic kidney disease patients, OPG was declared a marker of cardiovascular events and mortality [18–20]. Hemodialysis patients were also studied and OPG was associated with mortality [21, 22]. A recent research on HD patients demonstrated no significant link between OPG and mortality [23]. All these results may seem a medical paradox, as it was demonstrated that OPG is an anti-calcification factor [24] and low OPG maybe associated with poor prognosis in some HD patients [25]. Studies have also demonstrated that denosumab, an endogenous RANKL inhibitor which mimics the natural action of OPG, may suppress the progression of arterial calcifications [26, 27]. Further studies are warranted before a sound hypothesis for this seeming contradiction can be set.

We obtained other important results related to impact on outcome. Our patients with no AVF, using a central venous catheter as the vascular access for HD had an elevated all-cause and cardiovascular mortality; this is a result consistent some studies [28], but not with others [29]. Best survival belonged to patients with AVF.

The relationship between smoking and outcomes in HD patients is not well understood. In our study, smokers and diabetes had higher mortality rate. A retrospective cohort study analyzed death at 2 years in HD patients and reported that incidence rate of mortality for active smokers with diabetes was high and that death probabilities increased with greater exposure to smoking [30].

We demonstrated also that AAI < 1 is associated with all-cause and cardiovascular mortality. A low ankle-arm index is a marker of atherosclerosis, and it can suggest stenosis or thrombosis of the arteries in the lower limbs. Therefore, this result indicates that atherosclerotic peripheral arterial disease is a risk factor for death in ESRD patients. Studies were generally inconsistent regarding this issue, but a newer publication showed promising and similar results [31].

Treatment with Ca salts offered protection against death in our HD patients. This is a controversial area, some authors detected an increased risk of cardiovascular death [32], but our results may be explained by a possible trend to better control of mineral metabolism due to this treatment, even thou the specific markers were not significantly correlated with mortality.

There are also several *limitations in this study*. First, including patients with both AIC and AMC could be misleading. Second, relatively reduced number of patients impede us to make a general statement about these results. Finally, a single measurement of OPG, AIC, AMC and AAI do not offer the possibility to assess their progression in relation with mortality. Further studies are necessary to address this issue.

In conclusion, we assessed AIC and AMC on ultrasound, OPG and other data with regards to predicting survival in HD patients. Univariate analysis showed that high AIC and OPG, age, smoking, diabetes, low AAI and lack of AVF are associated with all-cause and cardiovascular mortality in all study patients. In multivariate analysis, AIC scoring and OPG predicted all-cause and cardiovascular mortality. To improve the survival of dialysis dependent patients, it is critical to understand the contributions of potentially modifiable risk factors. Hence, identifying AIC on ultrasound and measuring OPG may provide benefit in survival prediction in HD patients.

Acknowledgements We would like to thank the patients and personnel involved in the creation of this cohort; their collaboration made the study possible.

Funding There is no specific funding for this research involved in collection, analysis, interpretation of data and in writing of the manuscript.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical standards This study was approved by the Ethics Committee for Scientific Research of the University of Medicine and Pharmacy "Iuliu Haţieganu", Cluj-Napoca, Romania; the study respected the ethical standards of the Declaration of Helsinki.

Informed consent All included patients agreed to participate in the study and signed the informed consent.

References

1. Modi ZJ et al (2019) Risk of cardiovascular disease and mortality in young adults with end-stage renal disease: an analysis of the US renal data system. JAMA Cardiol 4(4):353–362
2. Ohtake T, Kobayashi S (2017) Impact of vascular calcification on cardiovascular mortality in hemodialysis patients: clinical significance, mechanisms and possible strategies for treatment. Renal Replace Therapy 3(1):13
3. Gungor O, Kocyigit I, Yilmaz MI, Sezer S (2018) Role of vascular calcification inhibitors in preventing vascular dysfunction and mortality in hemodialysis patients. Semin Dial 31(1):72–81
4. Mesquita M et al (2009) Plasma osteoprotegerin is an independent risk factor for mortality and an early biomarker of coronary vascular calcification in chronic kidney disease. Clin Chem Lab Med (CCLM) 47(3):339–346
5. Tschiderer L, Willeit J, Schett G, Kiechl S, Willeit P (2017) Osteoprotegerin concentration and risk of cardiovascular outcomes in nine general population studies: literature-based meta-analysis involving 26,442 participants. PLoS ONE 12:e0183910
6. Znorko B, Oksztulska-Kolanek E, Michałowska M, Kamiński T, Pawlak K (2017) Does the OPG/RANKL system contribute to

the bone-vascular axis in chronic kidney disease? A systematic review. Adv Med Sci 62(1):52–64
7. Düsing P et al (2021) Vascular pathologies in chronic kidney disease: pathophysiological mechanisms and novel therapeutic approaches. J Mole Med 22:1–14
8. Moldovan D et al (2015) Role of osteoprotegerin in vascular disorders of the end-stage renal disease patients. Biomarkers 20(2):116–122
9. London GM (2011) Arterial calcification: cardiovascular function and clinical outcome. Nefrología (English Edition) 31(6):644–647
10. Bellasi A et al (2021) Predictive value of measures of vascular calcification burden and progression for risk of death in incident to dialysis patients. J Clin Med 10(3):376
11. Chung WS et al (2020) Progression of aortic arch calcification is associated with overall and cardiovascular mortality in hemodialysis. Dis Markers. https://doi.org/10.1155/2020/62931852020
12. Asicioglu E et al (2021) Baseline carotid intima media thickness is associated with cardiovascular morbidity and mortality in peritoneal dialysis patients. Ther Apher Dial. https://doi.org/10.1111/1744-9987.13629
13. Tian J et al (2020) Different ultrasound scoring methods for assessing medial arterial calcification: association with diabetic complications. Ultrasound Med Biol 46(6):1365–1372
14. Chen NC, Hsu CY, Chen CL (2017) The strategy to prevent and regress the vascular calcification in dialysis patients. Biomed Res Int 2017:1–11
15. Suzuki S, Suzuki M, Hanafusa N, Tsuchiya K, Nitta K (2020) Denosumab recovers aortic arch calcification during long-term hemodialysis. Kidney Internat Rep. https://doi.org/10.1016/j.ekir.2020.12.002
16. Moldovan D et al (2017) Osteoprotegerin and uremic osteoporosis in chronic hemodialysis patients. Int Urol Nephrol 49(5):895–901
17. Kacso I et al (2015) Adiponectin predicts cardiovascular events in diabetes dialysis patients. Clin Biochem 48(13–14):860–865
18. Yilmaz MI et al (2016) Osteoprotegerin in chronic kidney disease: associations with vascular damage and cardiovascular events. Calcif Tissue Int 99:121–130
19. Marques GL et al (2021) Osteoprotegerin is a marker of cardiovascular mortality in patients with chronic kidney disease stages 3–5. Sci Rep 11:2473
20. Alderson HV et al (2016) FGF-23 and Osteoprotegerin but not Fetuin-A are associated with death and enhance risk prediction in non-dialysis chronic kidney disease stages 3–5. Nephrology 21(7):566–573
21. Winther S et al (2013) Osteoprotegerin and mortality in hemodialysis patients with cardiovascular disease. Clin Nephrol 80(3):161–167
22. Huang QX et al (2020) Elevated osteoprotegerin concentration predicts increased risk of cardiovascular mortality in patients with chronic kidney disease: a systematic review and meta-analysis. Kidney Blood Press Res 45(4):565–575
23. Fitzpatrick J et al (2020) Calcification biomarkers, subclinical vascular disease, and mortality among multiethnic dialysis patients. Kidney internat Rep 5(10):1729–1737
24. Zhou S et al (2013) Osteoprotegerin inhibits calcification of vascular smooth muscle cell via down regulation of the Notch1-RBP-Jκ/Msx2 signaling pathway. PLoS ONE 8(7):e68987
25. Yokoyama K et al (2008) Low blood osteoprotegerin levels are a predictor to poor prognosis in Japanese patients on hemodialysis due to diabetic nephropathy. Ther Apher Dial 12(3):259–260
26. Chen CL et al (2020) Impact of denosumab on cardiovascular calcification in patients with secondary hyperparathyroidism undergoing dialysis: a pilot study. Osteoporos Int 31:1507–1516
27. Singh A, Tandon S, Tandon C (2021) An update on vascular calcification and potential therapeutics. Mol Biol Rep 48:887–896
28. Gil Giraldo Y, Muñoz Ramos P, Ruano P, Quiroga B (2020) Vascular access-related mortality in hemodialysis patients during and after hospitalization. Ther Apher Dial 24(6):688–694
29. Yu Y et al (2020) Vascular access type was not associated with mortality and the predictors for cardiovascular death in elderly Chinese patients on hemodialysis. Blood Purif 49(1–2):63–70
30. Li NC et al (2018) Association of smoking status with mortality and hospitalization in hemodialysis patients. Am J Kidney Dis 72(5):673–681
31. Hsu PC et al (2021) Usefulness of ankle-brachial index calculated using diastolic blood pressure and mean arterial pressure in predicting overall and cardiovascular mortality in hemodialysis patients. Int J Med Sci 18(1):65
32. Tsai PH, Chung CH, Chien WC, Chu P (2020) Effects of calcium-containing phosphate binders on cardiovascular events and mortality in predialysis CKD stage 5 patients. PLoS ONE 15(10):e0241435

Int Urol Nephrol
DOI 10.1007/s11255-015-1106-x

NEPHROLOGY - ORIGINAL ARTICLE

Survival after parathyroidectomy in chronic hemodialysis patients with severe secondary hyperparathyroidism

Diana Moldovan[1,4] · Simona Racasan[2] · Ina Maria Kacso[1] · Crina Rusu[1] · Alina Potra[1] · Cosmina Bondor[3] · Ioan Mihai Patiu[2] · Mirela Gherman-Căprioară[1]

Received: 30 June 2015 / Accepted: 2 September 2015

Abstract
Introduction The life for end-stage renal disease patients has remarkably improved in the last years. Although mineral and bone disorders remain as unsolved complication, in severe secondary hyperparathyroidism (sHPT), the ultimate treatment is parathyroidectomy (PTX). It is an old treatment, but there are still insufficient data regarding survival after PTX. The study goals were to compare 2-year mortality and morbidity after PTX in surgically versus medically treated sHPT and to compare the efficacy and safety in subtotal versus total PTX in a cohort of patients receiving hemodialysis (HD).
Methods This prospective, longitudinal study was carried out on a cohort of chronic HD patients with severe sHPT (iPTH over 700 pg/ml). Among the overall HD population, 26 patients underwent PTX. This group was compared to a control group treated with specific drugs. Laboratory parameters, specific symptoms and mortality were registered after 24 months of follow-up for each group. The subgroups of subtotal and total PTX patients were also compared.
Results All average values of mineral markers were significantly reduced after PTX, as a proof that surgical treatment was effective. The reduction in mineral markers and the improvement in symptoms and mortality rates were similar after total and subtotal PTX. Bone pain was significantly lower in patients after PTX than in those drug treated ($p = 0.0005$), but not muscle weakness and itching. Survival at 2 years was better in patients surgically treated (PTX) despite significantly higher mean baseline values of iPTH, Ca and ALP compared to patients medically treated ($p = 0.03$).
Conclusions We compared clinical and laboratory outcomes in HD patients with severe sHPT. Mortality, bone pain and mineral markers were improved by PTX. Total and subtotal PTX had similar clinical outcomes.

Keywords Parathyroidectomy · Secondary hyperparathyroidism · Hemodialysis patients · Survival

Introduction

Chronic kidney disease-mineral and bone disorder (CKD-MBD) pathogenesis is multifactorial. It comprises metabolic abnormalities associated with chronic kidney disease, and, also, the secondary effects of medical interventions. There is no ideal drug for prevention and treatment and, usually, multiple interventions are necessary to break their pathophysiological chains.

There are immediate therapeutic targets such as prevention of parathyroid gland hyperplasia, maintaining normal serum levels of phosphorus (P) and calcium (Ca), skeletal status and improving cardiovascular outcomes. There are also long-term therapeutic targets such as reducing the risk of renal osteodystrophy, cardiovascular morbidity and mortality and avoiding the occurrence of calciphylaxis [1, 2].

✉ Diana Moldovan
dnmoldovan@yahoo.com

1 Department of Nephrology, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

2 Nefromed Dialysis Center, Cluj-Napoca, Romania

3 Department of Statistics, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

4 Emergency County Hospital, "Mihai Manasia" Nephrology and Dialysis Clinic, 3-5 Clinicilor Street, Cluj- Napoca, Romania

Published online: 16 September 2015

Therapies in use, such as diet, modification of dialysis characteristics, vitamin D and calcium-based phosphate binders, had a limited efficacy [3]. Recently, new drugs such as sevelamer hydrochloride, lanthanum carbonate, selective vitamin D receptor activators, calcimimetics have been included in our daily practice. They involve high costs and, sometimes, do not solve the problem. Thus, in order to make appropriate recommendations, we need to understand the effects of available treatments. Even if the surgical intervention is an "old" treatment for renal secondary hyperparathyroidism (sHPT), it needs further assessment regarding efficiency and impact on mortality.

According to KDIGO guidelines, CKD-MBD management is aimed to improve survival and bone outcomes. In this spirit, interventions on parathyroid glands are indicated if parathyroid hormone (iPTH) concentrations exceed 800 pg/mL and are associated with hypercalcemia and hyperphosphatemia refractory to medical treatment. However, this iPTH concentration represents an arbitrary cut-off value and, often, complications occur at lower levels of iPTH. There has been a trend to consider surgical intervention earlier in the course of sHPT, particularly if the imagistic estimated weight of a parathyroid gland exceeds 500 or 1000 mg (normal 30–40 mg), due to high probability of nodular hyperplasia and autonomous secretion. Available techniques are subtotal, total with implant or total parathyroidectomy (PTX) or ethanol percutaneous injection. In postoperatory period, monitoring and adequate treatment are required [1, 4, 5].

A lot of studies analyze the impact of increased serum levels of iPTH, P or of vascular calcifications on mortality in end-stage renal disease patients [6, 7]. Still, there are few data regarding survival after PTX. One of the most important studies addressing this issue was conducted by Kestenbaum et al. [8]. They compared two large groups of chronic dialysis patients with sHTP. First group underwent PTX, and the control group was treated conservatory. They concluded that short-term mortality was increased, and long-term mortality was reduced in PTX patients. They raise some concerns about PTX risks regarding adynamic bone disease and increased rate of recurrences. Also, hungry bone syndrome should be a reason for concern [9].

In our area, PTX represented an infrequent medical practice in the past. In recent years, due to surgical expertise in this domain, the PTX rate has increased. Now, every patient with severe sHPT can benefit of surgical approach. Therefore, we consider that a comparison of severe sHPT available treatment results is necessary.

The study goal was to evaluate clinical outcomes up to 2 year after parathyroidectomy in patients with severe sHTP receiving hemodialysis. The objectives were to assess the indication of PTX and the postoperatory evolution, to compare the long-term mortality in surgically versus medically treated sHPT and to compare the efficacy and safety through survival rate in subtotal versus total PTX.

Patients and methods

This prognostic study was carried out on a cohort of chronic hemodialysis patients with severe sHPT. The method of data collection was exposed–unexposed. This was a prospective, longitudinal study. Among the overall 220 HD patients population in the Nefromed Dialysis Center, 56 patients had an iPTH over 700 pg/ml associated with hypercalcemia and hyperphosphatemia or refractory to medical treatment. We have indicated parathyroidectomy to these patients. Twenty-six patients accepted the intervention. Eligibility criteria were patients prevalent in dialysis who accepted the study protocol and underwent PTX, age >18 years and iPTH over 700 pg/ml.

Exclusion criteria were previous renal transplant and refusal of the study protocol. All patients who met the criteria were recruited in the study as exposed. They were included in the study from the day when the surgical intervention was performed.

PTX was performed in the Department of Surgery of our Emergency County Hospital. The selection for PTX and the intervention period lasted 4 months. For the PTX group, the clinical and biochemical assessment was realized in the preoperative hours, after 2, 12 and 24 months. The type of intervention was mentioned, either total or subtotal PTX.

The unexposed group consisted in 26 patients with iPTH over 700 pg/ml, without surgical intervention and treated with specific drugs. The surgical intervention was proposed to these patients also, but they refused or postponed it due to personal reasons. The medically treated group was clinically and biochemically analyzed at the beginning of the study. Medication for decreasing iPTH consisted in calcium carbonate, calcium acetate, sevelamer hydrochloride and calcitriol. These drugs were prescribed and dose-adjusted according to serum Ca, P and iPTH levels.

These two groups were compared. Four patients refused to participate in the study.

Clinical data (age, gender, HD vintage, treatments with phosphate (P) binders and vitamin D derivates, medical history—the presence of diabetes mellitus, arterial hypertension and of the cardiac failure) were recorded. Serum calcium (Ca), inorganic phosphorus (P), alkaline phosphatase (ALP), intact parathyroid hormone (iPTH—Roche second-generation assay), albumin, C-reactive protein (CRP) and hemoglobin (Hb) were measured.

The follow-up period was 24 months for each group. The number of deaths was recorded. The symptoms such as bone pain, muscle weakness and pruritus were recorded

at the beginning of the study and in the follow-up period. The severity at the time of assessment of each symptom was rated from 0 to 10 on a numerical scale, 0 meaning that the symptom is absent and 10 that it is of the worst possible severity. The patient's opinion of the severity of the symptoms was the "gold standard" for symptom assessment.

Statistical analysis was performed using SPSS 19.0 program. Normal distribution was tested with the Shapiro–Wilk test. Values are expressed as mean ± standard deviation (SD) and median (interquartile range). For comparison of two means of independent samples, *t* test or Mann–Whitney test was used. As a method of multiple comparison of dependent variables, repeated-measure ANOVA (Hotelling's trace test or Greenhouse–Geisser test) or Friedman test was used. For comparison of qualitative variables, Chi-square test or Fisher's exact test was employed. Survival analysis was performed with Cox regression; survival curves were represented with Kaplan–Meier curve. Statistical significance threshold was considered $p < 0.05$.

The study was approved by the Ethical Committee of our University and is in accordance with the ethical standards of the Declaration of Helsinki of 1975 revised in 1983; each patient signed an informed consent.

Results

Mean age in PTX group was 51.62 ± 9.92 years with a minimum of 33 years old and a maximum of 67 years old; HD vintage was 110.42 ± 71.36 months with a minimum of 16 months and a maximum of 324 months; the group consisted in 14 females and 12 males. Medically treated group (non-PTX) was composed of 6 females and 20 males. It had a mean iPTH of 1416.2, with a minimum of 718 and a maximum of 2676 pg/ml (Table 1).

We compared PTX and non-PTX groups. PTX group included significantly more females and increased HD vintage. Serum iPTH, Ca and ALP levels were increased in PTX group; mean values for age, P, CRP, Hb and albumin were not significantly different.

In PTX group, mineral markers had significantly decreased after 24 months. These decreases of mineral markers were not significant in non-PTX group (Tables 2, 3). Two years after PTX, 3 patients (11.53 %) had iPTH within the target limits, 9 patients (34.61 %) had values of iPTH < 150 pg/ml, and 14 patients (53.84 %) had iPTH > 300 pg/ml. It can be observed that the mean value of iPTH at 24 months after intervention remained still high.

For PTX patients, the evolution of symptoms was as follows. Bone pain was present in all 26 patients (100 %). Improvement was observed in 20 patients (77 %). Muscle weakness was present in 21 patients (80.76 %), and it had been improved in 9 of 21 patients (42.85 %). Pruritus was present in 9 patients (34.61 %), and it had been improved in 5 of 9 patients (55.55 %). The evolution of symptoms for non-PTX patients was different. Bone pain was present in 25 patients (96.15 %), and it had been improved in 6 patients (24 %). Muscle weakness was present in 18 patients (69.2 %), and it had been improved in 6 of 18 patients (33.33 %). Pruritus was present in 12 patients (46.15 %), and it had been improved in 3 of 12 patients (33.33 %). The degree of improvement in symptoms was compared, and only bone pain was significantly decreased after PTX versus medically treated group ($p = 0.0005$). Differences between the two groups regarding the improvements in muscle weakness ($p = 0.78$) and pruritus ($p = 0.20$) were not statistically significant (Table 4).

Table 1 Baseline characteristics of the patients

Parameter	PTX treated group	Non-PTX treated group	*p*
Age (years)	51.62 ± 9.92	49.65 ± 11.49	0.51
Gender—no females (%)	14 (53.84 %)	6 (23.07 %)	**0.04**
Presence of DM (%)	6 (23 %)	8 (30.76 %)	0.38
Presence of HTA (%)	20 (76.93 %)	17 (65.38 %)	0.45
Presence of cardiac failure (%)	4 (15.38 %)	3 (11.53 %)	0.64
HD vintage (months)	105.50 (56.75–155.50)	41.50 (28.00–105.50)	**0.02**
iPTH (pg/ml)	2037 (1582.75–3146.75)	1282.5 (848.25–1901.5)	**0.001**
Ca (mg/dl)	9.34 ± 0.85	8.68 ± 0.94	**0.01**
P (mg/dl)	7.04 ± 1.83	6.58 ± 1.90	0.39
ALP (U/l)	612 (375–1179.5)	336 (241–552.5)	**0.004**
Albumin (g/dl)	4.03 ± 0.31	4.16 ± 0.29	0.11
Hb (g/dl)	11.32 ± 2.01	11.91 ± 0.93	0.27
CRP (mg/dl)	1.21 (0.5–2.32)	1.05 (0.34–1.90)	0.45

Data were expressed as mean ± SD for parametric variables, as median (25th–75th percentile) for nonparametric variables and as frequencies for qualitative variables

Statistically significance is marked with bold characters

Table 2 Evolution of biochemical parameters in PTX group

Parameter	Preop (*t*1)	Post-op at 2 months (*t*2)	Post-op in 12 months (*t*3)	Post-op in 24 months (*t*4)	*p*
iPTH (pg/ml)	2464.77 ± 1239.31	774.73 ± 1199.38 (c)	653.62 ± 935.18 (b)	746.14 ± 1141 (a)	**<0.0001**
Ca (mg/dl)	9.34 ± 0.85	8.40 ± 0.88 (c)	8.35 ± 1.47 (b)	8.36 ± 0.93 (a)	**0.001**
P (mg/dl)	7.04 ± 1.83	5.20 ± 1.96 (c)	5.71 ± 1.85 (b)	5.80 ± 1.46 (a)	**0.02**
ALP (U/l)	987.91 ± 1123.02	533.33 ± 434.34	191.13 ± 119.22 (b)	146.33 ± 114.35 (a)	**0.009**
Albumin (g/dl)	4.03 ± 0.31	4.32 ± 1.09	4.17 ± 0.37	4.24 ± 0.26	0.18

Data are expressed as mean ± SD. (a) = *t*1 vs *t*4 $p < 0.05$; (b) = *t*1 vs *t*3 $p < 0.05$; (c) = *t*1 vs *t*2 $p < 0.05$; (d) = *t*2 vs *t*4 $p < 0.05$; (e) = *t*2 vs *t*3 $p < 0.05$; (f) = *t*3 vs *t*4 $p < 0.05$

Statistically significance is marked with bold characters

Table 3 Evolution of biochemical parameters in PTX group versus non-PTX group

Parameter	PTX treated group			Non-PTX treated group			*p*
	*t*2	*t*3	*t*4	*t*2	*t*3	*t*4	
iPTH (pg/ml)	774.73 ± 1199.38 (c)	653.62 ± 935.18 (b)	746.14 ± 1141 (a)	1109 ± 332.44	887 ± 678.98	987.23 ± 1076.43	0.09
Ca (mg/dl)	8.40 ± 0.88	8.35 ± 1.47	8.36 ± 0.93	8.6 ± 1.1	8.45 ± 0.9	8.43 ± 0.8	0.45
P (mg/dl)	5.20 ± 1.96 (c)	5.71 ± 1.85 (b)	5.80 ± 1.46 (a)	5.34 ± 1.45	5.22 ± 1.34	5.25 ± 1.33	0.67
ALP (U/l)	533.33 ± 434.34	191.13 ± 119.22 (b)	146.33 ± 114.35 (a)	434.44 ± 324.12	378.2 ± 123.7	498.2 ± 432.4	0.76
Albumin (g/dl)	4.32 ± 1.09	4.17 ± 0.37	4.24 ± 0.26	4.12 ± 1.1	4.18 ± 0.56	4.22 ± 0.26	0.23

Data are expressed as mean ± SD. (a) = *t*2 vs *t*3 $p < 0.05$; (b) = *t*2 vs *t*4 $p < 0.05$; (c) = *t*3 vs *t*4 $p < 0.05$

Table 4 Clinical features after treatment for secondary hyperparathyroidism

Symptom	PTX (no. 26)	Non-PTX (no. 26)	*p*
Bone pain improvement—no pts (%)	20/26 (77 %)	6/25 (24 %)	**0.0005**
Muscle weakness improvement—no pts (%)	9/21 (80.76 %)	6/18 (55.55 %)	0.78
Pruritus improvement—no pts (%)	5/9 (55.55 %)	3/12 (33.33 %)	0.20

Statistically significance is marked with bold characters

Subtotal PTX was performed in 19 patients, including 12 females (70.58 %). Mean age in sPTX group was 51.15 ± 2.52 years, and mean HD vintage was 102.5 ± 56.2 months. Total PTX was performed in 7 patients. Total PTX was not followed by autotransplant. Total PTX group included 2 females (28.57 %); the mean age was 52.9 ± 6.72 years, and mean HD vintage was 131.9 ± 104.9 months. Biochemical features after total PTX versus subtotal PTX were not significantly different. Bone pain was alleviated in 9 of 19 patients (47.36 %) in sPTX group and in 5 of 7 patients (71.42 %) in tPTX group ($p = 0.39$). Muscle weakness improved in 4 of 16 patients (25 %) in sPTX group and in 2 of 5 patients (40 %) in tPTX group ($p = 0.59$). Pruritus improved in 3 of 6 patients (50 %) in sPTX group and in 2 of 3 (66.66 %) in tPTX group ($p = 0.89$).

At 24 months of follow-up, 12 non-PTX patients and 4 PTX patients deceased. Two-year survival rate was 84.6 % in PTX group (22 patients survived) and 53.8 % in non-PTX group (14 patients survived). The difference in survival between the two groups was analyzed with Cox regression test. Non-PTX group had a higher mortality risk than PTX group ($p = 0.043$; HR 3.27, 95 %CI 1.04–10.30). The other mineral parameters included in the study did not increase the mortality risk (Table 5). The presence of DM and increased levels of CRP represented also predictors for mortality. Kaplan–Meier curve illustrates survival difference (Fig. 1). In subtotal PTX subgroup, 3 from 19 patients deceased (survival of 84.2 %). In total PTX subgroup, 1 from 7 patients deceased (survival of 85.7 %). The difference between death rates was not significant ($p = 0.78$).

Discussion

Our study reached its goal and established that PTX offers a long-term survival advantage over medical therapy for severe sHPT. There are concerns that PTX can dramatically reduce serum iPTH and P levels, factors closely related to mortality. At the present time, no randomized clinical trial data demonstrated the benefits of PTX, and the relationship between PTX and mortality is not clear enough to become a guideline recommendation [10]. Survival analysis of our patients by therapeutic option revealed better survival

Table 5 Difference in mortality between PTX and non-PTX groups: multivariate analysis

Parameter	Univariate analysis[a]		Multivariate analysis[b]	
	HR (95 % CI)	*p*	HR (95 % CI)	*p*
Intervention (HR for non-PTX)	3.27 (1.04–10.3)	**0.043**	3.50 (1.09–11.18)	**0.035**
Age (years)	1.03 (0.98–1.08)	0.310	1.06 (0.90–1.18)	0.341
Gender	3.37 (0.95–11.95)	0.060	2.97 (0.98–10.45)	0.122
Presence of DM	3.63 (1.31–10.03)	**0.013**	3.85 (1.34–11.02)	**0.012**
Presence of HTN	1.44 (0.46–4.53)	0.531	1.22 (0.42–3.63)	0.643
Presence of cardiac failure	0.92 (0.33–2.53)	0.869	0.82 (0.45–1.5)	0.688
HD vintage (months)	1.00 (0.99–1.00)	0.334	1.00 (0.99–1.80)	0.243
iPTH (pg/ml)	1.00 (1.00–1.00)	0.168	1.02 (0.90–1.10)	0.230
Ca (mg/dl)	0.73 (0.44–1.22)	0.231	0.71 (0.38–1.18)	0.308
P (mg/dl)	1.20 (0.92–1.56)	0.180	1.24 (0.89–1.52)	0.228
ALP (U/l)	1.00 (1.00–1.00)	0.112	1.00 (1.00–1.00)	0.112
Albumin (g/dl)	0.43 (0.09–2.08)	0.294	0.51 (0.12–2.12)	0.314
Hb (g/dl)	0.74 (0.52–1.07)	0.111	0.70 (0.50–1.14)	0.123
CRP (mg/dl)	1.87 (1.23–2.83)	**0.030**	1.88 (1.11–3.19)	**0.019**

HR hazard ratio, *CI* confidence interval, *PTX* parathyroidectomy, *DM* diabetes mellitus, *HTN* hypertension, *HD* hemodialysis, *Ca* calcium, *P* phosphorus, *ALP* alkaline phosphatase, *Hb* hemoglobin, *CRP* C-reactive protein

[a] Cox univariate regression

[b] Cox multivariate regression (ENTER method) with intervention, DM and CRP as independent predictors

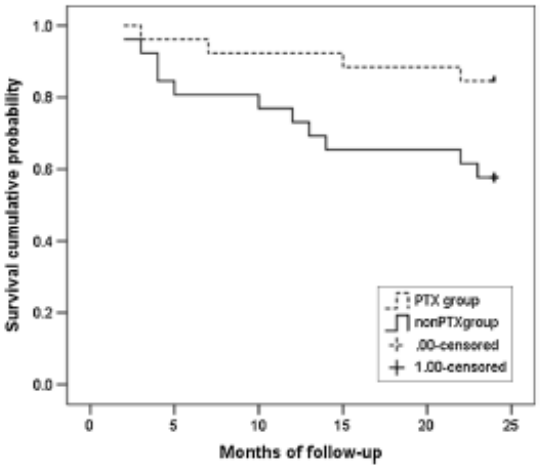

Fig. 1 Kaplan–Meier survival curve illustrates 24-month survival in PTX versus non-PTX group. Survival rate was 84.6 % in PTX group and 53.8 % in non-PTX group ($p = 0.01$)

at 2 years of surgical patients compared to those treated medically for sHPT. This survival advantage is even more important, while the mean values of serum iPTH, Ca and ALP were significantly higher in PTX group at baseline assessment. A recent study realized on nationwide sample of patients receiving HD assessed the clinical events up to 1 year after PTX. Morbidity was significant during the PTX hospitalization and the year after intervention comparing with the year before surgery. In conclusion, the authors recommend caution in the indication of PTX [11]. In contrast to the previous study, a Japanese nationwide case–control study evaluated dialysis patients with severe sHPT. It demonstrated that 1-year mortality rate was significantly higher among patients without a history of PTX comparing with those with a history of PTX [12]. Even if short-term mortality, mostly in immediate postoperative period, was increased, decreased long-term mortality was the main result in other studies [8, 13]. Trombetti et al. [14] suggest that the survival advantage of patients after PTX is because healthier patients with fewer comorbidities are selected for the intervention. Other studies report beneficial effects of protection against cardiovascular events and deaths that occur after PTX treatment [15–18].

The presence of DM and increased levels of CRP represented also predictors for mortality. These results are in concordance with previous studies [19, 20].

There are a number of dilemmas regarding PTX, in relation with the type of intervention, efficiency and safety. Some studies estimate PTX as efficient and safe [4, 18]. Other authors consider that the adverse events risk is too high and recommend medication as preferable [9, 21, 22]. In our study, mineral metabolism markers did not decrease after medication. We consider the surgical treatment as being efficient, because median values of all mineral metabolism markers (Ca, P, ALP and iPTH) have been significantly reduced after PTX. Although it is

worth noting that only a minority reached the targets for optimal iPTH, the rest had too small or remained with high values. This increased percent of patients with remnant sHTP could be due to higher number of sPTX and to its lower efficiency versus tPTX. These results emphasize the existing concerns about the effects of PTX [11, 21]. In terms of improving symptoms, bone pain has been significantly improved in patients after PTX than in those drug treated. Differences between the two groups on improving muscle weakness and alleviating pruritus were not statistically significant. Cheng et al. [23] study applied a 13 symptoms questionnaire in patients with sHPT. They demonstrated that PTX is associated with marked improvement in all symptoms and in quality of life.

The optimal surgical treatment of sHPT has not been clearly defined. Subtotal parathyroidectomy (sPTX) is the resection of 3½ parathyroid glands, with a remnant left in situ. Total parathyroidectomy (tPTX) is the resection of all four parathyroid glands and is often accompanied by autotransplantation of a portion of a normal-appearing gland, usually into the forearm [24]. Both sPTX and tPTX with autotransplant are acceptable surgical treatments [25, 26], although new techniques have been developed [27]. Studies recommend total PTX [28] or subtotal PTX [29, 30] considering recurrence rate, also the risk of adynamic bone disease, hungry bone syndrome or other electrolyte disturbances [7, 31]. Reductions in iPTH and ALP were significantly more important in the group with total PTX versus subtotal PTX. Differences regarding alleviating symptoms were not significant; total and subtotal PTX improved symptoms in a similar manner. Mortality rates were similar in the two subgroups of patients with total PTX and subtotal PTX. Results were similar with those from a large study of Kuo 2015 et al. [32] comparing 30-day morbidity after sPTX and tPTX. They found no difference in the 30-day morbidity, mortality or readmission rates between the two treatments.

sHPTH treatment is nowadays an issue, far from being solved. PTX is regarded as an ultimate therapeutic resource. It is used for refractory sHPT, but often turns out to be either too radical or relatively ineffective. Bringing harmony between pharmacological therapies and interventions on parathyroid glands, we should expect a more efficient management of CKD-MBD, leading to a decrease in mortality and morbidity in these patients.

In this study, it is worth noting that the long-term benefits of PTX versus medical treatment occur at a threshold of iPTH even lower than established by the guidelines as a recommendation to PTX. Given that bone and vascular complications are hardly reversible, when sHPT is severe and mineral and bone disorders are advanced, it makes sense to act not only medically, but also surgically from earlier stages of the disease.

Although prospective, the study has the limit of reduced number of patients. It restrains us to generalize the results. Other limit of the study is the increased number of patients outside the targeted levels of iPTH with high risk of adynamic bone disease or recurrence of the sHPT. The lack of possibility to use lanthanum carbonate, selective vitamin D receptor activators and calcimimetics represents also a limit of our study.

Conclusions

All average values of mineral markers were significantly reduced after PTX, as a proof that surgical treatment was effective. Total PTX was more effective than subtotal PTX regarding lowering iPTH and ALP, but the degree of improvement in symptoms and mortality was similar after both types of surgery. Survival was better in patients surgically treated (PTX) compared to patients medically treated. Thus, successful PTX may reduce the risk of mortality in HD patients with severe, uncontrolled sHPT.

Acknowledgments This paper was published under the frame of European Social Fund, Human Resources Development Operational Program 2007–2013, Project No. POSDRU/159/1.5/138776.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest.

References

1. National Kidney Foundation (2003) K/DOQI clinical practice guidelines for bone metabolism and disease in chronic kidney disease. Am J Kidney Dis 42(suppl 3):S1–S202
2. Eknoyan G, Levin A, Levin NW (2003) Bone metabolism and disease in chronic kidney disease. Am J Kidney Dis 42(4 Suppl 3):1
3. Al Aly Z, Gonzalez EA, Martin KJ et al (2004) Achieving K/DOQI laboratory target values for bone and mineral metabolism: an uphill battle. Am J Nephrol 24:422–426
4. Elder G (2005) Parathyroidectomy in the calcimimetic era. Nephrology 10:511–515
5. Kidney Disease: Improving Global Outcomes (KDIGO) CKD-MBD Work Group (2009) KDIGO clinical practice guideline for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Kidney Int Suppl 113:S1–S130
6. Fernández-Martín JL, Martínez-Camblor P, Dionisi MP et al (2015) Improvement of mineral and bone metabolism markers is associated with better survival in haemodialysis patients: the COSMOS study. Nephrol Dial Transplant 30(9):1542–1551
7. Tentori F, Blayney MJ, Albert JM et al (2008) Mortality risk for dialysis patients with different levels of serum calcium, phosphorus, and PTH: the Dialysis Outcomes and Practice Patterns Study (DOPPS). Am J Kidney Dis 52:519–530

8. Kestenbaum B, Andress DL, Schwartz SM et al (2004) Survival following parathyroidectomy among United States Dialysis patients. Kidney Int 66:2010–2016
9. Goldfarb M, Gondek SS, Lim SM, Farra JC, Nose V, Lew JI (2012) Postoperative hungry bone syndrome in patients with secondary hyperparathyroidism of renal origin. World J Surg 36:1314–1319
10. Covic A, Apetrii M, Heymann EP, Goldsmith D (2012) Medical and surgical management (including diet). Clin Rev Bone Miner Metab 10(3):174–183
11. Ishani A, Liu J, Wetmore JB et al (2015) Clinical outcomes after parathyroidectomy in a nationwide cohort of patients on hemodialysis. Clin J Am Soc Nephrol 10(1):90–97
12. Komaba H, Taniguchi M, Wada A et al (2015) Parathyroidectomy and survival among Japanese hemodialysis patients with secondary hyperparathyroidism. Kidney Int. doi:10.1038/ki.2015.72
13. Ma TL, Hung PH, Jong IC et al (2015) Parathyroidectomy is associated with reduced mortality in hemodialysis patients with secondary hyperparathyroidism. BioMed Res Int. doi:10.1155/2015/639587
14. Trombetti A, Stoermann C, Robert JH et al (2007) Survival after parathyroidectomy in patients with end-stage renal disease and severe hyperparathyroidism. World J Surg 15:1014–1021
15. Costa-Hong V, Jorgetti V, Henrique L et al (2007) Parathyroidectomy reduces cardiovascular events and mortality in renal hyperparathyroidism. Surgery 142(5):699–703
16. Dussol B, Morand P, Martinat C et al (2007) Influence of parathyroidectomy on mortality in hemodialysis patients: a prospective observational study. Ren Fail 29(5):579–586
17. Bleyer AJ, Burkart J, Piazza M et al (2005) Changes in cardiovascular calcification after parathyroidectomy in patients with ESRD. Am J Kidney Dis 46:464
18. Slinin Y, Foley R, Collins AJ (2005) Calcium, phosphorus, parathyroid hormone, and cardiovascular disease in hemodialysis patients: the USRDS waves 1, 3, and 4 study. J Am Soc Nephrol 16:1788–1793
19. Miskulin D, Bragg-Gresham J, Gillespie BW et al (2009) Key comorbid conditions that are predictive of survival among hemodialysis patients. Clin J Am Soc Nephrol 4:1818
20. Yeun JY, Levine RA, Mantadilok V, Kaysen GA (2000) C-reactive protein predicts all-cause and cardiovascular mortality in hemodialysis patients. Am J Kidney Dis 35:469
21. Mazzaferro S, Pasquali M, Farcomeni A et al (2008) Parathyroidectomy as a therapeutic tool for targeting the recommended NKF-K/DOQITM ranges for serum calcium, phosphate and parathyroid hormone in dialysis patients. Nephrol Dial Transplant 23(7):2319–2323
22. Tsai WC, Peng YS, Chiu YL et al (2015) Risk factors for severe hypocalcemia after parathyroidectomy in prevalent dialysis patients with secondary hyperparathyroidism. Int Urol Nephrol 47(7):1203–1207
23. Cheng SP, Lee JJ, Liu TP et al (2014) Parathyroidectomy improves symptomatology and quality of life in patients with secondary hyperparathyroidism. Surgery 155(2):320–328
24. Sadideen HM, Taylor JD, Goldsmith D (2012) Total parathyroidectomy without autotransplantation after renal transplantation for tertiary hyperparathyroidism: long-term follow-up. Int Urol Nephrol 44(1):275–281
25. Schlosser K, Veit JA, Witte S et al (2007) Comparison of total parathyroidectomy without autotransplantation and without thymectomy versus total parathyroidectomy with autotransplantation and with thymectomy for secondary hyperparathyroidism: TOPAR PILOT-Trial. Trials 8:22. doi:10.1186/1745-6215-8-22
26. Iwatomo N, Sato N, Nishida M et al (2012) Total parathyroidectomy improves survival of hemodialysis patients with secondary hyperparathyroidism. J Nephrol 25:755
27. He Q, Zhu J, Zhuang D, Fan Z et al (2015) Robotic total parathyroidectomy by the axillo-bilateral-breast approach for secondary hyperparathyroidism: a feasibility study. J Laparoendosc Adv Surg Tech 25(4):311–313
28. Tominaga Y, Katayama A, Sato T et al (2003) Re-operation is frequently required when parathyroid glands remain after initial parathyroidectomy for advanced secondary hyperparathyroidism in uraemic patients. Nephrol Dial Transplant 18(Suppl 3):iii65–iii70
29. Sharma J, Raggi P, Kutner N et al (2012) Improved long-term survival of dialysis patients after near-total parathyroidectomy. J Am Coll Surg 214(4):400–407 (**discussion 407–408**)
30. Tsai WC, Peng YS, Yang JY et al (2013) Short- and long-term impact of subtotal parathyroidectomy on the achievement of bone and mineral parameters recommended by clinical practice guidelines in dialysis patients: a 12-year single-center experience. Blood Purif 36(2):116–121
31. Yang YL, Lu HF, Chung KC, Jawan B, Chou FF (2015) Young age, male sex, and end-stage renal disease with secondary hyperparathyroidism as risk factors for intraoperative hyperkalemia during parathyroidectomy. J Clin Anesth 27(3):195–200
32. Kuo LE, Wachtel H, Karakousis G, Fraker D, Kelz R (2014) Parathyroidectomy in dialysis patients. J Surg Res 190(2):554–558

Int Urol Nephrol
DOI 10.1007/s11255-013-0422-2

NEPHROLOGY-ORIGINAL PAPER

FGF-23, vascular calcification, and cardiovascular diseases in chronic hemodialysis patients

Diana Moldovan · Ioan Moldovan · Crina Rusu · Ina Kacso · Ioan Mihai Patiu · Mirela Gherman-Caprioara

Received: 25 February 2013 / Accepted: 15 March 2013

Introduction Chronic hemodialysis (HD) patients have bad prognosis and cardiovascular diseases (CVD) represent their main threatening complication. Fibroblast growth factor (FGF-23) has been associated with all kinds of evil consequences, including cardiovascular morbidity, but some studies demonstrated the contrary. Therefore, it is important to know whether FGF-23 is associated with cardiovascular risk or protection. The purpose of this study was to assess the links between FGF-23 and intimal vascular calcification (VC) and with the presence of CVD in chronic HD patients.

Patients and methods This study was carried out on a cohort of randomly selected 88 prevalent HD patients. We recorded demographical, clinical, and biochemical data, including FGF-23. VC was evaluated on carotid ultrasound. CVD were registered.

Results The mean age was 59.68 ± 14.49 years, HD vintage was 59.61 ± 52.39 months, and 20 patients were diabetic (22.72 %). VC was present in 54 patients (61.4 %) and 25 patients (28.4 %) had CVD. FGF-23 correlated positively with HD vintage ($r = 0.37$; $p < 0.001$) and iPTH ($r = 0.21$; $p = 0.048$). FGF-23 did not correlate with VC score. Patients with CVD were older ($p = 0.006$), had lower FGF-23 ($p = 0.008$), higher VC score ($p = 0.009$), lower Hb ($p = 0.008$), albumin ($p = 0.003$), and creatinine ($p = 0.03$). Low FGF-23 was identified as a risk factor for CVD.

Conclusion We report on a novel association between low FGF23 and CVD in chronic HD patients and a lack of correlation of FGF-23 with VC. FGF-23 could play a role in cardiovascular protection that remains to be confirmed in larger studies.

Keywords Fibroblast growth factor 23 · Chronic hemodialysis · Vascular calcification · Cardiovascular diseases

D. Moldovan · C. Rusu · I. Kacso · M. Gherman-Caprioara
Nephrology Department of Emergency County Hospital, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

D. Moldovan (✉)
"Mihai Manasia" Nephrology Clinic, 3-5 Clinics Street, 400006 Cluj-Napoca, Romania
e-mail: dnmoldovan@yahoo.com

I. Moldovan
Military Hospital, Cluj-Napoca, Romania

I. M. Patiu
Nefromed Dialysis Center, Cluj-Napoca, Romania

Introduction

In spite of increasing research and care for end-stage renal disease patients, their mortality is still very high, exceeding 20 % per year [1]. The main cause of mortality is the cardiovascular diseases (CVD) which

Published online: 03 April 2013

was often associated with vascular calcification (VC) [2]. VC is highly prevalent in chronic dialysis patients as we have reported already [3]. Mineral and bone disorders frequently contribute to accelerated VC and CVD, and we have demonstrated that medial VC represents a consequence of increased intact parathyroid hormone levels (iPTH) [3]. Calcification of the media of the arteries and intimal calcification which affects the advanced atherosclerotic plaques can be distinguished and this may be important in view of their different clinical consequences [4]. From a prognostic point of view, the distinction between intima and media calcification appears to be useful as well. London et al. [5] have shown that maintenance hemodialysis (HD) patients with predominant intima calcification have a higher relative risk of mortality than those with predominant media calcification, whose relative risk in turn is much greater than in those with no calcification. Therefore, the study of factors affecting intimal VC is important as well.

Fibroblast growth factor 23 (FGF-23) is a novel discovered biomarker which increases renal phosphate excretion and decreases circulating 1,25-dihydroxyvitamin D concentrations. In recent years, there has been an increasing awareness of the regulatory role of FGF-23 in mineral metabolism [6] and its particular prominence in patients with chronic kidney disease (CKD). FGF-23 is a hormone secreted by osteocytes and osteoblasts and achieves target cell specificity through binding to FGF receptor–Klotho complexes. Some studies support that this bone-derived hormone is linked to early changes in vascular function, arterial stiffness, endothelial dysfunction [7], higher atherosclerosis score [8], predisposing to an increased cardiovascular risk [9] or to peripheral vascular calcification and coronary artery calcification score [10, 11]. Our previous work indicated also close pathogenetic links between bone disease and medial VC in chronic HD patients [12].

Emerging data suggest a potential of FGF-23 to identify CKD patients at high risk for cardiovascular disease and death [13–16] and those who might benefit from early phosphorus-related therapies, such as phosphate binders, active vitamin D, and cinacalcet [17, 18]. On the contrary, animal studies demonstrated that FGF-23 null mice develop VC and FGF-23 is protective against VC [19]. Also, there are clinical studies that have demonstrated the lack of any correlation between FGF-23 and VC [20]. Since alterations in mineral metabolism are associated with increased cardiovascular morbidity and mortality in CKD [10, 15], it is important to know whether FGF-23 is directly associated with cardiovascular risk or protection. In overall actual medical research literature, also, more evidence is required to clarify these issues.

The purpose of this study was to test the links between FGF-23 and intimal atherosclerotic plaques calcifications and between FGF-23 and the presence of cardiovascular diseases in chronic hemodialysis patients. We intended also to detect other risk factors for cardiovascular diseases in hemodialysis patients.

Patients and methods

This study was cross-sectional; it was carried out on a cohort of randomly selected HD patients treated in Nefromed Dialysis Center Cluj-Napoca. Among the overall HD population of 190 patients, 88 were recruited. Eligibility criteria: patients prevalent in dialysis, age >18 years, patients from the morning shift, acceptance to the study protocol. Exclusion criteria: life expectancy less than 6 months, parathyroidectomy, previous renal transplant. We recorded data regarding demographical and clinical characteristics (age, gender, HD vintage, presence of diabetes, dialysate calcium (Ca), HD prescription, treatments with phosphates (P) binders and vitamin D derivates; medical history, cardiovascular diseases). A panel of markers was measured: serum calcium (Ca), inorganic phosphorus (P), alkaline phosphatase (ALP), intact parathyroid hormone (iPTH—Roche second-generation assay), urea, albumin, and C-reactive protein (CRP); fibroblast growth factor 23 (Human FGF-23; ELISA; Uscn Life Science Inc., Wuhan, China). Blood samples for the biochemical evaluation were drawn prior to the HD session. Laboratory tests were performed in a central laboratory. All the samples were taken before a midweek session in the same week of the echographical study. Hemodialysis adequacy was assessed using the clearance of urea (spKt/V) and urea reduction ratio (URR). These were calculated using the next formulas: spKt/V = 2.4*(1−urea post-HD/urea preHD)−0.276 and URR = (1−urea post-HD/urea preHD) * 100.

Carotid vascular calcifications were evaluated on ultrasound, using the LogicScan 64 digital portable

ultrasound machine (Telemed, Lithuania) with a 5–10 MHz linear transducer. The ultrasound examiner was blinded to the clinical and laboratory data. We examined three segments of the arteries (bilateral): common carotid artery (1 cm proximal to the carotid bifurcation), bifurcation (1–2 cm), and internal carotid artery (1 cm distal to the bifurcation). The blood vessels were studied using real-time and color Doppler sonography through longitudinal and transversal sections. The intra-observer reproducibility of this assessment was assured using 2 consecutive ultrasound exams; in case of discordance, a third examination was performed in order to settle the right result. The VC was considered to be calcified atheroma plaques, represented by hyperechoic images with posterior shadows in the arterial walls. The VC's presence was recorded. Among patients with VC, a VC score was counted ranging from 0 (no calcification) to 6 (calcification of all artery sites examined from both sides).

Cardiovascular diseases (CVD) were defined by ischemic heart disease (acute myocardial infarction, angor pectoris, and positive coronarography), heart failure, stroke, heart failure, arrhythmia, aortic aneurysm, and peripheral artery disease. The CVD diagnosis was established by a specialist (cardiologist, neurologist) and the consultation's document was attached to each patient's chart.

Statistics

For continuous factors, data were expressed as mean ± standard deviation (SD) if variables had normal distribution and as median (interquartile range) if the distribution was not normal. Data were expressed as frequencies for qualitative variables. For continuous variables, the statistical comparison was performed using *t* test or Mann–Whitney Rank Sum Test. Chi square or Fisher exact test evaluated the relation between qualitative variables. The Kolmogorov–Smirnov test was employed for the continuous variables to compare the observed cumulative distribution function with the normal distribution. Parametric (Pearson) and nonparametric (Spearman) correlations were determined. Multivariable regression, stepwise method, and logistic models were performed to examine the relationship between the CVD/VC with FGF-23 and clinical and biochemical parameters. A p value <0.05 was taken as statistically significant. All statistical analyses were performed using SPSS 13.0 statistics packages.

Ethical issues: All patients gave their informed consent. Their privacy was respected. The study protocol was approved by the University Ethics Committee.

Results

The overall HD population in Nefromed Dialysis Center consisted in 190 HD patients. Among them, 19.12 % had diabetes. Eighty-eight patients entered in the study, 45 were males (51.13 %) and 20 patients were diabetic (22.72 %). The mean age was 59.68 ± 14.49 years, ranging from 20 to 87 years. They had been on HD for between 3 and 253 months, with a mean of 59.61 ± 52.39 months (Table 1). They achieved mean spKt/V of 1.53 ± 0.28, being on a standard hemodialysis schedule of 3 × 4 h/week.

Table 1 Demographical, clinical and biochemical parameters of the total study population

Parameter	All 88 patients
Age (years)	61 (53–71.75)
HD vintage (months)	48 (29–65.5)
Male gender (%)	51.13
DM (%)	22.72
FGF-23 (pg/ml)	43.5 (23.1–72.7)
VC score	2 (0–5)
URR (%)	75.96 (69.54–80.16)
spKt/V	1.54 (1.39–1.64)
Bicarbonate (mEq/l)	23.45 ± 3.12
Calcium (mg/dl)	9.06 (8.68–9.46)
Phosphate (mg/dl)	4.44 ± 1.31
iPTH (pg/ml)	267.6 (149.8–552.1)
ALP (UI/l)	75.96 (56.09–100.97)
Hb (g/dl)	11.32 ± 1.4
Ferritin (ng/ml)	564.04 (334.62–807.88)
CRP (mg/dl)	0.66 (0.30–1.36)
Albumin (g/dl)	3.86 (3.62–4.04)
Creatinine (mg/dl)	8.43 ± 2.24

HD hemodialysis, *DM* diabetes mellitus, *FGF-23* fibroblast growth factor 23, *VC score* vascular calcification score, *URR* urea reduction ratio, *spKt/V* urea clearance, *iPTH* parathyroid hormone, *ALP* alkaline phosphatase, *Hb* hemoglobin, *CRP* C-reactive protein

Dialisate Ca had a concentration of 1.25 mmol/l in 31 patients (35.2 %), 1.5 mmol/l in 45 patients (51.1 %), and 1.75 mmol/l in 12 patients (13.6 %). Regarding treatments for mineral metabolism, 47 patients received calcium-based phosphates binders (53.4 %), 20 patients received sevelamer hydrochloride (22.7 %) and 20 patients received vitamin D compounds (22.7 %). Twenty-five patients (28.4 %) had CVD. Hypertension was diagnosed and treated in 63 patients (71.6 %). Carotidal VC was present in 54 patients (61.4 %). Serum FGF-23 levels ranged from 7.6 to 290.8 pg/ml.

FGF-23 correlated positively with HD vintage ($r = 0.37$; $p < 0.001$) and iPTH ($r = 0.21$; $p = 0.048$). FGF-23 did not correlate with the severity of VC score ($r = -0.06$; $p = 0.57$) or serum *P* levels ($r = -0.10$; $p = 0.37$). VC scores correlated positively with age ($r = 0.50$; $p < 0.001$) and negatively with albumin ($r = -0.21$; $p = 0.05$).

A comparison between the patients with and without CVD was performed. Serum FGF-23 levels were significantly lower in the group of HD patients with CVD ($p = 0.008$).

Patients with CVD were older ($p = 0.006$), had significantly more calcified plaques (higher mean VC score) ($p = 0.009$), and were more anemic (lower Hb) ($p = 0.008$). The patients with CVD were more affected by protein malnutrition (had lower albumin ($p = 0.003$) and creatinine levels ($p = 0.03$)). The number of diabetics was significantly higher in the group with CVD (Table 2). The HD efficacy was not different between the two studied groups.

All the studied parameters were introduced in logistic regression stepwise method for CVD: age, FGF-23, and albumin entered in the equation. The enter method introduced in logistic regression only factors significant on univariate analysis and important factors for CVD; it found only FGF-23 as significant predictor for CVD (Table 3).

We also compared the characteristics of patients with VC (VC score > 0) and those without VC (VC score = 0). The patients with VC had significantly increased age, HD vintage, and creatinine and lower ALP (Table 4). It was a trend for a higher FGF-23 in VC group versus the group without VC ($p = 0.05$). In the multiple regression models, stepwise method considered VC as dependent variable and all the other studied parameters as independent variables. Age, HD vintage, and serum creatinine were found to be significant predictors for VC's presence. We also used the enter method for logistic regression; only age remained predictive for the development of VC (Table 5).

Discussion

In the current study, we for the first time report an association between low circulating FGF-23 and the presence of cardiovascular diseases in chronic HD patients. Therefore, increased serum FGF-23 levels could be a protective factor against CVD development. Some authors proposed the FGF-23 measurement in CKD as it could predict faster progression of renal disease in CKD patients [21]. Indeed, a positive relationship between FGF-23 and cardiovascular risk and mortality in HD patients and across the spectrum of CKD has been the result for some studies [22–25]. But this view over the data may not be reliable regarding FGF-23, because it has been discovered as a protection factor against vascular damage. Animal studies demonstrated that FGF-23 null mice display extensive VC and these results support the idea of VC inhibition properties of FGF-23 [19, 26]. Consistent with our study and in contrast with the above-mentioned clinical studies, Ashikaga et al. [27] indicated that FGF-23 was a negative predictor of an increase in carotid intima-media thickness, as marker of atherosclerosis. The FGF-23 level was also an independent negative predictor of peripheral VC, but not aortic medial VC, and FGF-23 behavior was independent of serum phosphate (P) levels, leading to conclusion that FGF-23 had a protective effect on VC [28]. In a cohort of men without CKD, plasma FGF-23, and incident coronary heart disease had not been associated [29] and some authors demonstrated that FGF-23 is not associated with mortality in HD patients, only if they selected a special subgroup [30]. Interestingly, in the study of Gutierrez et al. [7], the association between FGF-23 and cardiovascular pathology (assessed by left ventricular hypertrophy and left ventricular mass index as surrogate markers) lost its significance after multivariable adjustment. Genetically modified animal models have provided valuable insights into the role of FGF-23 in health and disease. Interestingly, FGF-23$^{-/-}$ mice develop a widespread phenotype resembling human premature aging, including muscle wasting, infertility, atrophy of

Table 2 Comparison of clinical and biochemical profiles of hemodialysis patients with and without cardiovascular diseases

Parameter	No CVD (63 pts)	CVD (25 pts)	*p*
Age (years)	58 (49–70)	65 (60–74)	**0.006**
HD vintage (months)	50 (29–80)	39 (27.5–55)	0.13
Male gender (%)	34 pts (53.9 %)	11 pts (44 %)	0.48
DM (%)	11 pts (17.46 %)	9 pts (36 %)	**<0.001**
HTA (%)	48 pts (76.2 %)	15 pts (60 %)	0.10
FGF-23 (pg/ml)	47.2 (26.1–94.9)	30.1 (15.05–45.5)	**0.008**
VC (%)	36 pts (57.1 %)	18 pts (72 %)	0.14
VC score	2 (0–4)	4 (0–6)	**0.009**
URR	76.88 (69.84–81.03)	71.56 (67.04–78.19)	0.36
spKt/V	1.56 (1.40–1.66)	1.44 (1.33–1.60)	0.36
Bicarbonate (mEq/l)	23.1 ± 3.27	24.31 ± 2.55	0.10
Calcium (mg/dl)	9.17 (8.76–9.54)	8.03 (8.52–9.40)	0.21
Phosphate (mg/dl)	4.55 ± 1.32	4.18 ± 1.26	0.23
iPTH (pg/ml)	279.3 (149.8–564.5)	192.1 (109.97–494.57)	0.21
ALP (UI/l)	75.8 (56.07–99.56)	76.13 (56.07–113)	0.54
Hb (g/dl)	11.57 ± 1.39	10.70 ± 1.25	**0.008**
Ferritin (ng/ml)	569.9 (319.24–816.89)	498.51 (359.04–780.77)	0.77
CRP (mg/dl)	0.63 (0.25–1.33)	0.77 (0.35–2.64)	0.39
Albumin (g/dl)	3.89 (3.69–4.07)	3.75 (3.36–3.94)	**0.003**
Creatinine (mg/dl)	8.75 ± 2.36	7.64 ± 1.69	**0.03**
Ca-based P binders use (%)	32 pts (50.8 %)	15 pts (60 %)	0.29
Sevelamer use (%)	16 pts (25.4 %)	4 pts (16 %)	0.25
Vitamin D use (%)	14 pts (22.22 %)	6 pts (24 %)	0.85

HD hemodialysis, *DM* diabetes mellitus, *HTA* hypertension, *FGF-23* fibroblast growth factor 23, *VC* vascular calcification, *URR* urea reduction ratio, *spKt/V* urea clearance, *iPTH* parathyroid hormone, *ALP* alkaline phosphatase, *Hb* hemoglobin, *CRP* C-reactive protein, *Ca* calcium, *P* phosphate

Table 3 Multiple regression analysis of factors associated with the presence of cardiovascular diseases in chronic hemodialysis patients

Independent variable	*p*	OR	95.0 % CI for OR	
			Lower	Upper
Age	0.10	1.05	0.99	1.11
Gender	0.48	1.63	0.42	6.25
HD vintage	0.34	1.01	0.99	1.03
DM	0.05	0.25	0.06	0.99
FGF-23	0.02	0.97	0.94	1.00
VC score	0.20	1.20	0.90	1.60
Hb	0.06	0.51	0.26	1.02
Albumin	0.35	0.35	0.04	3.26
Creatinine	0.64	0.92	0.64	1.32

HD hemodialysis, *DM* diabetes mellitus, *FGF-23* fibroblast growth factor 23, *VC* vascular calcification, *Hb* hemoglobin

multiple organ systems, pulmonary emphysema, osteoporosis, atherosclerosis, extensive soft tissue calcifications, and a severely shortened lifespan [19]. Genetic restoration of the systemic actions of human FGF-23 in FGF-23-knockout mice reverses hyperphosphatemia to hypophosphatemia and prevents aging associated complications, including cardiovascular diseases [31].

Hyperphosphatemia increases death risk in HD patients [1], and usually, FGF-23 and serum P display a positive relationship in CKD [21]. FGF-23 decreases serum P, and lower P levels are inevitably linked to a reduction in cardiovascular risk in HD patients. Therefore, higher FGF-23 should decline the cardiovascular risk. In spite of the fact that, in our study, FGF-23 did not correlate with serum P levels, high FGF-23 was associated with lower rate of CVD. It is

Table 4 Comparison of clinical and biochemical profiles of chronic hemodialysis patients with and without carotid vascular calcifications

Parameter	No VC (34 pts)	VC (54 pts)	*p*
Age (years)	54 (38.75–60)	65 (58.75–73)	**0.01**
HD vintage (months)	36.5 (13.75–60.05)	50 (36–78.75)	**0.04**
Male gender (%)	18 pts (52.94 %)	27 pts (50 %)	0.48
DM (%)	7 pts (20.58 %)	13 pts (24.07 %)	0.70
HTA (%)	22 pts (64.70 %)	41 pts (75.92 %)	0.25
FGF-23 (pg/ml)	41.1 (18.5–64.52)	43.8 (22.97–80.42)	**0.05**
URR	76.33 (70.31–81.86)	74.19 (68.38–79.71)	0.57
spKt/V	1.55 (1.41–1.68)	1.50 (1.36–1.63)	0.57
Bicarbonate (mEq/l)	23.41 ± 3.68	23.46 ± 2.74	0.93
Calcium (mg/dl)	9.08 (8.59–9.48)	9.06 (8.75–9.47)	0.83
Phosphate (mg/dl)	4.42 ± 1.38	4.44 ± 1.27	0.94
iPTH (pg/ml)	215.2 (140.57–608.82)	286.8 (154.1–541.45)	0.65
ALP (UI/l)	69.51 (45.29–97.12)	77.98 (63.69–110.47)	**0.003**
Hb (g/dl)	10.98 ± 1.51	11.53 ± 1.29	0.27
Ferritin (ng/ml)	542.56 (307.74–824.97)	564 (371.1–794.48)	0.88
CRP (mg/dl)	1.57 (0.18–1.29)	0.68 (0.30–1.40)	0.17
Albumin (g/dl)	3.95 (3.73–4.07)	3.79 (3.61–3.97)	0.24
Creatinine (mg/dl)	8.29 ± 2.72	8.45 ± 1.90	**0.01**
Ca-based P binders use (%)	16 pts (47 %)	31 pts (54 %)	0.34
Sevelamer use (%)	8 pts (23.50 %)	12 pts (22.22 %)	0.88
Vitamin D use (%)	6 pts (17.64 %)	14 pts (25.92 %)	0.36

HD hemodialysis, *DM* diabetes mellitus, *HTA* hypertension, *FGF-23* fibroblast growth factor 23, *VC* vascular calcification, *URR* urea reduction ratio, *spKt/V* urea clearance, *iPTH* parathyroid hormone, *ALP* alkaline phosphatase, *Hb* hemoglobin, *CRP* C-reactive protein, *Ca* calcium, *P* phosphate

Table 5 Multiple regression analysis of factors associated with the presence of carotid vascular calcifications in chronic hemodialysis patients

Independent variable	*p*	OR	95.0 % CI for OR	
			Lower	Upper
Age	<0.0001	1.21	1.11	1.32
HD vintage	0.07	1.02	1.00	1.04
DM	0.65	0.67	0.12	3.86
FGF-23	0.31	1.01	0.99	1.02
Creatinine	0.13	1.32	0.92	1.90
ALP	1.00	1.00	0.99	1.01
URR	0.16	0.96	0.90	1.02
Calcium	0.83	0.88	0.28	2.75
Phosphate	0.50	1.21	0.69	2.14
iPTH	0.46	1.00	1.00	1.00

HD hemodialysis, *DM* diabetes mellitus, *FGF-23* fibroblast growth factor 23, *ALP* alkaline phosphatase, *URR* urea reduction ratio, *iPTH* parathyroid hormone

noteworthy a very recent paper by Shalhoub et al. [32]. In order to reduce PTH, monoclonal antibodies against FGF-23 were administered to a rat model. The authors reported that mortality increased with decreasing FGF-23. This study illustrates the danger in leaping from epidemiologic studies that associate elevated FGF-23 with adverse consequences to thinking that if we reduce these levels, we will improve possible consequences [33].

In our study, the patients with CVD were older, with more extended VC. They were significantly more affected by protein malnutrition and anemia. Advanced age and low albumin were recognized as additional risk factors for CVD.

FGF-23 correlated positively with iPTH; similar evidence has derived from Nakanishi et al. [34, 35] who reported that FGF-23 is a predictor of secondary hyperparathyroidism.

Current evidence on the association of FGF-23 with VC is mixed; some studies demonstrated a positive and independent association with aortic [11], peripheral [10] or coronary calcification in HD patients [36]. Other studies reported negative correlations. Tamei et al. [37] had studied the relation of FGF-23 with the progression of VC; they demonstrated that FGF-23 level in repressors was significantly higher than in non-progressors and progressors. Inaba et al. [28] showed that FGF-23 is linked to peripheral VC in prevalent HD patients. But, in our study, FGF-23 was significantly higher with increasing HD vintage; VC score increased with age. Our study failed to demonstrate any correlation between FGF-23 and the severity of VC (VC score). Similar results had Roos et al. [20], reporting that FGF-23 did not correlate with coronary artery calcification in patients with normal renal function. Testing the relationship between FGF-23 and aortic calcification, Kojima et al. [38] also demonstrated a lack of significance.

FGF-23 was higher in the group of patients with VC than those without VC, but this association became non-significant after multivariable adjustment. Increasing age was found as risk factor for VC in the present study, in conformity with others [10].

At the present time, the exact mechanisms of FGF-23 influence on VC and CVD development remains uncertain. Additional studies are warranted to evaluate whether low FGF-23 can predict bad cardiovascular outcomes, and whether it is a modifiable risk factor. There were a few limitations of our study, such as its observational and its cross-sectional nature. Our analysis was limited by the study population and the subjective manner of ultrasound examination.

In conclusion, we report on a novel association between low FGF-23 and CVD in chronic HD patients. To our knowledge, only few investigations have explored the relationship between FGF-23 and the presence of CVD in the HD patients. The use of FGF-23 as a clinical marker or for predicting cardiovascular outcomes has to be established subsequently. It remains to be determined whether FGF-23 plays a direct role in cardiovascular protection, or whether FGF-23 intervenes in atherosclerotic complications indirectly through regulation of mineral metabolism.

Acknowledgments We thank the medical staff of Nefromed Dialysis Center for the help in collecting medical records, Rodica Rahaian for determining plasma FGF-23 levels and Cosmina Bondor for assistance in the statistical analysis. This work was supported by a research grant from "Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca.

Conflict of interest Dr D Moldovan, Dr Kacso and Prof Dr Gherman have served as speakers for Amgen. Dr Ioan Moldovan has served as a speaker for GlaxoSmithKline and Boehringer Ingelheim. Dr Rusu and Dr Patiu have served as speakers for Abbott.

References

1. Block GA, Klassen PS, Lazarus JM, Ofsthun N, Lowrie EG, Chertow GM (2004) Mineral metabolism, mortality, and morbidity in maintenance hemodialysis. J Am Soc Nephrol 15(8):2208–2218
2. Adragao T, Pires A, Lucas C et al (2004) A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. Nephrol Dial Transpl 19(6):1480–1488
3. Moldovan D, Rusu C, Patiu IM et al (2010) Could the serum parathormone be a predictive marker for peripheral vascular calcification in chronic dialysis patients? Acta Endo (Buc) 1:43–55
4. Druke T (2008) Arterial intima and media calcification: distinct entities with different pathogenesis or all the same? Clin J Am Soc Nephrol 3:1583–1584
5. London GM, Guérin AP, Marchais SJ, Métivier F, Pannier B, Adda H (2003) Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. Nephrol Dial Transpl 18(9):1731–1740
6. Razzaque MS (2009) The FGF23–klotho axis: endocrine regulation of phosphate homeostasis. Nat Rev Endocrinol 5:611–619
7. Mirza MA, Larsson A, Melhus H et al (2009) Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. Atherosclerosis 207: 546–551
8. Mirza MA, Hansen T, Johansson L et al (2009) Relationship between circulating FGF23 and total body atherosclerosis in the community. Nephrol Dial Transpl 24:3125–3131
9. Bernheim J, Benchetrit S (2011) The potential roles of FGF23 and Klotho in the prognosis of renal and cardiovascular diseases. Nephrol Dial Transpl 26(8):2433–2438
10. Jean G, Bresson E, Terrat JC et al (2009) Peripheral vascular calcification in long-haemodialysis patients: associated factors and survival consequences. Nephrol Dial Transpl 24:948–955
11. Nasrallah MM, El-Shehaby AR, Salem MM et al (2010) Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification in haemodialysis patients. Nephrol Dial Transpl 25(8):2679–2685
12. Moldovan D, Moldovan I, Rusu C et al (2011) Vascular calcifications and renal osteodystrophy in chronic hemodialysis patients: what is the relationship between them? Int Urol Nephrol 43:1179–1186
13. Seiler S, Reichart B, Roth D, Seibert E et al (2010) FGF-23 and future cardiovascular events in patients with chronic kidney disease before initiation of dialysis treatment. Nephrol Dial Transpl 25(12):3983–3989

14. Kirkpantur A, Balci M, Gurbuz OA et al (2011) Serum fibroblast growth factor-23 (FGF-23) levels are independently associated with left ventricular mass and myocardial performance index in maintenance haemodialysis patients. Nephrol Dial Transpl 26(4):1346–1354
15. Gutierrez OM, Januzzi JL, Isakova T et al (2009) Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. Circulation 119:2545–2552
16. Hsu HJ, Wu MS (2009) Fibroblast growth factor 23: a possible cause of left ventricular hypertrophy in hemodialysis patients. Am J Med Sci 337:116–122
17. Oliveira RB, Cancela ALE, Graciolli FG et al (2010) Early Control of PTH and FGF23 in Normophosphatemic CKD Patients: a New Target in CKD-MBD Therapy? Clin J Am Soc Nephrol 5:286–291
18. Ford ML, Smith ER, Tomlinson LA et al (2012) FGF-23 and osteoprotegerin are independently associated with myocardial damage in chronic kidney disease stages 3 and 4. Another link between chronic kidney disease–mineral bone disorder and the heart. Nephrol Dial Transpl 27(2):727–733
19. Shimada T, Kakitani M, Yamazaki Y et al (2004) Targeted ablation of FGF23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest 113:561–568
20. Roos M, Lutz J, Salmhofer H et al (2008) Relation between plasma fibroblast growth factor-23, serum fetuin-A levels and coronary artery calcification evaluated by multislice computed tomography in patients with normal kidney function. Clin Endocrinol 68(4):660–665
21. Fliser D, Kollerits B, Neyer U et al (2007) Fibroblast growth factor 23 (FGF23) predicts progression of chronic kidney disease: the Mild to Moderate Kidney Disease (MMKD) Study. J Am Soc Nephrol 18(9):2600–2608
22. Gutierrez OM, Mannstadt M, Isakova T et al (2008) Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 359:584–592
23. Seiler S, Cremers B, Rebling NM et al (2011) The phosphatonin fibroblast growth factor 23 links calcium-phosphate metabolism with left-ventricular dysfunction and atrial fibrillation. Eur Heart J 32(21):2688–2696
24. Jean G, Terrat JC, Vanel T et al (2009) High levels of serum fibroblast growth factor (FGF)-23 are associated with increased mortality in long haemodialysis patients. Nephrol Dial Transpl 24:2792–2796
25. Isakova T, Xie Huiliang, Yang Wei et al (2011) Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. JAMA 305(23):2432–2439
26. Stubbs JR, Liu S, Tang W et al (2007) Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. J Am Soc Nephrol 18:2116–2124
27. Ashikaga E, Honda H, Suzuki H et al (2010) Impact of fibroblast growth factor 23 on lipids and atherosclerosis in hemodialysis patients. Ther Apher Dial 14(3):315–322
28. Inaba M, Okuno S, Imanishi Y et al (2006) Role of fibroblast growth factor-23 in peripheral vascular calcification in non-diabetic and diabetic hemodialysis patients. OsteoporosInt 17(10):1506–1513
29. Taylor EN, Rimm EB, Stampfer MJ, Curhan GC (2011) Plasma fibroblast growth factor 23, parathyroid hormone, phosphorus, and risk of coronary heart disease. Am Heart J 161(5):956–962
30. Olauson H, Qureshi AR, Miyamoto T et al (2010) Relation between serum fibroblast growth factor-23 level and mortality in incident dialysis patients: are gender and cardiovascular disease confounding the relationship? Nephrol Dial Transpl 25(9):3033–3038
31. DeLuca S, Sitara D, Kang K et al (2008) Amelioration of the premature aging-like features of FGF-23 knockout mice by genetically restoring the systemic actions of FGF-23. J Pathol 216:345–355
32. Shalhoub V, Shatzen EM, Ward SC et al (2012) FGF23 neutralization improves chronic kidney disease-associated hyperparathyroidism yet increases mortality. J Clin Invest 122:2543–2553
33. Moe OW (2012) Fibroblast growth factor 23: friend or foe in uremia? J Clin Invest 122:2354–2356
34. Nakanishi S, Kazama J, Nii-Kono T et al (2005) Serum fibroblast growth factor-23 levels predict the future refractory hyperparathyroidism in dialysis patients. Kidney Int 67:1171–1178
35. Schoppet M, Hofbauer LC, Brinskelle-Schmal N et al (2012) Serum level of the phosphaturic factor FGF23 is associated with abdominal aortic calcification in men: the STRAMBO study. J Clin Endocrinol Metab 97(4):E575–E583
36. Coen G, De Paolis P, Ballanti P et al (2011) Peripheral artery calcifications evaluated by histology correlate to those detected by CT: relationships with fetuin-A and FGF-23. J Nephrol 24(3):313–321
37. Tamei N, Ogawa T, Ishida H et al (2011) Serum fibroblast growth factor-23 levels and progression of aortic arch calcification in non-diabetic patients on chronic hemodialysis. J Atheroscler Thromb 18:217–223
38. Kojima F, Uchida K, Ogawa T et al (2008) Plasma levels of fibroblast growth factor-23 and mineral metabolism in diabetic and non-diabetic patients on chronic hemodialysis. Int Urol Nephrol 40:1067–1074

International Journal of
Molecular Sciences

MDPI

Review

The Spectrum of Minimal Change Disease/Focal Segmental Glomerulosclerosis: From Pathogenesis to Proteomic Biomarker Research

Yuriy Maslyennikov [1], Andrada Alina Bărar [1], Crina Claudia Rusu [1], Alina Ramona Potra [1], Dacian Tirinescu [1], Maria Ticala [1], Alexandra Urs [1], Ioana Ecaterina Pralea [2], Cristina Adela Iuga [2,3], Diana Tania Moldovan [1,*] and Ina Maria Kacso [1]

[1] Department of Nephrology, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, 400012 Cluj-Napoca, Romania; maslyennikov_yuriy@elearn.umfcluj.ro (Y.M.); barar_andrada_alina@elearn.umfcluj.ro (A.A.B.); claudia.rusu@umfcluj.ro (C.C.R.); alina.potra@umfcluj.ro (A.R.P.); tirinescu.dacian@umfcluj.ro (D.T.); cosa.maria@umfcluj.ro (M.T.); alexandra.urs@elearn.umfcluj.ro (A.U.); maria.kacso@umfcluj.ro (I.M.K.)
[2] Department of Personalized Medicine and Rare Diseases, MedFuture—Research Centre for Biomedical Research, "Iuliu Haţieganu" University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania; pralea.ioana@umfcluj.ro (I.E.P.); cristina.iuga@medfuture.ro (C.A.I.)
[3] Department of Drug Analysis, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy, 400349 Cluj-Napoca, Romania
* Correspondence: diana.moldovan@umfcluj.ro; Tel.: +40-264-592-202

Abstract: Podocyte injury plays a central role in both focal segmental glomerulosclerosis (FSGS) and minimal change disease (MCD). Pathogenic mechanisms are diverse and incompletely understood, partially overlap between FSGS and MCD, and are not reflected by kidney biopsy. In order to optimize the current variable response to treatment, personalized management should rely on pathogenesis. One promising approach involves identifying biomarkers associated with specific pathogenic pathways. With the advancement of technology, proteomic studies could be a valuable tool to improve knowledge in this area and define valid biomarkers, as they have in other areas of glomerular disease. This work attempts to cover and discuss the main mechanisms of podocyte injury, followed by a review of the recent literature on proteomic biomarker studies in podocytopathies. Most of these studies have been conducted on biofluids, while tissue proteomic studies applied to podocytopathies remain limited. While we recognize the importance of non-invasive biofluid biomarkers, we propose a sequential approach for their development: tissue proteomics could first identify proteins with increased expression that may reflect underlying disease mechanisms; subsequently, the validation of these proteins in urine or plasma could pave the way to a diagnostic and prognostic biomarker-based approach.

Keywords: proteomics; minimal change disease; focal segmental glomerulosclerosis; podocytopathies; biomarkers

Academic Editors: Flávio Reis, Alice Santos-Silva and Susana Coimbra

Received: 18 January 2025
Revised: 4 March 2025
Accepted: 6 March 2025
Published: 9 March 2025

Citation: Maslyennikov, Y.; Bărar, A.A.; Rusu, C.C.; Potra, A.R.; Tirinescu, D.; Ticala, M.; Urs, A.; Pralea, I.E.; Iuga, C.A.; Moldovan, D.T.; et al. The Spectrum of Minimal Change Disease/Focal Segmental Glomerulosclerosis: From Pathogenesis to Proteomic Biomarker Research. *Int. J. Mol. Sci.* **2025**, *26*, 2450. https://doi.org/10.3390/ijms26062450

1. Background

While genomics has provided critical insights into the genetic foundations of diseases, proteomics, supported by recent technological advancements, offers a more immediate and dynamic understanding of disease mechanisms, biomarker identification, and treatment responses. This capability is particularly valuable in the context of personalized

medicine, where tailored treatment strategies depend on a comprehensive understanding of an individual's molecular profile. As such, proteomics serves as a vital complement to genomics in advancing modern pathology.

In the past two decades, in some glomerular diseases, tissue proteomics have clarified pathogenic mechanisms and led to the development of diagnostic and prognostic biofluid biomarkers promptly embedded in clinical practice [1]. In an area with an urgent need for pathogenic clarification and reclassification, such as minimal change disease (MCD) and focal segmental glomerulosclerosis (FSGS), proteomic studies have the potential to make a significant impact.

2. Podocyte Injury in the Continuum of MCD/FSGS

Minimal change disease and FSGS are both podocyte disorders primarily characterized by nephrotic syndrome associated with various degrees of foot process effacement (FPE) observed on electron microscopy (EM). The key distinction lies in light microscopy (LM) where FSGS presents with patterns of focal and segmental sclerosis while LM is typically normal in MCD. Additionally, MCD is associated with negative immunofluorescence staining, whereas in FSGS, low intensity complement deposition and/or IgM may be observed in the sclerotic areas, traditionally regarded as non-specific (trapping) [2,3]. From a clinical and therapeutic perspective, current guidelines suggest a classification of FSGS into primary, genetic, secondary and FSGS of undetermined cause [4]. Primary FSGS is characterized by diffuse FPE (generally over 80%) nephrotic syndrome; a specific cause cannot usually be identified. Secondary FSGS mostly presents with important proteinuria, possibly in the nephrotic range but without overt hypoalbuminemia; it has less abundant FPE (25–40%) and is usually due to adaptive mechanisms to focal podocyte injury. In genetic causes of FSGS, the degree of FPE is variable, from extensive in certain monogenic causes (e.g., NPHS 1) to focal in other instances (e.g., ACTN4 mutation that cause cytoskeletal structural changes that decrease podocyte resistance to injury or mechanical stress). FSGS of undetermined cause refers to forms without extensive FPE but without an identifiable secondary or genetic cause [5,6]. From a clinical point of view, it is important to distinguish between primary FSGS (amenable to immunosuppression) and secondary forms, treated conservatively. However, it is now widely recognized that this morphology-based classification is insufficient and does not fully capture the complexity of podocyte diseases.

It is generally accepted that FSGS is a pattern of response to various etiopathogenic factors, but it becomes increasingly clear that MCD, traditionally viewed as a distinct disorder, may also be included in this spectrum of diseases with different triggers and patterns of response. In fact, a unifying approach has been considered for the MCD/FSGS spectrum [2]. This perspective is reinforced by evidence that, in certain cases, progression from MCD to FSGS has been documented, with sampling error and the earlier stage of the disease potentially explaining an increased likelihood for observing an MCD pattern [2,7]. However, the most compelling argument for considering these diseases together lies in the shared podocyte injury and its direct consequences, namely, FPE and proteinuria.

A podocyte is a highly specialized and ontogenetically conserved cell with a primordial role in maintaining permselectivity of the glomerular filtration. Tertiary interdigitating foot processes (FPs) from neighboring podocytes are linked through a highly specialized protein structure—the slit diaphragm (SD). In its structure, several proteins form a ladder-like structure, which plays a crucial role in the glomerular filtration process by preventing protein loss. Additionally, podocytes are covered with anion charge, assured by the glycocalyx of the cells, which enhances further the filtration barrier [5,8].

The stability of a podocyte is assured through its anchoring to the glomerular basement membrane (GBM) via a specialized molecular assembly which is called focal

adhesion (FA). These are intricate protein structures which link extracellular matrix (ECM) and GBM to the podocyte cytoskeleton. FA includes integrin as one of the most important transmembrane anchoring proteins [9]; importantly, FAs, like SD, do not just act as a mechanical anchor but also as a signaling hub, mediating the podocyte response to various stimuli. They play a critical role in regulating cell behavior and motility [9,10], influencing processes like cell migration survival, which are important for maintaining glomerular integrity and function.

Podocyte function is highly dependent on the integrity of its cytoskeleton that is mainly composed of actin fibers in the foot processes and of microtubules in the cellular body. The organization of the actin cytoskeleton is fine-tuned by proteins that control the polymerization and depolymerization of actin filaments as well as proteins that link these filaments into bundles or networks—referred to as actin-binding proteins. Key regulatory molecules, including RHO-GTPases such as Rac1, Rho A, and Cdc42, as well as other intracellular pathways, are responsible for regulating the actin cytoskeleton dynamics by interacting with signals from the SD and FA [9,10].

Noteworthy, podocytes are highly specialized and differentiated cells that do not divide. Lethal or sublethal injury to podocytes results in exposed areas of GBM, leading to the disruption of filtration barrier, proteinuria, and potentially subsequent inflammatory and profibrotic changes.

As a direct consequence of structural and functional alterations within podocytes and their actin cytoskeletons, the interdigitating structure of the FP becomes distorted, leading to FPE and compromising the permselectivity of the glomerular filtration barrier, resulting in proteinuria. However, the mechanisms underlying these morphological and functional changes differ significantly, both from an etiologic point of view and from a pathogenetic perspective.

First, an important differentiation is to be made regarding cytoskeleton rearrangement and subsequent FPE as a reactive process or as the initial culprit in podocyte disease [11–13]. In the first scenario, when there is focal (limited and not widespread) injury to the podocytes, there is an attempt to limit the degree of damage by structural and functional changes in the affected and neighboring cells that occur as an adaptative process. This might be the case in hyperfiltration-induced podocyte damage when FPE is the price paid for strengthened attachment at the level of FA; in this case, the podocyte sacrifices permselectivity in order to prevent further podocyte loss by improving adherence. On the other hand, in the second scenario, extensive and diffuse structural and functional changes in the cytoskeleton are the initial cause of FPE and proteinuria, resulting from genetic disease, antibody- or other immune-mediated injury, or yet unknown causes; in this case, the etiology and mechanisms are also very different [11,13,14]. The key pathways involved in this process are listed below and depicted in Figures 1–3, which detail the intricate molecular interactions that contribute to podocyte dysfunction in the scenarios mentioned above.

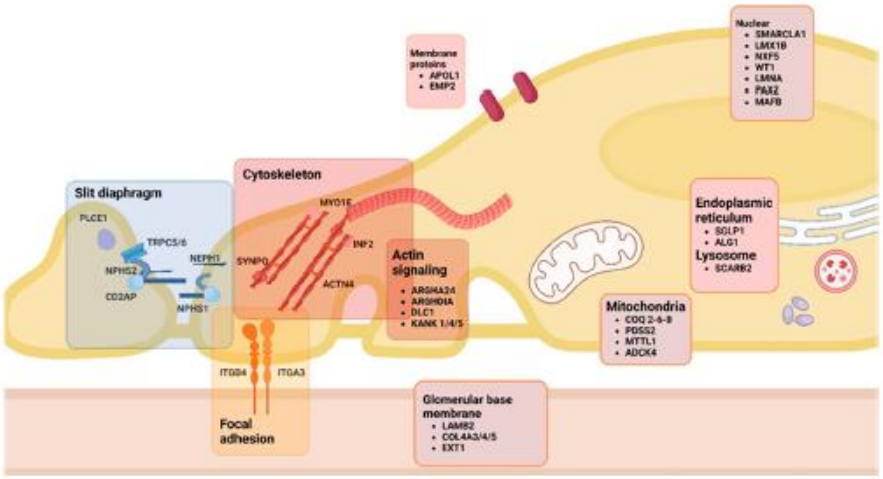

Figure 1. Schematic representation of podocyte pathogenic genes. Gene variants are grouped according to cellular structure. PLCE1, phospholipase C epsilon 1; TRPC 5/6, transient receptor potential channel 5/6; NPHS1, nephrin; NPHS2, podocin; CD2AP, CD2-associated protein; NEPH1, Kin of IRRE-like protein 1; MYO1E, myosin 1E; SYNPO, synaptopodin; INF2, inverted-formin2; ACTN4, alpha actinin 4; ITGB4, integrin ß4; ITGA3, integrin α3; ARGHA24, Rho-GTPase-activating protein 24; ARGHDIA, Rho-GDP-dissociation inhibitor 1; DLC1, Rho GTPase-activating protein 7; KANK 1/4/5, KN motif and ankyrin repeat domains 1/4/5; LAMB2, laminin subunit beta 2; COL4A3/4/5, collagen type IV aplha 3/4/5 chain; APOL1, Apolipoprotein A1; EMP2, epithelial membrane protein2; COQ 2/6/8, coenzime Q2/6/8; PDSS2, decaprenyl-diphosphate synthase subunit 2; MTTL1, mitochondrially encoded tRNA leucine 1; ADCK4, coenzyme Q8B; SGLP1, sphingosine-1-phosphate lyase 1; ALG1, chitobiosyldiphosphodolichol beta-mannosyltransferase; SCARB2, lysosomal integral membrane protein-2; SMARCLA1, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein 1; LMX1B, LIM homeobox transcription factor 1-beta; NXF5, nuclear RNA Export Factor 5; WT1, Wilms tumor 1; LMNA, lamin A/C; PAX2, paired box gene 2; MAFB, transcriptor factor MafB. This figure was created with BioRender.com.

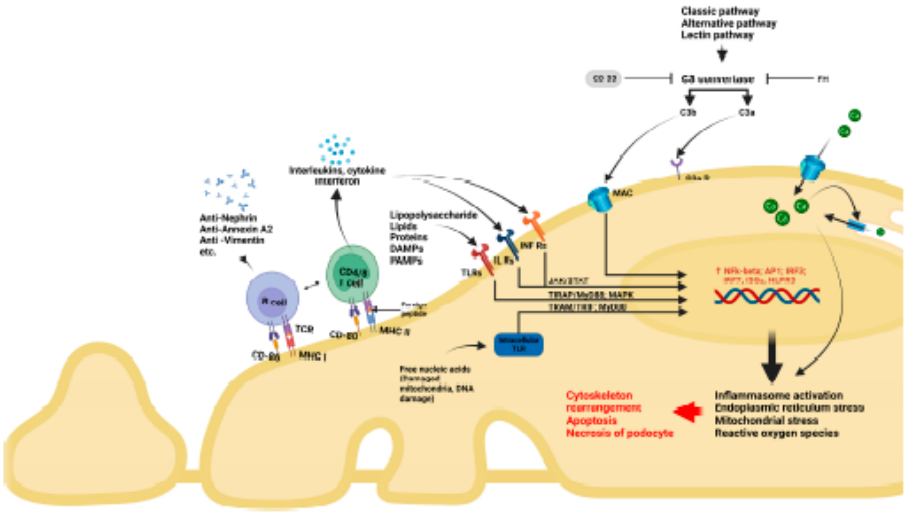

Figure 2. Schematic representation of immune-mediated podocyte injury. Podocytes interact with immune cells through major histocompatibility complex type I (MHC I) and II (MHC II) but also possess co-stimulatory molecules for B lymphocytes (CD86) and for T lymphocytes (CD80). Imbalance between effector and regulatory T lymphocytes, interleukin/ cytokine synthesis, and B cell-mediated antibody synthesis against podocyte structures can occur due to podocyte interaction with immune cells. Toll-like receptors (TLRs) can detect signals like pathogen-related molecular patterns (PAMPs) and damage-related molecular pattern (DAMPs) both from outside (e.g., lipopolysaccharide, lipids, and proteins) and within the cell (e.g., free nucleic acids). Upon binding TLRs, increased transcription of inflammatory genes (NF-kß, IRF3, IRF7, and AP1) and inflammasome activation are mediated through intracellular signaling proteins such as TIRAP, MyD88 and MAPK, ultimately resulting in cytoskeleton rearrangement and apoptosis. Interleukin receptors (IL Rs) and interferon receptors (INF Rs) also increase the transcription of inflammatory and profibrotic factors through the JAK/STAT signaling pathway. Complement-mediated podocyte injury can occur due to sublytic amounts of membrane attack complex (MAC). The activation of the NF-kß pathway and a rapid increase in intracellular Ca^{2+} induce NFk-ß-mediated inflammatory response, mitochondrial stress, oxidative stress, and endoplasmic reticulum stress. The lack of inhibitor factors such as CD55 or complement factor H (FH) can trigger and intensify MAC production. This figure was created with BioRender.com.

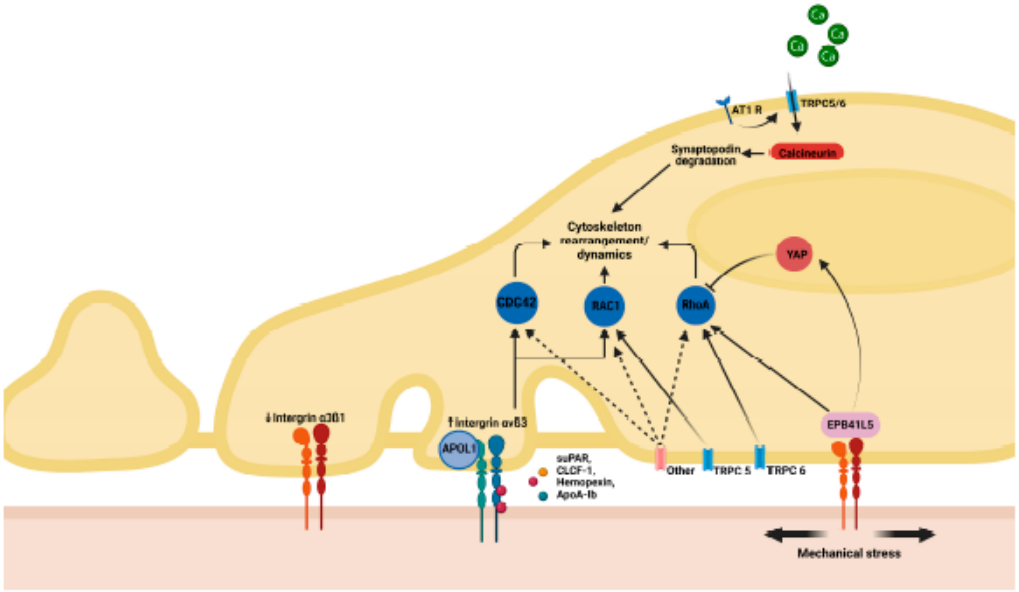

Figure 3. Schematic representation of mechanical stress-related podocyte injury. Angiotensin II receptor 1 (AT1 R) upon stimulation causes increased intracellular calcium influx through transient receptor potential channel 6 (TRPC6), followed by increased calcineurin activity and increased degradation of synaptopodin, an actin-binding protein. Cytoskeletal rearrangements are controlled by Rho GTPases: cell division control protein 42 (CDC42), Rac family small GTPase 1 (RAC1), and Ras homolog family member A (RhoA). Mechanical stress is sensed and transmitted by B integrins to erythrocyte membrane protein band 4.1 like 5 (EPB41L5). EPB41L5 activates intracellular pathways which regulate the translation of proteins that influence RhoA activity and cytoskeletal rearrangement. Yes-associated protein (YAP) is a transcription co-regulator factor stimulated by EPBB41L5 activity. YAP nuclear translocation results in the translation of proteins that regulate podocyte Rho GTPase activity. Membrane proteins like TRPC5/6 also can directly regulate the activity of Rho GTPases, other membrane proteins may be also involved in Rho GTPase regulation (dashed arrows). Permeability factors (soluble urokinase plasminogen activator receptor, suPAR; hemopexin, HPX; and cardiotrophin-like cytokine factor 1, CLCF1) interfere with FA; in particular, suPAR binds and activates αvß3 B integrin, further modulating cytoskeleton rearrangements via CDC42 and RAC1. APOL1 expression increases the linking of suPAR. This figure was created with BioRender.com.

2.1. Structural Deficiencies

Structural deficiencies of the SD, actin cytoskeleton, and FA can be the cause of podocyte dysfunction. Currently, more than 70 monogenic causes of proteinuric kidney disease have been identified, many of which comprise proteins of the SD structure (such as NEPH1, NPHS1, and NPHS2), as well as some proteins involved in the regulation of the actin cytoskeleton (ACTN4, INF2, etc.). Additionally, defects in myosin proteins and Rho-GTPase signaling pathways have been linked to podocyte dysfunction. Moreover, variants in genes encoding structures from GBM and ECM, like laminins and collagen, can lead to the dysfunction of filtration processes [15,16]. Podocyte pathogenic variants are depicted in Figure 1.

2.2. Virus-Induced Lesions

Other potential drivers of podocyte injury are virus-induced factors. Studies have demonstrated that podocytes possess sensors for double-stranded RNA, a byproduct of viral replication, and can lead to an innate response. This response includes an increase in the expression of toll-like receptors (TLRs) and other signaling molecules, resulting in the altering of podocyte SD structures including proteins such as NPHS1, NPHS2, and CD2AP. These changes, in turn, promote increased cytokine production and inflammation via the NF-κB pathway [17]. Investigations in murine models showed that interferon beta, a major component of antiviral response, can lead to podocyte loss [18]. In the context of HIV-associated nephropathy, it was shown that viruses are present in podocytes, directly damaging the actin cytoskeleton and contributing to podocyte dysfunction [19].

Sometimes, these mechanisms can act in concert or could be additive. For example, the two distinct polymorphisms in the apolipoprotein L1 (APOL1) gene (G1 and G2) that are frequent in West African patients and have monoallelic or biallelic inheritance (e.g., G1/G1, G1/G2, or G2/G2) are risk factors for FSGS, with the highest risk in people presenting two risk variants—some studies suggesting an up to 17-fold increase in risk [20,21]. However, a "second podocyte hit", such as HIV-associated viral infection, increases this risk even more [22].

2.3. Permeability Factors

Several studies have proposed that permeability factors may play a crucial role in the pathogenesis of certain podocyte diseases. This hypothesis is sustained by several observations. For instance, patients with primary FSGS develop recurrence shortly after kidney transplantation, suggesting the presence of circulating factors that may drive podocyte injury. Furthermore, these patients often experience significant reduction in proteinuria after plasmapheresis, which provides additional evidence for the existence of a circulating permeability factor. Also, it was reported that murine models developed proteinuria following the injection of sera from patients with FSGS [23]. These findings collectively suggest that permeability factors could play a crucial role in driving podocyte injury. Hemopexin [24], circulating ANGPTL4 [25], suPAR [26], CLCF1 [27], and Apolipoprotein A-Ib (ApoA-Ib) [28,29] have been suggested as potential permeability factors. Research has also identified several intracellular pathways involved in the action of these factors. For instance, CLCF1 has been shown to activate intracellular cascades such as Janus Kinase 2 (JAK2) and Signal Transducer and Activator of Transcription 3 (STAT3), which ultimately affect cytoskeleton polymerization and can interfere with ß1 integrins binding at the FA level [30]. Apolipoprotein A-Ib is a form of Apolipoprotein A-I that was found to be associated with recurrence of FSGS in kidney-transplant patients; the pathogenic pathway is unknown, yet one proposed mechanism suggests an abnormal activity of a protease, producing both ApoA-Ib and podocyte dysfunction [31].

2.4. Immune-Mediated Injury

Immune mechanisms are an important component undermining podocyte integrity, and several distinct pathways may be involved in this process (Figure 2):

- T lymphocytes have long been suspected of being involved in podocyte dysfunction, particularly in MCD. Podocytes express major histocompatibility factor I/II proteins which are involved in immune recognition, as well as B7-1 (CD80), which are T cell co-stimulatory molecules. These influence the activation of lymphocytes, trigger imbalance between effector and regulatory T lymphocytes, as well as interleukin (IL) and other cytokine synthesis by T lymphocytes. For example, IL-17 synthesis by CD17 lymphocytes, with subsequent TNF-α-induced inflammatory cascade [32] and

inflammatory responses [33], are all consequences of podocyte/immune cell interactions. More recently, the role of B lymphocyte-driven mechanisms in podocyte injury has gained attention. Podocytes also express B7-2 (CD86), a co-stimulatory molecule that plays a key role in the activation of B lymphocytes and antigen-presenting cells [34]. Recent studies have convincingly documented antibody-mediated podocyte injury, providing evidence for the pathogenic role of nephrin antibodies in steroid-responsive MCD [35]. In addition to nephrin, several antibodies, such as those against annexin A2 [36], or other components of the cytoskeleton [37] have been suggested as etiological factors in certain subsets of podocyte diseases.

- Podocytes also express recognition receptors such as TLRs, which can recognize pathogen-associated molecular patterns (PAMPs), including DNA/RNA fragments. Upon recognition, TLRs activate intracellular pathways that ultimately lead to generation of reactive oxygen species (ROS), mitochondrial stress, and endoplasmic reticulum stress [38]. Additionally, podocytes can respond to metabolic stimuli (e.g., hyperglycemia) and toxic environment stimuli (e.g., puromycin aminoxide toxicity). Such stimuli lead to the activation of intracellular pathways such as NF-κB or MAPK, triggering the production of inflammatory cytokines (IL-1 and IL-18) and TGF-ß, increase oxidative stress, and activate the inflammasome, ultimately resulting in podocyte damage and apoptosis [34,39,40].
- Podocytes are increasingly recognized for their role in regulating the complement system. They are able to synthetize components of the complement system including C1q, C1r, C2, C3, C3a receptor (C3aR), C5a receptor (C5aR), and C7, as well as their inhibitors such as CD47, CD55, CD59, soluble complement factor I (CFI), and complement factor H (CFH). This enables podocytes to precisely regulate inflammatory responses triggered by complement, thereby supporting glomerular homeostasis and reducing damage caused by complement activation. However, podocytes can also become targets of complement-mediated injury under certain instances. For example, in an elegant experimental model of adriamycin-induced podocyte injury, the lack of a C3 convertase inhibitor (CD55) exacerbated podocyte injury and proteinuria, suggesting that complement regulation is essential for podocyte protection [41]. Moreover, podocytes are heavily dependent on factor H protection against excessive complement activation, as complement factors can leak into the GBM, especially under proteinuric conditions. Factor H is able to inactivate them by N terminal binding while being anchored through the C terminal fragment to components of the GBM. Additionally, studies have shown that puromycin/immunotoxin-induced podocyte injury leads to increased factor H expression in podocytes, which correlates with the clearance of subendothelial immune complex deposits [42]. Finally, recent evidence suggests that the activation of the lectin pathway of the complement system may contribute to the pathogenesis of FSGS [43].
- Recent studies have increasingly recognized that sub-lytic levels of the membrane attack complex (MAC) can induce podocyte injury without causing cell death. When internalized, excess MAC can influence calcium (Ca^{2+}) entry into the cell, leading to a cascade of downstream effects. One major consequence is the activation of the NF-κB-mediated inflammatory response, which can play a critical role in podocyte dysfunction. Additionally, excessive calcium influx can dysregulate oxidative stress pathways and cause endoplasmic reticulum and mitochondrial stress [44].

2.5. Toxic-Induced Podocyte Damage

Puromycin and adryamicin are among the most commonly used substances to induce FSGS and MCD in murine models. Both puromycin aminonucleoside nephrosis and adriamycin-induced nephropathy have a highly proteinuric early phase, resembling the

MCD model characterized by complete FPE. This is followed by the progressive development of segmental sclerosis lesions, resembling FSGS. In humans, one of the most frequent toxic causes of podocyte injury is the use of anabolic-androgenic steroids [45], which disrupts SD structure through excess complement activation [43] and increases the production of profibrotic cytokines (TGF-ß) and inflammation [46].

2.6. Mechanical Stress

As previously mentioned, an important source of podocyte dysfunction is mechanical stress. Podocytes play a crucial role in maintaining glomerular filtration, and their intricate cytoskeletal structure is continuously adapting to the environmental changes sensed by FA and SD complexes. However, under conditions of hemodynamic stress and/or structural deficiencies, mechanical forces can overwhelm the adaptability of the podocyte, resulting in FPE or even podocyte detachment. Increased glomerular capillary pressure results in the enlargement of capillaries and causes stress to foot processes, enabling elongation and/or shape modification. Glomerular hypertrophy, a universal adaptative response mechanism after kidney injury, is primarily mediated through the activation of tubulo-glomerular feedback and serves as a major driver of mechanical podocyte injury [10,44]. Recent studies have identified several pathways involved in regulating cytoskeletal conformation under mechanical stress. For example, the interaction of suPAR leads to the increased expression of αvß3 B integrin, especially in patients with the APOL1 gene variant. This interaction activates Rho GTPases like CDC42, RhoA, and RAC1, contributing to cytoskeleton dysfunction. Additionally, tensile stress is transmitted by ß integrins to the erythrocyte membrane protein band 4.1 like 5 (EPB41L5), and through multiple signaling molecules (e.g., YAP), it modulates Rho-GTPase activity. The activation of TRPC channels by angiotensin receptors or mechanical stress leads to intracellular calcium influx, which, in turn, activates calcineurin, resulting in synaptopodin degradation. Additionally, increased intracellular calcium promotes the production of reactive oxygen species and mitochondrial and endoplasmic reticulum stress, and can lead to NF-κB and NFAT pathway activation, leading to inflammation and further podocyte damage. Moreover, RhoA1, Rac1, and CDC42 can be directly regulated by the activity of TRPC channels and other ligands [10].

Given the fact that proteinuria and FPE in podocyte diseases are primarily the result of cytoskeleton rearrangements but triggered by different etiologies and mediated by various intracellular pathways, we propose that a classification system based on etiopathogenetic mechanisms—rather than morphology—would be more adequate for this spectrum of diseases. This approach could also potentially lead to more precise diagnostic and therapeutic strategies, ultimately improving patient outcomes by targeting the underlying molecular pathways driving podocyte dysfunction.

3. From Pathogenesis to Therapeutic Approach

The choice of therapy for podocyte diseases should be tailored to the pathogenetic mechanism underlying the condition, as the effectiveness of the therapeutic approaches is often contingent on the underlying cause.

For instance, steroid resistance occurs in 8–25% of MCD and FSGS cases, with resistance being more common in FSGS than in MCD. Additionally, steroid resistance is the most important prognostic factor for kidney failure [47].

Corticosteroids exert their effects by binding to steroid receptors on podocytes, leading to the suppression of proinflammatory mechanisms including the synthesis of ILs and TNF-α [48]. Furthermore, corticosteroids help stabilize actin filaments, prevent apoptosis, and increase the expression of the nephrin gene, thereby stabilizing the SD structure [49]. In cases of corticosteroid resistance, cyclophosphamide may be used as it enhances CD4+

T-cell activation, modulates the TLR/MyD88/MAPK pathway, and reduces Th17 generation, thus exerting immunosuppressive effects that help manage podocyte injury [50].

Calcineurin inhibitors, such as ciclosporin and tacrolimus, not only act as immunosuppressants, but also directly stabilize the cytoskeleton in podocytes primarily by inhibiting synaptopodin degradation, regulating podocyte dynamics, and reducing cell apoptosis [51–53].

With the discovery of anti-nephrin antibodies (and other antibodies targeting the cytoskeleton) as a culprit in the pathogenesis of MCD/FSGS, B-cell suppression using rituximab has emerged as an interesting therapeutic option, allowing for more prolonged remissions, and is highlighted as a promising approach in a recent position paper of the Immunology European Renal Association Working Group [47]. However, several other potential therapies are under investigation that are directed against some of the pathogenic mechanisms mentioned. These include anti-TNFα therapy, B-cell targeted therapy, and the blockade of TRPC 5/6 channels to regulate intracellular calcium entry. Also, targeting intracellular proinflammatory pathways is a future strategy being explored [47]. Complement inhibition strategies in the current effervescent era of anti-complement therapy validated in different glomerular diseases could also be an option for research, but more data and study trials are required [54].

The current management and diagnosis strategy for MCD and FSGS is based on kidney biopsy. Putting aside its invasive nature, the main shortcoming of the current approach is its inability to identify the underlying etiology and pathophysiological mechanisms at play, and hence its inability to guide a targeted treatment approach. Additionally, biopsy findings often fail to differentiate clearly between primary (amenable to immunosuppression) and secondary/genetic forms of FSGS [5]. Hence, reliance on histology solely must be reevaluated.

A more personalized and optimized approach is clearly needed. Such approaches should be guided by a deeper understanding of the specific molecular mechanisms underlying each patient's condition, enabling more precise diagnosis and tailored treatment options.

4. Advancements in Biomarker Discovery: Insights from Proteomic Approaches

A potential way to guide therapy is the identification of the pathogenic pathway involved by means of biomarkers. This approach could not only help to pinpoint the underlying mechanisms involved in individual cases, but also aid in selecting patients included in clinical trials of targeted therapy in order to maximize the chance of discovering efficient treatment strategies.

Therefore, research on biofluid (urine and plasma) proteomic biomarkers that can both identify the disease (MCD/FSGS) and point to a specific pathogenic pathway—regardless of the morphological form of podocyte injury—offers a promising approach. Furthermore, these biomarkers could potentially predict the response to treatment. Several attempts have been made over the past decades to identify panels of biomarkers that allow for the non-invasive characterization of MCD/FSGS in both experimental and human diseases. These studies build upon previous findings from genomics and transcriptomics, which have offered insights into podocyte disease mechanisms. However, we do not dwell on these data as they are beyond the scope of the current review, especially since increased gene expression does not necessarily result in protein translation or directly influence disease phenotype.

The main advantage of biofluid proteomics is represented by its non-invasive nature. Moreover, urinary proteomics has the potential to directly reflect modifications within the kidney. However, there are also potential downsides due to the nature of the disease;

namely, plasma-filtered proteins in these heavily proteinuric conditions may affect urinary findings or, conversely, the accumulation of plasma proteins secondary to decreased glomerular filtration rate can confound the findings. A summary of key proteomic studies and their main findings is provided in Table 1 and discussed below.

Table 1. Current proteomic research results relevant to podocyte diseases and their main findings.

Study	Groups	Tissue/Biofluid	Method	Significance
Shui et al. [55], 2008	(Murine model) FSGS and healthy controls	Urine	2DE, MALDI-TOF MS	FSGS—upregulated proteins involved hemodynamic disturbance, apoptosis, ECM protein deposition, and sclerosis (COL4A1, ECM-1, KLK, KNG1 precursor, ANXA1, CDH1, and ADAM32)
Sedic et al. [56], 2014	12 INS, 12 healthy controls	Urine	LC-MS	INS—74 proteins upregulated, 9 potential biomarkers Oxidative stress may be a pathogenic mechanism
Candiano et al. [57], 2006	10 MCD, 7 FSGS 6 MN, 10 healthy controls	Urine/Plasma	2DE, MALDI-TOF/MS	MCD/FSGS/MN—upregulated Albumin fragments and SERPINA1 in urine and plasma compared to healthy controls
Nafar et al. [58], 2014	11 FSGS,6 IgAN, 8 healthy controls	Urine	nano-LC/MS	FSGS—upregulated CD59, CD44, IBP7, Robo4, and DPEP1
Muruve et al. [59], 2022	15 MCD, 37 MN, 20 healthy controls	Plasma	SOMAscan	MCD—70-protein signature; serpin family proteins downregulated compared to MN/healthy controls (SERPINA10, SERPINA4, SERPINC1, SERPINF2, and SERPINF1); complement system and coagulation pathway downregulated MCD—upregulated immune and growth factor signaling proteins (STAT1, STAT3, CD40LG, and FGF16); carbohydrate and lipid metabolism (GAPDH, GSK3A/B, PKM2, HK2, CHST6, LRP1B, APOE, and APOA1) compared to MN/healthy controls
Choi et al. [60], 2017	Discovery cohort: 4 MCD, 4 FSGS, 4 MN, 4 healthy controls Validation cohort: 13 MCD, 5 FSGS, 26 MN, 9 IgAN, 8 healthy controls	Urine	SDS-PAGE, LC-MS	MCD—upregulated CD14, C9, and SERPINA1 FSGS—upregulated CDH26, RNASE1, and DIS3L
Araumi et al. [61], 2021	14 MCD, 11 DN, 23 MN	Urine/Plasma	nano-LC/MS	DN—upregulated urinary RBP4 and SH3BGRL3 compared to MCD
Navarro-Muñoz et al. [62], 2012	9 FSGS, 3 MCD, 9 IgAN, 6 MN, 7 healthy controls	Urine	HPLC-MS/MS	MCD/FSGS—upregulated SERPINA1 and downregulated UMOD compared to healthy controls
Perez et al. [63], 2014	22 FSGS, 22 MCD	Urine	MALDI-TOF MS	UMOD and SERPINA1 can differentiate between FSGS and MCD
Perez et al. [64], 2017	25 FSGS, 24 MCD	Urine	2D-DIGE; MALDI-TOF MS	MCD—upregulated SERPINA1, PTAFR, CCNY, TF, HTN3, and MRPL17 FSGS—upregulated CALB2
Chebotareva et al. [65], 2022	30 FSGS, 9 MCD	Urine	LC-MS	"Severe" FSGS vs. "mild" FSGS/MCD—upregulated complement activity (C4b, C9, CFB, and CFI); upregulated podocyte damage (VTN, HPX, GSN, and APOA1); upregulated ECM accumulation (CST3, DBP, RBP4, AHSG, SERPING1, LUM, and CLU)
Suresh et al. [66], 2016	55 INS: 5 SRNS MCD, 5 SRNS FSGS, 2 SRNS MN	Urine	iTRAQ LC/MS	SRNS FSGS vs. SRNS MCD—upregulated A2M and ORM2
Catanese et al. [67], 2023	19 primary FSGS, 44 secondary FSGS	Urine	CE-MS	Primary FSGS vs. secondary FSGS—upregulated collagen fragments, SERPINA1, UBE3A, RNF146, complement C3, and PLG; Downregulated fragment of PIGR.

Hellin et al. [68], 2009	15 idiopathic FSGS, 11 genetic FSGS	Plasma	2DE, MALDI-TOF MS, Western blot, LC-ES-MS	Three very low-molecular-mass albumin fragments in plasma of patients with genetic FSGS vs. idiopathic FSGS/ healthy controls
Zhao et al. [69], 2014	(Murine model) FSGS	Urine	LC-MS	FSGS—urine protein change pattern in time: upregulation of AFM and CP; downregulation of CDH2 and ACAN; distinct pattern of FETUB and B2M
Bai et al. [70], 2012	9 SRNS, 32 SSNS	Urine	Chip-MS	SRNS—upregulated SAMDC1, FKBP1A, and rpsK SRNS—downregulated rpmF
Kalantari et al. [71], 2014	5 mild FSGS, 5 advanced FSGS	Urine	nano-LC/MS	Mild FSGS—upregulated DNASE2 Advanced FSGS—upregulated HP Complement and coagulation pathways activated in FSGS
Chhuon et al. [28], 2023	4 recurrent FSGS, 4 non-INS controls; post-transplant	Plasma	nano-LC-MS/MS	Recurrent FSGS—upregulated neutrophil degranulation; downregulated platelet degranulation and lipid-binding proteins; dysregulation of mTOR pathway
Lopez-Hellin et al. [29], 2012	6 recurrent FSGS, 34 non-recurrent FSGS; post-transplant	Urine	2DE/MALDI-TOF/LC-ESI-MS/MS	Urinary ApoA-Ib associated with recurrent FSGS
Andersen et al. [72], 2012	4 INS remission/active disease	Plasma/Urine	nano-LC/MS	Active disease—downregulated urinary CDH1, CDH3, KLKB1, HPX
Piyaphanee et al. [73], 2011	19 SRNS, 15 SSNS, 10 healthy controls	Urine	MALDI-TOF/MS	A1BG is associated with SRNS FSGS
Kalantari et al. [74], 2014	6 SS FSGS, 4 SR FSGS	Urine	LC-MS	SS—upregulated APOA1 SR—upregulated MXRA8 Acute inflammatory response was the predominant biological process (CLUS, A1AG2, AACT, and TRFE)
Dong et al. [75], 2023	3 MCD, 11 IgAN, 19 LN. 5 MN, 8 healthy controls	Tissue	LCM + nano-LC-MS/MS	MCD/IgAN vs. LN/MN—downregulated CD59; A2M upregulated in every group but not in MCD; downregulated FLII (regulatory cytoskeleton protein) in glomerular disease versus control
Bărar et al. [76], 2023	6 MCD, 9 FSGS, 3 healthy controls	Tissue	LC-MS/MS	58 significant proteins between the 3 groups
Bărar et al. [12], 2023	5 MCD, 3 healthy controls	Tissue	LC-MS/MS	MCD—upregulated ANXA2 and NID1 MCD—downregulated ZO-1, MYO1C, ITGA3, ACTR3B, and NES
Merchant et al. [77], 2020	Collapsing FSGS 7, NOS-FSGS 6, healthy controls	Tissue/Urine	LC-MS/MS	Collapsing FSGS—distinct pattern of sclerosis compared to other form of FSGS; upregulated cathepsin B and cathepsin C in tissue

Abbreviations used: FSGS—Focal Segmental Glomerulosclerosis; MCD—Minimal Change Disease; MN—Membranous Nephropathy; IgAN—Immunoglobulin A Nephropathy; SRNS—Steroid-Resistant Nephrotic Syndrome; INS—Idiopathic Nephrotic Syndrome; SS—Steroid-Sensitive; SR—Steroid-Resistant; LN—Lupus Nephritis.

4.1. MCD/FSGS Versus Other Nephrotic Syndromes/Healthy Controls

One potential use of urinary and plasma proteomics is to identify a panel of proteins that are able to non-invasively differentiate primary podocytopathies (MCD vs. FSGS) from other nephrotic conditions or healthy controls. When compared to controls, the urinary proteomic profile of the murine model of FSGS showed upregulated proteins involved in hemodynamic disturbance, apoptosis, ECM protein deposition, and sclerosis including COL4A1, ECM-1, KLK, KNG1 precursor, ANXA1, CDH1, and ADAM32 [55]. Additionally, molecules involved in oxidative stress were differentially expressed in the urine of children with idiopathic nephrotic syndrome compared to controls [56]. Another study showed that albumin fragments and α1-antitrypsin can differentiate MCD/FSGS/membranous glomerulopathy (MN) from controls [57]. In a separate study, a

comparison of urine samples from 11 patients with FSGS, 6 patients with IgA nephropathy (IgAN), and 8 healthy controls revealed that CD59, CD44, IBP7, Robo4, and DPEP1 were the most significantly differentially expressed proteins for FSGS [58]. In another study, a panel of 65 plasma proteins was shown to be differentially expressed in MCD versus MN or healthy controls, with members of the serpin family identified as a signature of MCD [59]. Elevated urinary SERPINA1, alongside CD14 and C9, was suggested to be associated with primary podocyte diseases and proposed as a signature panel for MCD [60]. In the same line of research, RBP4 and SH3BGRL3 were found to differentiate between MCD and diabetic nephropathy [61]. Moreover, downregulated urinary UMOD and upregulated SERPINA1 were found to differentiate MCD and FSGS from healthy controls [62].

4.2. MCD Versus FSGS

A complementary approach aims to differentiate MCD from FSGS, and several contributions are noted in this regard. In a study by Perez et al. [63], using the MALDI-TOF proteomic technique, discovered that urinary proteinsUMOD, SERPINA1, B2M, and ALB can help differentiate MCD from FSGS. In a distinct study, it was observed that SERPINA1, TF, HTN3, and MRPL17 levels were reduced in FSGS when compared to MCD, whereas CALB2 levels were elevated [64]. Additionally, Apolipoprotein A1, Alpha 2 macroglobulin (A2M), and ORM2 could differentiate steroid-resistant MCD from steroid-resistant FSGS. In the same study, significantly higher values of retinol-binding protein 4 (RBP4) were observed in patients with FSGS compared to those with MCD [66]. Another study identified ApoA4, HPX, VTN, GSN, and components of the complement system (C4b and factors B and I), along with retinol- and vitamin D-binding proteins, as proteins able to differentiate MCD from FSGS (differentiated according to the severity of the disease and response to treatment [65]). Notably, in the same study, patients classified with the "severe" form of FSGS exhibited increased urinary proteins including components of the MAC (C8a and C9) and downregulation of the protective factor CD59.

4.3. Primary Versus Secondary FSGS

Another interesting and contributive approach is aimed at differentiating primary from secondary FSGS. This is important from a clinical and therapeutic point of view as the therapeutic approach is fundamentally different. In this regard, Hellin et al. [68] described three very low-molecular-weight albumin fragments which were present in the plasma of patients with genetic FSGS as opposed to idiopathic FSGS, offering a potential biomarker for distinguishing between these two etiologies. Furthermore, a comprehensive study has described a panel of 93 proteins, including upregulated collagen fragments, SERPINA1, UBE3A, RNF146, complement C3, and PLG, as well as a downregulated fragment of PIGR, which collectively serve as biomarkers to differentiate primary from secondary FSGS [67].

4.4. Prognosis and Response to Treatment

The proteomic characterization of biofluids can be used as a non-invasive approach for predicting response to treatment and prognosis in podocyte diseases. There are several attempts published in this area, with one notable investigation using a murine model of adriamycin-induced FSGS. This study showed specific trends in the urine proteome pattern as disease progressed: the upregulation of proteins such as AFM and CP and the downregulation of CDH2 and ACAN were observed throughout the course of the disease. In contrast, distinct trends for FETUB and B2M were noted in different stages of FSGS in mice [69]. Several studies have identified differentially expressed proteins that may serve as biomarkers to differentiate between subtypes of nephrotic syndrome or predict

prognosis. For instance, differentially expressed proteins such as rpmF, SAMDC1, FKBP1A, and rpsK were claimed to differentiate between steroid-sensitive nephrotic syndrome and steroid-resistant nephrotic syndrome [70]. Also, decreased urinary CDH1and CDH3 were observed in patients in remission from nephrotic syndrome, suggesting their potential contribution to pathogenesis [72]. Kalantari et al. [71] also identified a panel of urinary prognostic biomarkers in patients with FSGS, with DNASE2 and HP showing the greatest fold change in terms of overrepresentation and underrepresentation in FSGS patients with the best and worse prognosis. A particular form of Apolipoprotein, Apo-A1b, was first increased in the urine of FSGS patients with relapsing disease but not in genetic or non-relapsing forms [29]. Chhuon et al. [28] shed light on some potential mechanisms involved in FSGS recurrence, by comparing plasma proteomic findings (including soluble proteins and extracellular vesicle proteomic profile) of post-transplant recurrent FSGS patients to non-INS patients and healthy controls: upregulated proteins were involved in neutrophil degranulation and downregulated proteins involved in platelet degranulation and lipid-binding (including APOA1). In the same study, proteomic findings of podocytes exposed to the plasma of patients with recurrent FSGS revealed the dysregulation of mTOR pathway and significant differences in proteins involved in cytoskeleton organization. Another study reported APOA1 and MXRA8 as the most significant proteins with the highest fold-change differentiating steroid-resistant and steroid-sensitive FSGS [74]. Additionally, alpha-1-B glycoprotein was found to be associated with steroid-resistant FSGS [73].

The current biomarker research snapshot for podocyte disease can seem confusing at this point. Differences can partly be explained by the targeted patient population and by the differences in proteomic technique. Also, the untargeted approach to biofluid biomarkers can be influenced by pathogenesis, proteinuria, and/or reduced renal function; all these factors need to be addressed in order to improve prediction by biomarkers.

5. Proteomic Biomarkers: The Way Forward?

Proteomic analysis has changed over time: as more advanced technologies, such as high-resolution mass spectrometry and refined protein quantification methods, continue to evolve, they provide greater sensitivity and accuracy in detecting low-abundance proteins and post-translational modifications. These advancements enable a more comprehensive and detailed analysis of biofluids, increasing the potential to identify novel biomarkers. Accordingly, the success of biomarker discovery is in enhancing the sensitivity and specificity of proteomic analyses. Effective sample preparation is critical in improving the detection of low-abundance proteins. An option for biological fluids would be affinity-based depletion tools which selectively remove high-abundance proteins; in tissue samples, Laser Capture Microdissection (LCM) can be a pertinent option. LCM enables the isolation of specific tissue regions and structures (such as the isolation of glomeruli), improving both the sensitivity and tissue specificity, thus providing insights into localized disease processes.

Bead-based sample preparation methods have gained attention (SP3 approach—Hughes), which is an excellent option for scenarios involving low amounts of samples. In the context of proteomic studies on kidney disease, a combination of the above-mentioned sample preparation strategies was employed by Höhne et al. [78]. The authors proposed an optimized sample preparation protocol for the analysis of the proteome of a single glomerulus, enabling them to quantify the proteomes in kidney nephron segments consisting of as few as 200 cells and monitor podocyte marker proteins from as few as 80 podocytes per glomerulus independently of antibodies.

The need to analyze low sample amounts underscores the growing requirement to dissect complex biological systems into their individual components, particularly

individual cells, in order to dissect their intrinsic heterogeneity. Advancement in techniques has allowed single-cell proteome characterization, which was able to achieve a depth profile of thousands of proteins per cell [79]. One of the most recent technique developments resulted in deep visual proteomics, which merges artificial intelligence image analysis, automated laser microdissection, and high-sensitivity mass spectrometry while maintaining the spatial information of the single-cell proteomic findings [80]. While single-cell proteomics in renal tissues is still in its early stages, there have been promising advancements enhancing all aspects of MS-based proteomics analytical framework—from sample preparation to MS data acquisition and processing.

An important area of proteomics is the study of post-translational modifications (PTMs). These modifications (phosphorylation, glycosylation, etc.) play a crucial role in regulating protein function, stability, localization, and interactions and, subsequently, can dramatically impact cellular processes. In the context of kidney diseases, these modifications have been found to be integral to pathogenesis, as they are associated with cellular responses to injury, fibrosis, and inflammation in renal tissues. The authors refer the reader to the excellent, comprehensive overview of Liu et al. [81] which details the molecular mechanisms by which PTMs contribute to the pathogenesis of renal diseases. In FSGS, the study by Chhuon et al. [28] provides critical insights into the early signaling events that occur in podocytes after exposure to plasma from rFSGS patients. The authors highlighted that, following the exposure of podocytes to rFSGS plasma, several phosphoproteins involved in cytoskeleton rearrangement and mTOR activation were significantly modified. The ability to analyze these PTMs at a cellular level, particularly through advancements in single-cell proteomics, offers a promising avenue for understanding the complexities of kidney disorders.

Bioinformatic tools have significantly enhanced proteomic study outcomes by enabling the identification of connections at the pathway level, improving the interpretation of complex biological data, and facilitating the discovery of key biomarkers and pathways involved in various diseases. These tools also aid in visualizing large-scale data generated from proteomic experiments and in integrating results from other omics fields while covering several stages of analysis: from MS raw data analysis and protein identification and annotation to the post-processing steps, including functional and pathway analysis, protein–protein interaction networks, statistical analysis and validation, integration with other omics data, and data visualization. While the increased availability of computational resources has made these tools more accessible, it has also made it easier to make mistakes, leading to inflated or misleading results. This issue is compounded by the lack of standardized guidelines in the field regardless of the level at which these tools are applied, further increasing the potential for errors.

Furthermore, evolving data analysis techniques, including machine learning algorithms, may contribute to more precise interpretation of complex proteomic data, enabling better classification and prediction of disease subtypes and treatment responses. Another direction is the integration of multi-omics approaches, such as mass spectrometry-based metabolomics, as it can provide a more holistic understanding of disease pathways. These advancements bring us closer to personalized medicine in podocyte diseases, where tailored therapeutic strategies can be developed based on individual biomarker profiles and underlying pathogenic mechanisms. These advancements are one of the reasons for which proteomic research holds significant promise as a way towards personalized medicine in this field, pending several amendments.

In our view, it is difficult to assess the value of a broad biomarker panel or an untargeted biofluid proteomic approach applied indiscriminately to the entire spectrum of MCD/FSGS. It would be of incomparably greater value if the proposed biomarkers were directly linked to a specific pathogenic pathway, hence enabling the identification of a

subset of patients with a defined mechanism for podocyte injury, potentially amenable to a specific therapeutic approach.

Based on pathogenesis, some contributions of targeted proteomics approaches can be listed:

- Complement activation has emerged as an important mechanism in the pathogenesis of FSGS and is strongly associated with more severe morphological injury and poorer prognosis. Decreased plasma C3 levels were associated with loss of kidney function and, more importantly, proteinuria and tubulointerstitial injury, as well as a progressive renal disease in FSGS [82]. Furthermore, both plasma and urine complement proteins, including MAC, correlate inversely with kidney function and directly with proteinuria and histologic findings in FSGS [83,84]. Recent reports have indicated that urinary C5a and MAC may serve as useful biomarkers for distinguishing FSGS from MCD [85].
- B lymphocyte-mediated injury, as evidenced by the presence of nephrin antibodies, annexin antibodies, and other antibodies [36,37,75], has been identified as a key contributor to disease in certain subsets of patients within the MCD/FSGS spectrum.
- T lymphocyte-mediated mechanisms have been identified through the increased CD80 levels in the urine of nephrotic MCD patients, a marker not observed in patients in remission from MCD or in FSGS patients [86]. This suggests that CD80 may serve as a potential biomarker for active disease and could play a role in the pathogenesis of nephrotic syndrome in certain patient subsets.
- Proinflammatory and profibrotic pathways can be reflected by elevated urinary TGF-ß levels, which are reported to be higher in FSGS compared to MCD [87].

A step forward in identifying the pathogenic mechanisms involved and developing more accurate biofluid biomarkers is the untargeted proteomic glomerular study. In recent decades, the advancement of proteomics techniques, coupled with the ability to separate glomerular tissue from formalin-fixed, paraffin-embedded biopsies, has led to tremendous progress in the characterization of several glomerular diseases, like MN, C3 glomerulonephritis, and amyloidosis. These studies have, in many cases, resulted in the reclassification of diseases (membranoproliferative glomerulonephritis and amyloidosis) or prompted a reevaluation of diagnosis and management in MN [1]. However, attempts to characterize the spectrum of MCD/FSGS through these methods are surprisingly scarce. One study, published in abstract form, suggests that secondary FSGS is associated with upregulated complement pathways, while the primary form of FSGS shows upregulated proteins involved in cell-to-cell and matrix adhesion compared to MCD [88]. Furthermore, one study performed proteomic analysis on whole FFPE renal tissue and discovered upregulated LAMP1 and ACSL4 in pediatric resistant FSGS, and their finding also support complement activation in FSGS [89]. Upregulated complement alongside fibrinogen pathway and protease inhibitors (SERPINA1 and SERPINA3) were also described in a different study by the characterization of ECM in a murine model of FSGS [90].

Another study suggested that complement-regulating proteins, such as CD59, were substantially decreased in MCD when compared to other glomerular diseases known for complement-related inflammation (MN or IgAN) [75]. Our own research identified 58 significantly different proteins when comparing MCD, FSGS, and healthy controls; the pathway analysis of these proteins was associated with cytoskeleton dynamics and nephrin interactions [76]. Furthermore, we identified ANXA2 as one of the significantly increased proteins in MCD compared to controls [12].

We believe that efforts focusing on tissue proteomics in the FSFS/MCD spectrum has to be amplified for at least two reasons. First, given the considerable heterogeneity of pathophysiological mechanisms in MCD/FSGS, proteomic findings can vary significantly from one patient to another. In our own experience, differentially expressed proteins can differ

within the FSGS or MCD groups, which is eventually a reason to reclassify patients within the MCD/FSGS spectrum. For instance, in one of our previous findings, the proteomics profiles of certain MCD patients were more closely related to controls, while others resembled FSGS profiles (as shown in Figure 4, which presents a heatmap of differentially expressed proteins in MCD/FSGS and healthy controls [76]). To enhance the value of proteomic findings, more uniform pathogenesis-based cohorts with larger numbers of patients are needed. Glomerular proteomic studies should be able to ultimately distinguish between specific pathways of disease. This is elegantly illustrated in a study which shows that collapsing forms of FSGS are driven by enzymes such as cathepsin B and cathepsin C, derived from activated parietal cells infiltrating glomerular tufts that result in distinctive patterns in matrix production and/or degradation, not found in non-collapsing forms. The parietal epithelial cell signature in collapsing FSGS is illustrated by an increase in specific proteins, such as annexin A3, alongside cathepsin B and cathepsin C [77].

Second, once one or more significantly upregulated proteins have been identified in glomerular tissue, an attempt can be made to measure them in urine or plasma. Of course, tissue, plasma, and urine have different proteomic signatures as they are different compartments and an upregulated tissue protein is not necessarily found in urine (and/or plasma). However, certain proteins with possible pathogenic roles could derive from plasma (e.g., cytokines, enzymes, and permeability factors) and, more importantly, urine proteins can reflect shedding of tissue proteins. For example, C4d is found in glomeruli of FSGS even before sclerosis develops [43], and increased urinary and plasma C5b-9 and C5a in FSGS have been reported [84,85]. Urinary nephrin and podocalyxin are early markers of proteinuric glomerular diseases, reflecting podocyte injury [91,92].

Biofluid-directed proteomic studies offer the advantage of being non-invasive, but, as mentioned, they can be influenced by several factors: renal function can result in an increase in plasma proteins and proteinuria, and influence the abundance of urinary proteins, not necessarily reflecting pathogenic process within the glomeruli (accidental findings), and the tubular compartment can further confound results (protein reabsorption or tubular source of proteins). The complexity of the relation of tissue proteomics to urinary findings is reflected in the aforementioned study by Merchant et al. [77], in which some, but not all, of the urinary proteins found to be increased in collapsing FSGS are correlated to the tissue abundance of proteins. However, the value of biofluid proteomic findings would be significantly enhanced if studied within a prospective cohort guided by tissue proteomics findings.

Such biomarkers could be evaluated at the time of first presentation and repeated during remission and during relapse in order to certify that they are influenced by the pathogenic process of podocyte injury and are not an accidental finding. A similar approach would determine whether they also have a prognostic value in terms of predicting response to therapy or relapsing course. This approach would enhance the clinical applicability and accuracy of biofluid biomarkers for monitoring and choice of therapy. A potential workflow is suggested in Figure 5.

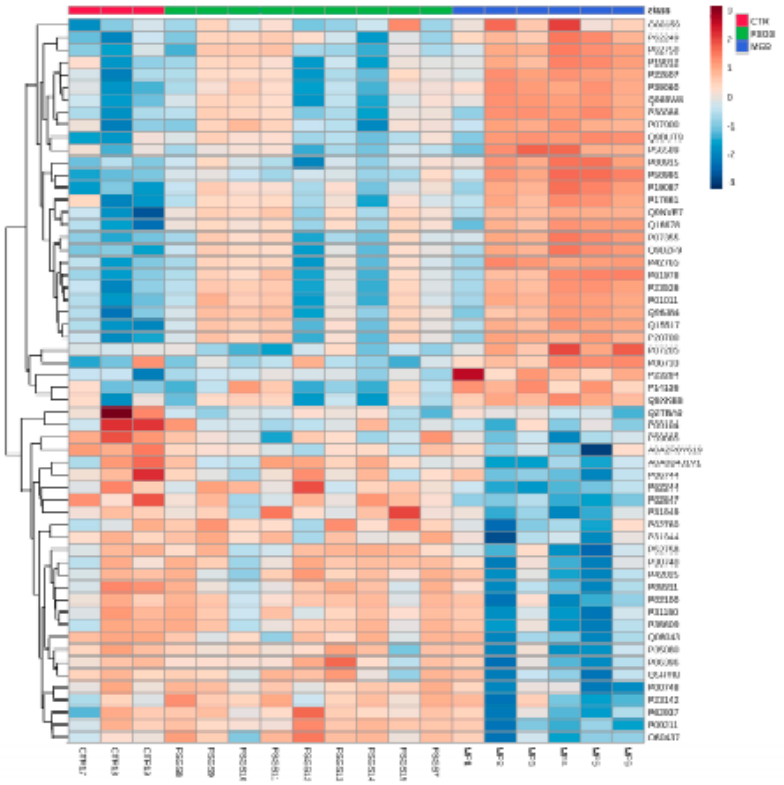

Figure 4. Differentially expressed proteomic signatures of patients with FSGS, patients with MCD, and healthy controls. Reprinted with permission from Bărar et al. [76].

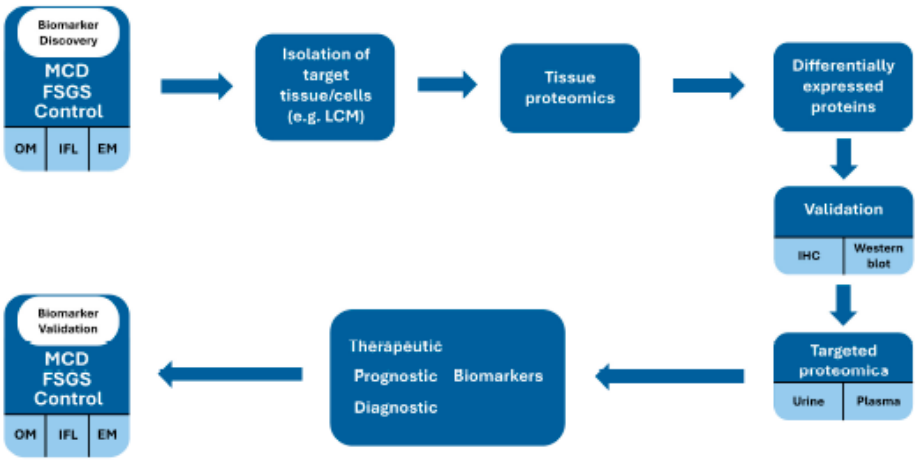

Figure 5. Proposed approach for biomarker development.

6. Concluding Remarks/Future Perspectives

The spectrum of MCD/FSGS needs urgent reassessment based on pathogenetic mechanisms in order to allow personized treatment and improve prognosis. A merely

histological approach is insufficient whereas increasingly refined proteomic techniques might be an important tool for a personalized, fine-tuned diagnostic and therapeutic approach.

Our proposed approach in biomarker research aims to transition from tissue proteomics, which, as mentioned before, most closely reflects the underlying pathological mechanisms and structural changes within the podocyte diseases, to urine or plasma protein profiling.

In this way, a patient or subgroup of patients exhibiting the same pathophysiology of podocyte injury, as identified by tissue proteomic profiling, could potentially be characterized by biofluid biomarker approaches. This would offer significant value for non-invasive diagnostic perspectives and, most importantly, for prognostic evaluation and therapeutic decision-making.

Author Contributions: Conceptualization, I.M.K. and Y.M.; methodology, D.T.M. and I.M.K.; Validation, A.R.P., D.T., M.T., C.C.R. and A.U.; Investigation, Y.M. and A.A.B.; Resources, Y.M.; Writing—original draft, I.M.K. and Y.M.; Writing—review and editing, I.E.P., C.A.I., D.T.M. and I.M.K.; Supervision, I.M.K. and D.T.M.; Visualization, Y.M., A.U. and A.A.B.; Project administration, I.M.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received funding by "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca under internal grant 32153/2/16.12.2024, grant title: "Analiza proteomică avansata in podocitopatiile primare: corelarea analizei tisulare glomerulare cu biomarkeri biofluidici cu potenţial teragnostic".

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MCD	Minimal change disease
FSGS	Focal segmental glomerulosclerosis
FPE	Foot process effacement
EM	Electron microscopy
LM	Light microscopy
FP	Foot process
SD	Slit diaphragm
FA	Focal adhesion
ECM	Extracellular matrix
GBM	Glomerular basement membrane
TLRs	Toll-like receptors
ILs	Interleukins
MAC	Membrane attack complex
IgAN	IgA nephropathy
MN	Membranous nephropathy
rFSGS	Recurrent focal segmental glomerulosclerosis
MS	Mass spectrometry

References

1. Caster, D.J.; Hobeika, L.; Klein, J.B.; Powell, D.W.; McLeish, K.R. Changing the Concepts of Immune-mediated Glomerular Diseases through Proteomics. *Proteomics Clin. Appl.* **2015**, *9*, 967–971. https://doi.org/10.1002/prca.201400159.
2. Sim, J.J.; Smoyer, W.E.; Schachter, A.D. Minimal Change Disease and FSGS Are a Spectrum of a Single Disease within Immune-Mediated Nephrotic Syndrome. *Kidney360* **2024**, *5*, 1197–1199. https://doi.org/10.34067/KID.0000000000000499.
3. Ravaglia, F.; Melica, M.E.; Angelotti, M.L.; De Chiara, L.; Romagnani, P.; Lasagni, L. The Pathology Lesion Patterns of Podocytopathies: How and Why? *Front. Cell Dev. Biol.* **2022**, *10*, 838272. https://doi.org/10.3389/fcell.2022.838272.

4. Rovin, B.H.; Adler, S.G.; Barratt, J.; Bridoux, F.; Burdge, K.A.; Chan, T.M.; Cook, H.T.; Fervenza, F.C.; Gibson, K.L.; Glassock, R.J.; et al. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. *Kidney Int.* **2021**, *100*, S1–S276. https://doi.org/10.1016/j.kint.2021.05.021.
5. Ishizuka, K.; Miura, K.; Hashimoto, T.; Kaneko, N.; Harita, Y.; Yabuuchi, T.; Hisano, M.; Fujinaga, S.; Omori, T.; Yamaguchi, Y.; et al. Degree of Foot Process Effacement in Patients with Genetic Focal Segmental Glomerulosclerosis: A Single-Center Analysis and Review of the Literature. *Sci. Rep.* **2021**, *11*, 12008. https://doi.org/10.1038/s41598-021-91520-9.
6. De Vriese, A.S.; Sethi, S.; Nath, K.A.; Glassock, R.J.; Fervenza, F.C. Differentiating Primary, Genetic, and Secondary FSGS in Adults: A Clinicopathologic Approach. *J. Am. Soc. Nephrol.* **2018**, *29*, 759–774. https://doi.org/10.1681/ASN.2017090958.
7. Maas, R.J.; Deegens, J.K.; Beukhof, J.R.; Reichert, L.J.; ten Dam, M.A.; Beutler, J.J.; van den Wall Bake, A.W.L.; Rensma, P.L.; Konings, C.J.; Geerse, D.A.; et al. The Clinical Course of Minimal Change Nephrotic Syndrome with Onset in Adulthood or Late Adolescence: A Case Series. *Am. J. Kidney Dis.* **2017**, *69*, 637–646. https://doi.org/10.1053/j.ajkd.2016.10.032.
8. Husain, S. Role of Podocyte in Kidney Disease. *Front. Biosci. -Landmark* **2024**, *29*, 250. https://doi.org/10.31083/j.fbl2907250.
9. Blaine, J.; Dylewski, J. Regulation of the Actin Cytoskeleton in Podocytes. *Cells* **2020**, *9*, 1700. https://doi.org/10.3390/cells9071700.
10. Haydak, J.; Azeloglu, E.U. Role of Biophysics and Mechanobiology in Podocyte Physiology. *Nat. Rev. Nephrol.* **2024**, *20*, 371–385. https://doi.org/10.1038/s41581-024-00815-3.
11. Kriz, W.; Shirato, I.; Nagata, M.; LeHir, M.; Lemley, K.V. The Podocyte's Response to Stress: The Enigma of Foot Process Effacement. *Am. J. Physiol. -Ren. Physiol.* **2013**, *304*, F333–F347. https://doi.org/10.1152/ajprenal.00478.2012.
12. Bărar, A.A.; Pralea, I.-E.; Maslyennikov, Y.; Munteanu, R.; Berindan-Neagoe, I.; Pîrlog, R.; Rusu, I.; Nuțu, A.; Rusu, C.C.; Moldovan, D.T.; et al. Minimal Change Disease: Pathogenetic Insights from Glomerular Proteomics. *Int. J. Mol. Sci.* **2024**, *25*, 5613. https://doi.org/10.3390/ijms25115613.
13. Somlo, S.; Mundel, P. Getting a Foothold in Nephrotic Syndrome. *Nat. Genet.* **2000**, *24*, 333–335. https://doi.org/10.1038/74139.
14. Huber, T.B.; Benzing, T. The Slit Diaphragm: A Signaling Platform to Regulate Podocyte Function. *Curr. Opin. Nephrol. Hypertens.* **2005**, *14*, 211–216. https://doi.org/10.1097/01.mnh.0000165885.85803.a8.
15. Mann, N.; Sun, H.; Majmundar, A.J. Mechanisms of Podocyte Injury in Genetic Kidney Disease. *Pediatr. Nephrol.* **2024**. https://doi.org/10.1007/s00467-024-06551-x
16. Kopp, J.B.; Anders, H.-J.; Susztak, K.; Podestà, M.A.; Remuzzi, G.; Hildebrandt, F.; Romagnani, P. Podocytopathies. *Nat. Rev. Dis. Primers* **2020**, *6*, 68. https://doi.org/10.1038/s41572-020-0196-7.
17. Yamashita, M.; Millward, C.A.; Inoshita, H.; Saikia, P.; Chattopadhyay, S.; Sen, G.C.; Emancipator, S.N. Antiviral Innate Immunity Disturbs Podocyte Cell Function. *J. Innate Immun.* **2013**, *5*, 231–241. https://doi.org/10.1159/000345255.
18. Migliorini, A.; Angelotti, M.L.; Mulay, S.R.; Kulkarni, O.O.; Demleitner, J.; Dietrich, A.; Sagrinati, C.; Ballerini, L.; Peired, A.; Shankland, S.J.; et al. The Antiviral Cytokines IFN-α and IFN-β Modulate Parietal Epithelial Cells and Promote Podocyte Loss. *Am. J. Pathol.* **2013**, *183*, 431–440. https://doi.org/10.1016/j.ajpath.2013.04.017.
19. Li, J.; Das, J.R.; Tang, P.; Han, Z.; Jaiswal, J.K.; Ray, P.E. Transmembrane TNF-α Facilitates HIV-1 Infection of Podocytes Cultured from Children with HIV-Associated Nephropathy. *J. Am. Soc. Nephrol.* **2017**, *28*, 862–875. https://doi.org/10.1681/ASN.2016050564.
20. Gbadegesin, R.A.; Ulasi, I.; Ajayi, S.; Raji, Y.; Olanrewaju, T.; Osafo, C.; Ademola, A.D.; Asinobi, A.; Winkler, C.A.; Burke, D.; et al. *APOL1* Bi- and Monoallelic Variants and Chronic Kidney Disease in West Africans. *N. Engl. J. Med.* **2025**, *392*, 228–238. https://doi.org/10.1056/NEJMoa2404211.
21. Kopp, J.B.; Nelson, G.W.; Sampath, K.; Johnson, R.C.; Genovese, G.; An, P.; Friedman, D.; Briggs, W.; Dart, R.; Korbet, S.; et al. APOL1 Genetic Variants in Focal Segmental Glomerulosclerosis and HIV-Associated Nephropathy. *J. Am. Soc. Nephrol.* **2011**, *22*, 2129–2137. https://doi.org/10.1681/ASN.2011040388.
22. Daneshpajouhnejad, P.; Kopp, J.B.; Winkler, C.A.; Rosenberg, A.Z. The Evolving Story of Apolipoprotein L1 Nephropathy: The End of the Beginning. *Nat. Rev. Nephrol.* **2022**, *18*, 307–320. https://doi.org/10.1038/s41581-022-00538-3.
23. Wada, T.; Nangaku, M. A Circulating Permeability Factor in Focal Segmental Glomerulosclerosis: The Hunt Continues. *Clin. Kidney J.* **2015**, *8*, 708–715. https://doi.org/10.1093/ckj/sfv090.
24. Bakker, W.W.; Borghuis, T.; Harmsen, M.C.; Van den Berg, A.; Kema, I.P.; Niezen, K.E.; Kapojos, J.J. Protease Activity of Plasma Hemopexin. *Kidney Int.* **2005**, *68*, 603–610. https://doi.org/10.1111/j.1523-1755.2005.00438.x.
25. Clement, L.C.; Macé, C.; Avila-Casado, C.; Joles, J.A.; Kersten, S.; Chugh, S.S. Circulating Angiopoietin-like 4 Links Proteinuria with Hypertriglyceridemia in Nephrotic Syndrome. *Nat. Med.* **2014**, *20*, 37–46. https://doi.org/10.1038/nm.3396.

26. Wei, C.; El Hindi, S.; Li, J.; Fornoni, A.; Goes, N.; Sageshima, J.; Maiguel, D.; Karumanchi, S.A.; Yap, H.-K.; Saleem, M.; et al. Circulating Urokinase Receptor as a Cause of Focal Segmental Glomerulosclerosis. *Nat. Med.* **2011**, *17*, 952–960. https://doi.org/10.1038/nm.2411.
27. Reiser, J.; Nast, C.C.; Alachkar, N. Permeability Factors in Focal and Segmental Glomerulosclerosis. *Adv. Chronic Kidney Dis.* **2014**, *21*, 417–421. https://doi.org/10.1053/j.ackd.2014.05.010.
28. Chhuon, C.; Herrera-Marcos, L.V.; Zhang, S.-Y.; Charrière-Bertrand, C.; Jung, V.; Lipecka, J.; Savas, B.; Nasser, N.; Pawlak, A.; Boulmerka, H.; et al. Proteomics of Plasma and Plasma-Treated Podocytes: Application to Focal and Segmental Glomerulosclerosis. *Int. J. Mol. Sci.* **2023**, *24*, 12124. https://doi.org/10.3390/ijms241512124.
29. Lopez-Hellin, J.; Cantarell, C.; Jimeno, L.; Sanchez-Fructuoso, A.; Puig-Gay, N.; Guirado, L.; Vilariño, N.; Gonzalez-Roncero, F.M.; Mazuecos, A.; Lauzurica, R.; et al. A Form of Apolipoprotein A-I Is Found Specifically in Relapses of Focal Segmental Glomerulosclerosis Following Transplantation. *Am. J. Transplant.* **2013**, *13*, 493–500. https://doi.org/10.1111/j.1600-6143.2012.04338.x.
30. Taherkhani, A.; Farrokhi Yekta, R.; Mohseni, M.; Saidijam, M.; Arefi Oskouie, A. Chronic Kidney Disease: A Review of Proteomic and Metabolomic Approaches to Membranous Glomerulonephritis, Focal Segmental Glomerulosclerosis, and IgA Nephropathy Biomarkers. *Proteome Sci.* **2019**, *17*, 7. https://doi.org/10.1186/s12953-019-0155-y.
31. Jacobs-Cachá, C.; Puig-Gay, N.; Helm, D.; Rettel, M.; Sellarès, J.; Meseguer, A.; Savitski, M.M.; Moreso, F.J.; Soler, M.J.; Seron, D.; et al. A Misprocessed Form of Apolipoprotein A-I Is Specifically Associated with Recurrent Focal Segmental Glomerulosclerosis. *Sci. Rep.* **2020**, *10*, 1159. https://doi.org/10.1038/s41598-020-58197-y.
32. Zhai, S.; Sun, B.; Zhang, Y.; Zhao, L.; Zhang, L. IL-17 Aggravates Renal Injury by Promoting Podocyte Injury in Children with Primary Nephrotic Syndrome. *Exp. Ther. Med.* **2020**, *20*, 409–417. https://doi.org/10.3892/etm.2020.8698.
33. Bhargava, R.; Tsokos, G.C. The Immune Podocyte. *Curr. Opin. Rheumatol.* **2019**, *31*, 167–174. https://doi.org/10.1097/BOR.0000000000000578.
34. Liu, R.; Wen, X.; Peng, X.; Zhao, M.; Mi, L.; Lei, J.; Xu, K. Immune Podocytes in the Immune Microenvironment of Lupus Nephritis (Review). *Mol. Med. Rep.* **2023**, *28*, 204. https://doi.org/10.3892/mmr.2023.13091.
35. Hengel, F.E.; Dehde, S.; Lassé, M.; Zahner, G.; Seifert, L.; Schnarre, A.; Kretz, O.; Demir, F.; Pinnschmidt, H.O.; Grahammer, F.; et al. Autoantibodies Targeting Nephrin in Podocytopathies. *N. Engl. J. Med.* **2024**, *391*, 422–433. https://doi.org/10.1056/NEJMoa2314471.
36. Ye, Q.; Zhang, Y.; Zhuang, J.; Bi, Y.; Xu, H.; Shen, Q.; Liu, J.; Fu, H.; Wang, J.; Feng, C.; et al. The Important Roles and Molecular Mechanisms of Annexin A2 Autoantibody in Children with Nephrotic Syndrome. *Ann. Transl. Med.* **2021**, *9*, 1452. https://doi.org/10.21037/atm-21-3988.
37. Ye, Q.; Zhou, C.; Wang, D.; Fu, H.; Wang, J.; Mao, J. Seven Novel Podocyte Autoantibodies Were Identified to Diagnosis a New Disease Subgroup-Autoimmune Podocytopathies. *Clin. Immunol.* **2021**, *232*, 108869. https://doi.org/10.1016/j.clim.2021.108869.
38. Chang, A.; Ko, K.; Clark, M.R. The Emerging Role of the Inflammasome in Kidney Diseases. *Curr. Opin. Nephrol. Hypertens.* **2014**, *23*, 204–210. https://doi.org/10.1097/01.mnh.0000444814.49755.90.
39. Sameer, A.S.; Nissar, S. Toll-Like Receptors (TLRs): Structure, Functions, Signaling, and Role of Their Polymorphisms in Colorectal Cancer Susceptibility. *Biomed. Res. Int.* 2021, *2021*. https://doi.org/10.1155/2021/1157023.
40. Aranda-Rivera, A.K.; Srivastava, A.; Cruz-Gregorio, A.; Pedraza-Chaverri, J.; Mulay, S.R.; Scholze, A. Involvement of Inflammasome Components in Kidney Disease. *Antioxidants* **2022**, *11*, 246. https://doi.org/10.3390/antiox11020246.
41. Angeletti, A.; Cantarelli, C.; Petrosyan, A.; Andrighetto, S.; Budge, K.; D'Agati, V.D.; Hartzell, S.; Malvi, D.; Donadei, C.; Thurman, J.M.; et al. Loss of Decay-Accelerating Factor Triggers Podocyte Injury and Glomerulosclerosis. *J. Exp. Med.* **2020**, *217*, e20191699. https://doi.org/10.1084/jem.20191699.
42. Zoshima, T.; Hara, S.; Yamagishi, M.; Pastan, I.; Matsusaka, T.; Kawano, M.; Nagata, M. Possible Role of Complement Factor H in Podocytes in Clearing Glomerular Subendothelial Immune Complex Deposits. *Sci. Rep.* **2019**, *9*, 7857. https://doi.org/10.1038/s41598-019-44380-3.
43. Zagorec, N.; Horvatić, I.; Šenjug, P.; Sović, S.; Galešić, K.; Galešić Ljubanović, D. The Lectin Pathway–A Dominant Pattern of the Complement System Activation in Primary Focal Segmental Glomerulosclerosis? *Kidney Int. Rep.* **2024**, *9*, 1925–1926. https://doi.org/10.1016/j.ekir.2024.02.1441.
44. Nagata, M. Podocyte Injury and Its Consequences. *Kidney Int.* **2016**, *89*, 1221–1230. https://doi.org/10.1016/j.kint.2016.01.012.
45. Herlitz, L.C.; Markowitz, G.S.; Farris, A.B.; Schwimmer, J.A.; Stokes, M.B.; Kunis, C.; Colvin, R.B.; D'Agati, V.D. Development of Focal Segmental Glomerulosclerosis after Anabolic Steroid Abuse. *J. Am. Soc. Nephrol.* **2010**, *21*, 163–172. https://doi.org/10.1681/ASN.2009040450.

46. Albano, G.D.; Amico, F.; Cocimano, G.; Liberto, A.; Maglietta, F.; Esposito, M.; Rosi, G.L.; Di Nunno, N.; Salerno, M.; Montana, A. Adverse Effects of Anabolic-Androgenic Steroids: A Literature Review. *Healthcare* **2021**, *9*, 97. https://doi.org/10.3390/healthcare9010097.
47. Mirioglu, S.; Daniel-Fischer, L.; Berke, I.; Ahmad, S.H.; Bajema, I.M.; Bruchfeld, A.; Fernandez-Juarez, G.M.; Floege, J.; Frangou, E.; Goumenos, D.; et al. Management of Adult Patients with Podocytopathies: An Update from the ERA Immunonephrology Working Group. *Nephrol. Dial. Transplant.* **2024**, *39*, 569–580. https://doi.org/10.1093/ndt/gfae025.
48. Broek, M. van den; Smeets, B.; Schreuder, M.F.; Jansen, J. The Podocyte as a Direct Target of Glucocorticoids in Nephrotic Syndrome. *Nephrol. Dial. Transplant.* **2022**, *37*, 1808–1815. https://doi.org/10.1093/ndt/gfab016.
49. Schijvens, A.M.; ter Heine, R.; de Wildt, S.N.; Schreuder, M.F. Pharmacology and Pharmacogenetics of Prednisone and Prednisolone in Patients with Nephrotic Syndrome. *Pediatr. Nephrol.* **2019**, *34*, 389–403. https://doi.org/10.1007/s00467-018-3929-z.
50. Jiang, H.; Shen, Z.; Zhuang, J.; Lu, C.; Qu, Y.; Xu, C.; Yang, S.; Tian, X. Understanding the Podocyte Immune Responses in Proteinuric Kidney Diseases: From Pathogenesis to Therapy. *Front. Immunol.* **2024**, *14*, 1335936. https://doi.org/10.3389/fimmu.2023.1335936.
51. Li, X.; Zhang, X.; Li, X.; Wang, X.; Wang, S.; Ding, J. Cyclosporine A Protects Podocytes via Stabilization of Cofilin-1 Expression in the Unphosphorylated State. *Exp. Biol. Med.* **2014**, *239*, 922–936. https://doi.org/10.1177/1535370214530365.
52. Chiba, Y.; Inoue, C.N. Once-Daily Low-Dose Cyclosporine A Treatment with Angiotensin Blockade for Long-Term Remission of Nephropathy in Frasier Syndrome. *Tohoku J. Exp. Med.* **2019**, *247*, 35–40. https://doi.org/10.1620/tjem.247.35.
53. Liao, R.; Liu, Q.; Zheng, Z.; Fan, J.; Peng, W.; Kong, Q.; He, H.; Yang, S.; Chen, W.; Tang, X.; et al. Tacrolimus Protects Podocytes from Injury in Lupus Nephritis Partly by Stabilizing the Cytoskeleton and Inhibiting Podocyte Apoptosis. *PLoS ONE* **2015**, *10*, e0132724. https://doi.org/10.1371/journal.pone.0132724.
54. Vivarelli, M.; Barratt, J.; Beck, L.H.; Fakhouri, F.; Gale, D.P.; Goicoechea de Jorge, E.; Mosca, M.; Noris, M.; Pickering, M.C.; Susztak, K.; et al. The Role of Complement in Kidney Disease: Conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int.* **2024**, *106*, 369–391. https://doi.org/10.1016/j.kint.2024.05.015.
55. Shui, H.-A.; Huang, T.-H.; Ka, S.-M.; Chen, P.-H.; Lin, Y.-F.; Chen, A. Urinary Proteome and Potential Biomarkers Associated with Serial Pathogenesis Steps of Focal Segmental Glomerulosclerosis. *Nephrol. Dial. Transplant.* **2007**, *23*, 176–185. https://doi.org/10.1093/ndt/gfm587.
56. Sedic, M.; Gethings, L.A.; Vissers, J.P.C.; Shockcor, J.P.; McDonald, S.; Vasieva, O.; Lemac, M.; Langridge, J.I.; Batinić, D.; Pavelić, S.K. Label-Free Mass Spectrometric Profiling of Urinary Proteins and Metabolites from Paediatric Idiopathic Nephrotic Syndrome. *Biochem. Biophys. Res. Commun.* **2014**, *452*, 21–26. https://doi.org/10.1016/j.bbrc.2014.08.016.
57. Candiano, G.; Musante, L.; Bruschi, M.; Petretto, A.; Santucci, L.; Boccio, P. Del; Pavone, B.; Perfumo, F.; Urbani, A.; Scolari, F.; et al. Repetitive Fragmentation Products of Albumin and A1-Antitrypsin in Glomerular Diseases Associated with Nephrotic Syndrome. *J. Am. Soc. Nephrol.* **2006**, *17*, 3139–3148. https://doi.org/10.1681/ASN.2006050486.
58. Nafar, M.; Kalantari, S.; Samavat, S.; Rezaei-Tavirani, M.; Rutishuser, D.; Zubarev, R.A. The Novel Diagnostic Biomarkers for Focal Segmental Glomerulosclerosis. *Int. J. Nephrol.* **2014**, *2014*, 1–10. https://doi.org/10.1155/2014/574261.
59. Muruve, D.A.; Debiec, H.; Dillon, S.T.; Gu, X.; Plaisier, E.; Can, H.; Otu, H.H.; Libermann, T.A.; Ronco, P. Serum Protein Signatures Using Aptamer-Based Proteomics for Minimal Change Disease and Membranous Nephropathy. *Kidney Int. Rep.* **2022**, *7*, 1539–1556. https://doi.org/10.1016/j.ekir.2022.04.006.
60. Choi, Y.W.; Kim, Y.G.; Song, M.-Y.; Moon, J.-Y.; Jeong, K.-H.; Lee, T.-W.; Ihm, C.-G.; Park, K.-S.; Lee, S.-H. Potential Urine Proteomics Biomarkers for Primary Nephrotic Syndrome. *Clin. Proteom.* **2017**, *14*, 18. https://doi.org/10.1186/s12014-017-9153-1.
61. Araumi, A.; Osaki, T.; Ichikawa, K.; Kudo, K.; Suzuki, N.; Watanabe, S.; Watanabe, M.; Konta, T. Urinary and Plasma Proteomics to Discover Biomarkers for Diagnosing between Diabetic Nephropathy and Minimal Change Nephrotic Syndrome or Membranous Nephropathy. *Biochem. Biophys. Rep.* **2021**, *27*, 101102. https://doi.org/10.1016/j.bbrep.2021.101102.
62. Navarro-Muñoz, M.; Ibernon, M.; Bonet, J.; Pérez, V.; Pastor, M.C.; Bayés, B.; Casado-Vela, J.; Navarro, M.; Ara, J.; Espinal, A.; et al. Uromodulin and Antitrypsin Urinary Peptide Analysis to Differentiate Glomerular Kidney Diseases. *Kidney Blood Press. Res.* **2012**, *35*, 314–325. https://doi.org/10.1159/000335383.
63. Pérez, V.; Ibernón, M.; López, D.; Pastor, M.C.; Navarro, M.; Navarro-Muñoz, M.; Bonet, J.; Romero, R. Urinary Peptide Profiling to Differentiate between Minimal Change Disease and Focal Segmental Glomerulosclerosis. *PLoS ONE* **2014**, *9*, e87731. https://doi.org/10.1371/journal.pone.0087731.
64. Pérez, V.; López, D.; Boixadera, E.; Ibernón, M.; Espinal, A.; Bonet, J.; Romero, R. Comparative Differential Proteomic Analysis of Minimal Change Disease and Focal Segmental Glomerulosclerosis. *BMC Nephrol.* **2017**, *18*, 49. https://doi.org/10.1186/s12882-017-0452-6.

65. Chebotareva, N.V.; Vinogradov, A.; Brzhozovskiy, A.G.; Kashirina, D.N.; Indeykina, M.I.; Bugrova, A.E.; Lebedeva, M.; Moiseev, S.; Nikolaev, E.N.; Kononikhin, A.S. Potential Urine Proteomic Biomarkers for Focal Segmental Glomerulosclerosis and Minimal Change Disease. *Int. J. Mol. Sci.* **2022**, *23*, 12607. https://doi.org/10.3390/ijms232012607.
66. Suresh, C.P.; Saha, A.; Kaur, M.; Kumar, R.; Dubey, N.K.; Basak, T.; Tanwar, V.S.; Bhardwaj, G.; Sengupta, S.; Batra, V.V.; et al. Differentially Expressed Urinary Biomarkers in Children with Idiopathic Nephrotic Syndrome. *Clin. Exp. Nephrol.* **2016**, *20*, 273–283. https://doi.org/10.1007/s10157-015-1162-7.
67. Catanese, L.; Siwy, J.; Wendt, R.; Amann, K.; Beige, J.; Hendry, B.; Mischak, H.; Mullen, W.; Paterson, I.; Schiffer, M.; et al. Differentiating Primary and Secondary FSGS Using Non-Invasive Urine Biomarkers. *Clin. Kidney J.* **2024**, *17*, sfad296. https://doi.org/10.1093/ckj/sfad296.
68. Hellin, J.L.; Bech-Serra, J.J.; Moctezuma, E.L.; Chocron, S.; Santin, S.; Madrid, A.; Vilalta, R.; Canals, F.; Torra, R.; Meseguer, A.; et al. Very Low-Molecular-Mass Fragments of Albumin in the Plasma of Patients With Focal Segmental Glomerulosclerosis. *Am. J. Kidney Dis.* **2009**, *54*, 871–880. https://doi.org/10.1053/j.ajkd.2009.07.011.
69. Zhao, M.; Li, M.; Li, X.; Shao, C.; Yin, J.; Gao, Y. Dynamic Changes of Urinary Proteins in a Focal Segmental Glomerulosclerosis Rat Model. *Proteome Sci.* **2014**, *12*, 42. https://doi.org/10.1186/1477-5956-12-42.
70. BAI, Y.; LIU, W.; GUO, Q.; ZOU, Y. Screening for Urinary Biomarkers of Steroid-Resistant Nephrotic Syndrome in Children. *Exp. Ther. Med.* **2013**, *5*, 860–864. https://doi.org/10.3892/etm.2012.875.
71. Kalantari, S.; Nafar, M.; Samavat, S.; Rezaei-Tavirani, M.; Rutishauser, D.; Zubarev, R. Urinary Prognostic Biomarkers in Patients With Focal Segmental Glomerulosclerosis. *Nephrourol. Mon.* **2014**, *6*. https://doi.org/10.5812/numonthly.16806.
72. Andersen, R.F.; Palmfeldt, J.; Jespersen, B.; Gregersen, N.; Rittig, S. Plasma and Urine Proteomic Profiles in Childhood Idiopathic Nephrotic Syndrome. *Proteom. Clin. Appl.* **2012**, *6*, 382–393. https://doi.org/10.1002/prca.201100081.
73. Piyaphanee, N.; Ma, Q.; Kremen, O.; Czech, K.; Greis, K.; Mitsnefes, M.; Devarajan, P.; Bennett, M.R. Discovery and Initial Validation of α 1-B Glycoprotein Fragmentation as a Differential Urinary Biomarker in Pediatric Steroid-resistant Nephrotic Syndrome. *Proteom. Clin. Appl.* **2011**, *5*, 334–342. https://doi.org/10.1002/prca.201000110.
74. Kalantari, S.; Nafar, M.; Rutishauser, D.; Samavat, S.; Rezaei-Tavirani, M.; Yang, H.; Zubarev, R.A. Predictive Urinary Biomarkers for Steroid-Resistant and Steroid-Sensitive Focal Segmental Glomerulosclerosis Using High Resolution Mass Spectrometry and Multivariate Statistical Analysis. *BMC Nephrol.* **2014**, *15*, 141. https://doi.org/10.1186/1471-2369-15-141.
75. Dong, J.; Zheng, F.; Liu, F.; He, J.; Li, S.; Pu, W.; Xu, H.; Luo, Z.; Liu, S.; Yin, L.; et al. Global-Feature of Autoimmune Glomerulonephritis Using Proteomic Analysis of Laser Capture Microdissected Glomeruli. *Front. Immunol.* **2023**, *14*, 1131164. https://doi.org/10.3389/fimmu.2023.1131164.
76. Bărar, A.A.; Pralea, I.E.; Berindan-Neagoe, I.; Pirlog, R.; Nutu, A.; Maslyennikov, Y.; Potra, A.R.; Iuga, C.A.; Kacso, I.M. Proteomic Patterns in Glomerular Research, a Laser Capture Microdissection and Liquid Chromatography-Tandem Mass Spectrometry Approach. *Rev. Rom. Med. Lab.* **2023**, *31*, 263–274. https://doi.org/10.2478/rrlm-2023-0029.
77. Merchant, M.L.; Barati, M.T.; Caster, D.J.; Hata, J.L.; Hobeika, L.; Coventry, S.; Brier, M.E.; Wilkey, D.W.; Li, M.; Rood, I.M.; et al. Proteomic Analysis Identifies Distinct Glomerular Extracellular Matrix in Collapsing Focal Segmental Glomerulosclerosis. *J. Am. Soc. Nephrol.* **2020**, *31*, 1883–1904. https://doi.org/10.1681/ASN.2019070696.
78. Höhne, M.; Frese, C.K.; Grahammer, F.; Dafinger, C.; Ciarimboli, G.; Butt, L.; Binz, J.; Hackl, M.J.; Rahmatollahi, M.; Kann, M.; et al. Single-Nephron Proteomes Connect Morphology and Function in Proteinuric Kidney Disease. *Kidney Int.* **2018**, *93*, 1308–1319. https://doi.org/10.1016/j.kint.2017.12.012.
79. Truong, T.; Kelly, R.T. Whatʼs New in Single-Cell Proteomics. *Curr. Opin. Biotechnol.* **2024**, *86*, 103077. https://doi.org/10.1016/j.copbio.2024.103077.
80. Mund, A.; Coscia, F.; Kriston, A.; Hollandi, R.; Kovács, F.; Brunner, A.-D.; Migh, E.; Schweizer, L.; Santos, A.; Bzorek, M.; et al. Deep Visual Proteomics Defines Single-Cell Identity and Heterogeneity. *Nat. Biotechnol.* **2022**, *40*, 1231–1240. https://doi.org/10.1038/s41587-022-01302-5.
81. Liu, Z.; Yang, J.; Du, M.; Xin, W. Functioning and Mechanisms of PTMs in Renal Diseases. *Front. Pharmacol.* **2023**, *14*, 1238706. https://doi.org/10.3389/fphar.2023.1238706.
82. Liu, J.; Xie, J.; Zhang, X.; Tong, J.; Hao, X.; Ren, H.; Wang, W.; Chen, N. Serum C3 and Renal Outcome in Patients with Primary Focal Segmental Glomerulosclerosis. *Sci. Rep.* **2017**, *7*, 4095. https://doi.org/10.1038/s41598-017-03344-1.
83. Thurman, J.M.; Wong, M.; Renner, B.; Frazer-Abel, A.; Giclas, P.C.; Joy, M.S.; Jalal, D.; Radeva, M.K.; Gassman, J.; Gipson, D.S.; et al. Complement Activation in Patients with Focal Segmental Glomerulosclerosis. *PLoS ONE* **2015**, *10*, e0136558. https://doi.org/10.1371/journal.pone.0136558.

84. Huang, J.; Cui, Z.; Gu, Q.; Zhang, Y.; Qu, Z.; Wang, X.; Wang, F.; Cheng, X.; Meng, L.; Liu, G.; et al. Complement Activation Profile of Patients with Primary Focal Segmental Glomerulosclerosis. *PLoS ONE* **2020**, *15*, e0234934. https://doi.org/10.1371/journal.pone.0234934.
85. Cambier, A.; Patey, N.; Royal, V.; Gougeon, F.; Genest, D.S.; Brachemi, S.; Bollée, G.; Merlen, C.; Bonnefoy, A.; Lapeyraque, A.-L.; et al. A Prospective Study on Complement Activation Distinguishes Focal Segmental Glomerulosclerosis from Minimal Change Disease. *Kidney Int. Rep.* **2024**, *9*, 661–670. https://doi.org/10.1016/j.ekir.2023.12.015.
86. Garin, E.H.; Mu, W.; Arthur, J.M.; Rivard, C.J.; Araya, C.E.; Shimada, M.; Johnson, R.J. Urinary CD80 Is Elevated in Minimal Change Disease but Not in Focal Segmental Glomerulosclerosis. *Kidney Int.* **2010**, *78*, 296–302. https://doi.org/10.1038/ki.2010.143.
87. Woroniecki, R.P.; Shatat, I.F.; Supe, K.; Du, Z.; Kaskel, F.J. Urinary Cytokines and Steroid Responsiveness in Idiopathic Nephrotic Syndrome of Childhood. *Am. J. Nephrol.* **2008**, *28*, 83–90. https://doi.org/10.1159/000109396.
88. Almaani, S.; Madhavan, S.M.; Shapiro, J.P.; Satoskar, A.A.; Ayoub, I.; Rovin, B.H.; Parikh, S.V. Comparison of Glomerular Proteomics Profiles of Healthy Human Kidney and Minimal Change Disease Identifies Distinct Targets and Pathways. *J. Am. Soc. Nephrol.* **2021**, *32*, 445. https://doi.org/10.1681/ASN.2021321051445a.
89. Ni, J.; Tian, S.; Bai, L.; Lv, Q.; Liu, J.; Liu, J.; Fang, Y.; Zhai, Y.; Shen, Q.; Rao, J.; et al. Comparative Proteomic Analysis of Children FSGS FFPE Tissues. *BMC Pediatr.* **2022**, *22*, 707. https://doi.org/10.1186/s12887-022-03764-7.
90. Bukosza, E.N.; Kornauth, C.; Hummel, K.; Schachner, H.; Huttary, N.; Krieger, S.; Nöbauer, K.; Oszwald, A.; Razzazi Fazeli, E.; Kratochwill, K.; et al. ECM Characterization Reveals a Massive Activation of Acute Phase Response during FSGS. *Int. J. Mol. Sci.* **2020**, *21*, 2095. https://doi.org/10.3390/ijms21062095.
91. Akankwasa, G.; Jianhua, L.; Guixue, C.; Changjuan, A.; Xiaosong, Q. Urine Markers of Podocyte Dysfunction: A Review of Podocalyxin and Nephrin in Selected Glomerular Diseases. *Biomark. Med.* **2018**, *12*, 927–935. https://doi.org/10.2217/bmm-2018-0152.
92. Kostovska, I.; Trajkovska, K.T.; Topuzovska, S.; Cekovska, S.; Labudovic, D.; Kostovski, O.; Spasovski, G. Nephrinuria and Podocytopathies. In *Advances in Clinical Chemistry;* Elsevier: Amsterdam, The Netherlands, 2022; pp. 1–36.

Int Urol Nephrol
DOI 10.1007/s11255-010-9841-5

NEPHROLOGY – ORIGINAL PAPER

Vascular calcifications and renal osteodystrophy in chronic hemodialysis patients: what is the relationship between them?

Diana Moldovan · Ioan Moldovan · Crina Rusu · Simona Racasan · Ioan M. Patiu · Adrian Brumboiu · Cosmina Bondor · Liliana Parvu · Ina Kacso · Remus Orasan · Mirela Gherman-Caprioara

Received: 17 May 2010 / Accepted: 27 August 2010

Abstract
Introduction Vascular calcifications (VCs) and renal osteodystrophy (ROD) are frequently seen together and represent the major causes of morbidity and mortality in hemodialysis (HD) patients. Some studies suggest a pathogenic link between them, but there is no consensus as yet regarding this issue. The main objective of our study was to establish whether there is any relation between VCs and ROD in our HD patients. We evaluated the prevalence of VCs and ROD and the relationship between VCs and some clinical and biochemical characteristics of HD patients.
Methods We examined radiological signs of VCs and ROD on hands and pelvis bone radiographs in 81 chronic HD patients, and we calculated a VC score on this basis.
Results We found a significant relation between radiological signs of ROD and those of VC ($P = 0.019$). The patients with ROD had a higher mean VC score ($P = 0.02$). By linear regression, the VC score correlated directly with serum calcium (Ca), phosphorus (P), intact parathyroid hormone (iPTH) and CaxP product and inversely with serum albumin. The logistic regression model revealed that ROD, male gender and treatment with calcium salts were predictive of VCs development. There were no associations between VCs and age, HD vintage, diabetes, dialysate Ca concentration, vitamin D treatment, spKt/V, URR and C-reactive protein (CRP) levels.
Conclusion There seems to be a pathogenetic link between bone and artery diseases in chronic HD patients. Both VCs and ROD have a high prevalence. ROD, male gender and treatment with calcium salts are risk factors for VCs.

Keywords Hemodialysis · Vascular calcifications · Renal osteodystrophy

D. Moldovan (✉) · C. Rusu · L. Parvu · I. Kacso · M. Gherman-Caprioara
"Mihai Manasia" Nephrology and Dialysis Clinic Cluj-Napoca, University of Medicine and Pharmacy Cluj-Napoca, Nephrology Clinic. 3-5 Clinics Street, 400006 Cluj-Napoca, Romania
e-mail: Diana.Moldovan@umfcluj.ro

I. Moldovan
Emergency Military Hospital, Cluj-Napoca, Romania

S. Racasan · I. M. Patiu · R. Orasan
Nefromed Dialysis Center, Cluj-Napoca, Romania

A. Brumboiu
Radiology and Imagistic Department Emergency County Hospital Cluj-Napoca, University of Medicine and Pharmacy, Cluj-Napoca, Romania

C. Bondor
Medical Informatics and Biostatistics Chair "Iuliu Hatieganu", University of Medicine and Pharmacy, Cluj-Napoca, Romania

Introduction

Chronic kidney disease (CKD), renal replacement therapies and various treatments induce complex

Published online: 23 September 2010

biochemical disturbances of the calcium–phosphate metabolism with a wide spectrum of bone, vascular and soft tissue abnormalities. Changes in mineral metabolism and bone structure are an almost universal finding in progressive chronic kidney disease [1–3].

The cardiovascular diseases are highly prevalent in hemodialysis (HD) patients. They are induced and favored greatly by vascular calcifications (VCs) [4, 5].

These cardiovascular and bone complications represent major causes for morbidity, impaired autonomy, decreased quality of life and eventually death in these patients [6, 7].

Renal osteodystrophy (ROD) represents a heterogeneous pattern of bone disturbances associated with CKD and concomitant diseases, including osteitis fibrosa cystica (OFC) induced by secondary hyperparathyroidism (SHPTH), osteomalacia (OM), adynamic bone disease (ABD), mixed uremic osteodystrophy, osteoporosis, aluminum bone disease, amyloid bone disease and metastatic calcifications [3]. Since 1943, when the term "ROD" was introduced [8], the diagnosis was based mostly on radiographic findings. Although numerous new diagnosis modalities such as PTH levels, bone mass density, and bone biopsy have been introduced in clinical practice [9–11], plain film radiography, especially fine quality hand radiography, still plays a role [12]. The main radiographic findings are induced by HPTH and are located especially in hands, clavicles, sacrum-iliac and pubic bones. OM and ABD are painful complications, and their main clinical and radiological manifestations are bone fractures [3, 12–15]. Recently, the "Kidney Disease: Improving Global Outcomes" (KDIGO) foundation recommended that the term "ROD" should be used exclusively for alterations in bone morphology assessed by biopsy associated with chronic kidney disease [2]. But bone biopsy is a laborious and painful procedure, it allows the analysis of a single bone site and it has limited indications in clinical practice. Therefore, bone radiographs remain a key method for the diagnosis of ROD.

Vascular calcifications can also be diagnosed by plain radiography, numerous studies demonstrating a good sensitivity and specificity compared with CT-techniques [16, 17]. Plain radiology is used in order to investigate various arterial sites situated in the pelvis, thigh [7, 18, 19], hands [4], abdominal aorta [17], feet [7] and even knee, arm and skull [5]. The KDIGO guideline for chronic kidney disease—mineral and bone disorders (CKD-MBD)—recommends only radiography for the detection of VCs as a screening tool [2, 20].

Numerous studies have demonstrated associations between atherosclerosis and osteoporosis in the general population [21–23]. This association between bone and vascular disorders was also observed in patients with CKD, concerning the two main types of CKD-MBD, high bone turnover (SHPTH) and low bone turnover (ABD) diseases [24, 25], but this association is not consistent in all studies. The coexistence of abnormal bone and VCs represents a double threat for dialysis patients. Today experts use the terms "kidney—bone—vascular axis" or "bone—arteries cross-talk in CKD" [26]. The KDIGO proposed this new term, CKD-MBD, in the attempt to bring together alterations in mineral metabolism, bone changes and vascular or other soft tissue calcifications [2, 20].

The main objective of our study was to see whether there is a link between VCs and ROD in a cohort of HD patients, using radiography for diagnosis. Secondary objectives were to evaluate the prevalences of ROD and VCs, as well as the relationship between VCs and several clinical and biochemical characteristics of HD patients, especially mineral metabolism markers.

Patients and methods

This cross-sectional study has been carried out in a cohort of randomly selected HD patients treated in Nefromed Dialysis Center, Cluj-Napoca, Romania. Eligibility criteria were dialysis duration $\geq$6 months, age >18 years, and patients' agreement to undergo radiological examination. Exclusion criteria were previous parathyroidectomy, previous renal transplant, and other known bone disease. The scoring ranged from 0 (meaning no calcification) to 8 (meaning bilateral calcification of all the arteries).

The data regarding demographical and clinical characteristics (age, gender, HD vintage, presence of diabetes, dialysate Ca concentration, and treatments with calcium-based phosphates binders and vitamin D) were recorded. The patients were treated with conventional HD, 12 h a week, with polysulphone dialyzer membranes. They received calcium carbonate or calcium acetate as phosphate binders and vitamin D

(calcitriol). Blood samples for the biochemical evaluation were drawn prior to the HD session.

The plain radiographic films of hands and pelvis evaluated all bone abnormalities (Figs. 1, 2). ROD was defined based on the following diagnostic criteria: subperiosteal bone erosions located on the radial border of the middle phalanges of the index and long fingers and on the femoral neck; resorbtion of the terminal phalanges tuft (acroosteolysis); juxta-articular bone erosions around the metacarpo-phalangeal joints, appearing as small lucent defects (cysts); subchondral bone resorption of the sacrum-iliac and the pubic joints with a widened and irregular joint space and sometimes vertical joint subluxation; erosive enthesopathy, and brown tumors. We considered all the above as typical features of SHPTH-associated bone disease. Looser zones on pubic branches, iliac bones, femoral neck and long bones are rare, but suggestive for osteomalacia. Osteoporosis and fractures were considered also to be signs of ROD.

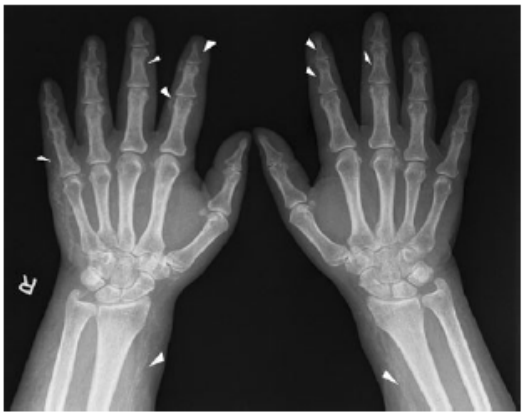

Fig. 1 Hands radiograph. Subperiosteal bone resorbtion on the radial border of the middle phalanges of the index and medius; acroosteolysis in the distal phalanges. Radial and digital arteries calcifications

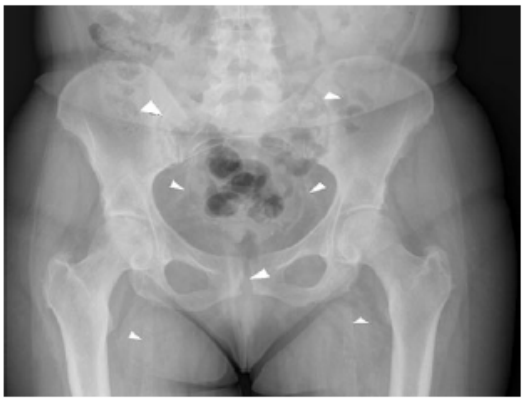

Fig. 2 Pelvis radiograph. Subcondral resorbtion in the sacro-iliac and public joints. Iliac and femoral arteries calcifications

Peripheral VCs were evaluated on the same radiographic films. The hands films were used to evaluate digital and radial arteries (Fig. 1), and pelvic films to evaluate iliac and femoral vessels (Fig. 2). We used the Adragao score for VCs [4], whose methodology is described in detail elsewhere [27]. The study protocol was approved by the institutional ethics committee.

The radiological findings were analyzed by two physicians, a radiologist and a nephrologist, both blinded to the clinical and laboratory data.

We measured serum calcium (Ca), inorganic phosphorus (P), alkaline phosphatase (ALP), intact parathormone (iPTH), urea, albumin and C-reactive protein (CRP). Calcium–phosphate product (CaxP), $\text{spKt/V} = 2.4 \times (1 - \text{urea postHD/urea preHD}) - 0.276$ and $\text{URR} = (1 - \text{urea postHD/urea preHD}) \times 100$ were calculated.

Statistics

Data were expressed as mean ± standard deviation (SD) for continuous factors, as frequencies for qualitative variables. For continuous variables, the statistical comparison was made using *t*-test or Mann–Whitney rank sum test. Chi-square or Fisher exact test was used to evaluate the relation between qualitative variables. We compared groups with and without VCs and constructed regression models (logistic for VC as binary variable and linear for VC as continuous variable) with VC as dependent variable to assess the association with ROD and other markers of CKD-MBD. When building a multivariable regression model, the enter method was used. A $P < 0.05$ was taken as significant. All statistical analyses were performed using Sigma Stat and SPSS 13.0 statistic packages.

Results

The study cohort consisted of 81 hemodialysis (HD) patients, 35 women and 46 men, among which 10

patients had diabetes mellitus (DM). Seventy-two patients (88.9%) were treated with calcium salts and 43 patients (53%) with vitamin D compounds. No patient received aluminum-containing phosphate binders. The descriptive statistics results are presented in Table 1.

Subperiosteal bone erosions was the main radiographic finding, located on the radial border of the middle phalanges of the index and medius, in 22 patients. Acroosteolysis was found in 10 patients. Erosions were located also on other fingers and metacarpal bones. There was a wide range of bone and joint changes, such as carpal, metacarpal, cubital and radial styloid cysts, recent and old bone fractures in 3 patients, hip prostheses in 2 patients, pelvis subchondral cysts, subchondral sclerosis in 7 patients, chronic osteoporosis in 31 patients, erosions and widening of sacrum-iliac joints in 35 patients and of pubic joints in 16 patients, juxta-articular soft tissue calcifications in 16 patients and pelvic calcified enthesopathy in 5 patients. No Looser zones or brown tumors were observed.

Vascular calcifications were found in the following locations: radial artery in 42 patients (31 bilateral), digital arteries in 19 patients (19 bilateral), iliac artery in 44 patients (38 bilateral), and femoral artery in 41 patients (38 bilateral).

The VC score distribution was as follows: score 0 in 24 patients; score 1 in 6 patients; score 2 in 12 patients; score 3 in 3 patients; score 4 in 7 patients; score 5 in 3 patients; score 6 in 9 patients; score 7 in 1 patient; and score 8 in 16 patients.

The patients were divided into two groups: with and without VCs. We compared these two groups regarding the presence of ROD, clinical characteristics and biochemical parameters. The group with VCs consisted of 45 patients with ROD and 12 patients without ROD. In the group without VCs, there were 12 patients with ROD and 12 patients without ROD. We found a significant relation between the presence of radiological signs of ROD and VCs ($P = 0.019$).

We also divided the population in groups with and without ROD, comparing the mean VC scores. The patients with ROD had a higher mean VC score ($P = 0.02$) (Fig. 3); The serum iPTH levels in various groups are shown in Table 2.

In univariate analysis, the presence of VCs was associated with higher serum Ca, P, ALP levels (Table 3) and higher CaxP product. There were no correlations between VCs and age, gender, HD vintage, presence of diabetes, dialysate Ca concentration, vitamin D treatment, spKt/V, URR, and CRP (Table 3).

Table 1 Characteristics of the cohort

Feature	
Number of patients	81
ROD prevalence (%)	70.37
VCs prevalence (%)	70.37
Male gender (%)	56.8
Diabetes mellitus (%)	12.34
Mean age (years)	56.67 ± 12.03
Age range (years)	28–82
Mean HD vintage (months)	52.11 ± 49.58
HD time range (months)	7–231
Ca (mg/dl)	8.16 ± 1.06
P(mg/dl)	6.06 ± 1.76
CaXP (mg^2/dl^2.)	49.21 ± 18.35
ALP (U/l)	290.18 ± 180.75
iPTH (pg/ml)	610.69 ± 649.6
spKt/V	1.32 ± 0.22
URR	66.86 ± 9.56
Albumin (g/dl)	4.09 ± 0.45
CRP (mg/dl)	1.27 ± 1.36

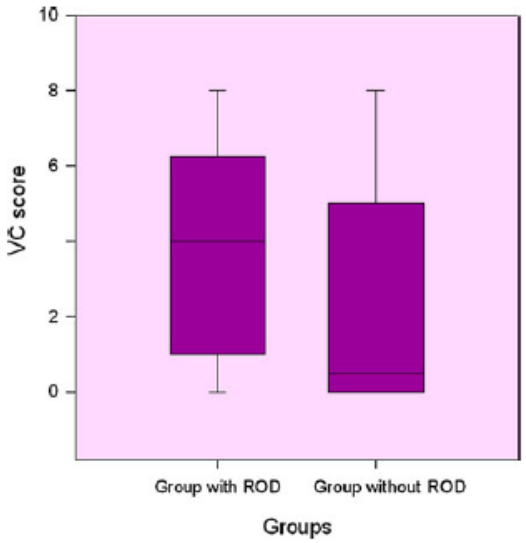

Fig. 3 The mean VC score in the groups with and without ROD ($P = 0.02$)

Table 2 The serum iPTH levels in the studied population

	iPTH(pg/ml)			Total patients
	<150	150–300	>300	
Group with ROD	10	12	35	57
Group without ROD	9	3	12	24
Group with VC	12	11	34	57
Group without VC	7	4	13	24
Total HD patients	19	15	47	81

By linear regression, the VC score as a continuous variable correlated directly with serum Ca, P, iPTH and CaxP and inversely with serum albumin (Table 4).

In order to assess the influence of the studied variables on the presence of VCs, we introduced the data in logistic regression. The independent variables were age, gender, diabetes, dialysate Ca, Ca and vitamin D treatments, serum Ca, P, CaxP, iPTH, ALP, spKt/V, URR, serum albumin and CRP. The created model revealed that ROD ($P = 0.029$; OR = 7.704; 95% CI = 1.239–47.916), gender ($P = 0.036$; OR = 7.226; 95% CI = 1.138–45.882) and oral treatment with calcium salts ($P = 0.04$; OR = 1.006; 95% CI = 1–1.011) are predictive for VCs presence (Table 5).

Discussion

The prevalence of mineral and skeletal complications in long-term HD patients increases as the life expectancy of these patients improves [2, 3, 28]. Most of the patients treated for more than 10 years experience skeletal complications that may impair their autonomy. On the other hand, cardiovascular disease represents the most important cause of death in these patients, vascular calcifications playing an important role [4, 5, 29].

Our study demonstrated there is a link between bone and vascular disease in HD patients. The presence and the severity of VCs were significantly related to ROD occurrence. We also found a high prevalence of these two complications in our HD patients.

The association between radiographic ROD and VCs could shed a light on the pathophysiologic links between bone and vascular disease. The osteoporosis–VC association can be observed in the general population in the absence of overt mineral

Table 3 Comparison between the two groups of patients: with and without VCs

Feature	Patients without VC	Patients with VC	*P*
Number of patients	24	57	
ROD (%)	50	78.94	**0.019**
Age (years)	57.4 ± 12.3	56.4 ± 11.9	0.72
HD vintage (months)	26.5 (15.5–43.5)	37 (19.5–94.3)	0.12
Male gender (%)	10 pts (41.66%)	36 pts (63.15%)	0.44
Diabetes (%)	2 pts (8.33%)	8 pts (14.03%)	0.71
Dialysate Ca (mmol/l)	1.5 (1.5–1.63)	1.5 (1.25–1.5)	0.048
Calcium salts treatment (g/year)	317.9 ± 196.1	307.9 ± 167.6	0.81
Vitamin D treatment (mcg/year)	22.5 (0–67.5)	5.75 (0–37.5)	0.10
Serum Ca (mg/dl)	7.77 ± 1.17	8.33 ± 0.98	**0.029**
Serum P (mg/dl)	5.47 ± 1.34	6.31 ± 1.88	0.05
CaxP (mg^2/dl^2)	40.4 ± 14.2	52.9 ± 18.7	**0.004**
ALP (U/l)	188 (160.5–246)	258 (183–349.5)	**0.009**
iPTH (pg/ml)	305.5 (139.2–535.8)	392 (198.2–1091.2)	0.12
Spkt/V	1.4 (1.25–1.5)	1.3 (1.2–1.4)	0.17
URR	70.2 (63.5–74.3)	66.6 (61.2–71.5)	0.17
Albumin (g/dl)	4.24 (3.91–4.49)	4.16 (3.9–4.3)	0.17
CRP (mg/dl)	0.56 (0.26–1.33)	0.95 (0.5–1.59)	0.13

Note: Bold values indicate statistically significant

Table 4 Linear regression for VC as a continuous dependent variable

Independent variable	*P*
Age (years)	0.34
HD vintage (months)	0.86
Dialysate Ca (mmol/l)	0.10
Treatment Ca (g/year)	0.42
Treat vit D (mcg/year)	0.15
Serum Ca (mg/dl)	**0.02**; $R = 0.25$ (direct)
Serum P (mg/dl)	**0.03**; $R = 0.23$ (direct)
CaxP (mg^2/dl^2)	**0.002**; $R = 0.32$ (direct)
iPTH (pg/ml)	**0.05**; $R = 0.21$ (direct)
ALP (U/l)	0.08; $R = 0.19$
Spkt/V	0.13
URR	0.12
Albumin (g/dl)	**0.019**; $R = -0.26$ (inverse)
CRP (mg/dl)	0.42

Note: Bold values indicate statistically significant

metabolism disorders. In CKD or ESRD patients, the relationship between VCs and bone disorders is associated with deterioration of mineral and bone metabolism caused by changes in serum phosphate and calcium and disruption of endocrine pathways, which intervene in osteoblast-like transformation of vascular smooth muscle cells [30].

In our study, most of the bone X-ray changes belonged to OFC, secondary to HPTH. Osteoporosis and fractures could be due to any kind of disease included in the concept of ROD, but the exact subtype of ROD could not be specified in this study. Regarding osteoporosis, KDIGO recommends that the term should not be used to describe bone fragility in CKD patients, since bone is likely to be more severely affected by CKD than might be expected from normal aging, because of the extreme turnover and remodeling that occur in CKD [20].

The increased bone resorbtion seen in HPTH is frequently associated with VCs [31, 32]. A high frequency and extent of VCs are also observed in patients with bone demineralization and low bone turnover [18, 24, 25].

Some authors demonstrated an association between high serum iPTH levels and the severity of coronary calcifications in HD patients [33]. We previously described a positive relationship between peripheral VCs and mineral metabolism markers, with serum iPTH being predictive for VC severity [27].

Some studies suggest that serum Ca, P and CaxP are responsible for cardiovascular calcifications or for the link between bone and VCs [34–36]. We observed in our study that high serum Ca, P, CaxP and ALP were associated with VCs in univariable analysis; Ca, P, CaxP and iPTH correlated directly

Table 5 Logistic regression with VC as binary dependent variable

	P	OR	95.0% C.I.	
			Lower	Upper
Age (years)	0.878	1.005	0.940	1.075
Gender (%)	**0.036**	7.226	1.138	45.882
DM (%)	0.466	2.344	0.237	23.188
ROD	**0.029**	7.704	1.239	47.916
CaxP (mg^2/dl^2)	0.561	1.217	0.627	2.362
Ca (mg/dl)	0.977	0.945	0.021	42.421
P (mg/dl)	0.560	0.213	0.001	38.791
iPTH (pg/ml)	0.490	1.001	0.999	1.003
ALP (U/l)	0.130	1.007	0.998	1.015
URR	0.803	1.088	0.559	2.118
Ktv	0.882	0.120	0.000	177848456954.560
Albumin(mg/dl)	0.986	0.983	0.143	6.744
CRP(mg/dl)	0.730	00.921	0.575	1.474
Oral Ca salts (g/year)	**0.040**	1.006	1.000	1.011
Vit D treat (mcg/year)	0.289	0.991	0.975	1.008
Ca dialysate (mmol/l)	0.273	0.040	0.000	12.717

Note: Bold values indicate statistically significant

with VCs in linear regression analysis. The multivariable analysis revealed that beside ROD, oral treatment with calcium salts is a risk factor for VCs, which is in accordance with other studies [37, 38].

Low levels of serum albumin were correlated with the VC score, and male gender was a risk factor for VCs, similar to other studies [5], both being recognized as cardiovascular risk factors.

We cannot distinguish whether the ROD radiological findings belong to high or low turnover bone disease, but their presence means that the bone is damaged because of the CKD. The particularity of our study is that we used the old radiography as a tool with two edges: one is considered sharp for diagnosing VCs and the other is maybe unfairly considered blunt, for evaluating ROD. Since both ROD and VCs are chronic conditions, we used ROD to assess the pathogenetic link between bone and vasculature, as single serum markers like P, Ca and PTH may not be representative of abnormalities that develop over a longer period of time.

Possible limitations

The accuracy of radiography for classifying ROD findings as high or low bone turnover disease may not be satisfactory. It can only indicate that there is a bone problem. There are concerns about radiation exposure. With renal failure, some possible preexistent bone diseases (like senile osteoporosis) might be considered as ROD. This could be a misleading approach. This semiquantitative VC score may not be suited for assessing progression.

Conclusion

Our study demonstrated that ROD and VCs do not develop independently, but they seem to be linked with each other. The VC score correlates with high Ca, P, iPTH and CaxP and low albumin serum levels. ROD, male gender and oral treatment with calcium salts are risk factors for VCs. Without underestimating the value of new diagnostic tests, we suggest that, even if considered obsolete, radiography should not be abandoned, as it can provide valuable information about both of these important complications of CKD: bone and vascular disease. Along with biochemical markers, radiography can help nephrologists to get a better picture of this new nosologic entity: CKD-MBD.

References

1. Hruska KA, Teitelbaum SL (1995) Mechanisms of disease: renal osteodystrophy. N Engl J Med 333:166–174
2. Moe S et al (2009) KDIGO clinical practice guideline for the diagnosis, evaluation, prevention and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). Kidney Int 76(Suppl 113):S22–S49
3. Drueke T, Salusky I (2001) The spectrum of renal osteodystrophy. Oxford University Press, , pp 397–417
4. Adragao T, Pires A, Lucas C et al (2004) A simple vascular calcification score predicts cardiovascular risk in haemodialysis patients. Nephrol Dial Transplant 19:1480–1488
5. Jean G, Bresson E, Terrat JC et al (2009) Peripheral vascular calcification in long-haemodialysis patients: associated factors and survival consequences. Nephrol Dial Transplant 4(3):685–690
6. Moe SM (2006) Vascular calcification and renal osteodystrophy relationship in chronic kidney disease. Eur J Clin Invest 36(Suppl 2):51–62
7. An WS, Son YK, Kim SE et al (2009) Vascular calcification score on plain radiographs of the feet as a predictor of peripheral arterial disease in patients with chronic kidney disease. Int Urol Nephrol. doi:10.1007/s11255-009-9697-8
8. Lui S, Chu H (1943) Studies in calcium and phosphorus metabolism with special reference to pathogenesis and effects of dihydrotachysterol and iron. Medicine 22: 103–107
9. Schwarz C, Sulzbacher I, Oberbauer R (2006) Diagnosis of renal osteodystrophy. Eur J Clin Invest 36(Suppl 2):13–22
10. Martin KJ, Olgaard K, Coburn JW et al (2004) Diagnosis, assessment, and treatment of bone turnover abnormalities in renal osteodystrophy. Am J Kidney Dis 43:558
11. Ferreira MA (2000) Diagnosis of renal osteodystrophy: when and how to use biochemical markers and non-invasive methods; when bone biopsy is needed. Nephrol Dial Transplant 15(Suppl 5):8–14
12. Jevtic V (2003) Imaging of renal osteodystrophy. Eur J Radiol 46(Issue 2):85–95
13. Adams JE (1999) Renal bone disease: radiological investigation. Kidney Int 56(Suppl 73):S38–S41
14. Sampaio LPG, Malzac FF, Raimundo PJ et al (2009) Prevalence of radiological findings among cases of severe secondary hyperparathyroidism. Sao Paulo Med. J 127(2): 71–77
15. Tigges S, Nance EP, Carpenter WA, Erb R (1995) Renal osteodystrophy: imaging findings that mimic those of other diseases. AJR Am J Roentgenol 165:143–148
16. Honkanen E, Kauppila L et al (2008) Abdominal aortic calcification in dialysis patients: results of the CORD study. Nephrol Dial Transplant 23(12):4009–4015
17. Bellasi A, Ferramosca E, Muntner P et al (2006) Correlation of simple imaging tests and coronary artery calcium

measured by computed tomography in hemodialysis patients. Kidney Int 70:1623–1628
18. London GM, Marty C, Marchais SJ et al (2004) Arterial calcifications and bone histomorphometry in end-stage renal disease. J Am Soc Nephrol 15:1943–1951
19. Gelev S, Spasovski G, Dzikova S et al (2008) Vascular calcification and atherosclerosis in hemodialysis patients: what can we learn from the routine clinical practice? Int Urol Nephrol 40(3):763–770
20. Moe S, Drüeke T, Cunningham J et al (2006) Definition, evaluation, and classification of renal osteodystrophy: a position statement from kidney disease: improving global outcomes (KDIGO). Kidney Int 69(11):1945–1953
21. Kiel DP, Kauppila LI, Cupples LA et al (2001) Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham heart study. Calcif Tissue Int 68:271–276
22. Van der Klift M, Pols HA, Hak AE et al (2002) Bone mineral density and the risk of peripheral arterial disease: the Rotterdam study. Calcif Tissue Int 70:443–449
23. Carr JJ, Register TC, Hsu F-C et al (2008) Calcified atherosclerotic plaque and bone mineral density in type 2 diabetes: the diabetes heart study. Bone 42:43–52
24. London GM, Marchais SJ, Guérin AP et al (2008) Association of bone activity, calcium load, aortic stiffness, and calcifications in ESRD. J Am Soc Nephrol 19:1827–1835
25. Toussaint ND, Lau KK, Strauss BJ et al (2008) Association between vascular calcification, arterial stiffness and bone mineral density in chronic kidney disease. Nephrol Dial Transplant 23:586–593
26. London G (2009) Bone-vascular axis in chronic kidney disease: a reality? Clin J Am Soc Nephrol 4:254–257
27. Moldovan D, Rusu C, Patiu I et al (2010) Could the serum parathormone be a predictive marker for peripheral vascular calcifications in chronic dialysis patients? Experience of a single center in Transylvania. Acta Endo (Buc) VI(no 1): 43–55
28. Buargub MA, Nabulsi MF, Shafeh TA (2006) Prevalence and pattern of renal osteodystrophy in chronic hemodialysis patients: a cross-sectional study of 103 patients. Saudi J Kidney Dis Transplant 17(3):401–407
29. Blacher J, Guerin AP, Pannier B et al (2001) Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. Hypertension 38:938–942
30. Spasovski GB (2007) Bone health and vascular calcification relationships in chronic kidney disease. Int Urol Nephrol 39(4):1209–1216
31. Coen G, Ballanti P, Mantela D et al (2009) Bone turnover, osteopenia and vascular calcifications in hemodialysis patients. Am J Nephrol 29:145–152
32. Barreto DV, Carvalho Barreto F, Carvalho AB et al (2005) Coronary calcification in hemodialysis patients: the contribution of traditional and uremia-related risk factors. Kidney Int 67:1576–1582
33. Coen G, Manni M, Mantella D et al (2007) Are PTH serum levels predictive of coronary calcifications in haemodialysis patients? Nephrol Dial Transplant 22:3262–3267
34. Hruska KA, Saab G, Mathew S, Lund R (2007) Renal osteodystrophy, phosphate homeostasis, and vascular calcification. Semin Dial 20(4):309–315
35. Covic A, Kanbay M, Voroneanu L et al (2010) Vascular calcification in chronic kidney disease. Clin Sci 119: 111–121
36. Chertow GM, Raggi P, Chasan-Taber S et al (2004) Determinants of progressive vascular calcification in haemodialysis patients. Nephrol Dial Transplant 19:1489–1496
37. Galassi A, Spiegel DM, Bellasi A, Block GA, Raggi P (2006) Accelerated vascular calcification and relative hypoparathyroidism in incident haemodialysis diabetic patients receiving calcium binders. Nephrol Dial Transplant 21:3215–3222
38. Jamal SA (2009) The effects of calcium-based versus non-calcium-based phosphate binders on mortality among patients with chronic kidney disease: a meta-analysis. Nephrol Dial Transplant 24(10):3168–3174