TITLE: Chronic Kidney Disease - Mineral and Bone disorders: Pathogenesis and management.

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ABSTRACT

The key players of the Chronic Kidney Disease-Mineral and Bone disorders (CKD-MBD) are calcium, phosphorus, PTH, FGF23, and the vitamin D hormonal system. The progressive reduction of kidney function greatly modifies the tightly interrelated mechanisms that control these parameters. As a result, important changes occur in the bone and mineral hormonal axis, leading to changes in bone turnover with relevant consequences in clinical outcomes, such as decrease in bone mass with increased bone fragility and bone fractures and increased vascular and valvular calcification, also with great impact in the cardiovascular outcomes.

So far, the knowledge of the mineral and bone disorders in CKD and the increased variety of efficacious therapies should lead to a better prevention and management of CKD-MBD.

KEYWORDS: CKD-MBD, renal osteodystrophy, secondary hyperparathyroidism, bone disorders, vascular calcification, bone fractures, osteoporosis.

GENERAL ASPECTS AND PATHOPHYSIOLOGY

To understand the pathogenesis and management of the Chronic Kidney Disease-Mineral and Bone Disorders (CKD-MBD) implies to analyze the regulation of mineral and bone metabolism, which includes the regulation of calcium and phosphorus that depends on the action of the calciotropic hormones: parathormone (PTH), calcitriol or 1,25 $(OH)_2D_3$ (the natural most active form of the hormonal system of vitamin D), calcidiol or 25 $(OH)D_3$ (substrate of calcitriol), calcitonin and FGF23/klotho and their impacts on bone [1]. All of them interact by modulating the absorption, elimination and deposition of calcium and phosphorus and cooperate to maintain the serum levels of both elements in normal ranges. The organs and glands involved in this regulation are the bone, the parathyroid glands, the intestine, and the kidney [2, 3]. The latter plays a major role because it is the final effector of several aspects of bone metabolism where important adjustments are done to maintain calcium and phosphorous homeostasis, acting also metabolically producing and/or activating components of the hormonal axis that regulate mineral metabolism.

Calcium and phosphorous participate in important biological processes, which justifies that they have an exquisite regulatory system. Calcium is the body's most abundant cation, 99% of it is found in bone and the remaining 1% in blood, extracellular fluid and soft tissues. It participates in vital processes such as cardiac contractility, neuromuscular function, membrane permeability, blood coagulation, bone mineralization and in different mechanisms of activation and secretion of hormones and enzymes [4].

Phosphorus is an essential nutrient, 85% is found in bone, the remaining 15% in blood, extracellular fluid, and soft tissues. It is part of the phospholipids of the membranes, nucleotides and nucleic acids and has a fundamental role in the enzymatic activation. At the renal level, it has a complex and very effective tubular reabsorption system that allows, if necessary, to recycle and retain almost all of the filtered phosphorus [5].

The previous description allows us to understand the importance of the normal kidney function to maintain calcium and phosphorous homeostasis. This almost perfect balance undergoes important and progressive maladjustments with the decline in kidney function that have repercussions in the calcium and phosphorus metabolism that become relevant in the severe phases of kidney failure.

In chronic kidney disease (CKD), the main alterations occur in calcium, phosphorus, PTH, FGF23/Klotho and the vitamin D hormonal system (calcidiol and calcitriol) (Figure 1), which lead to important changes in bone and vascular metabolism with very negative clinical

consequences, such as decreased bone mass, increased fragility fractures, and increased vascular and valvular calcification [6].

For nearly six decades, the mineral and bone disorders of CKD patients were known as "renal osteodystrophy". In 2006, a new term was coined by KDIGO guidelines "CKD-MBD" [7] to group and describe a new ample and complex clinical syndrome which includes not only biochemical and bone histological abnormalities, but also other bone and cardiovascular complications, such as bone fragility fractures and vascular and valvular calcification. Within this new definition, the term "renal osteodystrophy" was reserved to describe the histological bone abnormalities associated with CKD, which requires to perform a bone biopsy in order to classify the histological abnormalities based on parameters of bone turnover, mineralization, and volume (TMV) [8, 9].

Alterations in the regulatory axis of bone and mineral metabolism significantly affect the regulation of bone modeling during growth and bone remodeling during adulthood. These progressive CKD-MBD alterations begin in early stages of CKD and find their maximum expression in stages 4 and 5 [10]. Vascular and valvular calcification, that affects patients with CKD as a result of the dysregulation of bone and mineral metabolism, increase the accelerated aging of both systems. As consequence of the inappropriate or excessive use of some therapies, CKD-MBD can also be facilitated and/or aggravated. Two factors with great negative repercussions are, the excessive use of calcium and vitamin D receptor activators (VDRAs), that favor the excessive suppression of PTH and the reduction of bone remodeling [6]. Several studies have shown association between the excess of calcium and VDRAs and a higher prevalence of bone fragility fractures, cardiovascular disease, vascular calcification and mortality [11-14].

It is currently accepted that increase in FGF23, the decrease of Klotho, and the reduction of renal mass are possibly the earliest events in the pathogenesis of CKD-MBD. These changes favor the reduction of 1-α-hydroxylase in the kidney, which results in low levels of calcitriol, the most active natural activator of VDR, a fact that reduces the absorption of calcium in the intestine and favors the decrease in serum calcium, which in turn acts as a stimulus for the synthesis and release of PTH. The increase in PTH favors bone turnover and resorption and stimulates the production of 1-α-hydroxylase. All of these mechanisms translate into compensatory increases in serum calcium. Furthermore, the increase in FGF23 and PTH favor urinary phosphate excretion, maintaining normal serum phosphate levels until stages 3-4 of CKD [6, 10].

Despite the fact that FGF23 and PTH have synergistic effects in relation to the renal excretion of phosphorus, they have opposite effects on calcitriol synthesis. FGF23 inhibits 1-α-hydroxylase by decreasing the synthesis of calcitriol, whereas PTH stimulates the production of 1-α-hydroxylase, increasing calcitriol synthesis (Figure 1). FGF23 exerts its tubular effect by binding to its Klotho co-receptor and activating its FGFR-1 FGFR-3 receptors, while PTH does so by binding to its specific receptor. Both increase the phosphate excretion by reducing the concentration of the sodium cotransporters NaPi2a and NaPi2c, through PKA and PKC-dependent pathways [6, 10].

Calcium and calcitriol act on parathyroid cells through their specific receptors, the calcium-sensing receptor (CaSR), and the vitamin D receptor (VDR), respectively (Figure 1). CaSR is a member of the G protein-coupled membrane receptor family, while VDR is a nuclear receptor that, when bound to its activator (any active form of vitamin D, VDRA), acts as a transcription factor. Differences in the nature of the two ligands and their receptors lead to different mechanisms of action with synergistic functions on parathyroid cells.

The CaSR detects small decreases in extracellular calcium concentrations, increasing in seconds or minutes the release of stored PTH, if the stimulus is the opposite, it reduces the hormone release. If the calcium decrease persists for longer periods (hours, days), the synthesis of PTH increases, this occurs at the post-transcriptional level, modifying the stability of the mRNA. Whereas the decrease in serum calcium reduces the degradation of PTH mRNA, increasing its stability and half-life; the increase in calcium has the opposite effect [15]. Phosphorus acts in a similar way, but in the opposite direction, increases in phosphorus increase the stability and half-life of PTH mRNA and vice versa. It has recently been described that the effect of phosphorus is also carried out through its action on the CaSR [16]. Unlike calcium and phosphorus, calcitriol binds to VDR and exerts its effect by reducing the transcription of the PTH gene and the synthesis of this hormone.

When kidney function progressively decreases, these complex and tightly interrelated mechanisms fail to adequately control the mineral metabolism, and despite increases in FGF23 and PTH, serum phosphorus increase and serum calcitriol decrease. In advanced stages of CKD-MBD, this situation worsens, and as a consequence of the decrease in calcium and calcitriol and the increase in phosphorus, the parathyroid gland is subjected to a permanent proliferative stimulus that triggers severe forms of secondary hyperparathyroidism with hyperplasia of the gland, first diffuse and then nodular, with a significant reduction in the CaSR, VDR and FGFR/Klotho expression which ends in a poor response of the parathyroid glands to calcium, VDR activators (VDRA) and FGF23 [17].

Despite the fact that all the previously discussed changes lead to stimulation of the parathyroid gland and therefore what we should expect in CKD patients stage 5 would be a high prevalence of secondary hyperparathyroidism with high bone turnover, nowadays the latter is not the most frequent findings within the CKD-MBD constellation. Due to several other important factors, such as the aging of the CKD population, the increase in the prevalence of diabetes as a cause of CKD, the aluminum load and the excess of calcium and vitamin D, in the recent decades the more frequent pattern of bone lesions has changed from high to low-bone turnover [18-23]. Although the histological pattern of both lesions is different and represent the opposite extremes of the renal osteodystrophy, both are associated with similar complications, such as a higher prevalence of vascular calcification and bone fragility fractures, leading to a higher association with mortality risk.

Cross-sectional studies have shown the pattern of abnormalities of the main biochemical parameters of CKD-MBD at different stages of CKD (Figure 2). Up to glomerular filtration rates (GFR) of around 30-40 ml/min, serum calcium and phosphate values usually remain within the normal range, the respective decrease and increase of both are manifested with GFR below 20-30 ml/min [24]. In contrast, calcitriol begins to decrease early in the course of CKD (GFR between 70 and 80 ml/min), calcidiol and PTH increase a bit later, from GFR of 60-70 ml/min (Figure 2).

BIOCHEMICAL ABNORMALITIES

The more important changes in the CKD-MBD biochemical parameters currently start in patients CKD stage 3b, thus, the routine assessment of the biochemical parameters of CKD should begin in stages 2-3a. The frequency of assessments, the pattern of decline of the GFR and the type, severity and duration of the identified abnormalities, should be evaluated individually to adapt the frequency of the assessments and the non-pharmacological and pharmacological interventions.

The laboratory diagnosis of CKD-MBD includes the quantification of serum calcium, phosphate, PTH, calcidiol, alkaline phosphatase (ALP) (total or bone-specific) and the acid-base status, together with other serum and urinary parameters used routinely in the follow-up of patients with CKD. Although much progress has been made in the mechanisms involved in the role of FGF23/Klotho, its usefulness in routine clinical practice is still very limited.

The interpretation of the results requires a careful and individualized analysis, the importance of one single abnormal value of any of bone and mineral biochemical markers

should not be determinant. The diagnosis and management of CKD-MBD should be based mainly on the trend of changes in these markers, this concept plays a key role particularly in the interpretation of the PTH values [6].

A great effort has been made to better define the "optimal and safest" values of the CKD-MBD biochemical markers at the different stages of CKD in order to guide therapeutic decisions. The international guidelines (KDOQI, KDIGO), and more recently the results of international studies have established different cut-off levels with optimal ranges of efficacy and safety for each of these parameters, mainly in CKD patients stage 5 in dialysis (stage 5D) [6, 8, 25-30]. Table 1 summarizes the values that could be considered as adequate or acceptable of the main biochemical markers according to degree of CKD-MBD. Most of the recommendations are supported by updated scientific rationale, but the degree of evidence based on randomized clinical trials is still low.

BONE ABNORMALITIES

Bone tissue has unique biomechanical properties: such as a great mechanical tension to torsional and tensile stress with a great flexibility. These excellent properties of bone explain why only a small proportion of bone fractures occur despite the great number of falls suffered throughout life. Bone acquires and maintains these excellent biomechanical properties thanks to the activity of bone remodeling units, which during the young adult life (20-40 years), allow the renewal of an average of 5 to 10% of the skeleton per year. However, the ability to renew bone tissue suffers a progressive decline between the fourth and fifth decade of life, especially in women after menopause. In addition to the changes related to aging and gender, which are suffered by the entire population, the two disorders that have the greatest impact on the quantity, quality and fragility of bones are osteoporosis and renal osteodystrophy.

Bone strength is determined by the density and quality of the bone. In CKD, as a result of the described bone remodeling alterations and other factors that we will detail more extensively in other papers of this special issue [31, 32], patients present a more fragile bone with a higher risk of fractures. Unfortunately, the measurement of bone mineral density (BMD) by bone densitometry (DXA) does not capture the quality of bone (cortical and trabecular microarchitecture), but it can be assessed using high-resolution quantitative peripheral computed tomography (HR-pQCT). In spite of the previous mentioned limitations, DXA is a good non-invasive marker predictive of fragility bone fractures in the general population, as well as in CKD patients [25, 33].

The bone turnover rate affects both the cancellous (trabecular) and the compact (cortical) bone, and it depends on the activity of the bone remodeling units regulated by multiple factors, including PTH, which plays a key role in the remodeling process. The cortical bone is the most abundant type (85% of the skeleton), however, the bone with the highest metabolic activity is the trabecular bone [6]. Both decrease in CKD patients, but due to the higher proportion of cortical bone and the sustained major effect of PTH on the cortical bone, in long term, the loss of cortical bone is predominant and leads to a thin, trabecularized and more porous cortex, which increases exponentially bone fragility, especially in long bones, where cortical bone integrity is fundamental for bone health.

High serum PTH levels accelerates bone turnover, PTH stimulates bone cell proliferation and the activity of each remodeling unit, and also increases the recruitment of new bone remodeling units [34]. As a result of these two mechanisms, bone activity increases, osteoid formation is abnormal, immature and non-lamellar, and although this abnormal matrix formed is mineralized, it results in a bone with a lower strength and a greater fragility.

The quantification of serum PTH, associated if possible with bone alkaline phosphatase, are the most widely used biochemical parameters to evaluate non-invasively bone activity, although PTH serum values has many limitations. Very high serum PTH levels, usually greater than 450 pg/ml, have a reasonable good histological predictive value of accelerated bone turnover, but moderately high PTH values (300-450 pg/ml) do not have good correlation with bone turnover [35]. In fact, within these ranges of PTH values, high, normal or even low bone turnover can be observed.

Low PTH levels are associated with poor cell activity and inadequate and insufficient bone turnover. In practice, low bone turnover is suspected when serum PTH levels are below those considered "normal" for CKD, these values vary according to the stage of CKD. In patients CKD stage 5D, PTH levels lower than 2 times the upper limit of the maximum value of the general population (around 120 pg/ml), can be predictive of low bone turnover. Thus, even though the measurement of serum PTH levels in CKD patients is the most current non-invasive method used to assess bone turnover, it has important limitations to judge bone turnover.

MANAGEMENT OF CKD-MBD BIOCHEMICAL ABNORMALITIES

In patients CKD stages 3-5, GFR progressively decreases and there is an increasing inability to properly excrete phosphorus and prevent its retention. This fact is of great importance, as epidemiological and experimental evidence have indicated that hyperphosphatemia is "a toxic factor" at multiple levels and it has become an important risk factor associated with accelerated aging and increased mortality. Recent results of COSMOS, a prospective 3-year study performed in 6,790 patients with CKD stage 5D from 20 European countries, using analysis that mimic a clinical trial, showed that the decrease in serum phosphate levels throughout six months periods, was associated with a significant reduction in mortality [22].

The available clinical evidence on the optimal levels of serum phosphate in CKD is based primarily on observational studies and indicates that in CKD, serum phosphate should be kept as close as possible to the normal range [8, 25]. In practice, the approaches to achieve this goal include three levels of action: reduction of dietary phosphorus intake, use of phosphate binding agents and increase phosphate removal by adding more hours or days of dialysis [6].

The factors affecting the gastrointestinal absorption of phosphorus include vitamin D, the phosphorus content of food and its bioavailability. The sources of phosphorus in the diet are protein-rich foods, which in a non-vegetarian western diet represent about 60% of dietary phosphorus. Foods rich in phosphorus are dairy products, meat, fish, sausages, legumes, nuts, and chocolates. However, phosphorus is also found in high quantities in inorganic forms, with high bioavailability, in the additives and preservatives commonly used in the food industry. This aspect is becoming a matter of great importance for public health, not only in CKD but also in the general population [36]. The reduction of dietary phosphorus intake coming from proteins has an important limitation, since it is necessary to guarantee an adequate protein intake to avoid malnutrition.

The use of diets with moderate phosphorus restriction in combination with phosphate binding agents is the most widely used strategy to avoid phosphorus accumulation in patients CKD stage 5. This combined therapy allows a more liberal diet that may help to obtain a better health status that could positively affect survival, as suggested by a recent epidemiological study [37]. All available phosphate binders are effective in reducing serum phosphate, although it must be taken into account that calcium-based phosphate binders can increase

vascular calcifications and those containing aluminum have shown to be dangerous [6] and they are the only type of phosphate binding agents not associated with survival benefits [37].

Several aspects should be taken into account in the selection of the phosphate binding agents, such as the stage of CKD and the presence of other components of CKD-MBD. Despite the fact it is known that phosphate load retention may precede the increase of the serum phosphate levels, in the CKD stages 4-5, the phosphate binders should not be routinely used as a preventive treatment if there is no hyperphosphatemia. In patients with a trend to hypercalcemia, vascular calcification, low bone turnover and persistent low levels of PTH, the use and the dose of calcium phosphate binders should be restricted and carefully evaluated.

To obtain a higher phosphate removal, the hours of dialysis can be increased, either by prescribing prolonged nocturnal hemodialysis or short more frequent hemodialysis (2.5-3.5 hours per dialysis session), or using peritoneal dialysis. These strategies are useful to control hyperphosphatemia, to reduce the dose of phosphate binder prescription, and control PTH levels in patients CKD stage 5D. However, even using these approaches, a non-negligible percentage of patients CKD stage 5D still need to use phosphate binders to control the hyperphosphatemia [37].

Regarding calcium, in addition to the limitations of calcium phosphate binders, the most appropriate approach to avoid calcium overload in patients CKD stage 5D is to use a dialysate calcium concentrations between 1.25 and 1.50 mmol/l (2.5 and 3.0 mEq/l). Calcium concentrations higher than those mentioned, combined with the use of calcium-containing phosphate binders, lead to calcium overload and excessive suppression of PTH, all with negative effects associated to morbidity and mortality [38].

In patients CKD stage 3-4, it is suggested to maintain PTH levels in the upper normal range. In patients CKD stage 4-5 before dialysis, is acceptable to allow values discretely above the upper limit of the normal values, in fact, this allows a necessary phosphaturic effect. In patients CKD stage 5D the situation is more complex and the criteria will be detailed later. In advanced stages of CKD (stages 4-5), biochemical measurements, including PTH, should be evaluated more frequently (1-3 months) in order to early detect progressive increases in PTH, as well as changes in calcium, phosphate, and calcidiol, and try to correct them towards normality (Table 1). According to the KDIGO guidelines, in all stages of CKD, serum calcidiol should be maintained within the safe and biologically optimal range (20-40 pg/mL), this strategy can help to control serum PTH values, mainly in the early stages of CKD, and could also provide positive pleiotropic effects (cardiovascular, immune, infections, muscular, cancer….) at all stages of CKD [39]. The recommendations for the use of calcitriol or any

other form of active vitamin D receptor activator differs, in CKD patients stages 3-5, the use of calcitriol and active vitamin D analogs is not recommended as routine and they are reserved for CKD patients stages 4–5 with severe and progressive hyperparathyroidism, though these limitations are not worldwide accepted [40]. In patients CKD stage 5D, the use of calcimimetics, calcitriol, or vitamin D analogs, or a combination of calcimimetics with calcitriol or vitamin D analogs is suggested [30].

In patients on dialysis (CKD stage 5D), the PTH values are best defined by the guidelines (Table 1). At that level, the KDIGO guidelines suggest to maintain serum PTH levels within a range of 2 to 9 times the upper limit of the normal range of the assay. However, as already mentioned, it is important to emphasize that the therapeutic decision should not be based on isolated values but on the “trend of changes in PTH” [8, 25, 30]. Treatment should be started or modified before reaching the limits of the ranges mentioned in any sense, since it is from these limits, - both, downward and upward-, that there is a greatest association with a higher relative risk of mortality. To implement these recommendations and try not to overcome these ranges, it is necessary to consider not only the concept of "trends", but also to bear in mind that, in several studies, in patients CKD stage 5D, the better outcomes have been associated with serum PTH values around 150-350 pg/ml [41, 42].

In patients CKD stage 5D, the initial suggested management to reduce PTH is the use of calcimimetics and/or VDRAs. Although both are independently effective through their action on different receptors (CaSR and VDR), the combined administration of these drugs has been shown to have a synergistic effect [43]. Selection of the initial drug for the treatment should be based not only on PTH levels, but also on serum calcium and phosphate levels and other aspects of CKD-MBD, such as the presence of vascular calcification. If VDRAs are used, the appearance of hyperphosphatemia or hypercalcemia requires the reduction or suspension of the VDRAs, as occurs with severe (<7 mg/dl) and / or symptomatic hypocalcemia with the use of calcimimetics. The association of VDRAs and calcimimetics not only has synergistic molecular effects, but also reduces the possibility of adverse effects on calcium and serum phosphate, since the effects of one can counteract the effect of the other. If PTH is drastically reduced to levels associated with low bone turnover, the use of both should be reduced or discontinued.

In patients with severe secondary hyperparathyroidism who do not respond adequately to these drugs, parathyroidectomy should be considered. Although there is no agreement on the serum levels of PTH to which a parathyroidectomy should be indicated [44], the most

current practice is to perform a parathyroidectomy if despite an adequate medical treatment for a period of at least 3-6 months, patients permanently maintain PTH levels above 800 pg/ml.

EPIDEMIOLOGY AND PATHOPHYSIOLOGY OF VASCULAR CALCIFICATION

CKD patients have a very high prevalence of vascular calcifications that can double the percentage observed in the general population of the same age, sex, and region [11, 14] leading to cardiovascular disease, decreased life expectancy, and mortality, even in the early phases of CKD. Furthermore, calcification of the cardiac valves involves a high risk of cardiovascular dysfunction.

The mechanisms by which vascular and valvular calcification occurs are complex. Apart from the physicochemical precipitation of calcium and phosphate; it also involves a highly regulated active and modifiable processes, such as decrease in the inhibitors and increase in the promoters of vascular calcification, and also the formation of vesicles that induce a phenotypic change from vascular smooth muscle cells to bone-like cells (Figure 3). The outcome is the formation of bone inside the artery wall [45, 46].

Among the promoters of vascular calcification, high serum phosphate is considered to be the most important uremia-related, non-traditional risk factor associated to vascular calcification in CKD patients [6]. Phosphorous is capable of acting as a secondary intracellular messenger, activating several molecular pathways related to bone formation. It reaches the intracellular space through a specific sodium-dependent channel called Pit1 and exerts important negative actions. High intracellular phosphorus levels have been shown to increase the bone-specific transcription factor Cbfa1, resulting in the activation of several osteogenic factors, including bone morphogenetic proteins (BMP) which lead to phenotypic [47-49].

Among the inhibitors of vascular calcification, pyrophosphates, fetuin A, osteoprotegerin (OPG) and matrix Gla protein (MGP) have been the most studied in tissues and serum. Pyrophosphates are located in the vascular matrix and preserve the vascular smooth muscle cell phenotype, inhibiting the calcium phosphate crystals formation and the change of vascular smooth muscle cells to osteoblast-like cells [46, 50, 51]. Fetuin A is a well-known inhibitor of osteogenesis, and OPG holds back osteoclast differentiation, modulating bone resorption through its action as a decoy receptor of RANKL, and it could also act as an inhibitor of vascular calcification [52]. FGF23 and its co-receptor Klotho have also been related to vascular calcification. In fact, knockout mice for FGF23 and Klotho develop low

bone mass and accelerated ageing with widespread tissue calcification. The mechanisms by which FGF23/Klotho impair bone health and promote vascular calcifications appear to be related to phosphate excretion, vitamin D synthesis, and also PTH regulation.

Most of the factors discussed above, either promoters or inhibitors of the vascular calcification process, have been related not only with vascular calcification but also with bone loss, a fact that reinforces the idea that there are common pathways between bone and vascular metabolism [11, 13, 52, 53]. Both are very common disorders that have been always associated with aging, in the general population as well as in CKD patients. Recent studies have suggested that, in addition to aging, there are other factors that link vascular calcification and bone health, including Wnt pathway inhibitors and the RANK/RANKL/OPG system [54]; which will be treated specifically in one of the papers of this issue [55]. Furthermore, it has been recently described that LGR4, a new RANKL receptor, could also be involved in these alterations [56].

TYPES AND MANAGEMENT OF VASCULAR CALCIFICATION

The diagnosis of CKD-MBD includes the study and detection of cardiovascular calcification. This has always been considered a life-threatening complication of CKD with little chance of acting on them. The better knowledge of bone and mineral metabolism in CKD and, consequently, the appearance of new drugs in this field, has arisen a great interest in this complication, which today is considered, at least partially preventable through better control of the alterations of bone and mineral metabolism from early stages of CKD.

Vascular calcifications can be found at different levels of the cardiovascular system, both in arteries and in heart valves. There are three types of arteries depending on their size and structure: elastic or large-caliber arteries, muscular or medium-caliber arteries, and small-caliber arteries [13]. Elastic or large-caliber arteries are responsible of conducting the blood to the distribution arteries; they show a relatively thin wall in proportion to their diameter and a rather thick tunica media that contains more elastic fibers than smooth muscle with a fairly thin adventitia, the aorta, the subclavian and the common carotid arteries belong to this group.

At the second level are the muscular or medium-caliber arteries, which are capable of adapting to vasodilation and vasoconstriction and to adjust the blood volume to the perfusion requirements. They have a tunica media containing a high proportion of smooth muscle, the axillary, brachial, radial, coronary, femoral, and tibialis arteries are included in this group. Finally, on the third level are the small-caliber arteries, they are responsible of regulating the

local blood flow and perfusion pressure through variations in luminal size caused by vasoconstriction and vasodilation. They are less than 2 mm thick and have a tunica media that only contains smooth muscle, this group includes, among others, the palmar arch and the digital arteries [11, 13].

Vascular calcification can occur in the intima and media layers. Intimal calcification begins and progresses throughout lifetime mainly under the influence of genetic and lifestyle circumstances. Intimal calcification is associated with atherosclerosis, including endothelial dysfunction, intimal edema, lipid cell formation, and blood cell migration, which can cause a ruptured plaque, leading to the formation of a thrombus. It is currently associated with chronic arterial inflammation exacerbated by risk factors such as hypertension, diabetes, hypercholesterolemia, obesity, smoking, and a family history of heart disease [57].

Calcification of the media occurs in the elastic lamina of large-caliber and medium/small size arteries. It can be independent of atherosclerosis, but it can coexist with it. This type of calcification was initially known as "Monckeberg sclerosis" [58]. It affects arteries that are less likely to develop atherosclerosis, such as visceral abdominal, thyroid, lung, limb and femoral arteries, but it is also extremely common in large-caliber arteries like the aorta. It is frequently observed in CKD patients and other disorders with metabolic abnormalities such as diabetes and hypervitaminosis D. Table 2 summarizes traditional and non-traditional risk factors related to vascular calcification in CKD patients [6].

Although there is no consensus on the subject, performing a lateral abdominal radiograph and an echocardiogram, which are simple and inexpensive procedures, can be effectively used to detect vascular and valve calcification. There are other determinations, very useful in some locations such as the heart, based on the quantification of calcium (score) using techniques based on computed tomography (CT), electron beam tomography (EBCT) and multi-slice computed tomography (MSCT), all of them quite sensitive methods for the detection and quantification of calcium in the vessels [53, 59-61]. However, these techniques are not widely available and the location and extension of vascular calcification can be reproducibly determined by X-ray. Several available algorithms such as the Kauppila or Adragao methods [62, 63] allow to quantify and determine vascular calcification, featuring a good correlation with CT-based gold standard techniques that also show a similar association to the most sensitive methods with survival results. The information provided from these studies is useful to assess risk and prognosis, but also to guide the therapeutic management of CKD patients [11, 12, 14, 62, 63].

A recent study in patients CKD stage 5D, has shown that vascular calcifications are frequently located in larger-caliber arteries such as the aorta (about 80%), medium-caliber arteries, including the coronary arteries (about 60-70 %), and in small-caliber arteries (20-30%) [11]. The time (years) spent on hemodialysis has been positively associated with vascular calcification, particularly in medium-caliber arteries. It has been estimated that each year on dialysis increases the risk of developing vascular calcification by 15% [13].

In CKD-MBD, it is very important to follow strategies able to minimize or prevent the progression of vascular calcification and to take into account primary prevention measures to control risk factors (Table 2). It is crucial to promote a healthy lifestyle, a balanced diet, regular physical exercise, avoid tobacco and maintain a low dose of alcohol consumption. When vascular calcification is already present, secondary prevention should aim to reduce its complications, intensifying the measures and treatments previously described [1]. Most strategies to reduce vascular calcifications focus on correcting the mentioned classic and non-classic risk factors as much as possible. Other strategies, such as the use of compounds that reduce vascular calcification through the inhibition of hydroxyapatite formation and others such as pyrophosphates have shown their effectiveness, but they have not yet been transferred to routine clinical practice [64, 65].

CALCIPHYLAXIS

Calciphylaxis, also called "calcific uremic arteriolopathy," is a very severe uncommon form of medial calcification of small cutaneous arteries. It is characterized by painful ischemic skin ulcerations that are frequently followed by superinfections. The pathogenesis of this form of severe vascular calcification is associated with poor control of mineral metabolism, mainly PTH, calcium and phosphate, although several other factors have been implicated [66, 67]. In addition, it can be due to uncontrolled mineral metabolism and the dysregulation of some calcification inhibitors such as fetuin A, MGP and vitamin K [68, 69]. This form of calcification is associated with a high risk of mortality.

The management of calciphylaxis remains a challenge. As in other forms of vascular calcification, the first step in the treatment of calciphylaxis is the effective control of all risk factors, especially serum phosphorus, calcium, and PTH using the most effective strategies, including parathyroidectomy, if necessary. Ulcerated skin lesions are frequently infected, so antibiotic therapy is indicated [66-68]. Some of the experimental compounds in the treatment of vascular calcification are also being evaluated as a treatment for calciphylaxis [64, 65].

There are positive results with the use of therapeutics such as sodium thiosulfate, bisphosphonates, pyrophosphates, [70], and anti-inflammatory strategies [64-67, 71-82].

BONE MINERAL DENSITY AND FRACTURES IN CKD PATIENTS

The CKD-MBD term also includes the presence of bone fragility fractures, which can be due to renal osteodystrophy and osteoporosis, the latter a highly prevalent alteration associated with age, whose importance has considerably increased due to the aging of the CKD population [83]. Though several aspects specifically related to bone mineral density and bone fractures will be extensively treated in other papers of this special issue [31, 32], we will summarize the general most important alterations.

In osteoporosis there is a reduction in bone mass without a specific defect in bone formation. This occurs because the balance between bone formation and bone resorption is lost, favoring the latter; as a result less new bone is formed. The densitometric definition of osteoporosis and the criteria for its diagnosis were first adopted by the World Health Organization (WHO) in 1994 [84]. It is defined as “a disease characterized by low bone mineral density and a microarchitecture deterioration leading to low bone strength and increased risk of fractures". Strictly speaking, the definition applies only for use in Caucasian postmenopausal women for diagnostic purposes, and not for treatment. However, its use progressively expanded to include men and also to help in the treatment decision process. The WHO definition of osteoporosis did not include the CKD condition [6].

The T-score of the DXA measurement is used for the assessment of BMD and for the definition of osteoporosis. Each T-score difference in BMD represents 1 standard deviation (SD) from the peak bone mass. Values up to -1 SD BMD below the mean peak bone mass are considered normal; values between -1 SD and -2.5 SD BMD are indicative of osteopenia, and values below -2.5 SD BMD are indicative of osteoporosis [83, 84]. BMD measurement plays an important diagnostic, preventive, and managerial role in the general population, and its utility has recently been expanded to patients with CKD [25, 85].

The BMD measured by «Dual-energy X-ray Absorptiometry» (DXA) does not measure bone quality, but it has an a predictive value in the assessment of the bone fragility fractures risk. There is an increasing evidence indicating that CKD patients have a higher risk of fracture and associated mortality than the general population [13, 25, 53]. This probably occurs because the mechanical properties of the bone are affected not only by aspects related to osteoporosis but also by the specific changes due to the renal osteodystrophy. This complex

association, in which it is difficult to separate the two components, -osteoporotic and renal osteodystrophy-, has sometimes been described as "uremic osteoporosis" [86, 87], trying to emphasize the existence of a specific and complex alteration that include changes due to CKD plus the alterations of the "classic" osteoporosis [88, 89]. In fact, it is possible that the high prevalence of fractures in the aging CKD population might be more related to the classic osteoporosis rather than the renal osteodystrophy [86, 90].

Until a few years ago, BMD was considered to have little diagnostic value in CKD patients [8], however, recent studies have indicated that in CKD patients, decreased BMD is predictive of fracture risk, as it is in the general population, in stages 4-5D, it may even underestimate the risk of fracture. Updates of the KDIGO guidelines (2017) [25, 30] suggest evaluation with BMD in CKD patients with evidence of CKD-MBD and / or osteoporosis risk factors, and whether it is possible, to quantify the risk of fracture with FRAX® in a similar way to general population, since both can influence therapeutic decisions.

Although drugs for the treatment of classic osteoporosis have been approved expressly excluding CKD patients -especially stages 3b-5-, in recent years several important studies have been reanalyzed providing information indicating that these drugs can be used prudently and after an individualized analysis in CKD population stages 3b-5, which are ultimately the patients with the major problems and those who need it most. Until recently, the use of these drugs was only accepted for patients CKD stages 1-3a, in patients without obvious biochemical alterations, in which therapeutic management should be the same as that used in the general population.

Adequate dietary calcium content, normalization of calcidiol levels, and antiresorptives (bisphosphonates or denosumab) have been recommended in these patients, which could be administered without the need for a bone biopsy [86, 90]. Many of these recommendations are now equally valid for more advanced CKD stages and for patients with previous clinical or morphometric fractures, and / or very high risk of fracture, as long as it is considered that there is no risk of low bone turnover. However, this area is under review and even this last aspect is also currently debated [90].

In patients CKD stage 4-5, before any specific antiosteoporotic treatment is prescribed, the first step should always be the adequate control of serum calcium and vitamin D deposits repletion [86, 87, 90]. After this first step, if antiresorptives are used, since it is not eliminated through the kidneys, denosumab is the best alternative. However, an important limitation has recently mentioned, because an increase in fractures risk has been obseved when the drug is suspended without continuing with other antiosteoporotic treatment. With bisphosphonates

there is greater uncertainty and there is no experience. According to the technical data sheet, zoledronate and risedronate would be contraindicated. Pamidronate, alendronate and ibandronate are not expressly contraindicated, but there is no experience with its use in advanced stages of CKD.

Teriparatide, an anabolic drug, could be safe and effective for up to 2 years and of special use in parathyroidectomized patients or those with high suspicion of low bone turnover disease. Raloxifene has been used in CKD patients without significant climacteric symptoms and without risk of thromboembolism, but experience is also limited [91]. Finally, romosozumab, a very effective sclerostin inhibitor used in the general population, still is not a proved valid option in CKD patients, since its effect could be positive for bone, but possibly harmful for vascular calcification [92, 93].

In summary, the pathogenesis and management of the mineral and bone disorders associated to CKD have undergone major changes in the past 50 years. Until 2006, when the term CKD-MBD was coined, the study of these alterations had focused on the study of bone and the biochemical parameters of the calcium-phosphorous-vitamin D-PTH axis. With the new definition this axis has enriched, including cardiovascular alterations, vascular/valvular calcification and bone fragility fractures, aspects that are nowadays focusing many of the efforts in the current management of CKD-MBD. Fortunately, today we have more and better information to understand the CKD bone and mineral metabolism disorders and better drugs to treat the CKD patients.

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AUTHOR´S CONTRIBUTION

J.C.A. and N.C.L. had the idea for the article, B.M.C and J.M.V performed the literature search and data analysis, N.C.L, C.A.M and J.C.A. drafted the article and J.R.C., J.B., N.C.L, C.A.M and J.C.A. critically revised the article.

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Table 1. Recommended values from KDIGO for the main serum bone and mineral markers according to CKD grade (Modified from Cannata-Andía et al. Mineral and Bone Disorders in Chronic Kidney Disease, pages 223-239 of the book Management of Chronic Kidney Disease, A clinician's guide with permission from Springer, Copyright © 2014)

	CKD Stages 3-5	**CKD Stage 5D**
Serum phosphate	Lowers elevated serum P levels towards the normal range.	Lowers elevated serum P levels towards the normal range.
Serum calcium	Avoids hypercalcemia.	Avoids hypercalcemia.
Serum PTH	Maintains serum PTH within the normal or slightly elevated range in CKD stages 4-5 (see text).	Maintains serum PTH within the range of 2 to 9 times more than normal range.
Serum calcidiol	Maintains serum calcidiol within the safe and biologically optimal range (20-40 pg/mL).	Maintains serum calcidiol within the safe and biologically optimal range (20-40 pg/mL).

Table 2. Traditional and non-traditional uremia-related risk factors for vascular calcification in CKD patients (IL-1, Interleukin 1; IL-6, Interleukin 6; TNF-α, tumor necrosis factor-alpha). (Modified from Cannata-Andía et al. Mineral and Bone Disorders in Chronic Kidney Disease, pages 223-239 of the book Management of Chronic Kidney Disease, A clinician's guide with permission from Springer, Copyright © 2014).

Traditional risk factors	**Non-traditional risk factors (uremia-related)**
Hypertension	Time in dialysis
Diabetes mellitus	Hyperphosphatemia
Tobacco	Hyperparathyroidism and hypoparathyroidism
Genetic	High dosage of vitamin D metabolites
Age	Low Fetuin A
Dyslipidemia	Poor nutrition (Low albumin)
History of premature coronary heart disease	Chronic inflammation (High IL-1, IL-6, TNF-α)
Vitamin K inhibitors (warfarin)	Hyperhomocysteinemia

FIGURE CAPTIONS

Figure 1. Interrelationships between calcium, phosphorus, PTH, FGF23/klotho and calcitriol. The calcium ability to increase FGF23 and the low and high phosphorus to increase and respectively decrease serum calcitriol are not shown. ((Modified from Cannata-Andía et al. Mineral and Bone Disorders in Chronic Kidney Disease, pages 223-239 of the book Management of Chronic Kidney Disease, A clinician's guide with permission from Springer, Copyright © 2014.)

Figure 2. Evolution of CKD-MBD parameters. a) The prevalence of hyperparathyroidism, hypocalcemia, and hyperphosphatemia by GFR levels. b) Median values of calcium, phosphorus and intact PTH by GFR levels. c) Median values of $1,25(OH)_2D_3$, $25(OH)D_3$ and intact PTH by GFR levels. (Republished by permission from MacMillan Publishers Ltd: Kidney International. 2007; 71: 31–38, Copyright © 2007.)

Figure 3. Promoters and inhibitors of vascular calcification. ALP, alkaline phosphatase; Ca, calcium; LDLox, oxidized low-density lipoprotein; MGP, matrix GLA protein; P, phosphate; PTHrP, parathyroid hormone-related protein; TNF-a, tumor necrosis factor-alpha; Vit D_3, calcitriol. (Republished with permission of Oxford University Press from Nephrol Dial Transplant. 2011; 26, 3429–3436).