Review Article

Diagnostic Workup for Disorders of Bone and Mineral Metabolism in Patients with Chronic Kidney Disease in the Era of KDIGO Guidelines

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KDIGO (Kidney Disease: Improving Global Outcomes) is an international nonprofit organization devoted to "improve the care and outcomes of kidney disease patients worldwide through promoting coordination, collaboration, and integration of initiatives to develop and implement clinical practice guidelines." The mineral and bone disorder (MBD) in patients with chronic kidney disease (CKD) has been the first area of interest of KDIGO international initiative. KDIGO guidelines on CKD-MBD were published in 2009 with the intent to modify the previous KDOQI guidelines that had failed to consistently change the global outcome of CKD patients. After the publication of KDOQI guidelines for bone metabolism and disease in 2003, a large number of observational data emerged in literature linking disordered mineral metabolism with adverse clinical outcomes. Notwithstanding this large body of observational data, a paucity of evidence from high-quality clinical trials was available for the development of KDIGO guidelines. Herein, a summary will be provided of the most important findings of KDIGO guidelines regarding the diagnostic workup and clinical monitoring of CKD-MBD patients.

1. Introduction

The nonprofit foundation KDIGO (Kidney Disease: Improving Global Outcomes) was established in 2003 with the aim to improve outcomes in CKD patients through the optimization of care.

The mineral and bone disorder (MBD) in CKD has been the first area of interest of the KDIGO international initiative. A KDIGO position statement published in 2006 defined CKD-MBD disease as due to either one or a combination of the following clinical situations: (a) abnormalities of calcium, phosphorus, parathyroid hormone (PTH), or vitamin D metabolism; (b) abnormalities in bone turnover, mineralization, volume, linear growth, or strength; (c) vascular or other soft tissue calcification [1]. The term renal osteodystrophy was limited to the diagnosis of alterations in bone morphology and their histomorphometric quantification by bone biopsy. With regard to the volume and quality of

evidence, a KDIGO Work Group of international experts and an Evidence Review Team (ERT) were set up to collaborate for the development of a set of new guidelines for the care of adults and children with CKD stages 3–5 and for patients on chronic dialysis therapy or with a kidney transplant. To this purpose, the end points of direct importance to patients were identified as follows: mortality, cardiovascular disease events, fractures, hospitalisation, and quality of life. Intermediate and biochemical end points were also identified: vascular calcification, bone mineral density and bone biopsy, and circulating levels of PTH, calcium, phosphorus, and alkaline phosphatases. Only the randomised controlled trials (RCTs) of at least a 6-month duration with a sample size of more than 50 patients were taken into consideration for systematic review. An exception was made for studies involving bone biopsy, in which smaller sample sizes were accepted. For the development of KDIGO guidelines, evidence from literature was graded according to the Grading of Recommendations

Table 1: KDIGO guideline for CKD-MBD: laboratory monitoring.

CKD stage	Calcium and phosphorus monitoring	PTH and ALP monitoring	25 (OH) D monitoring
3	Every 6–12 months	Based on baseline level	Based on baseline level
4	Every 6–12 months	Every 6–12 months	
5 and 5D	Every 1–3 months	Every 3–6 months	

ALP: alkaline phosphatase.

Assessment, Development, and Evaluation system (GRADE) [2, 3]. The strength of evidence was graded "strong" (level 1) and "weak" (level 2), indicating recommendation and suggestion, respectively, the quality of evidence was distinguished into A (high), B (moderate), C (low), and D (very low). KDIGO guidelines for CKD-MBD were published in August 2009, and they established targets and strategies for biochemical bone and vascular components of this syndrome [4]. Unfortunately, due to the limited volume of available high-quality evidence found in the literature by the ERT, in the guidelines, the suggestions were largely more frequent than the recommendations. KDIGO appeared extremely cautious mainly in the field of therapeutic interventions due to the almost complete lack of clinical trial data supporting specific therapeutic strategies. According to the KDIGO guidelines, the diagnostic workup of CKD-MBD should include both the evaluation of biochemical abnormalities and of bone and vascular involvement in a CKD patient.

2. Biochemical Abnormalities

The KDOQI guidelines recommend that laboratory monitoring should begin in CKD stage 3 in adults (1C) and earlier at CKD stage 2 in children (2D). Table 1 shows the frequency of monitoring of the main laboratory parameters for CKD-MBD in adults. The frequency of measurements can be increased in CKD patients receiving treatments for CKD-MBD or in those in which biochemical abnormalities have been identified. Target levels for biochemical parameters are illustrated in Table 2, together with those indicated previously by the KDOQI guidelines [5].

2.1. Calcemia and Phosphatemia. According to the KDIGO guidelines, the target for calcium and phosphorus is to maintain circulating levels at the normal laboratory range (2C and 2D, resp.) at all stages of CKD. In particular, in CKD stage 5, phosphorus levels should not be simply equal to the upper phosphorus level of 5.5 mg/dL, as suggested by KDOQI guidelines, but should be lowered as much as possible toward the laboratory limit, which is lower than 5.5 mg/dL. Importantly, some observational studies of patients with normal renal function have shown that higher levels of serum phosphorus, even within the normal range, are associated with increased cardiovascular morbidity and mortality [6]. Unfortunately, there are no prospective studies

that have specifically identified the inflection point at which the increase in phosphorus becomes significantly associated with reduced survival and increased cardiovascular morbidity and mortality in patients at all stages of CKD. However, there is an increasing awareness of the dangerousness of hyperphosphatemia which is accompanied by the current tendency to maintain circulating phosphorus levels as low as possible, in the absence of conditions of malnutrition.

Like phosphorus, calcium levels should also be maintained within the normal laboratory range. It has been observed that calcium levels >9.5 mg/dL (>2.38 mmol/L) or even higher are associated with increased mortality in CKD patients [7–11]. Conversely, there is not much evidence of an association between low levels of calcium and mortality, and data of literature are controversial [8]. In recent observational studies, however, low serum calcium levels were associated with increased mortality in time-varying analyses and in combination with higher serum phosphorus (>3.5 mg/dL) and PTH levels (>150 pg/mL) [12, 13]. On the other hand, there are two aspects to consider. The first aspect is methodological, and it concerns the use of calcium corrected for albumin by means of formulas, which is very common in scientific literature. Recent studies have demonstrated that, considering ionized calcium as gold standard, the "corrected calcium" formula does not offer any superiority over total calcium for diagnosis [14]. For this reason, the KDIGO Work Group felt that both corrected calcium measurements and total calcium should continue to be used if they are part of the routine clinical practice. The second aspect is the evaluation of the calcium-phosphorus product. The effectiveness of this mathematical parameter in the clinical practice has recently been objected when compared with the evaluation of calcium and phosphorus alone. Welcoming these objections, KDIGO guidelines no longer recommend the use of this parameter in the clinical practice or give any target to achieve.

2.2. Parathyroid Hormone. At present, the use of "second-generation" PTH assays, which are widely available, is still highly recommended in the clinical practice by the Work Group, also in consideration of the fact that "third-generation" PTH assays have not been shown convincingly to improve the predictive value for the diagnosis of underlying bone disease. Second-generation assays detect not only the entire 1–84 PTH molecule, but also large fragments 7–84 whose concentration increases in CKD [15]. Third-generation methods detect only the entire 1–84 PTH molecule. The combined use of second- and third-generation assays allows measuring the 1–84 PTH/7–84 ratio. In some observational studies, this ratio has proved to be a significant predictor of bone disease and mortality [16]. However, these data were not a sufficient basis for recommending the preferential use of third-generation methods, which are also not yet widely available. The Work Group affirms that PTH target levels for patients with CKD stages 3–5 not on dialysis are still unknown; for this reason, it was found reasonable to suggest that in patients not yet on dialysis as well as in those with levels of intact PTH above the upper

TABLE 2: KDIGO guideline for CKD-MBD: laboratory target range.

CKD stage (mL/min)	PTH target	
	KDOQI	KDIGO
3 (59–30)	25–70	
4 (29–15)	70–110	Unknown
5 (<15)	150–300	
5D (dialysis)		In the range of 2–9 times the upper reference limit for the assay without marked changes over time
CKD stage (mL/min)	Phosphorus target (mg/dL)	
	KDOQI	KDIGO
3 (59–30)	2.7–4.6	
4 (29–15)		In the reference range
5 (<15)	3.5–5.5	
5D (dialysis)		Toward the reference range
CKD stage (mL/min)	Calcium target	
	KDOQI	KDIGO
3 (59–30)	In the reference range	
4 (29–15)		In the reference range
5 (<15)	8.4–9.5 (10.2)	
5D (dialysis)		

normal limit (2C) of the assay, calcium and phosphorus abnormalities be first evaluated and eventually treated. In fact, moderately elevated levels of PTH at this stage might express an adaptive mechanism which is still efficacious and requires only interventions on the causative factors represented by bone mineral disorders.

As for dialysis patients with CKD stage 5, instead, the Work Group suggests that only those patients for which the following double requirement of PTH levels is satisfied, be considered within the PTH target: PTH levels in the range of approximately two to nine times the upper normal limit for the assay (2C) and circulating levels remaining stable over the time. Actually, many observational studies have investigated the relationship between the circulating levels of PTH and adverse outcome, but the results obtained were often divergent, especially for low levels of PTH. In a large observational study, only intact PTH levels higher than 600 pg/mL were associated with an increased mortality risk [7]. Even more controversial is the relationship between mortality and low levels of PTH [17, 18]. It is possible that some interactive factors have a role in this relationship, such as comorbidities, which affect a great number of CKD and ESRD patients [18]. It is important to observe that in the KDIGO guidelines PTH targets have been anchored to the specific method applied. As a matter of fact, many different results from methods of the same generation have been reported [19]. This can be misleading in the application of guidelines in which target values refer to assays that are different from those used in the clinical practice. KDOQI guidelines suggested to maintain PTH levels between 150 and 300 pg/mL in CKD stage 5 patients, but this target range was based on studies where PTH was assayed with second-generation intact PTH Allegro Nichols IRMA, but that assay was no longer commercially available after KDOQI guidelines release. Referring target levels of PTH to the specific method applied is one of the great merits of KDIGO guidelines. Unfortunately, there is no information in these guidelines on the collection timing in relation to the frequency of hemodialysis and pulsatile PTH secretion, which is an area that has been little investigated in CKD and ESRD patients [20, 21].

2.3. Vitamin D. CKD patients are characterized not only by a deficit in renal 1 alpha hydroxylase, which causes low levels of 1,25 dihydroxyvitamin D (1.25 VD), but also by a high-frequency deficit in circulating 25-hydroxyvitamin D [22–24]. The circulating levels of 25 VD are present in multiple quantities compared to those of 1,25 VD, and 25 VD represents the nutritional form of a vitamin acting like an hormone. In fact, in addition to its established role in mineral homeostasis, vitamin D also seems to be crucially involved in other functions related to cellular response to environmental stimuli [25–27]. In particular, 25 VD seems to be hydroxylated by one extrarenal 1 alpha hydroxylase in many cells, even extrarenal cells. If activated locally in these cells, vitamin D is believed to be able to unblock genetic backgrounds and regulate important cell functions. These functions, called autocrine, could be helpful to explain the nontraditional effects of this hormone, in other words those effects which are not directly associated to its regulatory role in mineral metabolism. These nontraditional effects of vitamin D affect various fields related to pathology, from oncology to immunopathology and cardiovascular pathology; moreover, they seem to have a role in the protection

of survival even in non-CKD patients [27, 28]. In patients with stages 3–5D, the KDIGO guidelines suggest measuring circulating levels of 25 VD and repeating testing at intervals determined by the baseline values obtained (2C). However, it has also been emphasized that testing methods for 25 VD are not yet standardized, and definitions of "insufficiency" and "deficiency" need to be further validated in CKD patients. These definitions should also take into account the season and the geographical area where the assay is performed, as these parameters could influence the exposure and the consequent production of vitamin D.

2.4. Alkaline Phosphatases. The frequency of measurement of alkaline phosphatases is very similar to that of PTH and can provide additional information on bone turnover. In fact, as some comparative histological studies have demonstrated, PTH alone is not a good predictor of bone mineral disease with high and low turnover, except for respectively very high or very low values [29]. The Work Group believes that total alkaline phosphatases can contribute to correctly evaluating the status of skeletal metabolism, even in the presence of treatments interfering with bone turnover. However, in the presence of very high levels of total alkaline phosphatases, concomitant conditions such as hepatopathy and cholestasis must be excluded. Bone alkaline phosphatases can help to resolve interpretative doubts about the extra bone origin of alkaline phosphatases.

The importance of measuring alkaline phosphatases is emphasized by recent studies showing the association between total alkaline phosphatase levels and mortality in CKD and ESRD patients [30, 31]. It is recommended that for every laboratory test, all necessary information about the actual assay method in use be obtained by clinicians, who are also recommended to base therapeutic decisions on trend rather than on a single laboratory value.

The same laboratory tests and timing of monitoring are also applicable to transplanted patients (CKD stages 1–5 T), even to patients at the period immediately after kidney transplant the KDIGO guidelines recommend measuring serum calcium and phosphorus at least weekly, until these parameters have become stabilized.

3. Vascular and Bone Abnormalities

There is evidence in literature of the existence of a relationship between bone and vessels predisposing to the formation of vascular and soft tissue calcifications in CKD-MBD patients and in those with abnormal mineral metabolism [32]. Hypercalcemia and, above all, hyperphosphatemia may be the main responsible factors. Hyperphosphatemia is able to activate a sodium-phosphate cotransporter, Pit-1. This cotransporter causes the increase in intracellular phosphorus concentration in vascular smooth muscle cells, thus inducing an increase in the production of the core-binding factor alpha-1 [33], which is a transcription factor for osteoblastic differentiation of smooth muscle cells triggering an active vascular ossification process [34]. Hyperphosphatemia is associated with progressive coronary calcification in CKD

patients [35]. In these patients, coronary and noncoronary vascular calcifications are independent predictors of survival [36]. Vascular stiffness is considered to be the main cause of elevated mortality in patients with strong vascular calcification. On the basis of all these statements, KDIGO guidelines include the study of vascular calcifications and bone health in the diagnosis of CKD-MBD.

Quantitative evaluation of coronary artery calcification by electron beam computed tomography (EBCT) or by multislice computed tomography (MSCT) has been frequently used for research purposes, but it is still uncommon. For this reason, KDIGO guidelines suggest the use of other more easily available techniques, such as lateral abdominal radiograph for quantification of abdominal aortic calcifications at level L1–L4 (Kauppila method) [37], and echocardiogram for detection of valvular calcifications. The KDIGO Work Group also suggests that CKD-MBD patients with high presence of vascular calcifications should be considered at the highest cardiovascular risk in the clinical management of this pathology. Bone biopsy is the gold standard for the diagnosis of bone disease, and it should be limited to some specific settings in CKD patients: unexplained fractures, persistent bone pain, unexplained hypercalcemia, unexplained hypophosphatemia, possible aluminum toxicity, and prior to therapy with bisphosphonates. The detection of bone diseases through bone biopsy is possible through the evaluation of three parameters: turnover, measured as bone-formation rate, mineralization, measured as unmineralized osteoid, and bone volume (TMV classification). Osteitis fibrosa, characterized by high bone turnover, normal mineralization and increased bone volume, is still the most frequent form of uremic osteodystrophy in CKD patients not on dialysis and in hemodialysis patients, although in these patients it is progressively becoming less frequent compared to those on peritoneal dialysis. Adynamic bone disease, characterized by low bone turnover, normal mineralization, and low bone volume, is very common in patients on peritoneal dialysis and is becoming progressively more frequent also in CKD patients not on dialysis and in those on chronic hemodialysis. Osteomalacia, characterized by reduced bone turnover, abnormal mineralization, and little or unchanged bone volume, is not very frequent (<10%) in patients with CKD stages 3–5D. Mixed renal osteodystrophy, mainly affecting patients on chronic hemodialysis, is similar to osteitis fibrosa in terms of bone turnover and to osteomalacia for mineralization and bone turnover. Finally, renal osteodystrophy with mild lesions, mainly affecting patients on peritoneal dialysis, has lesions similar to those of osteitis fibrosa, but with lesser degree of changes in turnover and bone volume.

The KDIGO guidelines emphasize that the most accurate tools for the diagnosis of renal osteodystrophy are bone biopsy and TMV classification. Other indirect methods are less accurate, as in the case of BMD, whose ability to predict fracture risk in patients with CKD stages 3–5 as it does in the general population is not confirmed. BMD cannot certainly distinguish between different types of renal osteodystrophy. As mentioned above, measurements of circulating PTH do not provide adequate diagnostic

information on the type of bone disease, except for very high or very low levels. Not even the laboratory aid of bone alkaline phosphatases and biochemical markers of bone turnover increases sufficiently the predictive value of PTH for the underlying bone disease. Additionally, the measurement of bone mineral density (BMD) and biochemical markers of bone turnover should not be performed routinely in CKD patients stages 3–5. However, postmenopausal or age-related osteoporosis can be detected by BMD at earlier stages of CKD (until early stage 3). In later stages and in the presence of CKD-MBD, the underlying bone lesion should always be defined as renal osteodystrophy and not osteoporosis; the gold standard diagnosis is bone biopsy, as mentioned above. In transplanted patients with an estimated glomerular filtration rate greater than approximately 30 mL/min per $1.73 \, m^2$, measurement of BMD is suggested in the first 3 months after kidney transplant.

Bone fragility and especially vertebral and hip fractures are two of the main characteristics of renal osteodystrophy and are caused by abnormal bone quality [38]. These fractures are often unknown or difficult to detect; thus, a careful radiographic investigation is necessary given their important association with increased mortality and vascular calcification [39]. The encouragement to expand the investigation of bone fractures for diagnosis in CKD-MBD patients is clearly evident in the KDIGO guidelines.

4. Conclusions

KDOQI guidelines attribute a syndromic dimension to the abnormalities in the parathyroid-bone axis, which is responsible for worsening chronic renal failure. Alterations in mineral metabolism represent the core of this syndrome, and the KDOQI guidelines strongly recommend their therapeutic monitoring as the pivotal aspect for the correct clinical management of the disease at all CKD stages. This core is surrounded by parathyroid, bone, and vascular abnormalities. A correct diagnostic workup for CKD patients can no longer be limited to periodic laboratory measurements of circulating levels of PTH, calcium, and phosphorus. It should also include the measurement of total alkaline phosphatase and the detection of deficit of the nutritional form of vitamin D. Together with this, the search for bone fractures as well as for vascular calcifications and, in specific cases, the characterization of osteodystrophy through bone biopsy should also be considered. This extension of the diagnostic workup of CKD-MBD is aimed at the prognostic determination of the CKD stage in the patient, with consequences in the clinical management of the disease. As a matter of fact, patients with significant bone and/or vascular involvement are to be considered at higher risk and need to be treated. In these patients, any concomitant cardiovascular risk factors, both traditional and uremia related, if subject to possible changes, will require a particularly intensive treatment. However, KDIGO is extremely cautious in recommending such interventions, due to the almost complete lack of clinical trial data supporting specific strategies.

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