3b Detection and Evaluation of CKD-MBD

Title:

Is there a role for newer biomarkers in CKD-MBD management?

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Abstract

The current management of chronic kidney disease mineral bone disorder (CKD-MBD) relies largely on clinical judgement and assessment of biochemical parameters including serum calcium, phosphate and intact parathyroid hormone concentrations. In the past two decades, there has been a leap in the understanding of the pathophysiology of CKD-MBD, leading to the discovery of novel biomarkers. The potential utility of these markers in this clinical setting is an area of intense investigation. In the absence of any guidelines aiding the clinician's understanding and application of these markers, we summarise the current available literature surrounding fibroblast growth factor-23 (FGF23), α-Klotho, sclerostin, and serum calcification propensity testing and their respective assays in the context of CKD-MBD management.

Introduction

Chronic Kidney Disease - Mineral Bone Disorder (CKD-MBD) describes an entity observed in CKD patients, involving derangements in mineral metabolism, which are often accompanied by bone remodelling abnormalities and accelerated vascular and tissue calcification[1], and which are intimately related to poor outcomes in this population.[2-6] The clinician's assessment of the presence and severity of CKD-MBD is largely based on the serum or plasma based basic biochemical biomarkers and to a certain extent, clinical judgement. With rapid scientific expansion within this niche in mineral metabolism research, the potential value, if any, of some of the newer parameters requires attention. Here, we present selected biomarkers that are most topical and relevant to CKD-MBD.

Markers and assays

Fibroblast Growth Factor-23 and α-Klotho

The independent discoveries of fibroblast growth factor-23 (FGF23)[7,8] and α-Klotho[9] have broadened our understanding of mineral metabolism. While the regulation of these hormones is not completely understood, both play crucial roles in phosphate homeostasis, with FGF23 also a key regulator of vitamin D. In the kidney, FGF23 acts on the FGF-Receptor/α-Klotho co-receptor complex to reduce apical expression of key sodium-phosphate co-transporters within the renal proximal tubules, inhibiting tubular reabsorption of phosphate to induce phosphaturia.[10] FGF23 also reduces 1,25-dihydroxyvitamin-D ($1,25(OH)_2D_3$) synthesis by downregulating 1α-hydroxylase

and upregulating 24-hydroxylase.[10] This in turn results in lowering $1,25(OH)_2D_3$-stimulated intestinal absorption of phosphate.

α-Klotho is not only an obligate co-receptor for physiological FGF23 signalling and appears essential for FGF23-mediated phosphate regulation,[11] but soluble-Klotho (sKl), its cleaved component, can act as a paracrine or endocrine mediator. sKl displays enzymatic activity, important in regulating ion channels such as the renal outer medullary potassium (ROMK) channel and Transient Receptor Potential Vanilloid (TRPV5) ion channel.[12-14] sKl has also been implicated in growth factor signalling as well as demonstrating anti-insulin, anti-fibrotic and anti-oxidant activities.[15,16] FGF23, α-Klotho and their respective physiological actions have been reviewed in further detail elsewhere.[17,18] As direct regulators of phosphate metabolism, it is conceivable that both FGF23 and α-Klotho are potential biomarkers that may improve current clinical practice in the management of CKD-MBD. Further to their physiological roles, both have been independently linked to mortality and outcomes.

FGF23

FGF23 has consistently demonstrated strong associations with adverse outcomes, increased cardiovascular burden and poor prognoses in those with CKD, independent of renal function, traditional cardiovascular risk factors and other

mineral parameters.[18-22] Specifically, high FGF23 levels have been strongly associated with heart failure, left ventricular dysfunction, elevated left ventricular mass index and left ventricular hypertrophy.[23-28] Experimental studies provide convincing evidence that FGF23 could be pathogenic on cardiomyocytes[29] supporting the strong relationship seen in clinical studies. However, attempts at neutralising FGF23 in animal models have resulted in hyperphosphataemia, increased vascular calcification and excess mortality,[30] not only limiting the further development of such therapy but also highlighting the complexities of mineral metabolism that is currently incompletely understood.

There is also evidence that FGF23 is an earlier and more sensitive marker of disordered mineral metabolism and thus, superior to the current available markers of serum phosphate and parathyroid hormone (PTH).[31,32] Given this, there is strong advocacy for routine measurement of FGF23 in the setting of CKD and CKD-MBD, with the expected advantages of aiding clinicians in risk-stratifying and targeting management in those that may warrant most attention. The specific utility of FGF23 testing in those with CKD has also been elegantly reviewed.[33]

Available FGF23 Assays

FGF23 is a predominantly bone-derived hormone. Circulating FGF23 can be either the mature, physiologically active intact FGF23 (iFGF23) or the cleaved forms of n-

and c-terminal fragments (cFGF23).[34] There are numerous commercially available assays that measure iFGF23 alone or both iFGF23 and cFGF23 fragments (Table 1). While these assays have their respective advantages, they are not directly comparable with each other and have unresolved pre-analytical and analytical issues.[33] Further to that, the kits/measurements that have been employed in the relevant clinical studies cannot be used *en masse* and would be uneconomical if applied to large clinical settings, while the newer kits that boast high-throughput capability require further evaluation.[33]

α-Klotho

α-Klotho is the less studied of the two markers intimately linked to phosphate metabolism. Nonetheless, an inverse association to mortality has been reported, with higher circulating levels of soluble-Klotho (sKl) associated with increased survival in the general population[35] as well as those with dialysis-dependent kidney disease.[36] sKl has also been linked to improved cardiovascular outcomes, with levels reported to have an inverse associations to carotid artery intimal thickness[37] and coronary artery disease[38] in adults with normal renal function. Similarly in patients with CKD, sKl has shown inverse correlations to arterial stiffness,[39] carotid artery intimal thickness and left ventricular dysfunction.[40]

Available sKl Assays

α-Klotho exists in two forms - membrane-bound klotho and soluble klotho (sKl). mKl is variably expressed in tissues but abundantly in the kidney.[9] sKl is produced in two ways; first as a result of ectodomain cleavage of mKl and the second as a product of alternative splicing, leading to a shorter form of sKl. Proteomic analysis of various extracellular fluids suggests that the longer form of sKl, generated by cleavage is the major circulating species in humans.[41-43] Of the different commercially available immunoassays measuring sKl, the three most used in published studies include IBL (IBL International GmbH, Hamburg, Germany), Cusabio (Cusabio Biotech, Wuhan, China) and USCN (USCN Life Science Inc, Wuhan, China). Only the IBL kit provides information on epitope specificity. Heijboer *et al*. recently reported poor performance comparisons between these assays, with lack of unit standardization in readouts.[44] The IBL sKl assay performed better in comparisons[44] and remains by far the most widely reported assay. Although sKl measurement (with the IBL assay) does not seem to be subject to the same pre-analytical instability issues of FGF23 measurements,[45] the results reported with the IBL assay have still provided inconsistent results in the setting of CKD and reasons for this are as yet unclear.[17]

Sclerostin

Sclerostin is the transcriptional product of the SOST gene.[46] The protein is mostly produced by osteocytes, the most abundant cell type in bone.[47] It inhibits Wnt/β-catenin signaling, a cell surface receptor mediated pathway essential for osteogenesis.[48] Sclerostin is therefore considered a negative regulator of bone

growth. In patients with sclerosteosis, the mutant SOST gene results in the production of abnormal sclerostin, resulting in the phenotype of excessive bone growth. Despite its role as a negative regulator of bone growth, circulating sclerostin concentrations are positively correlated with bone mineral density (BMD) in post-menopausal women, which possibly reflects the increased number of osteocytes in people with higher BMD.[49,50]

Multiple cohorts have found that circulating sclerostin concentration are inversely correlated to the estimated glomerular filtration rate (eGFR).[51] The cause of this is unclear, with one study showing that urine sclerostin excretion was paradoxically increased in CKD.[52] Other studies suggest that increased bone production and synthesis by calcified vasculature may be responsible for the increased circulating sclerostin observed in CKD patients.[53,54] There are three studies that reported the association between circulating sclerostin and BMD in CKD patients. One study found that sclerostin is associated with BMD assessed by dual-energy x-ray absorptiometry (DXA) at the lumbar spine in a CKD stage 3b and 4 cohort,[55] another found a similar association between sclerostin and BMD by DXA at the distal radius in a haemodialysis cohort.[56] In a small haemodialysis cohort however, sclerostin was not associated with BMD measured at the distal radius by high resolution peripheral quantitative computed tomography (HR-pQCT).[57] The data on the association between sclerostin and vascular calcification in CKD is also conflicting with two

independent studies showing that patients with vascular calcification had increased circulating sclerostin, even after adjustment for confounders.[57,58] In a separate cohort, patients with calcification had increased circulating sclerostin using univariate analysis, but the relationship was inverse after multivariate adjustment.[59]

There are currently two commercially available sandwich ELISAs to measure serum or plasma sclerostin (TECO and Biomedica). A recent report demonstrated that there are significant differences in the absolute concentration between the two assays, therefore results assayed using kits from different manufacturers are not directly comparable.[60]

Serum Calcification Propensity Test (SCPT)/T_{50} test

Biomarkers in routine clinical practice frequently rely on the presence or concentration of a particular substance. Functional assays however are more appropriate in some instances, and example of this is the prothrombin time (PT). PT assesses the time taken for a plasma sample to clot after the addition of thromboplastin, an activator of the extrinsic coagulation cascade. The formation of a clot increases the opacity of the incubation vessel and therefore the time to clot formation can be determined optically. PT is therefore a functional study assessing the cumulative effect of the various pro- and anti-coagulants in plasma. Recently, a functional assay called the serum calcification propensity test (SCPT), also known as

the T50 test, has been developed to assess the ability of an individual's serum to resist crystal apatite formation when supraphysiological amount of calcium and phosphate ions are added to serum.[61] The biological and physical principles behind the test have been reviewed recently.[62] In essence, the test relies on the fact that the formation of nano-sized apatite crystals in serum is accompanied by an increased in the opacity of the serum mixture. Consequently, the time from the addition of calcium and phosphate ions until the formation of apatite crystals can be determined using a sensitive optical instrument.

T_{50} is analogous to PT in that it does not give information about any single substance, but rather reflects the balance between pro- and anti-calcific factors in serum, i.e. longer time to apatite formation suggests that serum contains lower levels of pro-calcific factors or more anti-calcific factors.[61] This test has been validated in one CKD cohort and two kidney transplant recipient cohorts.[63,64] In these studies, T_{50} has been shown to be an independent predictor of mortality. Currently, this assay is only commercially available through a centralised laboratory in Switzerland.

Current role of biomarker evaluation

The need for new biomarkers in CKD-MBD is not only of academic interest, but driven by the clinician and patient's desire to provide a more precise and detailed assessment of their disease. Ideally, such a biomarker would assist clinicians in

prompt diagnosis, aid in disease management and guide future direction for many if not all patients. Most importantly, the use of a new biomarker should aim for and lead to better outcomes for patients.

The biomarkers discussed above have the *potential* to be of clinical utility, but several issues require attention:

CKD-MBD is a descriptive umbrella which encompasses a range of biochemical, bone and vascular pathologies. An ideal biomarker that accurately predicts development or provides clinical guidance in one specific facet of CKD-MBD may perform poorly in other aspects of CKD-MBD. A comprehensive assessment of a biomarker's value against all facets of CKD-MBD is preferable to a limited assessment of a single endpoint.

Assay standardisation with an easily interpretable readout is important in any clinician's readiness to adopt the biomarker for routine use. Many of the novel biomarkers currently use the ELISA method. In some cases, there is significant variation between the different ELISA kit vendors, while in others, the variation between the different kits is simply unknown.

The current mineral parameters used routinely in day-to-day clinical practice, such as calcium, phosphate and PTH, have been the cornerstone of CKD-MBD diagnosis for the past decade because of their widespread availability. Equally important is the accessibility to therapies that directly modulate these parameters, i.e. a phosphate binder for a patient with hyperphosphataemia. At present, the novel biomarkers

discussed above do not correspond to marketed and approved targeted therapies. It is possible that the clinical utility of these biomarkers, if any, will only become apparent after the development of such targeted therapies.

Similar to drug development, the evolution of a biomarker from a research tool to adoption as a routine clinical test is a prolonged process. The existing knowledge provides the foundation for better future clinical decision making. An improved understanding on how biomarkers mediate disease or vice versa, more robust assay performance and the development of novel targeted therapies are all important in the "bench to clinic" translation of a biomarker.

Conclusion

Newer markers in the field of mineral research hold much promise in improving our understanding of CKD-MBD and further research of these markers may provide insight into mineral metabolism and its dysfunction, at the crux of CKD-MBD. However, to date none of these markers have been applied nor evaluated in the broader clinical setting as diagnostic or management tools in patients with CKD-MBD. Based on the currently available data, we cannot as yet support the wider clinical use of these markers. Clinically relevant questions that first need to be answered include: Is an improvement in biomarker read out directly translatable to an improvement in clinically relevant outcomes; and will the introduction of a new biomarker into clinical practice result in better clinical outcomes for patients?

Parallel to these clinical questions, further basic research is needed to elucidate the biological processes involved in modulating these biomarkers, which might lead to more targeted therapies.

Table 1. Summary of current literature on newer biomarkers in CKD-MBD

Biomarker	Commercial Assay Availability	Applicability of Commercial Assay	Associations with Hard Outcomes and Strength of Association
FGF23	Y	Multiple assays available, Assay costs high, No standardization of assays, High throughput setting not well evaluated	Y – strong
sKl	Y	Only 1 assay widely reported, Costs high, No standardization of assays, High throughput setting not well evaluated	Y – mild
Sclerostin	Y	2 ELISA assays available, the results of different assays are not directly comparable	No consistent association with outcome after adjustment
T_{50}	Y	Only performed at a single centre currently (now patented)	Y – strong

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