Potential for Biomarkers of Chronic Kidney Disease-Mineral Bone Disorder to Improve Patient Care

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Cardiovascular Disease in Chronic Kidney Disease versus the General Population

Chronic kidney disease (CKD) is an important public health problem, affecting around 10% of the UK adult population [1]. Cardiovascular disease is common among patients with CKD and is the most common cause of death in this population [2, 3].

Standard cardiovascular risk factors such as diabetes, hypertension and dyslipidaemia are common in CKD. However, these are not the only determinants of the high rates of cardiovascular disease associated with CKD, other factors include endothelial dysfunction, inflammation, arterial calcification and oxidative stress [1, 4–10]. The relative importance and interdependence of each of these factors in determining cardiovascular risk in CKD is largely unknown.

CKD-Mineral Bone Disorder and Vascular Risk

The CKD-mineral bone disorder (CKD-MBD) axis associates with abnormalities of bone, altered bone turnover and arterial calcification [11]. Arterial calcification results in reduced arterial elasticity, increased left ventricular afterload, left ventricular hypertrophy (LVH) and reduced...
coronary blood flow [12]. Increased arterial calcification is an independent predictor of cardiovascular mortality [4]. Although not all patients are affected equally, it can occur early in the evolution of CKD and progresses with declining glomerular filtration rate (GFR) [10, 13, 14].

Elevated levels of calcium phosphate product is a hallmark of arterial calcification [6, 15, 16], but is a relatively late marker. Imaging techniques such as computerized tomography (CT) or ultrasound scanning are able to detect arterial calcification but are unable to differentiate whether this is the intimal deposition seen in traditional atherosclerotic changes or the medial calcification observed with increased vascular stiffness in CKD [17]. As such, the current assessment of the CKD-MBD cardiovascular axis is imperfect and there is potential for biomarkers to contribute.

**Emerging Biomarkers**

Calcium, phosphate and parathyroid hormone are used in clinical practice to assess the CKD-MBD axis. There are numerous other biomarkers now available that have been related to the development and progression of CKD-MBD. There are an increasing number of studies that show varying utility of different biomarkers in relation to the outcomes of progression, cardiovascular events and death in CKD. However, many of these are association and not outcome studies and may be confounded by a lack of understanding of the biological actions and mutual interactions of these biomarkers.

Whether novel biomarkers related to CKD-MBD have the potential to enhance the risk stratification or clinical management of the CKD patient over and above traditional parameters and assessment tools needs to be considered before these markers can be adopted in clinical practice.

This review discusses novel biomarkers pertinent to the CKD-MBD and cardiovascular interface, first briefly describing their pathological associations and then the evidence for their usefulness in relation to important clinical endpoints. Serum markers that are already in use in clinical practice, for example calcium, phosphate, alkaline phosphatase and parathyroid hormone (PTH), will not be discussed.

**Biological Actions of CKD-MBD Biomarkers** (fig. 1)

**Promoters of Calcification in CKD**

**Osteoprotegerin**

Osteoprotegerin (OPG) is a natural inhibitor of calcification, it prevents the terminal differentiation and bone-resorbing action of osteoclasts [18]. A rapid development of arterial calcification and osteoporosis is seen in OPG knockout mice [19]. However, in CKD, elevated OPG levels are observed and these have been associated with aortic stiffness and markers of cardiovascular dysfunction such as raised serum troponin T (TnT) levels [20].

A positive association between OPG and development of arterial calcification exists in CKD. OPG binds to receptor activator of nuclear factor B ligand (RANKL) to inhibit osteoclastogenesis. An increased OPG to RANKL ratio is observed in a low bone turnover state and correlates with increased arterial calcification in CKD [21, 22].

**Osteocalcin**

Osteocalcin is a promoter of calcification secreted by osteoblasts. It has been located in atherosclerotic plaques and areas of calcification within vessels [23]. Osteocalcin may also be involved in insulin receptor expression in the metabolic syndrome [24].

**Inhibitors of Arterial Calcification in CKD**

**Fetuin-A**

Fetuin-A is synthesized in the liver and acts as an inhibitor of vascular calcification. Decreased levels are observed among CKD patients and are associated with the development of vascular calcification. Fetuin-A inhibits
activation of insulin receptors; elevated levels are associated with diabetes and insulin resistance [25]. Thus, fetuin-A activity may dichotomize into an increased risk for atherosclerosis and insulin resistance at high levels and risk for arterial calcification at low levels.

Matrix-Gla Protein

Matrix-Gla protein (MGP) is a potent inhibitor of calcification, preventing osteoblastic differentiation of vascular smooth muscle cells [17, 26]. MGP is produced by osteoclasts, chondrocytes and the smooth muscle cells of the arterial media. Different forms of MGP appear to have differing actions. In physiological processes, MGP undergoes vitamin K-dependent γ-glutamyl carboxylation, its carboxylated form is found in normal vessels and forms circulating complexes with fetuin-A [27]. The non-carboxylated form is present locally in areas of arterial calcification [28]. In mice that lack MGP, accelerated arterial calcification ensues and early death occurs due to extensive arterial calcification and vessel rupture [29].

Osteopontin

Osteopontin is expressed in mineralized tissues where it acts as an inhibitor of calcification. It is not present in normal vessels but may be expressed in calcified and atherosclerotic vasculature [30].

Other Factors

Fibroblast Growth Factor-23

Fibroblast growth factor-23 (FGF-23) is a ‘phosphatonin’ secreted by osteoblasts in response to an oral phosphate load and/or elevated levels of 1,25-dihydroxyvitamin D. It is believed to play a role in phosphate homeostasis, particularly preventing hyperphosphataemia by promoting phosphaturia and reducing renal production of 1,25-dihydroxyvitamin D [31]. Levels among CKD patients are higher than in the general population and are further elevated in the dialysis population [32]. FGF-23 levels correlate with serum phosphate, PTH and calcium [33]. It is postulated that these changes are a compensatory attempt to maintain normal phosphate balance in CKD [34]. It is thought that in advanced CKD, the capacity of FGF-23 to reduce phosphate levels is exceeded. Increasing FGF-23 levels then exhibit pathological effects, including promotion of more rapid progression of renal functional decline and the development of LVH [35].

Klotho

Klotho is a transmembrane protein, highly expressed in the kidney. It acts as a co-receptor for FGF-23 and is thus involved in the promotion of phosphaturia and reduction of arterial calcification. Secretion of the extracellular domain of klotho enables it to function as a humoral factor, promoting phosphaturia [36] and reducing phosphate uptake by vascular smooth muscle cells [37]. Animal studies have shown severe arterial calcification in klotho-deficient mice with CKD. In cross-sectional analysis, klotho levels in the kidney, serum and urine fall as CKD advances [37]. In a study of 312 patients with CKD stages 2–4 followed up for 2.2 ± 0.8 years, Seiler et al. [38] found no association between plasma klotho levels and progression to ESRD or death, although potential confounding by vitamin D and the lack of a control group caused some to question the validity of these results [39]. Further mechanistic studies, particularly examining the relationship between serum levels and tissue expression, and large-scale epidemiological studies are needed to answer these questions. Klotho has potential to be an early sensitive biomarker in CKD-MBD and there may be potential therapeutic benefit from preservation of klotho.

Vitamin D

The kidney is central to vitamin D metabolism, converting 25-hydroxy- to 1,25-dihydroxyvitamin D, its active form. Vitamin D has a beneficial effect on cardiac contractility, vascular tone and overall cardiac function [40]. Vitamin D deficiency is associated with an increased risk for cardiovascular disease, hypertension and diabetes; it is common in the general population and deficiency rates are significantly higher among CKD patients [41]. Hypervitaminosis D is associated with accelerated renal decline; however, it is likely that this is the result of hypercalcaemia [42]. Although vitamin D does not appear to initiate arterial calcification, hypercalcaemia and hyperphosphataemia induced by high vitamin D levels do mediate this process [43], and this has led to conflicting results from studies involving vitamin D.

Predictors of Progression

Progressive renal functional decline is associated with an increased risk for cardiovascular disease [3]. However, this risk of progression varies widely among people with CKD and there is therefore much interest in identifying risk factors for progression. Factors that have consistently been identified as determinants of progression in epidemiological studies include urine protein excretion and elevated mean arterial pressure
useful marker of progression in CKD. CKD-MBD biomarkers in this area.

There is little information to add to the understanding of progression, these are largely from association studies. Arterial calcification and vessel hypertrophy are important factors in determining vascular stiffness in CKD [12]. Arterial calcification is frequently used as a surrogate endpoint for an increased cardiovascular risk in biomarker studies.

Numerous studies have demonstrated a link between vascular disease and elevated OPG levels. Mesquita et al. [51] followed 77 patients (32 pre-dialysis, 45 haemodialysis) for 2 years. Coronary artery calcification was assessed by CT. Elevations in OPG correlated with the degree of calcification and were predictive of all-cause mortality. In a study looking at osteopontin levels in 36 haemodialysis patients and 35 matched healthy controls, osteopontin levels were higher in the dialysis group and correlated with degree of aortic calcification as assessed by CT [52]. A further observational study of 107 CKD stage 2–5 patients showed uncarboxylated MGP to be associated with increased aortic calcium scores on CT, levels were higher in later stages of CKD but over a median follow-up of 802 (±311) days were not significantly associated with increased mortality [53].

Ford et al. [54] carried out a 12-month observational study of 73 non-diabetic and 19 diabetic patients with CKD stages 3 and 4. Fetuin-A levels were measured at baseline and aortic pulse wave velocity (PWV) determined at time zero and 12 months. Fetuin-A levels were lower in the small non-diabetic CKD group. Lower fetuin-A levels were predictive of an increase in PWV over 1 year (β = 0.355, p < 0.001) as were age and systolic blood pressure. So, although limited by relatively small study numbers and a short period of follow-up, low fetuin-A measurement may be a useful biomarker of increasing arterial stiffness.

A complex inter-relationship between vitamin D, klotho, FGF-23 and osteopontin has been demonstrated in experimental mouse models. CKD-affected mice that underwent oral phosphate loading prior to treatment with active vitamin D (calcitriol) were less likely to develop aortic calcification than mice that did not receive vitamin D following phosphate loading. Furthermore, vitamin D was observed to increase klotho secretion, promote phos- phaturia, increase osteopontin expression and paradoxically reduce FGF-23 independent of PTH and calcium [55]. In a study of 140 CKD stage 2–5 patients, low 25-hy- droxyvitamin D levels were independently associated with all-cause mortality at 1 year. However, there was no asso- ciation between 25-hydroxyvitamin D levels and baseline coronary artery calcium scores or arterial stiffness deter- mined by PWV [56]. These studies provide information...
about the pathophysiological effects of vitamin D, the inter-relationship with other calcification co-factors and the potential benefits of vitamin D therapy, but it is less clear how endogenous vitamin D levels could translate into useful biomarkers of arterial calcification in CKD-MBD.

Studies examining the relationship between FGF-23 and arterial calcification have yielded conflicting results. In a study of 162 pre-dialysis patients and 58 controls with preserved renal function, coronary artery calcium scores were measured by CT in the CKD cohort. The association between FGF-23 and coronary artery calcification was not statistically significant (p = 0.38) [57]. In contrast, Desjardins et al. [58] did observe an association between FGF-23 and elevated aortic and coronary calcium scores in a cohort of 142 CKD stage 2–5 patients but no association between FGF-23 levels and PWV or bone mineral density. Levels of FGF-23 were again observed to increase before serum phosphate in early-stage CKD. Ford et al. [20] were unable to demonstrate an association between FGF-23 and PWV in 200 CKD stage 3–4 patients, although it was associated with increased levels of high-sensitivity TnT. Both FGF-23 and fetuin-A were correlated with baseline atherosclerosis scores on angiography in CKD stages 1–3 [59]. Scialla et al. [60] found no association between FGF-23 levels and coronary artery calcification scores in 1,501 CKD patients recruited from the CRIC study group. Furthermore, elevated FGF-23 levels did not induce arterial calcification in vitro. The results of this study are compelling as it combines observation with mechanistic data. The fact that most other studies are purely observational and open to confounding by factors such as serum phosphate levels, small study numbers, variations in stage of CKD and primary disease may account for their conflicting results.

Summary

Many CKD-MBD biomarkers are associated with arterial calcification and vascular stiffness. It is not clear whether these biomarkers can predict the development of calcification in its early stages and it is even less clear whether therapeutic intervention on the basis of these biomarkers could alter its clinical course.

Biomarkers of CKD-MBD and Cardiovascular Events and Death

Cardiovascular disease is common among CKD patients and its aetiology is complex. In clinical practice, assessment of traditional risk factors is used to determine the risk for cardiovascular disease and direct risk reduction therapies. Having recognized that arterial calcification and factors such as inflammation are important determinants of cardiovascular disease in CKD, there is potential for biomarkers associated with these processes to aid in the determination of risk for death and cardiovascular events in CKD-MBD.

Studies of fetuin-A in relation to mortality have shown conflicting results. In 822 patients with CKD stages 3–4 selected from the Modification of Diet in Renal Disease (MDRD) cohort, fetuin-A levels were not associated with cardiovascular or all-cause mortality [61], whereas in a cohort of 987 incident dialysis patients followed for a median of 2.8 years, an increase in baseline fetuin-A of 0.1 g/l was associated with a hazard ratio (HR) of 0.87 for all-cause mortality (95% CI 0.80–0.93, p = 0.0001) and less significantly an HR for cardiovascular mortality of 0.9 (95% CI 0.81–1, p = 0.09) [62]. Whether the discrepancies are due to threshold levels of fetuin-A not having been reached or differences in study group demographics is not clear. Further studies relating to cardiovascular outcomes and the pathophysiological mechanisms of action of this marker are needed.

In the haemodialysis population, increased FGF-23 levels are associated with an increased risk for cardiovascular mortality. In a prospective observational cohort of 219 maintenance haemodialysis patients, FGF-23 levels >8,400 RU/ml were associated with an increased mortality at 2 years, with an HR for death of 2.5 (CI 1.3–5, p = 0.007) [63]. In a nested case-control study of 400 haemodialysis patients, median baseline FGF-23 levels were 2,260 RU/ml in the 200 patients who died in comparison to 1,406 RU/ml in the survivors; this difference was significant (p < 0.001) [64].

There is interest in the pathophysiological mechanisms to explain the increased risk for death observed among those with elevated FGF-23 levels. Studies in mice have demonstrated a causal role for FGF-23 in the development of LVH that is independent of klotho [65]. Clinical studies have added to the evidence from animal models. Gutiérrez et al. [57] measured left ventricular mass by echocardiography in 162 pre-dialysis CKD patients and 58 subjects with preserved kidney function. Coronary artery calcium scores were also measured by CT in the CKD cohort. Elevated FGF-23 levels were universally associated with increased left ventricular mass and LVH even after adjustment for blood pressure and the presence of diabetes, with the association greatest among CKD patients. A 5% increase in left ventricular mass was observed for each 1-SD increase in log-transformed FGF-23.

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Table 1. Summary of outcome studies relating to CKD-MBD biomarkers

<table>
<thead>
<tr>
<th>Reference (first author)</th>
<th>Biomarker Population</th>
<th>Follow-up Type</th>
<th>Patients, n</th>
<th>Outcome</th>
<th>Clinical endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isakova [32]</td>
<td>FGF-23 CKD stages 2–4 (CRIC cohort)</td>
<td>3–5 years prospective cohort study</td>
<td>3,879</td>
<td>FGF-23 associated with increased risk for death; associated with increased risk for progression in those with eGFR &gt;30 ml/min</td>
<td>all-cause mortality progression to ESRF (defined as initiation of dialysis or transplantation)</td>
</tr>
<tr>
<td>Fliser [47]</td>
<td>FGF-23 non-diabetic CKD stages 2–5 (MMKD cohort)</td>
<td>median 53 months prospective cohort study</td>
<td>177</td>
<td>FGF-23 predicted progression of CKD</td>
<td>progression; doubling of baseline creatinine or ESRF</td>
</tr>
<tr>
<td>Arnlöv [73]</td>
<td>FGF-23 727 adult males (ULSAM study)</td>
<td>median 9.7 years population cohort</td>
<td>727</td>
<td>FGF-23 associated with CV mortality in eGFR &lt;60 ml/min group</td>
<td>cardiovascular and all-cause mortality</td>
</tr>
<tr>
<td>Gutiérrez [64]</td>
<td>FGF-23 incident haemodialysis patients</td>
<td>1 year nested case-control study; 200 survivors, 200 deaths</td>
<td>400</td>
<td>FGF-23 associated with increased mortality</td>
<td>all-cause mortality</td>
</tr>
<tr>
<td>Kanbay [59]</td>
<td>FGF-23 and fetuin-A CKD stages 1–3 baseline cross-sectional study</td>
<td>177</td>
<td>fetuin-A and FGF-23 correlated with Gensini score for CAD on angiography</td>
<td>severity of coronary atherosclerosis on angiography</td>
<td></td>
</tr>
<tr>
<td>Seiler [74]</td>
<td>FGF-23 CKD stages 2–5</td>
<td>4.8 years prospective cohort study</td>
<td>149</td>
<td>FGF-23 predictive of cardiovascular events</td>
<td>all-cause mortality; cardiovascular events: MI, angioplasty, CAGB, CVA, carotid endarterectomy of stenting, non-traumatic amputation of lower limb or PVD treated by surgery, angioplasty or stenting</td>
</tr>
<tr>
<td>Scialla [60]</td>
<td>FGF-23 CKD stages 2–4</td>
<td>376 days prospective cohort study</td>
<td>1,501</td>
<td>baseline FGF-23 not associated with calcification scores</td>
<td>baseline coronary artery calcium scores on CT</td>
</tr>
<tr>
<td>Desjardins [58]</td>
<td>FGF-23 CKD stages 2–5</td>
<td>baseline prospective cohort study</td>
<td>142</td>
<td>FGF-23 associated with calcification scores but not PWV</td>
<td>baseline aortic and coronary calcium scores on CT and PWV</td>
</tr>
<tr>
<td>Ford [20]</td>
<td>FGF-23/OPG CKD stages 3–4</td>
<td>baseline prospective cohort study</td>
<td>200</td>
<td>OPG associated with increased aortic stiffness</td>
<td>change in aortic pulse wave velocity</td>
</tr>
<tr>
<td>Sigrist [75]</td>
<td>OPG CKD 4, haemodialysis and peritoneal dialysis patients</td>
<td>40 months prospective cohort study</td>
<td>134</td>
<td>OPG associated with negative outcome/ increased risk for death</td>
<td>all-cause mortality</td>
</tr>
<tr>
<td>Mesquita [51]</td>
<td>OPG pre-dialysis CKD and haemodialysis patients</td>
<td>2 years prospective cohort study</td>
<td>77</td>
<td>OPG independent predictor of mortality in CKD group</td>
<td>all-cause mortality coronary artery calcium score</td>
</tr>
<tr>
<td>Ford [54]</td>
<td>fetuin-A CKD stages 3–4</td>
<td>1 year prospective cohort study</td>
<td>92</td>
<td>in non-diabetic patients fetuin-A associated with progressive arterial stiffness</td>
<td>change in aortic pulse wave velocity</td>
</tr>
<tr>
<td>Ix [61]</td>
<td>fetuin-A CKD stages 3–4 patients (MDRD group)</td>
<td>baseline observational study</td>
<td>822</td>
<td>fetuin-A not related to mortality</td>
<td>all-cause and cardiovascular mortality</td>
</tr>
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</table>
through the highest tertile of FGF-23 was associated with a 2.4-fold increase in risk for coronary artery calcification (95% CI 1.1–5.5), this was no longer significant after multivariable adjustment. FGF-23 appears to be predictive of cardiovascular events in patients with CKD stages 2–5 [66]. The association between FGF-23 and risk for death appears to exist even in early CKD, before traditional CKD-MBD markers become elevated. FGF-23 has potential to be used in clinical practice in the assessment of cardiovascular risk.

Other biomarkers may be associated with mortality. In a study of 825 incident haemodialysis patients, untreated vitamin D deficiency was associated with an increased risk for death at 90 days [67]. A further observational study of 50,987 incident haemodialysis patients demonstrated a 20% survival advantage after 90 days in the 37,123 patients who received vitamin D (HR 0.80, 95% CI 0.76–0.83, p < 0.001) [68]. Sigrist et al. [14, 75] demonstrated an association between raised OPG levels and all-cause mortality in 134 dialysis and CKD 4 patients followed for 40 months.

Troponin is associated with coronary artery disease and LVH. Smith et al. [69] examined the association between FGF-23, LVH and troponin in a group of 153 stable patients with CKD 3–4. Elevated FGF-23 levels were associated with elevated high-sensitivity cardiac troponin I...
and T (cTNI and cTNT), although the association was weaker when adjusted for left ventricular mass. Other studies have shown cTNT to be predictive of cardiovascular events and overall survival in the CKD population [70–72].

Summary
Cardiovascular disease is highly prevalent in CKD. Using biomarkers to predict the risk for cardiovascular events and death poses many challenges. Many of the studies looking at biomarkers in relation to cardiovascular endpoints are association studies. There is a paucity of data to demonstrate clear pathophysiological links between biomarkers and these endpoints. FGF-23 is an obvious therapeutic target for cardiovascular risk reduction in CKD, given its causal role in LVH, but not all biomarkers fulfil this brief (table 1).

References


Conclusion
The biomarkers discussed in this review provide varied information about arterial calcification, cardiac function and risk for progression in CKD-MBD. There are prospects for better vascular risk assessment in patients with advanced CKD, but as yet no single or suite of biomarkers is available for use in the clinical setting. Numerous barriers need to be overcome before CKD-MBD biomarkers can become part of routine clinical practice; many of the studies performed to date have been small association studies, carried out on select patient groups. Large-scale epidemiological data in diverse populations would enhance understanding of the potential clinical application of these biomarkers. Significantly, understanding of the pathophysiological roles of these biomarkers is patchy and there are many unanswered questions. More in vivo mechanistic studies and a greater understanding of the modes of action and interaction between these biomarkers are needed before they can be reliably applied in clinical practice.
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