

The Use of Fibroblast Growth Factor 23 Testing in Patients with Kidney Disease

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Abstract

The emergence of fibroblast growth factor 23 as a potentially modifiable risk factor in CKD has led to growing interest in its measurement as a tool to assess patient risk and target therapy. This review discusses the analytical and clinical challenges faced in translating fibroblast growth factor 23 testing into routine practice. As for other bone mineral markers, agreement between commercial fibroblast growth factor 23 assays is poor, mainly because of differences in calibration, but also, these differences reflect the variable detection of hormone fragments. Direct comparison of readout from different assays is consequently limited and likely hampers setting uniform fibroblast growth factor 23-directed targets. Efforts are needed to standardize assay output to enhance clinical use. Fibroblast growth factor 23 is robustly associated with cardiovascular and renal outcomes in patients with CKD and adds value to risk assessments based on conventional risk factors. Compared with most other mineral markers, fibroblast growth factor 23 shows better intraindividual temporal stability, with minimal diurnal and week-to-week variability, but substantial interindividual variation, maximizing discriminative power for risk stratification. Conventional therapeutic interventions for the CKD–mineral bone disorder, such as dietary phosphate restriction and use of oral phosphate binders or calcimimetics, are associated with variable efficacy at modulating circulating fibroblast growth factor 23 concentrations, like they are for other mineral metabolites. Dual therapy with dietary phosphate restriction and noncalcium-based binder use achieves the most consistent fibroblast growth factor 23–lowering effect and seems best monitored using an intact assay. Additional studies are needed to evaluate whether strategies aimed at reducing levels or antagonizing its action have beneficial effects on clinical outcomes in CKD patients. Moreover, a better understanding of the mechanisms driving fibroblast growth factor 23 elevations in CKD is needed to inform the use of therapeutic interventions targeting fibroblast growth factor 23 excess. This evidence must be forthcoming to support the use of fibroblast growth factor 23 measurement and fibroblast growth factor 23–directed therapy in the clinic.

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Introduction

Over the last decade, fibroblast growth factor 23 (FGF23) has emerged as one of the main bone-derived physiologic endocrine regulators of mineral metabolism in vertebrates, chiefly functioning as a counter-regulatory hormone for 1,25 dihydroxyvitamin D [1,25(OH)₂D] (1,2). The recognition that circulating FGF23 concentrations rise early in the progression of CKD and are not only strongly predictive of cardiovascular and renal end points (3–6) but, in particular, seem mechanistically linked to pathology (7) has driven optimism that earlier institution of therapies targeting FGF23 excess might improve patient outcomes. However, before FGF23 measurement can be incorporated into the standard laboratory testing repertoire and considered a *bone fide* clinical tool, it must be shown to have met key analytical and clinical benchmarks. The focus of this review is to appraise three such benchmarks: (1) ready availability of accurate and reproducible FGF23 assays suitable for testing in the modern clinical laboratory, (2) evidence that FGF23 measurement adds to or improves on existing testing strategies, and (3) evidence that the risk associated with FGF23 is modifiable or may help guide patient care.

Analytical Considerations of FGF23 Measurement

Intact FGF23 (approximately 32 kDa) contains a proteolytic cleavage site (R¹⁷⁶XXR¹⁷⁹) recognized by a family of serine endopeptidase proprotein convertases (8). Cleavage at this locus generates N- and C-terminal fragments (approximately 18 and 14 kDa, respectively) that, with intact hormone, enter the circulation (9). Two formats of immunometric FGF23 assay are available from several commercial manufacturers. Intact FGF23 (iFGF23) ELISAs, developed by Immutopics, Inc., Kainos Laboratories, and EMD Millipore, require simultaneous recognition of epitopes within the N- and C-terminal domains flanking the proteolytic cleavage site and detect only full-length hormone (10). Although different iFGF23 assays yield closely correlated results, they show poor absolute numerical agreement because of calibration differences (11,12). iFGF23 assays are also variably affected by instability issues caused by *ex vivo* proteolytic degradation of intact hormone, necessitating the collection of protease-protected plasma samples in circumstances where sample processing is delayed (>2 hours) (13).

The C-terminal FGF23 (cFGF23) ELISA from Immutopics, Inc., uses antibodies that bind two distinct epitopes within the C-terminal portion of the protein, thus detecting both intact and C-terminal fragments (14). The most recent generation of the cFGF23 assay seems least affected by preanalytical stability issues (11). It is important to recognize, however, that cFGF23 measurements do not necessarily reflect changes in FGF23 bioactivity, particularly in situations where FGF23 processing is altered. Patients with iron-deficiency anemia (15), fibrous dysplasia (16), or familial tumoral calcinosis (17) can display elevated cFGF23 concentrations, whereas intact hormone is normal or even low. The biologic activity of cFGF23 fragments remains a controversial topic. Early work by Berndt *et al.* (18) suggested that proteolytically generated peptides containing residues 180–205 retained the phosphaturic activity of intact hormone in opossum kidney cells but that further truncation of this domain abolished such activity. Goetz *et al.* (19) later localized the minimal receptor binding epitope to this region but contrastingly, provided *in vitro* and *in vivo* evidence that the isolated cFGF23 domain dose-dependently antagonized the activity of the intact hormone by competing for occupancy of the receptor complex. In general, therefore, iFGF23 measurements represent a more homogenous signal and are likely to provide the most reliable readout of biologic effect.

iFGF23 and cFGF23 assays often show poor analytical agreement, particularly at near-physiologic concentrations (20). This discordance relates not only to calibration differences but also, the detection of C-terminal fragments normally present in plasma using the cFGF23 assay (20). Formally defined reference intervals for iFGF23 (12–49 pg/ml) and cFGF23 (22–91 RU/ml) assays have been published for adult and pediatric populations (20,21) but not for all commercial kits.

In health, iFGF23 shows significant diurnal variability (approximately 30%), with concentrations generally reaching their peak in the early morning (20,22). cFGF23 levels show minimal circadian cycling (20,23,24) and less week-to-week intraindividual variability than other conventional mineral parameters (20,25,26). Conversely, although interindividual variability for iFGF23 and parathyroid hormone (PTH) concentrations seems similar, interindividual variation for cFGF23 is markedly higher (20). This yields a high index of individuality for cFGF23 and, although helpful for risk stratification, imposes limits on clinical interpretation with conventional population-based reference intervals (27). The relatively high intraindividual variation of PTH and phosphate means that, although serial cFGF23 concentrations need to change by only 25% in an individual to be considered significant, PTH and phosphate levels need to change by more than 32% and 56%, respectively (20). In contrast, in patients undergoing hemodialysis, the intraindividual variation of plasma cFGF23 concentrations seems higher than for other bone mineral markers according to some (28,29) but not all reports (30).

Recently, Shimizu *et al.* (31) developed a fully automated chemiluminescent immunoassay using the monoclonal antibodies from the Kainos iFGF23 ELISA. Compared with commercial ELISAs, this assay boasts better analytical sensitivity, greater analytical range, shorter assay time, and it is relatively sample-sparing; thus, it seems better suited

to a high-throughput clinical laboratory. EMD Millipore has also released a Luminex-based multiplex immunoassay bone panel including FGF23, offering the opportunity for simultaneous measurement of FGF23 and other bone and inflammatory biomarkers (*e.g.*, PTH, sclerostin, and TNF- α) in a single sample aliquot. However, despite having the broadest working range of available assays and thus, diminishing the need for repeat testing at dilution in patients with high concentrations, the analytical limit of detection of this assay is relatively high (within the physiologic range), and the manufacturer's performance data indicate quite high interassay imprecision. The application of these newer analytical platforms to clinical cohorts is awaited, and independent validation is required.

Table 1 compares key performance characteristics of available FGF23 assays. Currently, commercial FGF23 assays have not been approved or validated for *in vitro* diagnostic use. Overall, marked calibration differences constrain direct interassay comparisons. It should be stressed, however, that problems with assay standardization are not unique to FGF23, and similar long-standing issues are well described for many other bone mineral markers, most notably PTH and 25 hydroxyvitamin D [25(OH)D], that have been measured routinely for decades (32,33). Nonetheless, such experience with other mineral metabolism analytes should prompt attempts to harmonize FGF23 assay readout.

Does FGF23 Measurement Add Value for Patients with CKD?

CKD as a State of FGF23 Excess

According to analysis of 3879 patients with stages 2–4 CKD in the Chronic Renal Insufficiency Cohort (CRIC) study (3), plasma cFGF23 concentrations increase early in disease (eGFR=58 ml/min per 1.73 m²), before elevations in PTH (eGFR=47 ml/min per 1.73 m²), and consistent with earlier studies (34), well before the onset of frank hyperphosphatemia (<30 ml/min per 1.73 m²). In CRIC, few patients (<6%) showed an isolated increase in PTH (>65 pg/ml but normal FGF23), emphasizing the primacy of elevations in FGF23 in this patient group (3). Consistent findings have been reported in pediatric CKD cohorts using the same assay (35) and other adult nondialysis-dependent CKD cohorts using the Kainos iFGF23 assay (36). This temporal sequence is also borne out in longitudinal studies in murine models of progressive CKD (37). FGF23 concentrations increase inexorably as renal function falls, where intact full-length hormone seems to be the predominant circulating species (20,38). In dialysis patients without residual renal function, concentrations can be 1000-fold above normal (39,40), and after kidney transplantation, FGF23 levels decline rapidly in patients with good allograft function (41).

FGF23 and Adverse Outcomes in CKD

Multiple prospective studies have shown a consistent link between higher FGF23 concentrations and increased risk of both death and cardiovascular events across the entire spectrum of renal disease (4,5,42–45). Most studies show a near-linear increase in risk with increasing FGF23 concentrations, with available (but limited) data suggesting comparable results between cFGF23 and iFGF23

Table 1. Performance characteristics of assays for FGF23 measurement

Characteristic	Kainos Laboratories		Immutopics, Inc. ^a		Intact FGF23		C-Terminal FGF23 (Immutopics, Inc. ^a)	
	ELISA/colorimetric	Unknown	Residues 51–69 Residues 206–222 EDTA plasma	ELISA/colorimetric	EMD Millipore	CLIA/chemiluminescent	FIA Luminesx/fluorescent	ELISA/colorimetric
Epitope binding region	Unknown		Residues 206–222 EDTA plasma	Unknown		Unknown	Unknown	Residues 206–222 Residues 225–244 EDTA plasma
Preferred sample type	Serum		EDTA plasma	EDTA plasma or serum		Serum	EDTA plasma or serum	EDTA plasma
Preamalytical stability issues	No	Yes (13)	Yes (13)	No		Not tested	Not tested	No
Sample volume ^b (μl)	100	100	100	100		20	50	100
Functional sensitivity	2 pg/ml	1.5 pg/ml	1.5 pg/ml	18 pg/ml		1 pg/ml	37 pg/ml	1.5 RU/ml
Analytical range	2–800 pg/ml	2–2200 pg/ml	2–2200 pg/ml	18–2400 pg/ml		1–15,000 pg/ml	37–150,000 pg/ml	2–1400 RU/ml
Interassay imprecision (%)	<5	<5	<5	<12		<5	<15	<4
Assay time	3–4 h	3–4 h	3–4 h	3–4 h		20 min	2–16 h	3–4 h
Multiplex capability	No	No	No	No		No	Yes	No
Independent analytical validation	Yes (11,140)	Yes for first generation kit (11,140)	Yes for first generation kit (11,140)	Yes (11)		No	No	Yes (11,140)
Reference interval ^c	No	Yes, for first generation kit (20)	Yes, for first generation kit (20)	No		No	No	Yes (20,21)
Clinical outcome studies ^d	Multiple (36,42,46,50,74,75)	Several (43,141)	Several (43,141)	None		None	None	Multiple (3–6,55,59,60,142)

FGF23, fibroblast growth factor 23; CLIA, chemiluminescence immunoassay; FIA, fluorescence immunoassay.

^aSecond generation kits.

^bFor duplicate determinations.

^cDefined according to Clinical and Laboratory Standards Institute guidelines (143).

^dDeath or progression to ESRD requiring RRT.

measurements (43). These relationships seem minimally confounded by renal risk factors, conventional cardiovascular risk factors, therapeutic interventions, or, perhaps most significantly, other mineral parameters as well. Even adjustment for measured GFR does not seem to materially attenuate the association (4,46). As summarized in Table 2, after full adjustment, effects remain sizeable and stable across a number of diverse and well powered observational CKD cohort studies. Conversely, substitution of FGF23 for other mineral exposures, including PTH, 25(OH)D, 1,25(OH)₂D, or fractional excretion of phosphate (FEP), in the same multivariate models has failed to consistently show the same monotonic relationship with outcome (Table 3) (4,5,42), suggesting an effect specific to FGF23 rather than a more general one reflecting mineral disturbances. Indeed, in CRIC and the Homocysteine in Kidney and End Stage Renal Disease studies, the mortality risk associated with FGF23 is reportedly even greater than for established CKD-specific factors, like eGFR and proteinuria (4,5). Hence, the magnitude of the risk of hard end points with very high FGF23 concentrations suggests that FGF23 may well present an important clinical target in such patients in the future. Incorporation of FGF23 measurements into cardiovascular risk models based on conventional risk factors (*e.g.*, Framingham risk factors) seems to yield a small but significant improvement in risk reclassification in both nondialysis-dependent CKD (7%) and post-transplant (7%–11%) cohorts (42,45).

With respect to renal outcomes, higher FGF23 concentrations have been associated with earlier initiation of chronic dialysis and significant decrements in renal function in patients with nondialysis-dependent CKD (4,5,36,46), again yielding improvements in event reclassification (19%) (47), and have variably predicted graft failure after kidney transplantation (44,45). Moreover, FGF23 predicts progression in disease-specific causes of renal failure, including IgA nephropathy, and those patients with heavy proteinuric diabetic kidney disease (48,49). Recently, Semba *et al.* (50) also reported an independent association between FGF23 concentrations and the risk of incident CKD in a cohort of elderly community-dwelling women.

In the acute setting, FGF23 concentrations are reportedly much higher in patients with AKI compared with controls (more than 5-fold), with higher levels being predictive of greater risk of death or requirement for dialysis (51). Other mineral parameters were not predictive of outcome. Furthermore, in analysis of 3241 participants from the Cardiovascular Health Study (CHS) (52), an elderly community-dwelling cohort mostly free from renal disease (mean eGFR=71 ml/min per 1.73 m²), those individuals in the highest cFGF23 quartile had an almost 2-fold higher risk of future AKI hospitalization (median 10-year follow-up) compared with those individuals in the lowest quartile after full adjustment for renal (baseline eGFR and albuminuria) and cardiovascular risk factors.

The added value of FGF23 measurements to conventional renal and cardiovascular risk factors is noteworthy, because risk prediction models derived from the general population generally perform poorly in CKD patients (53). On this basis, a strong argument for the assimilation of FGF23 measurements into standard risk prediction models could be made. Importantly, the association between

FGF23 and outcomes does seem modified by a number of factors, most notably, vitamin D status and FEP (54–56). Those patients with both high FGF23 and low 25(OH)D/1,25(OH)₂D or high FGF23 and low FEP seem to have the greatest risks of left ventricular remodeling (54), poor renal survival, and cardiovascular events (55). Thus, these measures may provide complementary information, and the use of biomarker panels may enhance risk stratification further. However, these findings need to be replicated in other studies.

FGF23—Direct Link to Pathology?

Although studies in animal CKD models attest to a protective role in early CKD (57), possibly as a long-term but indirect and adaptive response to a high-phosphate load (58), other evidence alludes to potentially harmful effects of chronic FGF23 excess on various organ systems, most notably the heart (7).

Accumulating epidemiologic evidence now points strongly to a relationship between FGF23 and cardiac failure rather than occlusive atherosclerotic vascular disease (6). Indeed, even in cohorts without significant renal dysfunction, such as the Heart and Soul Study (59) and the CHS (60), a similar pattern is manifest, with the association between FGF23 and death mainly driven by heart failure events rather than myocardial infarction or cerebrovascular injury. Likewise, in patients undergoing coronary angiography, again with minimal renal dysfunction (19% eGFR<60 ml/min per 1.73 m²), FGF23 has primarily been associated with left ventricular dysfunction (61). Moreover, although higher FGF23 concentrations have been associated with numerous intermediate markers of vascular disease in both adult and pediatric CKD cohorts, the most consistent associations by far are with left ventricular mass index (LVMI) and left ventricular hypertrophy (LVH) (7,61–65). Analysis of CRIC shows not only a strong independent association between FGF23 and LVMI and the prevalence of LVH but also, the ability of FGF23 levels to predict incident LVH as well (7). Given the high prevalence of LVH in CKD and its link to congestive heart disease, arrhythmia, and sudden cardiac death (66), all of which contribute appreciably to the high burden of cardiovascular death in CKD (67), this finding represents a particularly important potential pathway of risk in this population. Hence, of great relevance to these epidemiologic observations were the landmark findings of Faul *et al.* (7), showing that FGF23 can directly induce hypertrophic gene programs in the cardiomyocyte, leading to LVH at the elevated levels seen in some advanced CKD patients. Independent investigators have subsequently corroborated these finding, showing the hypertrophic effect of FGF23 on cultured cardiomyocytes, and suggested that FGF23 might also have additional effects on myocardial contractility (68). Notably, in the study by Faul *et al.* (7), these effects were apparently transduced by the FGF receptor independent of α -klotho, which, according to the current paradigm, is an obligate coreceptor for physiologic FGF23 signaling in the kidney tubules and parathyroid glands (69). Indeed, as much as CKD would seem to be a state of FGF23 excess, CKD could also be characterized as state of α -klotho deficiency, with parathyroid and particularly, renal expression being markedly downregulated in

Table 2. Association of FGF23 concentration with all-cause mortality and cardiovascular events in large prospective nondialysis-dependent CKD cohort studies

Study	Demographics: <i>n</i> ; Age (yr); Sex (% Men); Race	Renal Function (CKD; eGFR)	FGF23 Assay/ Median Level (IQR)	Outcome	Events (<i>n</i> , %)/ Median Follow- Up (yr)	Adjusted Hazard Ratio (95% CI) ^a	
						Continuous	Highest Versus Lowest Group
Isakova <i>et al.</i> (CRIC) (4)	3879; 58±11; 56; 42% African American	2–4; 43±14	cFGF23/146 (96–239)	All-cause mortality	266, 7/3.5	1.5 (1.3 to 1.7) per 1 SD included	3.0 (1.8 to 5.1)
Scialla <i>et al.</i> (CRIC) (6)	3860; 58±11; 56; 42% African American	2–4; 44±15	cFGF23/145 (96–239)	Atherosclerotic events; CHF events	287, 7; 360, 9/3.6	1.2 (1.1 to 1.4) per doubling; 1.5 (1.3 to 1.7) per doubling per 1 SD	1.8 (1.2 to 2.6); 3.0 (2.0 to 4.5)
Kendrick <i>et al.</i> (HOST) (5)	1099; 69±11; 98; 26% African American	4–5; 18±16	cFGF23/392 (216–945)	All-cause mortality	453, 41/2.9	1.6 (1.3 to 2.1) per 1 SD included	2.2 (1.6 to 3.1)
Scialla <i>et al.</i> (AASK) (46)	809; 55±10; 60; 100% African American	2–5; 45±18 (¹²⁵ I-iothalamate)	iFGF23/44 (31–64)	Composite of all-cause mortality and initiation of RRT	351, 43 (117, 14 died)/7.9	1.3 (1.2 to 1.5) per doubling	2.3 (1.3 to 3.8)
Nakano <i>et al.</i> (OVIDS-CKD) (42)	738; 64 (54–72); 66; Japanese	1–5; 35±19	iFGF23/50 (32–81)	CV events predialysis	215, 29/4.4	1.7 (1.1 to 2.7) per 1 SD included	Not reported
Wolf <i>et al.</i> (44)	984; 55±10; 57; European	Post- transplant; 51±21	cFGF23/28 (20–43)	Composite of all-cause mortality and allograft loss	182, 18; 87, 9 died/3.1	1.4 (1.3 to 1.7) per 1 SD included; 1.5 (1.3 to 1.8) per 1 SD included	2.2 (1.4 to 3.7); 2.2 (1.1 to 4.0)
Baia <i>et al.</i> (45)	593; 52±12; 54; European (95% white)	Post- transplant; 47±16	cFGF23/140 (95–219)	CV mortality; all-cause mortality	66, 11; 128, 22/7.0	1.9 (1.1 to 3.2) per 1 SD included; 1.9 (1.3 to 2.7) per 1 SD included	3.0 (1.1 to 8.3); 2.2 (1.1 to 4.5)

IQR, interquartile range; 95% CI, 95% confidence interval; CRIC, Chronic Renal Insufficiency Cohort; cFGF23, C-terminal FGF23; CHF, congestive heart failure; HOST, Homocysteine in Kidney and End Stage Renal Disease; AASK, African American Study of Kidney Disease and Hypertension; iFGF23, intact FGF23; OVIDS-CKD, Osaka Vitamin D Study in Patients with CKD; CV, cardiovascular.

^aHazard ratios for fully adjusted models (exact model covariates differ but include adjustment for potential conventional renal, CV, and mineral confounders).

Table 3. Independent association of conventional mineral markers with all-cause mortality and CV events in large prospective nondialysis-dependent CKD cohort studies

Study	End Point	Phosphate	PTH	25(OH)D	1,25(OH) ₂ D	FEP
CRIC (4,6)	All-cause mortality	NR	No	NA	NA	No
CRIC (4,6)	Atherosclerotic events	Yes; HR, 1.1 (95% CI, 1.0 to 1.2) per 0.5 mg/dl	No	NA	NA	No
CRIC (4,6)	Heart failure events	Yes; HR, 1.1 (95% CI, 1.0 to 1.2) per 0.5 mg/dl	No	NA	NA	NA
HOST (5,56)	All-cause mortality	No	No	No	No	NA
HOST (5,56)	CV events	Yes; HR, 1.2 (95% CI, 1.1 to 1.3) per mg/dl	No	No	Not independent of FGF23	NA
AASK (46)	All-cause mortality and initiation of RRT (composite)	Only at high levels >3.5 mg/dl; HR, 1.3 (95% CI, 1.2 to 1.5) per 0.5 mg/dl; not significant in tertile analysis	Only at high levels >39 pg/ml; HR, 1.3 (95% CI, 1.1 to 1.5) per doubling; not significant in tertile analysis	No	NA	NA
OVIDS-CKD (42)	CV events predialysis	NR	No	No; predictive of death but not independently of hemoglobin	No	NA
Wolf <i>et al.</i> (44)	All-cause mortality and allograft loss (composite)	Yes; HR, 1.2 (95% CI, 1.1 to 1.4) per 1 SD; not significant in tertile analysis or patients with eGFR=30–90 ml/min per 1.73 m ²	No	NA	NA	NA
Baia <i>et al.</i> (45)	CV mortality	No	No	No	No	No

CV, cardiovascular; PTH, parathyroid hormone; 25(OH)D, 25 hydroxyvitamin D; 1,25(OH)₂D, 1,25 dihydroxyvitamin D; FEP, fractional excretion of phosphate; NR, not reported; NA, not assessed; HR, hazard ratio.

early disease (70,71). This finding may be significant, because α -klotho deficiency could theoretically potentiate the effects of chronic FGF23 excess in advanced CKD by favoring nonspecific, low-affinity FGF receptor activation in non-physiologic tissues, like the heart, but also feedback to the osteocyte, driving further increments in production to maintain biologic effect.

Although the aforementioned effects of FGF23 on cardiomyocytes provide a credible explanation of how very high FGF23 concentrations might drive cardiac dysfunction in dialysis patients, it is difficult to attribute the same effect to concentrations only fractionally above normal levels (72,73). Indeed, FGF23 concentrations have been robustly associated with all-cause mortality and cardiovascular events in a number of community-dwelling elderly cohorts largely free from significant renal dysfunction (59,60,74,75). Thus, additional work is required to understand the nature of the increased risk in individuals with normal or minimally reduced renal function and determine whether FGF23 might be causally related to cardiovascular disease in this setting or whether FGF23 levels are providing a surrogate marker of other processes directly related to pathology.

Although FGF23 levels have also been fairly consistently associated with poor renal end points, there is no mechanistic evidence linking higher FGF23 with renal disease progression. Nonetheless, the fact that TGF- β and TNF- α pathways are activated in murine CKD models after the administration of exogenous FGF23 (76) invokes FGF23 as a possible protagonist in renal fibrosis and inflammation. Moreover, in human studies, higher FGF23 concentrations have been independently associated with systemic proinflammatory cytokine levels (IL-6 and TNF- α) (77), suggesting a role in inflammatory processes and providing another plausible but unexplored risk pathway through which FGF23 could operate.

Rise of FGF23 in CKD—Unanswered Questions

The trigger for FGF23 elevations in CKD and the etiology of the prodigious increase in levels as renal function fails are poorly understood. Although multiple factors in advanced CKD favor increased synthetic drive (hyperparathyroidism, iron deficiency, inflammation, active vitamin D therapy, acidosis, hypercalemia, and feedback from end organ resistance caused by α -klotho downregulation) (78), osteocytic FGF23 gene expression and protein levels seem only modestly increased (if at all) in early human CKD and animal models (37,79,80), and they do not seem commensurately increased with circulating levels in dialysis patients (79). Indeed, in longitudinal analysis of an early progressive CKD rodent model, the rise in circulating FGF23 concentrations preceded changes in FGF23 gene expression in bone by 4 weeks (37). The source of circulating FGF23, particularly in early CKD, would, therefore, seem uncertain. Theoretically, very rapid changes in FGF23 levels, which were recently reported in mice with folic acid-induced AKI (81), may reflect the release of preformed hormone from bone matrix rather than *de novo* cellular synthesis of new protein. An alternative explanation of these data is that extraskeletal production of FGF23 may also contribute to the circulating pool. Although some analyses clearly identify bone as the principal source of

FGF23 in uremic and nonuremic rodent models (82,83), low-level expression in human tissue has also been variously reported in the liver, thymus, lymph nodes, spleen, small intestine, kidney, and heart (84–86). Whether CKD might affect the expression of FGF23 in these tissues and whether local production, possibly as a compensatory response to tissue injury, significantly augments systemic hormone levels seem worthy of additional investigation. In this regard, it seems particularly noteworthy that renal FGF23 expression has been reported in rodent models of diabetic nephropathy and polycystic kidney disease (87,88).

Little is known about FGF23 degradation and clearance. A growing number of studies suggest that renal clearance contributes minimally to FGF23 elimination (39,89), and unlike other peptide hormones, the high levels in CKD do not seem to be caused by accumulation of inactive hormone fragments (20,38). Additional clues come from the study of murine AKI, where renal impairment was found to only have a relatively modest effect on plasma FGF23 half-life (approximately 50% reduction) (81). The fact that neither increased osteocytic synthesis nor reduced renal clearance seems to account for the high levels typically seen in CKD suggests that extraskeletal sites of FGF23 synthesis and/or extrarenal routes of elimination may be important determinants in the evolution of FGF23 levels in this setting. Although controversial, a clear understanding of mechanisms underlying the increase in FGF23 in kidney disease is vital to fully comprehend the significance of changes in measured concentrations and better inform the use of therapeutic interventions targeting FGF23 excess.

FGF23—a Modifiable Risk Factor?

The strong association between FGF23 and mortality and the evidence that FGF23 excess may directly harm the heart raise the fundamental question of whether lowering FGF23 concentrations or antagonizing its action would reduce risk and improve patient outcome. Indeed, can conventional CKD–mineral bone disorder (CKD-MBD) therapy be used to successfully target FGF23?

Dietary Phosphate Restriction and Oral Phosphate Binders

Lowering FGF23 by restricting dietary phosphate content or reducing intestinal absorption with oral phosphate binders (or a combination of the two) has been the most comprehensively evaluated conventional CKD-MBD approach to date, but it has yielded somewhat inconsistent results, particularly in randomized controlled trials of nondialysis-dependent CKD patients (Table 4). To some extent, the apparent discordance is likely to reflect a number of factors from differences in binder type or dietary control/content to the duration of follow-up, sample size, and the FGF23 assay. The source of dietary phosphate seems to have quite a profound effect on changes in FGF23, at least in the short-term, with meat-based protein diets yielding higher levels than plant-based protein diets (despite equivalent phosphate content) (90). Dietary phosphate restriction strategies alone have yielded very modest nonsignificant reductions in FGF23 (91–94), although quite extreme restriction with a very low-protein diet (<400 mg phosphate/d) has been associated with a significant reduction in circulating levels (92). Overall, reductions in FGF23

Table 4. Responsiveness of FGF23 concentration to oral phosphate binder use in randomized controlled trials of CKD and dialysis cohorts

Study	Duration	Sample Size (n); CKD	Diet/Phosphate Intake	Binder/Dose	FGF23 Assay	FGF23 and Mineral Response
Binder versus placebo Block <i>et al.</i> (99)	9 mo	148; CKD stages 3 and 4; serum P=3.5–6.0 mg/dl	Not controlled	Composite active group: LaCO ₃ , CaAc, SCO ₃ ; mean=2700, 5900, 6300 mg/d, respectively	cFGF23/iFGF23	cFGF23/iFGF23: no change in active versus placebo; cFGF23: no difference between binder types; iFGF23: decrease in patients on SCO ₃ , increase in patients on CaAc; modest decrease in serum P (4.2–3.9 mg/dl) and FEP (32%–26%) in active arm
Isakova <i>et al.</i> (91)	3 mo (2×2)	39; CKD stages 3 and 4 normophosphatemic	<i>Ad libitum</i> versus 900 mg	LaCO ₃ ; fixed 3000 mg/d	cFGF23	Neither LaCO ₃ nor 900 mg P diet alone reduced levels compared with placebo or <i>ad libitum</i> diet; variable reduction 35% (±32%) with dual LaCO ₃ and 900 mg P but baseline cFGF23 much higher in this group (mean=514 versus 99, 127, 120 RU/ml); no change in serum P or FEP
Chue <i>et al.</i> (107)	9 mo	109; CKD stage 3 normophosphatemic	Not controlled	LaCO ₃ ; fixed 1600 mg/d	iFGF23	No change on active therapy (median decrease=5 pg/ml); no change in serum P or FEP
Seifert <i>et al.</i> (144)	12 mo	38; CKD stage 3 normophosphatemic	Not controlled	LaCO ₃ ; fixed 3000 mg/d	iFGF23	No change in LaCO ₃ versus placebo; no change in serum or 24-h urine P

Table 4. (Continued)

Study	Duration	Sample Size (n); CKD	Diet/Phosphate Intake	Binder/Dose	FGF23 Assay	FGF23 and Mineral Response
Calcium-based binder versus noncalcium-based binder CKD						
<i>Olivera et al.</i> (145)	1.5 mo	40; CKD stages 3 and 4 normophosphatemic	Dietary monitoring; 739±253 mg/d	CaAc versus SHCl; titrated, doubled every 2 wk	iFGF23	No change with CaAc (although trend to reduction); reduction by week 6 with SHCl (median decrease=49 pg/ml, 48%); no change in serum P but decrease in FEP in both groups No change with CaAc (mean increase=4%); mean 27% reduction with SHCl (–33% to –9%); greater reduction in serum P with SHCl (mean decrease=31% versus 15%)
<i>Yilmaz et al.</i> (105)	2 mo	100; CKD stage 4 hyperphosphatemic	Not controlled	CaAc versus SHCl; titrated to serum P<5.5 mg/dl	iFGF23	Nonsignificant trend to reduction with SCO_3 and increase with CaCO_3 ; significant decrease with SCO_3 treatment in patients with baseline concentration >70 pg/ml; no change in serum P but decrease in 24-h urine P No reduction with CaCO_3 but significant decrease with LaCO_3 (mean decrease=36%); no change in serum P but decrease in FEP in LaCO_3 arm
<i>Vlassara et al.</i> (106)	2 wk (crossover)	22; CKD stages 2–4; diabetic	Dietary monitoring; 967±479 mg/d	CaCO_3 versus SCO_3 ; fixed 3600 versus 4800 mg/d	iFGF23	Reduction with CaCO_3 +SHCl but not with CaCO_3 alone; levels increased in 8 of 26 patients on SHCl+ CaCO_3 therapy
<i>Soriano et al.</i> (146)	4 mo	32; CKD stages 4 and 5 hyperphosphatemic	Standardized diet: 1 g/kg per day protein; <800 mg P	CaCO_3 versus LaCO_3 ; titrated to serum P (<4.5 mg/dl)	iFGF23	No reduction with CaCO_3 but significant decrease with LaCO_3 (mean decrease=36%); no change in serum P but decrease in FEP in LaCO_3 arm
Hemodialysis <i>Koivumäki et al.</i> (147)	1 mo	46; prevalent HD	Not controlled	CaCO_3 versus SHCl+ CaCO_3 ; fixed 3000 mg/d CaCO_3 versus 3000 mg/d SHCl +3000 mg/d CaCO_3	iFGF23	Reduction with CaCO_3 +SHCl but not with CaCO_3 alone; levels increased in 8 of 26 patients on SHCl+ CaCO_3 therapy

Table 4. (Continued)

Study	Duration	Sample Size (n); CKD	Diet/Phosphate Intake	Binder/Dose	FGF23 Assay	FGF23 and Mineral Response
<i>Cancela et al.</i> (148)	12 mo	71; prevalent HD	Not controlled	CaAc versus SHCl; titrated to achieve serum P, iCal, iPTH targets	iFGF23	Substantial reduction in patients receiving SHCl over 1 yr (median decrease=17,508–4627 pg/ml) but no change in patients on CaAc No change with CaCO ₃ but reduction with LaCO ₃ (mean decrease=46%)
<i>Toida et al.</i> (149)	3 mo (crossover)	42; prevalent HD	Not controlled	CaCO ₃ versus LaCO ₃ ; titrated to achieve serum P, iCal, iPTH targets	iFGF23	

P, phosphate; LaCO₃, lanthanum carbonate; CaAc, calcium acetate; SCO₃, sevelamer carbonate; SHCl, sevelamer hydrochloride; CaCO₃, calcium carbonate; HD, hemodialysis; iCal, ionized calcium; iPTH, intact PTH.

have been seen most consistently in dialysis cohorts and treatment groups receiving noncalcium-based binders. Of note, the use of calcium-based phosphate binders is theoretically undesirable given the stimulating effect of calcium on FGF23 synthesis (95), which might negate any beneficial effect on phosphate balance in addition to possibly promoting extrasosseous mineral accrual (96). Assessment of FGF23 modulation using the cFGF23 assay has registered mostly null results and may be relatively insensitive to changes in bioactive levels (97–100). Combined strategies of dietary phosphate restriction (<700–750 mg/d) in addition to an oral binder seem to be more effective in achieving significant reductions in FGF23 than either approach in isolation (91,93,94), but the magnitude of this decrease is quite variable and not observed consistently in all patients on treatment (like for serum phosphate concentration and urinary phosphate excretion). However, the inconsistent response to such therapeutic interventions may also simply reflect the modest and variable effect that dietary phosphate has on FGF23 in CKD. Indeed, in an analysis of 880 individuals from the Heart and Soul Study (mean eGFR=71±22 ml/min per 1.73 m²), after adjustment for demographic factors and eGFR, Palomino *et al.* (101) failed to detect any significant association between 24-hour urinary phosphate excretion (a measure of dietary phosphate intake) and cFGF23 concentrations. Conversely, Gutierrez *et al.* (102) did report a significant association between cFGF23 and dietary phosphate intake (assessed by food logs) in 1261 individuals enrolled in the Health Professionals follow-up study. However, the magnitude of this effect was still modest: every 500-mg/d increase in dietary phosphate intake was associated with only a 3.4-RU/ml increase in cFGF23 (102). Even data from animal CKD models are unpersuasive, particularly in early progressive models of disease. Some studies either show a lack of response to phosphate restriction altogether (80) or relate improvement in cardiovascular function to changes in phosphate rather than FGF23 (103). While extreme, phosphate restriction (zero phosphate diet), however, has been associated with a paradoxical increase in plasma FGF23 (104).

The only tentative evidence of benefit associated with FGF23 lowering with an oral phosphate binder in the predialysis setting comes from a randomized, open-label study by Yilmaz *et al.* (105). An 8-week intervention with sevelamer hydrochloride in 47 hyperphosphatemic stage 4 CKD patients was associated with a 27% reduction in iFGF23 and a modest improvement in flow-mediated vasodilatation, independent of changes in serum phosphate. However, such an effect has yet to be replicated in other studies and remains mechanistically ambiguous: it may, in part, relate to the known pleiotropic effects of the drug (*e.g.*, anti-inflammatory) (106). Other similarly sized trials in normophosphatemic CKD patients have failed to show efficacy in FGF23 lowering or in the case of the Chronic Renal Impairment in Birmingham-Phosphate study (107), improvement in outcome (LVMI) over 9 months of therapy.

On theoretical grounds, one might challenge the notion that phosphate modulation would significantly alter FGF23 levels given that phosphate, unlike 1,25(OH)₂D or PTH, seems to have no direct effect on FGF23 production in cultured osteoblasts (2). Likewise, in healthy adults, intravenous infusion of phosphate fails to stimulate a response

in FGF23 (108), and there is minimal acute postprandial change in FGF23 in response to dietary intake (23). Thus, any effect of phosphate on FGF23 would be modest (if existent at all) and sluggish, possibly caused by indirect and delayed effects on bone mineralization or mediated by secondary changes in $1,25(\text{OH})_2\text{D}$ or PTH. Regardless, if the desired effect of dietary phosphate restriction is to lower FGF23 concentrations, then the lack of a homogeneous and reproducible effect casts doubt on its clinical efficacy as a solitary intervention.

Vitamin D

Expectedly, given the stimulating effect of $1,25(\text{OH})_2\text{D}$ on FGF23 transcription (2), use of active vitamin D or its analogs (paracalcitol and alphacalcitol) has been associated with increased plasma FGF23 concentrations in patients with secondary hyperparathyroidism (109,110). The long-term consequence of this increase with respect to cardiovascular risk has not been explored, but strong evidence relating higher FGF23 with worse outcome possibly cautions against excessive active vitamin D use. Indeed, although observational studies of hemodialysis patients suggest a survival advantage with active vitamin D therapy (111) (albeit attenuated at high doses) (112) and studies in non-CKD animal models attest to the protective properties of vitamin D receptor agonists (VDRAs) on cardiac hypertrophy (113,114), recent randomized controlled trials in CKD patients have failed to show any improvement in left ventricular structure or function with paracalcitol versus placebo (115,116). Thus, in the setting of CKD, the benefit of VDRAs on the heart seems uncertain. Regarding FGF23, however, the response to vitamin D therapy is highly variable and unrelated to pretreatment levels, and in a subset of individuals, the change seems negligible. Although it is not clear whether the efficacy of the therapy might differ in such patients, it remains possible that those individuals showing minimal change in FGF23 might be those patients that benefit most from treatment with VDRAs.

With respect to nutritional vitamin D therapy, high-dose ergocalciferol (300,000 IU) in osteoporotic individuals with vitamin D insufficiency but without renal disease yields a substantial increase in plasma FGF23 concentrations that is secondary to increases in phosphate and $1,25(\text{OH})_2\text{D}$ (117). Lower-dose therapy (<50,000 IU) with either ergocalciferol or cholecalciferol, however, has not been associated with significant changes in FGF23 in either hemodialysis patients or healthy postmenopausal women (118,119).

An alternative but hypothetical possibility is that, as an inducer of α -klotho expression (120), vitamin D therapy may help to restore the native FGF23–klotho axis in the kidney and parathyroid glands and hence, diminish end organ resistance and feedback. Theoretically, it might reduce systemic levels and attenuate some of the toxic effects of FGF23 excess. Renal $1,25(\text{OH})_2\text{D}$ depletion may also potentiate α -klotho deficiency through activation of the renin-angiotensin system and generation of angiotensin II (121). Interestingly, several reports of renin-angiotensin system inhibition in rodent CKD models suggest that such interventions may have beneficial effects on the FGF23–klotho axis by restoring renal α -klotho expression (122) and augmenting phosphate excretion while moderating the increase in systemic FGF23 levels with disease

progression (87). Likewise, in CKD mice, VDRAs were found to increase soluble klotho levels in the serum and urine (but not the kidney), increase phosphaturia, and lower serum FGF23 concentration (123). Currently, however, clinical data in support of this hypothesis are lacking.

Calcimimetics

Use of cinacalcet or experimental calcimimetic compounds (*e.g.*, velcalcetide) has been associated with reductions in FGF23 concentration in dialysis patients with secondary hyperparathyroidism (124–127). A similar reduction has also been observed in rats with CKD equivalent to stages 3 and 4 in humans (128). Given *in vitro* and *in vivo* data indicating a direct stimulating effect of PTH on FGF23 synthesis through PTH receptor-1 signaling pathways (129–131), one might expect the lowering effect of cinacalcet on FGF23 to be mediated by changes in PTH. Surprisingly, in dialysis patients, the effect of cinacalcet on FGF23 seems to be independent of changes in PTH (124–127) but instead, associated with changes in serum phosphate and calcium in most studies (124,126,127), although not consistently (125). Indeed, not all studies support a direct role for PTH in regulating FGF23 (132), and infusion of (1–34) PTH peptide in humans and mice has been shown to result in acute reductions in circulating FGF23 rather than the expected increase in levels (133,134). Thus, a detailed mechanistic appraisal of how cinacalcet lowers FGF23 levels and the role of PTH [either directly or indirectly through changes in $1,25(\text{OH})_2\text{D}$ and/or local processes in bone] (133,135,136) is certainly needed. The response in FGF23 levels to cinacalcet therapy is, like for PTH, quite variable: approximately one half of patients show little (if any) change in concentrations in FGF23 (124,126), and responsiveness seems unrelated to pretreatment levels (137).

Despite the disappointment of recent data from the Evaluation of Cinacalcet Hydrochloride Therapy to Lower Cardiovascular Events trial (138), yielding a negative primary end point (a composite of all-cause mortality and nonfatal cardiovascular events), the biochemical profile associated with cinacalcet therapy seems favorable compared with active vitamin D therapy. Whether reductions in FGF23 with cinacalcet can be related to improvement in specific outcome measures (*e.g.*, LVH) has yet to be reported. Theoretically, concurrent use of cinacalcet with a low-dose VDRA may be particularly useful in advanced CKD, combining the potentially beneficial cardiovascular effects of activation of the vitamin D receptor while lowering FGF23 concentrations. Again, however, such a positive effect has yet to be demonstrated in the clinical arena.

Experimental Approaches—FGF23 Neutralization and FGF Receptor Blockade

Administration of neutralizing FGF23 antibodies to 5/6-nephrectomized rats (with renal function equivalent to CKD stages 2–4 in humans) fed a high-phosphate diet, although normalizing calcium, $1,25(\text{OH})_2\text{D}$, and PTH and improving some skeletal mineralization and volume parameters, results in profound hyperphosphatemia, extensive vascular calcifications, and reduced survival (57). Pan-FGF receptor inhibition in rats also gives rise to a tumoral calcinosis-like phenotype (139). Thus, in patients with nondialysis-dependent CKD,

Table 5. Performance of FGF23 and other systemic bone mineral markers against key analytical and clinical benchmarks

Benchmark	Calcium	Phosphate	25(OH)D	1,25(OH) ₂ D	PTH	FGF23
Analytical criteria Biologic stability (diurnal, postprandial, week to week, interracial)	Small magnitude diurnal variation and postprandial changes (23); minimal week-to-week variability (24,25,151)	Substantial diurnal and week-to-week variability (15%-35%) (24,150,152,153) and marked postprandial and postexercise excursions (23)	Marked seasonal (>100%) (26) and interracial variation (lower in African Americans than whites) (154,155)	Modest diurnal and week-to-week variability (26,156)	Very short plasma half-life (<4 min) (157), substantial diurnal (bimodal) and week-to-week variability (20%-40%) (153,158), greater variability in dialysis patients (25), significant interracial differences (higher in African Americans) (154,155)	iFGF23: short plasma half-life (<60 min) (159), significant diurnal (30%) and week-to-week variability (20%) (20,22); cFGF23: minimal intraindividual variability (20,39), minimal postprandial response (23,91); significant interracial differences (lower in African Americans than whites) (46,154)
Preanalytical issues	Total: venous stasis/hemoconcentration effects; free: pH and temperature dependence	Sample type differences; artifactual rise with delayed separation and hemolysis	Negligible	Negligible	Sample type-, method-, and time-dependent stability (160,161)	Sample type-, method-, and time-dependent stability (11,13)
Measure and homogeneity	Good	Good	Total D ₂ +D ₃ or D ₃ -specific methods; vitamin D binding protein bound and unbound fractions (bioavailable) (162)	Poorly characterized protein binding profile	Multiple fragments, method-dependent detection (32); partially characterized	C-terminal fragments variably present (20); poorly characterized

Benchmark	Calcium	Phosphate	25(OH)D	1,25(OH) ₂ D	PTH	FGF23
Between-method agreement	Good	Good	Poor; calibration differences; matrix effects caused by serum binding proteins (164); variable cross-reactivity with other hydroxylated vitamin D metabolites (33); isobaric interferences in MS methods (33) Yes	Uncharacterized	Poor (165,166); calibration differences; no reference method; variable antibody specificity and affinities for different PTH peptides (167); cross-reactivity of intact assays for PTH (7–84)—overestimates bioactive fraction (168) Yes	Poor; calibration differences (11,12); no reference method or ISP; variable fragment detection (20)
High-throughput testing	Yes	Yes	Yes	No	Yes	No
Cost per test (US) ^a	\$8	\$8	\$30	\$40	\$30	\$40
Clinical criteria						
Temporality of change in CKD (3,34–36)	Variable	CKD stages 4 and 5	Variable	CKD stages 2 and 3	CKD stages 3 and 4	CKD stages 2 and 3
Robust and reproducible independent association with hard outcomes in multiple CKD studies ^b	No (169)	Multiple studies link higher P to increased risk for CV disease and mortality (169–171) but not consistently (172) (Table 3)	Several studies report association of low levels with and mortality (173), although not consistently (Table 3); few assessing outcome with bioavailable fraction	Inconsistent in the small number of available studies, infrequently measured (Table 3)	Inconsistent (169) (Table 3); few studies assessing outcome with whole/bioactive levels	Consistent linear association with CV events, mortality, and progression to ESRD in multiple large prospective cohort studies; minimally confounded; substantial adjusted effect size (Table 2)

Benchmark	Calcium	Phosphate	25(OH)D	1,25(OH) ₂ D	PTH	FGF23
Specific clinicopathological correlates	Uncertain	Vascular toxin (174)? (vascular calcification; endothelial dysfunction); LVH (175)? Tubular toxicity (163)?	Deficiency linked to glomerular dysfunction (podocyte loss, glomerulosclerosis, albuminuria) and LVH (176)?	Uncertain	Variable association with bone histomorphometry (bone formation rate) except at extreme values (32)	LVMi and LVH (7,61–65)
Adds to or superior performance over existing tests ^c	—	—	Potential risk modifier (36)	Potential risk modifier (54,56)	—	Yes, improves discrimination and reclassification of risk associated with CV events and renal failure (42,45,47)
Validated decision limits in CKD			None			
Modifiable risk in CKD			Uncertain			
Marker-guided therapy improves outcome in CKD			Variable response to therapy Need to individualize therapy No evidence			

MS, mass spectrometry; ISP, International Standard Preparation; CV, cardiovascular; LVH, left ventricular hypertrophy; LVMi, left ventricular mass index.

^aApproximate indicative commercial cost (including overheads).

^bDeath or progression to ESRD requiring RRT.

^cExisting tests: calcium, phosphate, and PTH.

complete blockade of FGF23 action would seem undesirable. Whether neutralization in patients with ESRD, in whom FGF23 concentrations are often substantially higher and there is a plausible mechanism of direct FGF23 toxicity to the heart, would retard LVH progression and yield clinical benefit remains questionable but may provide a rationale for testing such interventions in this setting. Partial antagonism of circulating FGF23 or more selective inhibition of specific FGF receptor isoforms that block harmful effects on the heart (but permit signaling in the kidney and modulation of phosphate handling) might be more widely applicable but have yet to be tested.

Summary and Conclusions

Table 5 appraises how FGF23 performs alongside other bone mineral markers with respect to important analytical and clinical benchmarks using current state of the art methodology. Several characteristics may favor the use of FGF23 in CKD over conventional mineral markers, like PTH and phosphate: (1) temporal stability of the signal, (2) temporality of increase, and (3) greater discriminative power. Critically, unlike most other bone mineral markers, higher concentrations seem robustly associated with hard outcome measures, yielding large and stable effect sizes. Moreover, unlike other mineral metabolites, FGF23 seems reproducibly related to specific clinical pathologic correlates (e.g., LVH), which provides a strong rationale for the assessment of interventions aimed at lowering circulating FGF23 levels. In general, however, conventional CKD-MBD therapies (e.g., oral phosphate binders or calcimimetics) result in variable and unpredictable changes in FGF23 levels, similar to the changes in other markers of mineral metabolism. Moreover, mechanistic aspects of how FGF23 lowering is achieved with these therapies remain poorly defined and need to be delineated. Additional specific strategies for controlling FGF23 should be considered.

Seemingly, one of the key obstacles to incorporating FGF23 measurement into current clinical practice is the lack of evidence that lowering FGF23 results in benefit to the patient. However, equivalent data showing improvement in outcome associated with modulating other contemporary

mineral measures (e.g., phosphate and PTH) are also lacking. For instance, in CKD patients, there remains no definitive evidence that serum phosphate lowering with oral binder therapy is associated with improvement in survival or for that matter, that restricting intestinal phosphate absorption even has the intended effect on overall phosphate balance. Indeed, in a recent study of stages 3 and 4 CKD patients treated with oral calcium carbonate, binder use was found to have no net effect on phosphate balance, at least in the short term (137). Thus, it could be argued that needing evidence of attenuated risk with FGF23 lowering demands substantially more from FGF23 than we ask of the mineral markers currently in use. Nonetheless, other questions regarding the use of FGF23 measurements have yet to be addressed. What is the goal of FGF23-directed therapy? Should it be to normalize FGF23 levels or restore a functional FGF23–klotho axis? Should we aim to neutralize the action of FGF23 or treat the underlying factors leading to its increase? What magnitude of reduction in FGF23 is required for a significant benefit? Given the substantial economic and practical implications of integrating a new biomarker into CKD management protocols, it seems prudent to rigorously assess the credentials of FGF23 measurement and explicitly define its clinical value in addition to or as an alternative to contemporary testing strategies. Indeed, perhaps it is timely to revisit the evidence base (or lack thereof) supporting the use of other routinely and frequently measured bone mineral markers. Assuming that FGF23 is toxic at high concentrations and that interventions to lower FGF23 yield clinical benefit, a number of potential applications for FGF23 measurement can be envisaged (Table 6).

In aggregate, although FGF23 shows considerable promise as a biochemical target for guiding patient management and therapy, several gaps in our knowledge provide a barrier to the use of FGF23 testing in the clinic. Chief among these gaps are the lack of evidence linking FGF23 lowering with improved outcome and to some extent, the lack of a proven therapy to specifically and effectively target FGF23 excess. Fundamentally, however, we have yet to fully understand the mechanisms driving FGF23 elevations in CKD or the means by which therapies, like calcimimetics or phosphate restriction, act to lower circulating levels in

Table 6. Potential applications of FGF23 measurement in clinical nephrology

Application	Example
Diagnostic	Early biochemical detection of the CKD-MBD; renal dysfunction (e.g., tubulointerstitial injury)
Risk stratification	CV: cardiac dysfunction, LVH, and death; high risk: high FGF23/low FEP/low 1,25(OH) ₂ D; renal: rapid progressors (eGFR decline >3–5 ml/min per year), ESRD, and requirement for RRT; high risk: high FGF23/low 25(OH)D
Therapeutic selection ^a	Early CKD: noncalcium-based oral P binders plus low P dietary prescription; vitamin D therapy; ACEi/ARB; advanced CKD: calcimimetic use or concurrent calcimimetic plus low-dose VDRA; postrenal transplantation; P/calcitriol supplementation
Monitor therapeutic response	As above; limit VDRA dose

CKD-MBD, CKD–mineral bone disorder; CV, cardiovascular; P, phosphate; ACEi/ARB, angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker; VDRA, vitamin D receptor activator.

^aIn addition to standard indications.

some patients. Although a better mechanistic understanding of these pathways may also help reveal alternative or improved avenues for therapeutic intervention, such considerations seem essential to validate FGF23 as a genuine clinical target.

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