Fibroblast Growth Factor-23 and Cardiovascular Events in CKD

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ABSTRACT

An elevated level of fibroblast growth factor-23 (FGF-23) is the earliest abnormality of mineral metabolism in CKD. High FGF-23 levels promote left ventricular hypertrophy but not coronary artery calcification. We used survival analysis to determine whether elevated FGF-23 is associated with greater risk of adjudicated congestive heart failure (CHF) and atherosclerotic events (myocardial infarction, stroke, and peripheral vascular disease) in a prospective cohort of 3860 participants with CKD stages 2–4 (baseline estimated GFR [eGFR], 44±15 ml/min per 1.73 m²). During a median follow-up of 3.7 years, 360 participants were hospitalized for CHF (27 events/1000 person-years) and 287 had an atherosclerotic event (22 events/1000 person-years). After adjustment for demographic characteristics, kidney function, traditional cardiovascular risk factors, and medications, higher FGF-23 was independently associated with graded risk of CHF (hazard ratio [HR], 1.45 per doubling [95% confidence interval (CI), 1.28 to 1.65]; HR for highest versus lowest quartile, 2.98 [95% CI, 1.97 to 4.52]) and atherosclerotic events (HR per doubling, 1.24 [95% CI, 1.09 to 1.40]; HR for highest versus lowest quartile, 1.76 [95% CI, 1.20 to 2.59]). Elevated FGF-23 was associated more strongly with CHF than with atherosclerotic events (P=0.02), and uniformly was associated with greater risk of CHF events across subgroups stratified by eGFR, proteinuria, prior heart disease, diabetes, BP control, anemia, sodium intake, income, fat-free mass, left ventricular mass index, and ejection fraction. Thus, higher FGF-23 is independently associated with greater risk of cardiovascular events, particularly CHF, in patients with CKD stages 2–4.


CKD is an international public health epidemic that increases risk of premature death due to cardiovascular disease. Rates of atherosclerotic disease are high in CKD, but risk of congestive heart failure is even more striking, with hazards approximately 3-fold higher than in non-CKD populations. Excess risk of congestive heart failure in CKD is often attributed to hypertension and anemia. However, aggressive control of these risk factors has not significantly improved heart failure outcomes in patients with CKD, suggesting additional mechanisms of disease.

Fibroblast growth factor-23 (FGF-23) is secreted by osteocytes and regulates phosphate and vitamin D homeostasis by stimulating phosphaturia and...

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inhibiting activation of vitamin D in the kidney.\textsuperscript{13} FGF-23 levels rise as kidney function declines, and higher levels are strongly associated with greater risk of death.\textsuperscript{14–19} As a potential explanatory mechanism of FGF-23–associated mortality, multiple studies consistently demonstrated that higher FGF-23 levels are independently associated with greater risk of prevalent and incident left ventricular hypertrophy,\textsuperscript{20–22} which is an important mechanism of cardiovascular disease in patients with CKD.\textsuperscript{9} In support of a causal role for elevated FGF-23 in the pathogenesis of left ventricular hypertrophy, FGF-23 stimulated pathologic hypertrophy of isolated cardiac myocytes and induced left ventricular hypertrophy in animals, independent of BP.\textsuperscript{20} In contrast, observational studies reported conflicting results on the association of FGF-23 with arterial calcification, which is another prominent pattern of cardiovascular injury in CKD.\textsuperscript{23} In the largest study to date, FGF-23 was not independently associated with coronary artery calcification in patients with CKD stages 2–4,\textsuperscript{24} and laboratory studies failed to demonstrate a procalcification effect of FGF-23 on vascular smooth muscle cells.\textsuperscript{24,25} These data suggest that direct effects of FGF-23 on cardiac remodeling, rather than the arterial vasculature, may underlie its association with mortality. We tested the hypotheses that elevated FGF-23 is a risk factor for cardiovascular disease events in patients with CKD stages 2–4 enrolled in the Chronic Renal Insufficiency Cohort (CRIC) study, and that FGF-23 is more strongly associated with risk of congestive heart failure compared with atherosclerotic events.

\textbf{RESULTS}

The study included 3860 participants with CKD from the CRIC study. Mean age of study participants ( \pm SD) was 58 \pm 11 years, the mean estimated GFR (eGFR) was 44 \pm 15 mL/min per 1.73 m\textsuperscript{2}, and median FGF-23 was 145.4 RU/mL (interquartile range, 96.0–238.8 RU/mL). Traditional cardiovascular risk factors were more prevalent in those with higher FGF-23 levels (Table 1).

\textbf{Atherosclerotic Events}

During a median follow-up of 3.6 years (interquartile range, 2.5–4.7 years), 287 participants experienced an atherosclerotic event that was adjudicated as possible, probable, or definite (22 total events/1000 person-years; 139 myocardial infarctions, 83 cerebrovascular accidents, 63 peripheral vascular disease procedures, 2 concurrent events). For the primary analysis, 490 participants were censored for onset of ESRD and 228 for death. Among the 287 events, 116 incident events (40\%) occurred in the 2681 participants without a history of atherosclerotic disease (12 incident events/1000 person-years).

In unadjusted Cox models, each doubling of FGF-23 levels was associated with a 37\% increased risk of an atherosclerotic event, and ascending FGF-23 quartiles were associated with a stepwise increase in risk (Table 2). The hazard ratio (HR) comparing the highest versus the lowest quartile was 2.75 (95\% confidence interval [CI], 2.19 to 3.46). Elevated FGF-23 remained significantly associated with greater risk of atherosclerotic events after sequential multivariable adjustment (Table 2). Each doubling of FGF-23 was associated with a 24\% higher risk in the primary model that adjusted for demographic characteristics, kidney function, traditional cardiovascular risk factors, and cardiovascular medications (Figure 1A). In models that included other mineral metabolites and nontraditional risk factors, including inflammatory markers and hemoglobin, the highest versus lowest quartile of FGF-23 was associated with a 1.63-fold greater risk of atherosclerotic events (95\% CI, 1.03 to 2.59), and a 23\% higher risk per doubling of FGF-23. In these models, higher serum phosphate (HR, 1.09 per 0.5 mg/dL; 95\% CI, 1.02 to 1.16) and calcium (HR, 1.09 per 0.5 mg/dL; 95\% CI, 1.01 to 1.17) were also associated with atherosclerotic events, but parathyroid hormone (PTH) was not. The relationship between FGF-23 and atherosclerotic events modestly attenuated with additional adjustment for N-terminal pro-B–type natriuretic peptide (NT-proBNP; \( n = 3317 \)); HR, 1.19 per doubling; 95\% CI, 1.01 to 1.40), but the point estimate was similar after additional adjustment for left ventricular mass index despite a reduction in power due to fewer participants with available measurements (\( n = 2523 \)); HR, 1.24 per doubling; 95\% CI, 0.96 to 1.61).

\textbf{Congestive Heart Failure Events}

During a median follow-up of 3.7 years (interquartile range, 2.5–4.7 years), 360 participants were hospitalized for adjudicated congestive heart failure events that were classified as probable or definite (27 events/1000 person-years). For the primary analysis, 415 participants were censored for onset of ESRD and 215 for death. Among the 360 total events, 230 incident events (64\%) occurred in the 3487 participants without a history of congestive heart failure (19 incident events/1000 person-years).

In unadjusted Cox models, each doubling of FGF-23 levels was associated with a 75\% increased risk of congestive heart failure hospitalization, and ascending FGF-23 quartiles were associated with a stepwise increase in risk (Table 2). The HR comparing the highest versus the lowest quartile was 8.18 (95\% CI, 5.47 to 12.24). The significant graded risk of congestive heart failure hospitalization across the spectrum of FGF-23 levels persisted in all multivariable models (Table 2). Each doubling of FGF-23 was associated with a 45\% higher risk in the primary model that adjusted for demographic characteristics, kidney function, traditional cardiovascular risk factors, and cardiovascular medications (Figure 1B). In the model that included other mineral metabolites and nontraditional risk factors, the highest versus lowest quartile of FGF-23 was associated with a 2.64-fold greater risk of congestive heart failure (95\% CI, 1.59 to 4.39), and a 39\% higher risk per doubling of FGF-23. Higher serum phosphate was also associated with congestive heart failure (HR, 1.10 per 0.5 mg/dL; 95\% CI, 1.01 to 1.19), but calcium and PTH were not. The relationship between FGF-23 and congestive heart failure events remained
Table 1. Baseline characteristics of the study population by quartiles of FGF-23

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>FGF-23 Quartile</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;96.0 RU/ml</td>
<td>96.0–145.4 RU/ml</td>
</tr>
<tr>
<td></td>
<td>(n=964)</td>
<td>(n=966)</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>56.1 ± 11.2</td>
<td>58.2 ± 10.9</td>
</tr>
<tr>
<td>Women</td>
<td>347 (36.0)</td>
<td>383 (39.7)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>450 (46.7)</td>
<td>419 (43.4)</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>399 (41.4)</td>
<td>369 (38.2)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>71 (7.4)</td>
<td>136 (14.1)</td>
</tr>
<tr>
<td>Other</td>
<td>44 (4.6)</td>
<td>42 (4.4)</td>
</tr>
<tr>
<td>Annual income &lt; $20,000^</td>
<td>184 (19.1)</td>
<td>268 (27.7)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>727 (75.4)</td>
<td>839 (86.9)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>290 (30.1)</td>
<td>440 (45.6)</td>
</tr>
<tr>
<td>Prior atherosclerotic CVD</td>
<td>198 (20.5)</td>
<td>275 (28.5)</td>
</tr>
<tr>
<td>Myocardial infarction or revascularization</td>
<td>144 (14.9)</td>
<td>209 (21.6)</td>
</tr>
<tr>
<td>Stroke</td>
<td>76 (7.9)</td>
<td>79 (8.2)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>32 (3.3)</td>
<td>41 (4.2)</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>33 (3.4)</td>
<td>66 (6.8)</td>
</tr>
<tr>
<td>Kidney function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated GFR (ml/min per 1.73 m²)</td>
<td>54.5 ± 14.0</td>
<td>47.2 ± 13.1</td>
</tr>
<tr>
<td>Urine albumin-to-creatinine ratio (µg/mg)^b</td>
<td>15 (5, 121)</td>
<td>31 (7, 268)</td>
</tr>
<tr>
<td>Traditional CVD risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking</td>
<td>75 (7.8)</td>
<td>97 (10.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>30.6 ± 6.7</td>
<td>31.5 ± 7.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>103.0 ± 14.7</td>
<td>106.5 ± 15.3</td>
</tr>
<tr>
<td>Women</td>
<td>100.9 ± 17.6</td>
<td>103.5 ± 19.1</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)^b</td>
<td>5.8 (5.5, 6.5)</td>
<td>6.1 (5.6, 7.2)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>184 ± 39</td>
<td>182 ± 41</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>107 ± 33</td>
<td>102 ± 33</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>49 ± 16</td>
<td>48 ± 16</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dl)^b</td>
<td>112 (78, 160)</td>
<td>128 (88, 183)</td>
</tr>
<tr>
<td>Nontraditional risk factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.5 ± 1.6</td>
<td>12.8 ± 1.7</td>
</tr>
<tr>
<td>Serum albumin (mg/dl)</td>
<td>4.1 ± 0.4</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)^b</td>
<td>1.9 (0.9, 4.6)</td>
<td>2.2 (1.0, 5.0)</td>
</tr>
<tr>
<td>Serum phosphate (mg/dl)</td>
<td>3.4 ± 0.5</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td>Serum calcium (mg/dl)</td>
<td>9.2 ± 0.4</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>Intact PTH (pg/ml)^b</td>
<td>40 (29, 59)</td>
<td>47 (33, 74)</td>
</tr>
<tr>
<td>N-terminal proBNP (pg/ml)^b</td>
<td>78 (35, 179)</td>
<td>116 (54, 281)</td>
</tr>
<tr>
<td>Echocardiography^c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ejection fraction &lt;50%</td>
<td>129 (15.5)</td>
<td>159 (18.9)</td>
</tr>
<tr>
<td>Left ventricular mass index (g/m²)^c</td>
<td>47 ± 12</td>
<td>50 ± 13</td>
</tr>
<tr>
<td>Medication use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiplatelet</td>
<td>392 (40.7)</td>
<td>446 (46.2)</td>
</tr>
<tr>
<td>β-Blocker</td>
<td>344 (35.7)</td>
<td>446 (46.2)</td>
</tr>
<tr>
<td>ACE inhibitor/ARB</td>
<td>577 (59.9)</td>
<td>689 (71.3)</td>
</tr>
<tr>
<td>Statin</td>
<td>431 (44.7)</td>
<td>537 (55.6)</td>
</tr>
<tr>
<td>Loop diuretic</td>
<td>175 (18.2)</td>
<td>303 (31.4)</td>
</tr>
</tbody>
</table>

Unless otherwise noted, values are n (%) or means ± SDs. CVD, cardiovascular disease; ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker.

^Sixteen percent of participants declined to respond.

*bMedian (interquartile range).

^Ejection fraction was available in 3225 participants and left ventricular mass index in 2880 participants.
Congestive heart failure hospitalization

| Variable                        | <96.0 RU/ml | 96.0–145.4 RU/ml | 145.5–238.9 RU/ml | ≥239.0 RU/ml | Continuous per Doubling
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerotic events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events (n)</td>
<td>47</td>
<td>62</td>
<td>78</td>
<td>100</td>
<td>–</td>
</tr>
<tr>
<td>Incidence per 1000 person-years</td>
<td>12.5</td>
<td>17.8</td>
<td>24.0</td>
<td>35.1</td>
<td>–</td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>Reference</td>
<td>1.41 (1.08 to 1.85)</td>
<td>1.90 (1.36 to 2.66)</td>
<td>2.75 (2.19 to 3.46)</td>
<td>1.37 (1.25 to 1.50)</td>
</tr>
<tr>
<td>Adjusted for demographic variables/kidney function</td>
<td>Reference</td>
<td>1.27 (0.97 to 1.66)</td>
<td>1.54 (1.12 to 2.13)</td>
<td>2.13 (1.68 to 2.69)</td>
<td>1.30 (1.16 to 1.46)</td>
</tr>
<tr>
<td>Adjusted for traditional risk factors</td>
<td>Reference</td>
<td>1.32 (0.90 to 1.92)</td>
<td>1.47 (1.14 to 1.90)</td>
<td>1.76 (1.20 to 2.59)</td>
<td>1.24 (1.09 to 1.40)</td>
</tr>
<tr>
<td>Adjusted for nontraditional risk factors</td>
<td>Reference</td>
<td>1.34 (0.90 to 1.98)</td>
<td>1.46 (1.11 to 1.91)</td>
<td>1.63 (1.03 to 2.59)</td>
<td>1.23 (1.04 to 1.45)</td>
</tr>
<tr>
<td>Congestive heart failure hospitalization</td>
<td>Reference</td>
<td>30</td>
<td>52</td>
<td>96</td>
<td>182</td>
</tr>
<tr>
<td>Events (n)</td>
<td>7.9</td>
<td>14.7</td>
<td>29.5</td>
<td>65.9</td>
<td>–</td>
</tr>
<tr>
<td>Incidence per 1000 person-years</td>
<td>Reference</td>
<td>1.85 (1.12 to 3.05)</td>
<td>3.69 (2.11 to 6.46)</td>
<td>8.18 (5.47 to 12.24)</td>
<td>1.75 (1.64 to 1.88)</td>
</tr>
<tr>
<td>Adjusted for demographic variables/kidney function</td>
<td>Reference</td>
<td>1.45 (0.88 to 2.39)</td>
<td>2.23 (1.23 to 4.03)</td>
<td>4.40 (2.96 to 6.53)</td>
<td>1.58 (1.51 to 1.65)</td>
</tr>
<tr>
<td>Adjusted for traditional risk factors</td>
<td>Reference</td>
<td>1.36 (0.78 to 2.38)</td>
<td>1.74 (1.06 to 2.86)</td>
<td>2.98 (1.97 to 4.52)</td>
<td>1.45 (1.28 to 1.65)</td>
</tr>
<tr>
<td>Adjusted for nontraditional risk factors</td>
<td>Reference</td>
<td>1.29 (0.69 to 2.40)</td>
<td>1.57 (0.86 to 2.87)</td>
<td>2.64 (1.59 to 4.39)</td>
<td>1.39 (1.22 to 1.58)</td>
</tr>
</tbody>
</table>

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**Table 2.** Risk of first atherosclerotic and congestive heart failure events in the overall population by baseline levels of FGF-23

When dually analyzed in a single model that adjusted for demographic characteristics, kidney function, traditional cardiovascular risk factors, and medications, FGF-23 was significantly more strongly associated with congestive heart failure than with atherosclerotic events ($P=0.02$). The association of FGF-23 with cardiovascular events was qualitatively similar across strata of severity of kidney disease, proteinuria, prior cardiovascular disease, diabetes, BP control, anemia, 24-hour urinary sodium, income, baseline fat-free mass assessed by bioelectrical impedance as a surrogate of edema, left ventricular systolic function, and left ventricular hypertrophy (Figure 2).

### Incident Events

Point estimates for the association of FGF-23 with incident atherosclerotic events were similar to the analysis of total events but did not reach statistical significance in adjusted models (Table 3). In contrast, higher FGF-23 remained independently associated with incident congestive heart failure in all analyses (Table 3). The results were qualitatively unchanged when we introduced a 1-year lag between measurement of FGF-23 and assessment of outcomes to ensure that elevated FGF-23 preceded incident cardiovascular disease rather than occurred as a consequence of it: congestive heart failure—HR, 1.52 per doubling of FGF-23 (95% CI, 1.26 to 1.84); atherosclerotic events—HR, 1.26 (95% CI, 0.89 to 1.80).

### Sensitivity Analyses

We performed a series of sensitivity analyses to verify the associations between FGF-23 and cardiovascular events (Table 4). To minimize the effect of potential misclassification of outcomes, we repeated the multivariable analyses restricted to definite events only. To account for out-of-hospital deaths that may have been due to cardiovascular causes, we reanalyzed each cardiovascular event subtype in a composite with death. To account for the possible influence that occurrence of one cardiovascular event type could have on the subsequent hazard of the other, we modeled history of atherosclerotic disease and congestive heart failure as time-varying covariates, updating these at the time of an incident event. Because missing data on individual covariates resulted in 10% loss of sample size in full models, we repeated the analysis after multiply imputing missing covariate data. The association between FGF-23 and congestive heart failure hospitalization was robust in all analyses. In contrast, analyses of “definite” atherosclerotic events were partially attenuated although CIs were also wider because of fewer events (Figure 3, Table 4). All associations were unchanged when we adjusted for 25-hydroxyvitamin D.
FGF-23 is associated with atherosclerotic and congestive heart failure events. Multivariable-adjusted hazard ratios of cardiovascular events according to levels of FGF-23 on the arithmetic scale. (A) Atherosclerotic events. (B) Congestive heart failure events. Models are adjusted for age, sex, income, eGFR, urinary albumin-to-creatinine ratio; history of hypertension, hypercholesterolemia, atherosclerotic cardiovascular disease, congestive heart failure, and diabetes; control of BP <140/90 mmHg; hemoglobin A1c; smoking status; body mass index; waist circumference; serum triglycerides; LDL cholesterol; use of angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, β-blockers, statins, and loop diuretics; and the number of prescribed classes of BP medications. The median of the first quartile of FGF-23 (74 RU/ml) serves as the reference. Tick marks on the x-axis represent individual participants’ FGF-23 levels.

DISCUSSION

Cardiovascular disease is the leading cause of death in patients with CKD,26 and elevated FGF-23 is a powerful predictor of mortality.16,18,19 In the current prospective cohort study, we demonstrate that elevated FGF-23 is independently associated with occurrence of cardiovascular disease events in CKD stages 2–4 and that elevated FGF-23 is more strongly associated with risk of congestive heart failure than atherosclerotic events.

The strong association between FGF-23 and congestive heart failure may be a clinical consequence of the direct hypertrophic effects of FGF-23 on the myocardium,20 but other possibilities could also contribute to our results. Because FGF-23 correlates with GFR, it is possible that unmeasured confounding related to severity of CKD could mediate the association. However, our results were unchanged when we stratified across or adjusted for a comprehensive set of covariates, including directly measured GFR. Furthermore, other CKD-specific factors that correlated with GFR to a similar extent in the CRIC study, such as PTH,18 were not associated with risk of congestive heart failure. Residual confounding by cardiovascular risk factors that are associated with baseline FGF-23 levels is another possibility.27 However, our results were robust in subgroups with favorable risk factor profiles, including those with no history of heart disease, diabetes, or anemia, and those with well controlled BP, higher income, and relatively low 24-hour urine sodium. Finally, it is interesting to note that the association between FGF-23 and congestive heart failure remained significant after adjustment for left ventricular mass index and that the effect size was similar in the subgroup of patients without left ventricular hypertrophy. These results suggest that changes in left ventricular mass and geometry may not fully mediate the strong association between FGF-23 and congestive heart failure.

The etiology of congestive heart failure events is particularly complex in patients with CKD, who are prone to volume overload because of reduced GFR or nephrotic syndrome, and in patients with ESRD in whom an inadequate dialysis prescription or poor adherence may contribute. Nevertheless, the association between elevated FGF-23 and greater risk of congestive heart failure events was similar in patients with earlier or later stages of CKD and among those with substantial or minimal proteinuria, and it was similarly robust regardless of whether we included or excluded events that occurred after onset of ESRD. Furthermore, congestive heart failure hospitalizations often represent exacerbations of an indolent disease process, such that the exact time of onset can be difficult to pinpoint. This natural history introduces the possibility of a “reverse causal” process in which elevated FGF-23 could reflect an early consequence rather than an upstream cause of heart failure.28 This scenario is also unlikely to explain our results because patients with a history of New York Heart Association class 3–4 heart failure were excluded from the CRIC study and our results were equally strong in lag analyses that exclusively considered incident heart failure events that occurred at least 1 year after FGF-23 was measured. Although our primary goal was to study novel disease mechanisms rather than to determine the predictive utility of FGF-23 as a biomarker of future heart failure risk, its independent association with congestive heart failure even after adjustment for NT-proBNP levels suggests that future studies should investigate FGF-23 in a panel of risk prediction biomarkers for congestive heart failure events.

Previous community-based studies similarly reported that higher levels of FGF-23 were associated with left ventricular hypertrophy, reduced ejection fraction, congestive heart failure events, and cardiovascular mortality.17,29–31 Furthermore, many of these associations were more pronounced among participants with CKD than in those without.17,29,30 The similarly strong association reported in this study of a large CKD population supports the notion that FGF-23 may be a particularly relevant risk factor for cardiovascular disease in patients.
with CKD. Because presence of CKD raises FGF-23 levels, it is possible that the stronger association of FGF-23 with cardiovascular disease in CKD reflects the effect of exposure to higher FGF-23 levels in this population, but additional mechanisms may contribute. For example, CKD is characterized by decreased renal expression of Klotho, which is the co-receptor that enhances the binding affinity of FGF-23 for FGF receptors in the kidney, where it exerts its classic effect on mineral metabolism. In contrast, the pro-hypertrophic effects of FGF-23 on the myocardium are FGF receptor dependent but occur in the absence of Klotho, which is not expressed by cardiac myocytes. Thus, the adverse effects of elevated FGF-23 on the heart may be exaggerated in CKD because of the combination of high FGF-23 levels and Klotho deficiency, which frees FGF-23 to increase binding to extrarenal FGF receptors, such as in the heart.

In contrast to the analyses of congestive heart failure events, the association between FGF-23 and atherosclerotic events was weaker. Although prior studies reported minimal diurnal variation in FGF-23 and highly stable FGF-23 levels over years in patients with stable kidney function, use of single baseline measurements of FGF-23 may have biased these findings toward the null. However, prior reports of the association between FGF-23 and atherosclerotic events were also conflicting. Similar to the current study, elevated FGF-23 was not associated with incident atherosclerotic events in

### Figure 2

FGF-23 is associated with cardiovascular events across strata of cardiovascular risk factors. HRs ratio (diamonds) and 95% CIs (horizontal bars) of cardiovascular events by levels of FGF-23 in selected subgroups. (A) Atherosclerotic events. (B) Congestive heart failure events. Models are adjusted for age, sex, income, eGFR, urinary albumin-to-creatinine ratio; history of hypertension, hypercholesterolemia, atherosclerotic cardiovascular disease, congestive heart failure, and diabetes; control of BP 140/90 mmHg; hemoglobin A1c; smoking status; body mass index; waist circumference; serum triglycerides; LDL cholesterol; use of angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, β-blockers, statins, and loop diuretics; and the number of prescribed classes of BP medications. History of cardiovascular disease refers to a history of atherosclerotic or congestive heart failure. Anemia is defined as hemoglobin <12 g/dl in women and <13 g/dl in men. Total N does not sum across groups to 3860 because of missing data for individual covariates that were included in the multivariable models.
the Health Professionals Follow-up Study, and previous studies that reported a significant association could not comprehensively adjust for baseline atherosclerotic risk factors that are associated with elevated FGF-23. Thus, the less robust association between atherosclerotic disease and FGF-23 in this study could represent residual confounding by incomplete ascertainment of the severity or duration of exposure to traditional atherosclerotic risk factors. Alternatively, the lower number of atherosclerotic events combined with our inability to ascertain out-of-hospital sudden cardiac death may have reduced the power of these analyses to detect a true association between FGF-23 and atherosclerotic events. In the absence of direct effects on the arterial vasculature, elevated FGF-23 levels could plausibly relate to atherosclerotic events indirectly through its associations with inflammation or Klotho deficiency, which promotes arterial calcification.

Interestingly, the association between FGF-23 and atherosclerotic events attenuated somewhat when we restricted the analysis to definite events. Although this could be due to fewer total events with a reduction in power, a less robust biologic relationship between FGF-23 and atherosclerotic events is also possible. According to the adjudication criteria, modestly elevated troponin levels without diagnostic electrocardiographic changes could have been classified as a possible or probable, but not a definite, myocardial infarction. Recent studies demonstrate that acute decompensated heart failure often manifests elevated cardiac troponins, possibly as a consequence of increased myocardial wall stress, and that low-grade troponin elevation strongly predicts incident heart failure. Low-grade troponin elevation is especially common among ambulatory patients with CKD, in whom it is also associated with left ventricular hypertrophy and elevated FGF-23 levels. The weaker association of FGF-23 with definite atherosclerotic events could have resulted from misclassification of low-grade troponin elevations as possible or probable atherosclerotic events when they were actually due to cardiac remodeling. Thus, certain participants’ low-grade troponin elevations may have been an early manifestation...
of their predisposition to congestive heart failure rather than coronary artery disease events.

The excess burden of congestive heart failure in CKD has been assumed to result from the high prevalence of atherosclerotic disease risk factors, anemia, and left ventricular pressure and volume overload due to hypertension and impaired sodium excretion that accompanies reduced GFR. In our study, FGF-23 was strongly associated with congestive heart failure independent of both estimated and measured GFR, and even among those without traditional and CKD-specific risk factors for cardiovascular disease, or those in whom these risk factors were well controlled. These findings suggest that FGF-23 could represent a novel mechanism of congestive heart failure that mediates at least a portion of excess cardiovascular disease risk attributable to CKD. Interventional studies are needed to determine whether reducing FGF-23 levels will prevent cardiovascular events in patients with CKD.

CONCISE METHODS

Study Design and Population
The CRIC [Clinical Research in CKD] study is a racially and ethnically diverse, multicenter prospective cohort study that aims to identify risk factors for cardiovascular disease and progression of CKD. The CRIC study enrolled 3939 adults aged 21–74 years with CKD stages 2–4 at 13 centers in the United States between 2003 and 2008. At enrollment, participants had an eGFR of 20–70 ml/min per 1.73 m². Major exclusion criteria included institutionalization, inability to provide informed consent, pregnancy, polycystic kidney disease, previous treatment with dialysis for >1 month, and New York Heart Association class 3–4 heart failure. Participants underwent annual study visits and biannual follow-up by telephone. The final study population for this analysis consisted of 3860 participants after exclusion of 79 with inadequate plasma samples for FGF-23 measurement or lack of follow-up for cardiovascular events. The protocol was approved by the human research committees at each participating center, and all participants provided written informed consent.

Table 3. Risk of incident atherosclerotic and congestive heart failure events among participants without a history of congestive heart failure (n=3487) or atherosclerotic disease (n=2681) by baseline levels of FGF-23

<table>
<thead>
<tr>
<th>Variable</th>
<th>FGF-23</th>
<th>&lt;96.0 RU/ml</th>
<th>96.0–145.4 RU/ml</th>
<th>145.5–238.9 RU/ml</th>
<th>≥239.0 RU/ml</th>
<th>Continuous per Doubling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerotic events</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events (n)</td>
<td></td>
<td>25</td>
<td>22</td>
<td>32</td>
<td>37</td>
<td>–</td>
</tr>
<tr>
<td>Incidence per 1000 person-years</td>
<td>8.4</td>
<td>8.6</td>
<td>14.6</td>
<td>20.4</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjustedab</td>
<td>Reference</td>
<td>1.03 (0.64 to 1.66)</td>
<td>1.73 (1.04 to 2.89)</td>
<td>2.42 (1.76 to 3.34)</td>
<td>1.34 (1.22 to 1.47)</td>
<td></td>
</tr>
<tr>
<td>Adjusted for demographic</td>
<td>Reference</td>
<td>0.93 (0.53 to 1.62)</td>
<td>1.39 (0.67 to 2.89)</td>
<td>2.01 (1.12 to 3.63)</td>
<td>1.27 (1.05 to 1.54)</td>
<td></td>
</tr>
<tr>
<td>variables/kidney function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for traditional risk factors</td>
<td>Reference</td>
<td>0.92 (0.51 to 1.64)</td>
<td>1.23 (0.63 to 2.41)</td>
<td>1.66 (0.82 to 3.38)</td>
<td>1.24 (0.96 to 1.60)</td>
<td></td>
</tr>
<tr>
<td>Adjusted for nontraditional risk factorsa</td>
<td>Reference</td>
<td>0.88 (0.47 to 1.65)</td>
<td>1.26 (0.58 to 2.73)</td>
<td>1.69 (0.68 to 4.18)</td>
<td>1.25 (0.92 to 1.69)</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure hospitalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Events (n)</td>
<td></td>
<td>25</td>
<td>31</td>
<td>70</td>
<td>104</td>
<td>–</td>
</tr>
<tr>
<td>Incidence per 1000 person-years</td>
<td>6.8</td>
<td>9.3</td>
<td>23.6</td>
<td>43.5</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjustedab</td>
<td>Reference</td>
<td>1.37 (0.85 to 2.21)</td>
<td>3.48 (2.16 to 5.59)</td>
<td>6.40 (4.37 to 9.38)</td>
<td>1.74 (1.58 to 1.92)</td>
<td></td>
</tr>
<tr>
<td>Adjusted for demographic</td>
<td>Reference</td>
<td>1.01 (0.62 to 1.65)</td>
<td>1.83 (1.00 to 3.33)</td>
<td>2.94 (2.01 to 4.30)</td>
<td>1.50 (1.36 to 1.66)</td>
<td></td>
</tr>
<tr>
<td>variables/kidney function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjusted for traditional risk factors</td>
<td>Reference</td>
<td>1.09 (0.60 to 1.97)</td>
<td>1.73 (1.04 to 2.86)</td>
<td>2.36 (1.63 to 3.41)</td>
<td>1.47 (1.27 to 1.69)</td>
<td></td>
</tr>
<tr>
<td>Adjusted for nontraditional risk factorsa</td>
<td>Reference</td>
<td>1.09 (0.63 to 1.87)</td>
<td>1.66 (1.00 to 2.75)</td>
<td>2.16 (1.49 to 3.13)</td>
<td>1.41 (1.21 to 1.64)</td>
<td></td>
</tr>
</tbody>
</table>

aRisk per 1-unit change in log2(FGF-23).
bAll models are clustered by clinical center.
cAdjusted for age, sex, race/ethnicity, income, eGFR, and urine albumin-to-creatinine ratio.
dAdjusted for covariates in the demographic and kidney function adjusted model, plus history of hypertension, hypercholesterolemia, history of atherosclerotic cardiovascular disease; history of congestive heart failure; diabetes; control of BP to <140/90 mmHg; hemoglobin A1c; smoking status; body mass index; waist circumference; serum triglycerides; LDL cholesterol; use of antplatelet medications, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, β-blockers, statins, and loop diuretics; and the number of prescribed classes of BP medications.
eAdjusted for covariates in the traditional risk factor– and medication-adjusted model, plus serum phosphate, calcium, albumin, C-reactive protein, PTH, and hemoglobin.

Data Collection
The CRIC study’s central laboratory measured baseline plasma C-terminal FGF-23 (ImmunoCytics, San Clemente, CA) in duplicate with intraassay coefficient of variation <10%. Demographic and clinical information were obtained at the baseline visit by questionnaires, interviews, and physical examination. Diabetes was defined by a fasting glucose≥126 mg/dl or use of insulin or oral hypoglycemic medications; hypertension by a systolic BP≥140 mmHg, diastolic
BP ≥90 mmHg, or use of antihypertensive medications; and hypercholesterolemia by a total serum cholesterol 200 mg/dl or use of cholesterol-lowering medications. History of cardiovascular disease was established by a self-reported history of congestive heart failure, myocardial infarction, coronary revascularization, cerebrovascular accident, peripheral artery revascularization, or amputation. Base-line laboratory covariates, including serum phosphate, calcium, albumin, total intact PTH (Scantibodies, Santee, CA), hemoglobin, high-sensitivity C-reactive protein, plasma NT-proBNP (Roche Diagnostics, Indianapolis, IN), and fasting lipid measures, were determined in a central laboratory. GFR was estimated from serum creatinine using the CKD-Epidemiology Collaboration equation and was directly measured as the clearance of 125I-iothalamate in a subset of 1414 participants. Body composition was assessed by body mass index, waist circumference, and bioelectrical impedance. Two-dimensional transthoracic echocardiography was performed within 2 years of study entry in 3323 participants (86%) at a median of 378 days from study baseline (interquartile range, 344–419 days). Left ventricular mass was indexed to height2.7, and ejection fraction was measured as previously described. Left ventricular hypertrophy was defined as a left ventricular mass index ≥50 g/m2.7 in men or ≥47 g/m2.7 in women.

Table 4. Adjusted HRs (95% CIs) of atherosclerotic event and congestive heart failure hospitalization by levels of FGF-23 in sensitivity analyses

<table>
<thead>
<tr>
<th>Model</th>
<th>Events (n)</th>
<th>FGF-23</th>
<th>&lt;96.0 RU/ml</th>
<th>96.0–145.4 RU/ml</th>
<th>145.5–238.9 RU/ml</th>
<th>≥239.0 RU/ml</th>
<th>Continuous per Doublingb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerotic event</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary modelc</td>
<td>261</td>
<td>Reference</td>
<td>1.32 (0.90 to 1.92)</td>
<td>1.47 (1.14 to 1.90)</td>
<td>1.76 (1.20 to 2.59)</td>
<td>1.24 (1.09 to 1.40)</td>
<td></td>
</tr>
<tr>
<td>Definite event</td>
<td>215</td>
<td>Reference</td>
<td>1.22 (0.89 to 1.66)</td>
<td>1.31 (0.98 to 1.76)</td>
<td>1.42 (0.90 to 2.23)</td>
<td>1.19 (1.01 to 1.41)</td>
<td></td>
</tr>
<tr>
<td>Event or death</td>
<td>468</td>
<td>Reference</td>
<td>1.28 (0.94 to 1.73)</td>
<td>1.61 (1.34 to 1.93)</td>
<td>2.36 (1.90 to 2.94)</td>
<td>1.38 (1.30 to 1.46)</td>
<td></td>
</tr>
<tr>
<td>Including post-ESRD events</td>
<td>306</td>
<td>Reference</td>
<td>1.41 (0.90 to 2.22)</td>
<td>1.58 (1.22 to 2.04)</td>
<td>1.89 (1.28 to 2.80)</td>
<td>1.24 (1.15 to 1.34)</td>
<td></td>
</tr>
<tr>
<td>CHF as time-varying covariate</td>
<td>287</td>
<td>Reference</td>
<td>1.31 (0.90 to 1.92)</td>
<td>1.46 (1.13 to 1.89)</td>
<td>1.74 (1.18 to 2.57)</td>
<td>1.23 (1.08 to 1.39)</td>
<td></td>
</tr>
<tr>
<td>Multiple imputation of missing covariates</td>
<td>287</td>
<td>Reference</td>
<td>1.12 (0.84 to 1.49)</td>
<td>1.26 (0.96 to 1.64)</td>
<td>1.59 (1.17 to 2.15)</td>
<td>1.21 (1.07 to 1.36)</td>
<td></td>
</tr>
<tr>
<td>Congestive heart failure hospitalization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary modelc</td>
<td>321</td>
<td>Reference</td>
<td>1.36 (0.78 to 2.38)</td>
<td>1.74 (1.06 to 2.86)</td>
<td>2.98 (1.97 to 4.52)</td>
<td>1.45 (1.28 to 1.65)</td>
<td></td>
</tr>
<tr>
<td>Definite event</td>
<td>235</td>
<td>Reference</td>
<td>1.40 (0.65 to 3.01)</td>
<td>1.85 (0.93 to 3.67)</td>
<td>3.30 (1.75 to 6.21)</td>
<td>1.50 (1.29 to 1.74)</td>
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<tr>
<td>Event or death</td>
<td>518</td>
<td>Reference</td>
<td>1.28 (1.07 to 1.53)</td>
<td>1.70 (1.19 to 2.42)</td>
<td>2.97 (2.59 to 3.40)</td>
<td>1.49 (1.40 to 1.58)</td>
<td></td>
</tr>
<tr>
<td>Including post-ESRD events</td>
<td>342</td>
<td>Reference</td>
<td>1.49 (0.87 to 2.56)</td>
<td>1.83 (1.12 to 2.99)</td>
<td>2.88 (1.80 to 4.62)</td>
<td>1.40 (1.23 to 1.60)</td>
<td></td>
</tr>
<tr>
<td>ASCVD as time-varying covariate</td>
<td>321</td>
<td>Reference</td>
<td>1.34 (0.76 to 2.36)</td>
<td>1.72 (1.04 to 2.84)</td>
<td>2.96 (1.95 to 4.48)</td>
<td>1.45 (1.28 to 1.64)</td>
<td></td>
</tr>
<tr>
<td>Multiple imputation of missing covariates</td>
<td>360</td>
<td>Reference</td>
<td>1.17 (0.68 to 2.00)</td>
<td>1.65 (1.03 to 2.65)</td>
<td>2.73 (1.89 to 3.95)</td>
<td>1.43 (1.30 to 1.58)</td>
<td></td>
</tr>
</tbody>
</table>

CHF, congestive heart failure; ASCVD, atherosclerotic cardiovascular disease.

The number of events represents events in adjusted models and may be lower than the overall number of events in the study population because of model-wise deletion.

Risk per 1-unit change in log₂(FGF-23).

Adjusted for age; sex; race/ethnicity; household income; eGFR; urine albumin-to-creatinine ratio; history of hypertension; hypercholesterolemia; history of atherosclerotic cardiovascular disease; history of congestive heart failure; diabetes; control of BP to <140/90 mmHg; hemoglobin A1c; smoking status; body mass index; waist circumference; serum triglycerides; LDL cholesterol; use of antiplatelet medications, angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, β-blockers, statins, and loop diuretics; and the number of classes of BP medications used. Clustered by center.

Figure 3. FGF-23 is more strongly associated with congestive heart failure compared to atherosclerotic events in overall, incident and definite event analyses. HRs (squares) and 95% CIs (vertical bars) for overall, incident, and definite atherosclerotic (A) and congestive heart failure events (B) by quartiles (Q) of FGF-23. Models are adjusted for demographic variables, kidney function, traditional cardiovascular risk factors, and medications. Q1 served as the reference group for all analyses.
Outcomes
The two primary adjudicated outcomes were first hospitalization for congestive heart failure and first atherosclerotic event, including hospitalization for myocardial infarction, cerebrovascular accident, or peripheral vascular disease, which occurred between enrollment and June 30, 2009. In addition to censoring for death, we censored our primary analyses for onset of ESRD because of difficulty in distinguishing congestive heart failure from volume overload and potential differences in the pathogenesis of cardiovascular events among patients undergoing dialysis.

Adjudication
Hospitalizations were self-reported by participants every 6 months by telephone or at in-person follow-up visits. Study personnel identified possible cardiovascular events by reviewing hospital billing codes. Two independent reviewers adjudicated cardiovascular events using hospital records and classified them as probable or definite, except for myocardial infarction, which was also classified as possible. Criteria for congestive heart failure included a combination of symptoms (dyspnea on exertion, paroxysmal nocturnal dyspnea, and orthopnea) accompanied by consistent findings on chest radiography (pulmonary edema, congestion) or physical examination (documentation of two or more of the following: pulmonary rales, S3 gallop, jugular venous distention $>5$ cm, peripheral edema). Criteria for myocardial infarction included a combination of chest pain, electrocardiography abnormalities, and elevated cardiac biomarkers. Peripheral vascular disease procedures were ascertained using International Classifications, Ninth Revision, codes. Two neurologists adjudicated cerebrovascular accidents. Further details of event adjudication are provided in the Supplemental Material.

Statistical Analyses
We calculated incidence rates of atherosclerotic and congestive heart failure events by FGF-23 levels modeled as a log-transformed continuous variable and in quartiles. Using Cox models, we separately evaluated time to each event type in the full study population and incident events in the population without a history of congestive heart failure ($n=3487$) or atherosclerotic disease ($n=2681$). Because initial analyses demonstrated linear relationships between log FGF-23 and log hazard of events, risks were reported on a linear continuous scale per unit of doubling of FGF-23. To further aid in interpretation of the results, FGF-23 was also analyzed in quartiles as we and others have done previously. To minimize the possibility that an ongoing subclinical cardiovascular disease process at baseline could have raised FGF-23 levels and hence inflated estimated risks of risk attributable to baseline FGF-23, we performed additional analyses that exclusively considered incident events that occurred $\geq 1$ year after FGF-23 was measured.

We adjusted Cox models sequentially for demographic variables (age, sex, race, ethnicity, and income), kidney function ($\text{eGFR}$ and urinary albumin-to-creatinine ratio), traditional cardiovascular risk factors (history of hypertension, hypercholesterolemia, atherosclerotic disease, congestive heart failure, and diabetes; control of BP $<140/90$ mmHg; hemoglobin $A1c$; smoking [never, former, or current]; body mass index; waist circumference; serum triglycerides; LDL cholesterol), use of cardiovascular medications (antiplatelet agents, angiotensin-receptor blockers or angiotensin-converting enzyme inhibitors, $\beta$-blockers, statins, loop diuretics), and the number of prescribed classes of antihypertensive drugs ($\leq 1, 2, 3, \text{ or } \geq 4$). We further adjusted for nontraditional risk factors that have been linked to cardiovascular disease in CKD populations, including serum phosphate, calcium, albumin, C-reactive protein, PTH, and hemoglobin. Secondary analyses separately adjusted for NT-proBNP, a biomarker of myocardial pressure and volume overload, and left ventricular mass index, a factor that may mediate an association between FGF-23 and cardiovascular events. All analyses were clustered by study center to correct standard errors for correlation structure. Because adjustment or stratification for study center did not qualitatively change results, these were not included in the final models.

To test whether elevated FGF-23 was more strongly associated with congestive heart failure than with atherosclerotic events, we included both outcomes in a single model allowing distinct baseline hazard functions and distinct covariate effects on each outcome. We compared the effects of FGF-23 on cause-specific hazards of each outcome in this model using the Wald chi-square test. The proportional hazards assumption was confirmed for all models using Schoenfeld residuals and log-log plots.

Subgroup Analyses
We investigated the association between FGF-23 and cardiovascular events in analyses stratified by covariates that may contribute to cardiovascular disease, including levels of kidney function ($\text{eGFR} \geq 45, 30-44$ and $<30$ ml/min per $1.73$ m$^2$) and proteinuria (urinary albumin-to-creatinine ratio $<300$ mg/g versus $\geq 300$ mg/g), history of cardiovascular disease, diabetes, BP control ($<140/90$ mmHg), anemia (hemoglobin $<13$ g/dl in men and $<12$ g/dl in women), 24-hour urine sodium ($<3000$ mg/d versus $\geq 3000$ mg/d), income ($<$120,000/year versus $\geq$120,000/year), categories of fat-free mass determined by bioelectrical impedance analysis as a surrogate of edema ($<60$ kg versus $\geq 60$ kg), left ventricular ejection fraction ($<50\%$ versus $\geq 50\%$), and presence of left ventricular hypertrophy.

Sensitivity Analyses
We performed the following sensitivity analyses: (1) analyses restricted to events meeting more stringent adjudication criteria and classified as “definite”; (2) analyses of the composite outcome of each cardiovascular event type with death; (3) analyses that included events that occurred after onset of ESRD; (4) analyses that incorporated atherosclerotic or congestive heart failure events that occurred after study enrollment as time-dependent covariates such that medical history was updated at the time of a new diagnosis; and (5) analyses in which missing covariates were multiply imputed using chained equations ($n=5$ cycles). In additional sensitivity analyses, we adjusted for $^{125}$I-iothalamate GFR in lieu of eGFR and for 25-hydroxyvitamin D levels in the subset of participants with these results ($n=1369$). Analyses were performed using Stata 11.1 (Stata Corp., College Station, TX), SAS 9.2 (SAS Institute, Inc., Cary, NC), and R, version 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria).
ACKNOWLEDGMENTS

Funding for the CRIC study was obtained under a cooperative agreement from the National Institute of Diabetes and Digestive and Kidney Diseases (U01DK060990, U01DK060984, U01DK061022, U01DK061021, U01DK061028, U01DK060980, U01DK060963, and U01DK060902). In addition, this work was supported in part by the University of Pennsylvania CTRC CTSA UL1 RR-024139 from the National Center for Advancing Translational Sciences component of the National Institutes of Health (NIH) and NIH roadmap for Medical Research, Michigan Institute for Clinical and Health Research UL1RR024986, University of Illinois at Chicago CTSAUL1RR029879, Tulane University Translational Research in Hypertension and Renal Biology P30GM103337, and Kaiser NIH/NCRR UCSF-CTSI UL1 RR-024134, Johns Hopkins University U11 RR-025005, University of Maryland GCRC M01 RR-16500, Clinical and Translational Science Collaborative of Cleveland, UL1TR000439 from the National Institute of Diabetes and Digestive and Kidney Diseases (U01DK081374, R01DK094796, K24DK093723, and U54TR000255, all from the NIH.

CRIC Study Investigators additionally include Lawrence J. Appel and Raymond R. Townsend

DISCLOSURES

T.J. has served as a consultant and received honoraria from Shire and Genzyme. M.W. has served as a consultant for or received honoraria from Abbott Laboratories, Amgen, Genzyme, Kai, and Lutipold.

REFERENCES


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CLINICAL EPIDEMIOLOGY

Fibroblast Growth Factor-23 and CVD

359


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This article contains supplemental material online at http://jasn.asnjournals.org/cgi/lookup/suppl/doi:10.1681/ASN.2013050465/-/DCSupplemental.
Fibroblast Growth Factor 23 and Cardiovascular Events in the Chronic Renal Insufficiency Cohort (CRIC) Study

Adjudication Supplement

Potential cardiovascular events were identified based on administrative hospital record review. Hospitalizations containing the following ICD-9/CPT codes were adjudicated:

<table>
<thead>
<tr>
<th>Event type</th>
<th>ICD-9 Codes</th>
<th>CPT Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrovascular Accident</td>
<td>430, 431, 432, 433, 434, 435, 436, 38.10</td>
<td>33572</td>
</tr>
<tr>
<td>Congestive Heart Failure</td>
<td>398.91, 402.01, 402.11, 402.91, 425, 428, 429, 514, 518.4</td>
<td>92986, 92987, 92990</td>
</tr>
</tbody>
</table>

During adjudication, hospital records were reviewed by two independent providers. In the case of cerebrovascular accident, both providers were neurologists. Disagreement between reviewers was resolved by consensus. Reviewers were blind to study data during adjudication of hospital records. The following worksheets served as a guide for the adjudication committee in determining event status. For adjudication of myocardial infarction, electrocardiograms (ECGs) were read by a central ECG Reading Center at Wake Forest University. ECGs during the index hospitalization were compared to the closest ECG prior to the admission date, such as those obtained at a prior CRIC study visit or at a previous hospitalization. Standard scoring criteria, Minnesota Code, were performed and results provided to adjudicators.
Myocardial Infarction Review Form

Tracking Number: ______ ______

Guidance for defining abnormality threshold for the lab using question #1. Please provide a response for each question:

1. Select the Troponin I threshold: (check only one)
   1. No Troponin I values available (got to Q#3)  
   2. Cannot determine the type of Troponin (go to Q#4)  
   3. 0.14 - No ULN available (go to Q#2)  
   4. 0.14 – Lab provides only a normal range and no indeterminate range and the ULN is ≥ 0.14. (go to Q#2)  
   5. If the lab provides only a normal range and no indeterminate range and the ULN is < 0.14.  
      Specify: 2x ULN = ___.___ ___ (go to Q#2)  
   6. If the lab provides an indeterminate category in addition to a normal range.  
      Specify: 2x ULN = ___.___ ___ (go to Q#2)

2. Select the Troponin I Determination: (check only one)
   1. Two or more values (~6 hours apart) ≤ ULN and < threshold = normal.  
   2. Any value ≥ threshold = abnormal  
   3. Only one value available < ULN = normal  
   4. One or more value and all > ULN and < threshold = equivocal.

3. Select the Troponin T Determination: (check only one)
   1. Any Troponin T ≤ 0.03 = normal  
   2. Any Troponin T > 0.03 = abnormal  
   3. No Troponin T

4. Select the Indeterminate Troponin: (check only one)
   2. Any Troponin ≥ 2x ULN = abnormal.  
   3. Any Troponin >ULN < 2x ULN = equivocal.  
   99. Not Applicable
5. Was there evidence of muscle trauma or surgery?  1 Yes  0 No

6. Guidance for interpreting CK and LDH results: *(check only one and go to Q#7)*

<table>
<thead>
<tr>
<th>MB-ULN Available, CK-MB Measured</th>
<th>No Muscle Trauma</th>
<th>Muscle Trauma</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB &gt; 99th percentile of ULN or if not available, &gt; 2x ULN</td>
<td>1 Abnormal</td>
<td>2 Equivocal</td>
</tr>
<tr>
<td>CK-MB &lt; 99th percentile of ULN or if not available, ≤ 2x ULN</td>
<td>3 Normal</td>
<td>4 Normal</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No MB ULN Available, CK-MB Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MB ≥ 10% CKTOT</td>
</tr>
<tr>
<td>CK-MB ≥ 5% and &lt;10% CKTOT</td>
</tr>
<tr>
<td>CK-MB &lt;5% CKTOT</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No CK-MB measured, CKTOT and LDH measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKTOT ≥ 2x ULN and LDH ≥ 2x ULN</td>
</tr>
<tr>
<td>[(CKTOT&gt;ULN and &lt; 2x ULN) AND (LDH&gt;ULN and &lt;2x ULN)]</td>
</tr>
<tr>
<td>[CKTOT&gt;2x ULN AND (LDH&lt;2x ULN and &gt; ULN)]</td>
</tr>
<tr>
<td>[(CKTOT&lt;2x ULN and &gt; ULN) AND (LDH&gt;2x ULN)]</td>
</tr>
<tr>
<td>[CKTOT&lt;ULN AND (LDH&lt;2x ULN and &gt;ULN)]</td>
</tr>
<tr>
<td>[(CKTOT&lt;2x ULN and &gt;ULN) AND LDH&lt;ULN]</td>
</tr>
<tr>
<td>CKTOT&lt;ULN AND LDH&lt;ULN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No CK-MB measured, No LDH, CKTOT measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKTOT ≥ 2x ULN</td>
</tr>
<tr>
<td>CKTOT &lt;2x ULN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No CK-MB measured, No CKTOT, LDH measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH ≥ 2x ULN</td>
</tr>
<tr>
<td>LDH &lt;2x ULN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No CK-MB measured, No CKTOT or LDH measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>33 Missing</td>
</tr>
</tbody>
</table>
7. Was cardiac pain present? 1 Yes (go to Q#8) 0 No (go to Q#9) 88 Uncertain (go to Q#8)

8. Guidance for myocardial infarction determination based on ECG data, cardiac biomarkers and cardiac pain documentation: (check only one and go to Q#10)

<table>
<thead>
<tr>
<th>ECG Pattern</th>
<th>Abnormal</th>
<th>Equivocal</th>
<th>Missing</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evolving Diagnostic ECG (Evolution of major Q-wave)</td>
<td>1 Definite MI</td>
<td>2 Definite MI</td>
<td>3 Definite MI</td>
<td>4 Definite MI</td>
</tr>
<tr>
<td>Positive ECG (Evolution of ST Elevation with or without Q-wave OR new LBBB)</td>
<td>5 Definite MI</td>
<td>6 Probable MI</td>
<td>7 Probable MI</td>
<td>8 No MI</td>
</tr>
<tr>
<td>Non-Specific ECG (Evolution of ST-T Depression/inversion alone OR evolution of minor Q-waves alone)</td>
<td>9 Definite MI</td>
<td>10 Possible MI</td>
<td>11 No MI</td>
<td>12 No MI</td>
</tr>
<tr>
<td>ECG Negative for Ischemia Normal, Absent, Uncodable, or Other</td>
<td>13 Definite MI</td>
<td>14 Possible MI</td>
<td>15 No MI</td>
<td>16 No MI</td>
</tr>
</tbody>
</table>

9. Guidance for myocardial infarction determination based on ECG data, cardiac biomarkers and absence of cardiac pain documentation: (check only one and go to Q#10)

<table>
<thead>
<tr>
<th>ECG Pattern</th>
<th>Abnormal</th>
<th>Equivocal</th>
<th>Missing</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evolving Diagnostic ECG (Evolution of major Q-wave)</td>
<td>1 Definite MI</td>
<td>2 Definite MI</td>
<td>3 Definite MI</td>
<td>4 Definite MI</td>
</tr>
<tr>
<td>Positive ECG* (Evolution of ST Elevation with or without Q-wave OR new LBBB)</td>
<td>5 Definite MI</td>
<td>6 Probable MI</td>
<td>7 Possible MI</td>
<td>8 No MI</td>
</tr>
<tr>
<td>Non-Specific ECG (Evolution of ST-T Depression/inversion alone OR evolution of minor Q-waves alone)</td>
<td>9 Definite MI</td>
<td>10 Possible MI</td>
<td>11 No MI</td>
<td>12 No MI</td>
</tr>
<tr>
<td>ECG Negative for Ischemia Normal, Absent, Uncodable, or Other</td>
<td>13 Definite MI</td>
<td>14 No MI</td>
<td>15 No MI</td>
<td>16 No MI</td>
</tr>
</tbody>
</table>
*10. What is your global impression of the final outcome using all available information in this medical record?

1  No MI
2  Possible MI
3  Probable MI
4  Definite MI
88  Can’t Determine

*11. What was the participant’s vital status at the discharge?

1  Alive
2  Dead
88  Unknown

*These responses must be concordant with the responses of the 2nd reviewer.

Note: If the participant’s discharge status is dead, please complete a death review form.

12. Did the patient undergo coronary revascularization?

1  Yes
0  No

a. If “Yes”: (Check only one answer)

1  Coronary angioplasty (including angioplasty with stenting, antherectomy)
2  Coronary artery bypass graft
98  Other (Specify): ____________________________

Comments:

[add comments here]
EVENT CLASSIFICATION FORM

1. Tracking number: ___ ___ ___
2. Date events reviewed: ___ / ___ / ___ (mm/dd/yyyy)

Please base your review on the documentation provided from this investigation. Your determination must be based on the criteria for CRIC outcome events defined in the “Outcomes MOP”.

3. Was this a cerebrovascular event? [ ] Yes [ ] No (Go to Q#5)

   3a. According to the CRIC outcome definitions, was this event (select only one)

   [ ] Intraparenchymal hemorrhage (IPH)?
   [ ] Subarachnoid hemorrhage (SAH)?
   [ ] Large-vessel cerebral infarction (LVCI)?
   [ ] Cardioembolic cerebral infarction (CCI)?
   [ ] Small-vessel cerebral infarction (SVCI)?
   [ ] Cerebral infarction not otherwise specified (CINOS)?

   3a1. Please categorize probability for the cerebrovascular event? [ ] Definite [ ] Probable [ ] Improbable [ ] Can’t Determine

4. Was there a second cerebrovascular event during this hospitalization? [ ] Yes [ ] No (Go to Q#5)

   If yes:

   4a. According to the CRIC outcome definitions, was this event (select only one)

   [ ] Intraparenchymal hemorrhage (IPH)?
   [ ] Subarachnoid hemorrhage (SAH)?
   [ ] Large-vessel cerebral infarction (LVCI)?
   [ ] Cardioembolic cerebral infarction (CCI)?
   [ ] Small-vessel cerebral infarction (SVCI)?
   [ ] Cerebral infarction not otherwise specified (CINOS)?

   4a1. Please categorize probability for the cerebrovascular event? [ ] Definite [ ] Probable [ ] Improbable [ ] Can’t Determine
EVENT CLASSIFICATION FORM

1. Tracking number: 
2. Date events reviewed: [mm/dd/yyyy]

5. Was the patient hospitalized?  
   If yes:  
   5a. What was the patient's vital status at discharge?  
      (select only one)  
      1. Alive (STOP)  
      2. Dead (STOP and complete a Death Review form)  
      88. Unknown (STOP)  

Comments:  
[Type comments here...]

Participant ID:  
Participant Initials:  
Clinical Center:  
Site:  
CRF Date: / /  
Visit Number:  
Reviewer ID:  

V4.0.20100607  
CVA_REVIEW
Figure 1. Flow Chart for Initial Stroke Evaluation

- **Stroke abstraction performed**
- **Autopsy or surgery proven subarachnoid hemorrhage**
  - Yes: **SAH**
  - No: **Sudden severe headache plus:**
    - 1. Evidence of subarachnoid blood on head CT.
    - OR
    - 2. A bloody or xanthochromic non-traumatic LP
  - Yes: **IPH**
  - No: **Autopsy or surgery proven intraparenchymal hemorrhage**
  - Yes: **IPH**
  - No: **Sudden severe headache plus evidence of intraparenchymal hematoma without subarachnoid hemorrhage seen on head CT or MRI.**
  - Yes: **IPH**
  - No: **Proceed to cerebral infarction determination**
Figure 2. Flow Chart for Cerebral Infarction Determination.

Stroke abstraction performed and the event is not an IPH or SAH

1 major or 2 minor stroke symptoms are present for >24 hours or until death

Yes

Improbable or No Stroke

No

1. Autopsy proven infarction, OR
2. CT or MRI demonstrate infarction

Yes

Definite stroke
Requires further subtyping (see below)

No

Probable Stroke
Requires further subtyping (see below)

Yes

Abrupt onset of clinical symptoms within a single

Yes

Autopsy, head CT, or MRI performed

No

No
Figure 3. Flow Chart for Cerebral Infarction Subtype Determination

Definite or probable stroke

- Autopsy or imaging show an infarction that is specifically described as a "lacune" OR "lacunar" OR as less than 1.5cm in size

  - Yes
    - There is evidence of a large vessel mechanism ipsilateral to the stroke or a cardioembolic mechanism
      - Yes
        - CINOS
      - No
        - SVC

  - No
    - There is evidence of a cardioembolic mechanism
      - Yes
        - CINOS
      - No
        - LVCI

- CINOS

- There is evidence of a large vessel mechanism ipsilateral to the stroke
  - Yes
    - CINOS
  - No
    - CCI
**IN-PATIENT HEART FAILURE EVENT REVIEWER FORM**

Instructions to Reviewers: *The data collected on this form should focus on admission and the first 48 hours of hospitalization.*

1. Tracking number:  

2. Is there documentation of clinical symptoms (dyspnea on exertion or rest, paroxysmal nocturnal dyspnea, and/or orthopnea)?
   - Yes
   - No
   - Uncertain

3. Do notes or radiology reports document radiographic evidence of pulmonary edema or pulmonary congestion?
   - Yes
   - Not Documented
   - Uncertain

4. Physical Exam findings to include at least two of the following:  
   *Provide one response to each item*
   - Inspiratory crackles ("rales") involving at least 1/3 of the lower lung fields
     - Yes
     - No
     - Not Documented
     - Uncertain
   - S3 gallop on auscultation
     - Yes
     - No
     - Not Documented
     - Uncertain
   - Jugular venous distension > 5cm
     - Yes
     - No
     - Not Documented
     - Uncertain
   - Peripheral edema
     - Yes
     - No
     - Not Documented
     - Uncertain
### In-Patient Heart Failure Event Reviewer Form

#### 5. Invasive hemodynamic or echocardiogram evidence including any of the following: **Check all that apply**

<table>
<thead>
<tr>
<th>Decision</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a. Pulmonary capillary wedge pressure &gt;18 mm Hg</td>
<td>0 No, 1 Yes, 87 Not Documented, 88 Uncertain</td>
</tr>
<tr>
<td>5b. Cardiac index &lt; 2.0 L/min/M²</td>
<td>0 No, 1 Yes, 87 Not Documented, 88 Uncertain</td>
</tr>
<tr>
<td>5c. Left ventricular ejection fraction ≤ 35%</td>
<td>0 No, 1 Yes, 87 Not Documented, 88 Uncertain</td>
</tr>
</tbody>
</table>

#### 6. *How would you characterize this event?*  
- 0 Not Heart Failure  
- 1 Definite Heart Failure  
- 2 Probable Heart Failure

#### 7. *What was the participant’s vital status at discharge?*  
- 0 Dead *(Complete a death review form)*  
- 1 Alive  
- 88 Unknown

*These responses must be concordant with the responses of the 2nd reviewer.*

#### 8. Did the patient undergo coronary revascularization?  
- 0 No  
- 1 Yes

a. **If “Yes”: (Check only one answer)**

- 0 No  
- 1 Coronary angioplasty (including angioplasty with stenting, antherectomy)  
- 2 Coronary artery bypass graft  
- 98 Other (Specify):

#### 9. Was an echocardiogram performed during this hospitalization?  
- 0 No  
- 1 Yes  
- 99 Can’t Determine

a. **If “YES”, what was the ejection fraction?**  

   ___ ___%

- 99 Unavailable
IN-PATIENT HEART FAILURE EVENT REVIEWER FORM

Comments:

See Below for Heart Failure Criteria

Heart Failure Criteria

Hospitalization for clinical symptoms (dyspnea on exertion or rest, paroxysmal nocturnal dyspnea, and/or orthopnea) with at least one of the following objective findings:

1. Radiographic evidence of pulmonary edema or pulmonary congestion

OR

2. Physical Exam findings consistent with CHF to include at least two of the following:
   a) Inspiratory crackles ("rales") involving at least 1/3 of the lower lung fields
   b) S3 gallop on auscultation
   c) Jugular venous distension > 5cm
   d) Peripheral edema.

OR

3. Invasive hemodynamic or echocardiogram evidence of CHF including any of the following:
   a) Pulmonary capillary wedge pressure >18 mm Hg
   b) Cardiac index < 2.0 L/min/M²
   c) Left ventricular ejection fraction ≤ 35%

9a. If there is more than one echo during this hospitalization, please list the ejection fraction of the first echo performed.
Peripheral vascular disease was defined by the presence of the following codes and not further adjudicated:

<table>
<thead>
<tr>
<th>Event type</th>
<th>ICD-9 Codes</th>
<th>CPT Codes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peripheral Vascular Disease</td>
<td>440, 441, 443, 444</td>
<td>24900, 25900, 25927, 26910, 27880, 33322, 33335, 33860, 33870, 35301, 35311, 35321, 35331, 35341, 35351, 35355, 35361, 35363, 35371, 35372, 35381, 35390, 35450, 35452, 35454, 35456, 35458, 35459, 35511, 35516, 35518, 35521, 35531, 35533, 35536, 35541, 35546, 35548, 35549, 35551, 35556, 35558, 35560, 35563, 35565, 35566, 35571, 35582, 35583, 35585, 35587, 35612, 35616, 35621, 35623, 35631, 35636, 35641, 35646, 35650, 35651, 35654, 35656, 35661, 35663, 35665, 35666, 35671, 35700, 35879, 75962, 75964, 75966, 75968, V49.7</td>
</tr>
</tbody>
</table>