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Association of Fibroblast Growth Factor-23 Levels and Angiotensin-Converting Enzyme Inhibition in Chronic Systolic Heart Failure

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ABSTRACT

OBJECTIVES The aim of this study was to evaluate the association of fibroblast growth factor (FGF)-23 with clinical and laboratory findings, the prognostic value of FGF-23, and the relationship between angiotensin-converting enzyme inhibitor (ACEi) therapy, FGF-23 levels, and outcomes in patients with chronic systolic heart failure (HF).

BACKGROUND FGF-23 is a bone-derived hormone regulating mineral metabolism. Higher FGF-23 levels are associated with an increased risk of cardiovascular mortality or HF development. Mechanisms leading to increased FGF-23 and its prognostic value have not been thoroughly studied in HF.

METHODS FGF-23 was measured in 369 patients (mean age 59 ± 11 years, 84% male) with systolic HF. Patients were followed for adverse events (e.g., death, urgent heart transplantation, ventricular assist device implantation).

RESULTS Tricuspid regurgitation severity, chronic kidney disease (CKD), alkaline phosphatase concentrations, inferior vena cava dilation, and absence of ACEi therapy were independently associated with FGF-23. FGF-23 was independently associated with outcomes in patients without CKD (hazard ratio [HR]: 1.43, 95% confidence interval [CI]: 1.14 to 1.78), but not in CKD patients (HR: 1.12, 95% CI: 0.87 to 1.45). In patients without CKD and with FGF-23 in the highest tertile, ACEi therapy was associated with a lower risk of adverse events (HR: 0.42, 95% CI: 0.21 to 0.81), whereas no association was seen in the remaining patients (HR: 1.18, 95% CI: 0.52 to 2.70).

CONCLUSIONS In systolic HF, elevated FGF-23 is an independent predictor of adverse events, particularly in patients with preserved renal function. The association of FGF-23 with adverse events likely reflects early alterations of renal hemodynamics and renin-angiotensin system activation. Increased FGF-23 may identify a subset of HF patients benefiting from ACEi therapy. (J Am Coll Cardiol HF 2015;3:829–39) © 2015 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

ACEi = angiotensin-converting enzyme inhibitor

BNP = B-type natriuretic peptide

CI = confidence interval

CKD = chronic kidney disease

EF = ejection fraction

eGFR = estimated glomerular filtration rate

FGF = fibroblast growth factor

HF = heart failure

HR = hazard ratio

IQR = interquartile range

RU = relative unit

RVD = right ventricular dysfunction

Sm = tissue systolic velocity

TAPSE = tricuspid annular plane systolic excursion

Fibroblast growth factor (FGF)-23 is a bone-derived hormone that primarily regulates renal phosphate handling and vitamin D metabolism (1). Several clinical and experimental studies suggested that higher FGF-23 concentrations are associated with cardiac dysfunction (2), left ventricular hypertrophy (3,4), and poor prognosis (5-7), particularly in patients with chronic kidney disease (CKD) (4,6). In the general population and in patients with stable ischemic heart disease, elevated FGF-23 increases the risk of heart failure (HF) development (8,9). However, only limited data are available on factors associated with FGF-23 level and its prognostic value among the patients with established HF (2,10). An increase in FGF-23 may reflect renal dysfunction, altered hemodynamics, or concomitant bone disease that is also common in patients with established HF (11).

SEE PAGE 840

The biological activity of FGF-23 is mediated by its binding to the canonical fibroblast growth factor receptor and its coreceptor protein Klotho. The Klotho protein exists in a membrane-bound and a soluble form. Soluble Klotho is derived from the extracellular part of membrane-bound Klotho. Whereas membrane-bound Klotho is a coreceptor for FGF-23 and is vital for its phosphaturic effect (12), soluble Klotho was implicated in vascular stiffness (13) and premature aging (14). Renin-angiotensin-aldosterone system activation reduces Klotho expression, which results in a compensatory FGF-23 increase (15). Whereas some authors have suggested that the effect of FGF-23 on the cardiovascular system is Klotho dependent (16) or that increased FGF-23 is just a measure of primary loss of Klotho function (17), others showed that the pathway is independent of Klotho (3,18).

Because FGF-23 increases in the earliest stages of renal impairment (3), even before creatinine and blood urea nitrogen (19), it was suggested to be an early marker of renal injury. In previous studies in patients with stable ischemic heart disease, FGF-23 (9) and other markers of renal dysfunction (20,21) identified patients who derived greater benefit from angiotensin-converting enzyme inhibitor (ACEi) therapy. Whether ACEi therapy in patients with HF and increased FGF-23 is associated with greater clinical benefit has never been tested.

The aims of the present study were to: 1) assess the factors associated with FGF-23 level; 2) assess the prognostic value of FGF-23; 3) determine whether the association between FGF-23 and outcome may be mediated through Klotho; and 4) test the interaction between ACEi therapy, FGF-23 levels, and outcomes among patients with HF.

METHODS

PATIENTS. Criteria for patient enrollment were described previously (22). In brief, the study enrolled patients with chronic (>6 months) moderate to advanced systolic HF (ejection fraction [EF] <50%) electively hospitalized at the Institute for Clinical and Experimental Medicine between November 1, 2007 and August 31, 2011 for consideration of advanced therapies (ventricular assist device implantation, cardiac transplantation, and implantable cardioverter-defibrillator or biventricular pacemaker implantation). Patients with acute HF decompensation, hemodynamic instability, reversible cardiac dysfunction (e.g., planned valve surgery or revascularization, tachycardia-induced cardiomyopathy), need for renal replacement therapy, active malignancy, or chronic infection were excluded. Medications at the time of enrollment were recorded. Definition of a low ACEi dose for different ACEi therapy used in this study is provided in Online Table 1. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Institute for Clinical and Experimental Medicine. Informed consent was obtained from all patients.

LABORATORY ANALYSES. Morning fasting blood samples were collected in ethylenediamine tetraacetic acid-anticoagulated and serum separator tubes and centrifuged. Serum and plasma aliquots were stored frozen at −80°C. Plasma samples were shipped to Boston, Massachusetts, on dry ice for FGF-23 and Klotho testing. FGF-23 levels were measured using the C-terminal human enzyme-linked immunosorbent assay (Immutopics, San Clemente, California). The interassay coefficients of variation were 11.8% at 29.3 relative units (RU)/ml and 5.6% at 285 RU/ml. Klotho concentrations were determined using the α-Klotho (soluble) solid-phase sandwich enzyme-linked immunosorbent assay (IBL America, Minneapolis, Minnesota). The interassay coefficients of variation were 8.0% at 773 pg/ml and 7.4% at 1,582 pg/ml.

All other biochemical analyses were performed in the Clinical Biochemistry Laboratory at the Institute for Clinical and Experimental Medicine. Plasma sodium, hepatic enzymes, glucose, and creatinine were measured in serum using the Architect

ci1600 analyzer (Abbott Laboratories, Chicago, Illinois). Estimated glomerular filtration rate (eGFR) was determined by the simplified CKD-EPI creatinine equation (23). CKD was defined as eGFR <60 ml/min/1.73 m^2. The B-type natriuretic peptide (BNP) concentrations were measured in serum using the Architect BNP microparticle immunoassay (Abbott Laboratories).

ECHOCARDIOGRAPHIC EXAMINATION. Echocardiographic examination was performed using an ultrasound system Vivid 7 (General Electric Healthcare, Wauwatosa, Wisconsin). Mitral and tricuspid regurgitations were assessed semiquantitatively and expressed in 3 grades (absent, insignificant, and significant). Right ventricular systolic pressure was estimated from the tricuspid regurgitation velocity (available in 75% of patients) and right atrial pressure estimate was based on the inferior vena cava diameter. Semiquantitative classification of right ventricular dysfunction (RVD) was derived from the apical 4-chamber view using tricuspid annular systolic excursion (M-mode tricuspid annular plane systolic excursion [TAPSE]) and tissue systolic velocity (Sm) with the following cutoffs: RVD = 0, normal: TAPSE, >20 mm; Sm, >12 cm/s; RVD = 1, mild impairment: TAPSE, 16 to 20 mm; Sm, 9 to 12 cm/s; RVD = 2, moderate: TAPSE, 10 to 15 mm; Sm, 6 to 8 cm/s; and RVD = 3, severe: TAPSE, <10 mm, Sm, <6 cm/s (as used before [22]).

ADDITIONAL EXAMINATIONS. To further examine factors associated with FGF-23 level, dual-energy x-ray densitometry (Lunar Prodigy, General Electric Healthcare, Little Chalfont, United Kingdom) and invasive hemodynamic examination were performed in a subset of patients. A balloon-tipped thermodilution catheter (Corodyn, Braun, Melsungen, Germany) and a standard data acquisition system (MacLab, General Electric Healthcare, Little Chalfont, United Kingdom) were used for invasive hemodynamic data acquisition.

PRIMARY OUTCOME. The primary outcome of this study was an adverse event defined as a combined endpoint of all-cause mortality, urgent heart transplantation, or implantation of ventricular assist device, whichever occurred first. Because the time to nonurgent transplantation reflects donor availability rather than the recipient's condition, patients receiving a nonurgent heart transplant were censored as having no outcome on the day of transplantation.

STATISTICAL ANALYSIS. Descriptive statistics are given as mean ± SD, median (interquartile range [IQR]), or frequency and percentage. Log-transformed values were used if the variables had skewed distribution. The patients were separated into tertiles according to FGF-23 levels. Linear trends across tertiles were assessed by analysis of variance, Wilcoxon rank sum, and chi-square tests for linear trends, as appropriate. All factors significantly associated with FGF-23 in the univariate analysis were included in the multiple stepwise linear regression model. Kaplan-Meier analysis was used to calculate cumulative event rates. Log-rank linear trend was tested across FGF-23 tertiles. The association of FGF-23 with hemodynamic and clinical variables was tested in a subset of patients with available hemodynamic data. To determine whether FGF-23 level contributes to the prediction of adverse events independently of other factors that may predict outcomes in patients with HF (age, sex, left ventricular EF, body mass index, New York Heart Association functional class, eGFR, BNP), the Cox proportional hazard model was used. Because the predictive value of FGF-23 may differ by CKD status, pre-specified interaction between FGF-23 level and CKD status was tested. The incremental value of FGF-23 to other clinical variables (age, sex, left ventricular EF, body mass index, New York Heart Association functional class, eGFR, BNP) was evaluated by calculating changes in C-statistics using the method proposed by DeLong et al. (24), integrated discrimination improvement and net reclassification improvement (25). Calculations were performed using SPSS version 19 software (Chicago, Illinois). A 2-sided p value <0.05 was considered statistically significant.

RESULTS

A total of 369 predominantly male (84%) patients 59 ± 11 years of age with advanced chronic systolic HF (median duration 6.5 years; IQR: 2.4 to 12.3 years) were enrolled. Optimal medical and device therapies (44% cardiac resynchronization therapy, 65% implantable cardioverter-defibrillator) were widely used. Population descriptive statistics are provided in **Table 1**. The median FGF-23 level was 147.6 (IQR: 85.8 to 359.3) RU/ml. FGF-23 was negatively associated with eGFR (Spearman r = −0.35; p < 0.0001) and positively with BNP (Spearman r = 0.48; p < 0.0001). In total, 138 patients (37%) had CKD, whereas 231 patients (63%) did not have CKD. Patients with CKD had significantly higher FGF-23 levels than patients without CKD (median, 206; IQR: 123 to 434 RU/ml vs. 120; IQR: 73 to 263 RU/ml; p < 0.0001).

TABLE 1 Descriptive Statistics by FGF-23 Tertiles

	1st Tertile	2nd Tertile	3rd Tertile	p for Linear Trend	Total
Age, yrs	58 ± 10	60 ± 11	59 ± 11	0.56	59 ± 11
Male	95 (78)	107 (88)	109 (87)	0.045	311 (84)
Body mass index, kg/m^2	27.9 ± 4.6	27.3 ± 4.2	27.9 ± 5.3	0.95	27.7 ± 4.7
Systolic BP, mm Hg	119 ± 19	114 ± 19	112 ± 19	0.002	115 ± 19
Diastolic BP, mm Hg	74 ± 11	72 ± 11	70 ± 11	0.02	72 ± 11
Heart rate, beats/min	78 ± 13	79 ± 13	84 ± 13	0.01	80 ± 13
Nonischemic etiology	61 (50)	47 (39)	60 (48)	0.76	168 (46)
Hypertension	58 (48)	62 (51)	67 (54)	0.34	187 (51)
Diabetes	29 (24)	41 (34)	55 (44)	0.001	125 (34)
LV ejection fraction, %	26 ± 7	25 ± 5	25 ± 8	0.04	25 ± 7
LV mass index, g/m^2	137 ± 35	146 ± 35	144 ± 38	0.16	142 ± 36
LV hypertrophy	91 (76)	100 (86)	99 (80)	0.44	290 (80)
RV dimension, mm	27 ± 7	30 ± 5	33 ± 7	<0.0001	30 ± 7
RV systolic pressure, mm Hg	42 ± 11	45 ± 12	49 ± 13	<0.0001	46 ± 12
Inferior vena cava diameter, mm	18 ± 5	19 ± 5	23 ± 6	<0.0001	20 ± 6
eGFR, ml/min/1.73 m^2	78 ± 18	67 ± 23	60 ± 22	<0.0001	68 ± 22
Glucose, mmol/l	6.1 ± 1.8	6.1 ± 1.8	6.7 ± 3.4	0.08	6.3 ± 2.5
International normalized ratio	1.10 ± 0.23	1.16 ± 0.25	1.32 ± 0.48	<0.0001	1.20 ± 0.35
BNP, pg/l	317 (157–656)	535 (317–1,056)	1057 (515–1,682)	<0.0001	560 (281–11,930)
Furosemide	105 (86)	117 (96)	118 (94)	0.02	340 (92)
Spironolactone	93 (76)	97 (80)	98 (78)	0.68	288 (78)
ACEi	96 (79)	84 (69)	80 (64)	0.01	260 (71)
ACEi dose, low/high	55 (57)/41 (43)	46 (55)/38 (45)	48 (60)/32 (40)	0.74	149 (57)/111 (43)
ARB	23 (19)	21 (17)	18 (14)	0.35	62 (17)
Beta-blockers	113 (93)	114 (93)	115 (92)	0.85	342 (93)
Right atrial pressure, mm Hg	6.58 ± 4.48	8.68 ± 5.4	12.52 ± 6.48	<0.0001	9.36 ± 6.01
Mean pulmonary pressure, mm Hg	30.54 ± 12.94	33.55 ± 11.4	37.81 ± 9.42	0.001	34.13 ± 11.57
Pulmonary wedge pressure, mm Hg	20.32 ± 10.08	22.94 ± 8.41	25.83 ± 6.63	0.001	23.16 ± 8.63
Transpulmonary gradient, mm Hg	10.2 ± 4.7	10.6 ± 5.0	12.0 ± 5.3	0.06	11.0 ± 5.0
Cardiac index, l/min/m^2	2.10 ± 0.42	1.96 ± 0.40	1.86 ± 0.44	0.003	1.97 ± 0.43
Mean BP – right atrial BP, mm Hg	86.8 ± 14.4	82.0 ± 11.3	73.7 ± 12.9	<0.0001	80.6 ± 13.7

Values are mean ± SD, frequency (%), or median (interquartile range).

ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker; BNP = B-type natriuretic peptide; BP = blood pressure; eGFR = estimated glomerular filtration rate; FGF = fibroblast growth factor; LV = left ventricular; RV = right ventricular.

TABLE 2 Multivariate Analysis of Factors Associated With FGF-23 Level in the Whole Population (N = 369, Model 1) and in Patients With Available Invasive Hemodynamic Data (n = 174, Model 2)

Model	Variable	Beta	sBeta	R^2 Change, %	p Value
1	Tricuspid regurgitation	0.26	0.18	21.4	<0.0001
	Chronic kidney disease*	0.54	0.23	7.7	0.003
	Alkaline phosphatase	0.40	0.22	6.0	0.006
	Inferior vena cava diameter	0.05	0.28	5.1	0.009
	Sodium level	−0.08	−0.19	3.0	0.039
	ACEi/ARB therapy	−0.43	−0.17	2.9	0.039
2	Mean BP – right atrial BP	−6.08	0.39	26.0	<0.0001
	Alkaline phosphatase	0.49	0.25	7.1	0.004
	Chronic kidney disease	0.55	0.23	6.5	0.004
	Sodium level	−0.07	−0.19	3.2	0.03

*Chronic kidney disease was defined as an estimated glomerular filtration rate <60 ml/min/1.73 m^2.

Abbreviations as in Table 1.

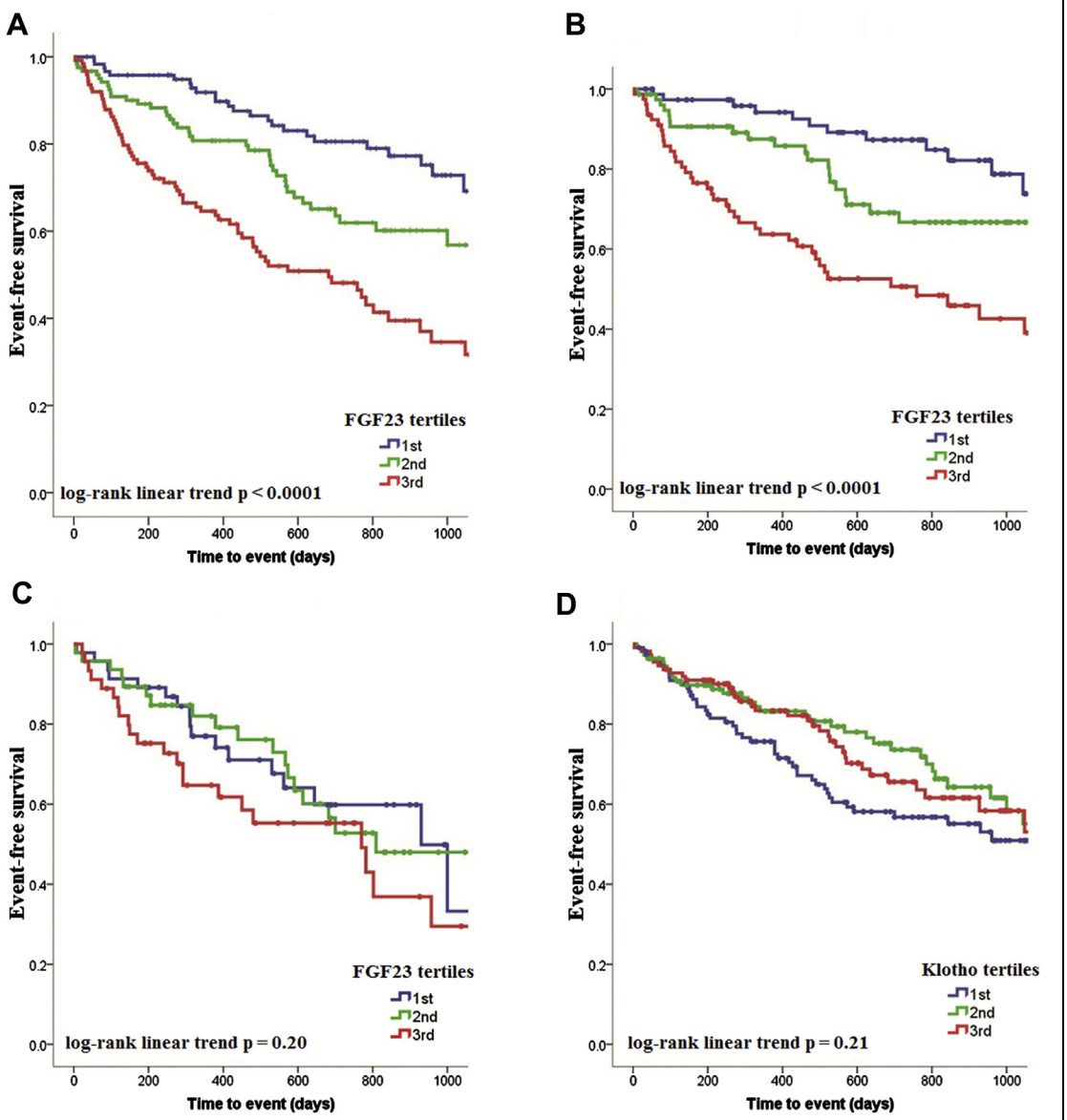
FACTORS ASSOCIATED WITH FGF-23 LEVEL. In the univariate analysis, with increasing FGF-23 tertile, there was a more severe functional impairment assessed by the New York Heart Association functional class, higher RV diameter, tricuspid gradient, inferior vena cava diameter, cholestatic liver enzymes (γ-glutamyl transferase and alkaline phosphatase), international normalized ratio, impaired renal function, and absence of ACEi (Table 1, Online Table 2). In the multivariate linear regression analysis, tricuspid regurgitation severity, CKD, alkaline phosphatase level, inferior vena cava diameter, absence of ACEi therapy, and hyponatremia remained independently associated with FGF-23 level (Table 2, Model 1).

FGF-23 LEVEL AND INVASIVE HEMODYNAMICS. Complete invasive hemodynamic data were available in

174 patients (47%). In the univariate analysis (Online Table 3), the decreased difference between mean arterial and right atrial pressure, which represents a proxy measure of glomerular perfusion pressure, together with increased right atrial pressure, showed the strongest association with increased FGF-23 level. In the multivariate analysis, the proxy measure of the pressure gradient across glomerulus remained independently associated with FGF-23 level and explained the largest proportion of FGF-23 variability (**Table 2**, Model 2).

FGF-23 AND DUAL-ENERGY X-RAY ABSORPTIOMETRY. Dual-energy x-ray absorptiometry was done in 152 patients (41% of all patients). Neither bone mass corrected for height ($r = 0.04$; $p = 0.60$) nor bone mineral density ($r = 0.06$; $p = 0.49$) was associated with FGF-23 level. FGF-23 was associated with lean

FIGURE 1 Kaplan-Meier Survival Curves by FGF-23 and Klotho Tertiles



Kaplan-Meier survival curves by FGF-23 tertiles in all patients **(A)**, in patients without chronic kidney disease **(B)**, in patients with chronic kidney disease **(C)**, and by Klotho tertiles in all patients **(D)**. FGF = fibroblast growth factor.

mass ($r = 0.20$; $p = 0.01$) but not fat mass ($r = -0.08$; $p = 0.33$).

FGF-23 AND OUTCOMES. During a median follow-up of 536 days (IQR: 254 to 850 days), 133 patients (36%) experienced an adverse event. Patients who had an adverse event had considerably higher FGF-23 than those without an event (249 RU/ml; IQR: 120 to 464 RU/ml vs. 122 RU/ml; IQR: 76 to 249 RU/ml; $p < 0.0001$). In the unadjusted analysis, the risk of adverse events increased linearly with FGF-23 tertiles (log-rank linear trend $p < 0.0001$) (Figure 1A). The risk of adverse events was 3.49 times higher (95% confidence interval [CI]: 2.21 to 5.50; $p < 0.0001$) in the upper FGF-23 tertile than in the lower tertile. In the multivariate analysis adjusted for age and sex, there was a significant interaction ($p < 0.05$) between FGF-23 tertile and the presence of CKD. To further explore this interaction, CKD-stratified analyses were performed.

FGF-23 STRATIFIED BY KIDNEY FUNCTION. In the univariate analysis, the risk of adverse events increased with increasing tertiles of FGF-23 among patients without CKD (log-rank linear trend $p < 0.0001$) (Figure 1B), but not among patients with CKD (log-rank linear trend $p = 0.20$) (Figure 1C). The risk of adverse events was 4.1 times higher (95% CI: 2.2 to 7.6; $p < 0.0001$) in the highest tertile than in the lowest FGF-23 tertile among patients without CKD, but the risk of adverse events in patients with CKD did not differ significantly between the first and third tertiles (hazard ratio [HR]: 1.5, 95% CI: 0.8 to 2.9; $p = 0.20$). In the multivariate Cox regression model adjusted for several clinical variables (Table 3, Model 2) and BNP (Table 3, Model 3), FGF-23 remained significantly associated with increased risk of adverse events among patients without CKD.

TABLE 3 Relative Hazard of Adverse Events in Relation to FGF-23 Tertile

	Model	HR* (95% CI)	p Value
No CKD	1†	4.07 (2.17–7.61)	<0.0001
	2‡	3.49 (1.77–6.87)	<0.001
	3§	2.56 (1.23–5.34)	0.01
CKD	1	1.51 (0.40–1.88)	0.20
	2	1.44 (0.72–2.91)	0.31
	3	0.86 (0.40–1.88)	0.71

*Hazard ratios for the 3rd tertile compared with the 1st tertile of FGF-23 are shown. †Model 1 is unadjusted. ‡Model 2 is adjusted for age, sex, body mass index, left ventricular ejection fraction, New York Heart Association functional class, and estimated glomerular filtration rate. §Model 3 includes Model 2 + B-type natriuretic peptide logarithm.

CI = confidence interval; CKD = chronic kidney disease (defined as estimated glomerular filtration rate <60 ml/min/1.73 m^2); FGF = fibroblast growth factor; HR = hazard ratio.

When FGF-23 was used as a continuous variable in the Cox model, results did not differ significantly. In the fully adjusted model, 1 SD increase in log-transformed FGF-23 was associated with 43% higher risk of adverse events among patients without CKD (HR: 1.43, 95% CI: 1.14 to 1.78; $p = 0.002$), but not in patients with CKD (HR: 1.12, 95% CI: 0.87 to 1.45; $p = 0.39$) (Online Table 4).

Among patients without CKD, an addition of FGF-23 to the clinical model led to a significant net reclassification improvement by 8.0% and integrated discrimination improvement by 3.2% (both $p < 0.05$), but did not significantly change the C-statistics (0.78, 95% CI: 0.72 to 0.84 vs. 0.80, 95% CI: 0.75 to 0.86; $p = 0.15$ for the model without vs. with FGF-23 included, respectively).

FGF-23 AND ACEI THERAPY AMONG PATIENTS WITHOUT CKD. FGF-23 level was higher in patients without ACEi therapy compared with patients receiving therapy ($p < 0.05$), whereas ACEi dose was not associated with FGF-23 level ($p = 0.53$). In the overall group, ACEi therapy was not associated with a lower risk of adverse events (HR: 0.65, 95% CI: 0.40 to 1.08; $p = 0.10$). However, among patients in the top tertile of FGF-23 (≥200 RU/ml), ACEi therapy was associated with a lower risk of adverse events (HR: 0.42, 95% CI: 0.21 to 0.81; $p = 0.01$) (Figure 2), whereas there was no association between ACEi dose and outcome ($p = 0.56$). On the other hand, ACEi therapy was not associated with lower risk of adverse events in the lower tertiles of FGF-23 (HR: 1.18, 95% CI: 0.52 to 2.70; $p = 0.69$).

FGF-23 AND KLOTHO. There was no association between FGF-23 and soluble Klotho concentrations ($r = 0.06$; $p = 0.25$). In the univariate analysis, Klotho was not associated with outcome when analyzed by Klotho tertiles (log-rank linear trend $p = 0.21$) (Figure 1D) or as a continuous variable ($p = 0.19$).

FGF-23 AND BNP. To further explore whether the prognostic value of FGF-23 among patients without CKD is additive to the information provided by BNP, the median values of FGF-23 (120 RU/ml) and BNP (502 pg/l) were used to stratify the population. The combination of FGF-23 and BNP identified subgroups with markedly different risks of adverse events ($p < 0.0001$) (Figure 3).

DISCUSSION

We demonstrated that FGF-23 is a strong predictor of adverse events in patients with chronic HF and preserved kidney function and that increased FGF-23

may identify a group of HF patients with greater benefit from ACEi therapy. Renal function and systemic hemodynamics were the main factors associated with FGF-23. In contrast, FGF-23 was unrelated to bone mass or Klotho level, and Klotho was not associated with outcomes, indicating that the link of FGF-23 with prognosis is unrelated to the Klotho-dependent pathway.

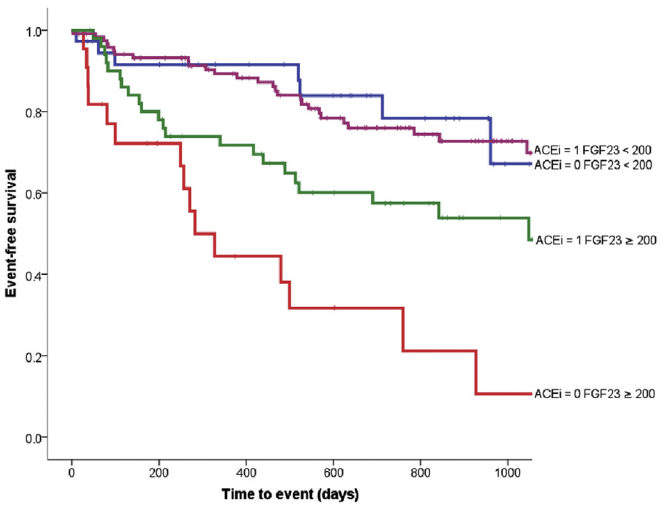
The predictive value of FGF-23 was previously reported in patients with stable HF with reduced EF (2,10,26). Similar to our results, these studies have shown that FGF-23 is related to the risk of adverse events. However, these studies were done in populations with largely preserved renal function; thus, the risk association with elevated FGF-23 among patients with CKD could not be evaluated. In the present study, we show that the predictive value of FGF-23 is confined to patients with a normal eGFR. Furthermore, in previous studies, factors associated with FGF-23 level have not been thoroughly examined. Identification of such associations is essential for understanding the pathways that may lead to FGF-23 elevation and to an increased risk of adverse events.

Several putative pathways by which FGF-23 may act in cardiorenal syndrome have been suggested. These include Klotho deficiency, a direct FGF-23 effect on the heart, vitamin D deficiency, bone and mineral metabolism disturbances or renin-angiotensin system activation.

Because FGF-23 suppresses Klotho expression, increased FGF-23 was hypothesized to be just a measure of primary loss of Klotho function (17). Decreased levels of soluble Klotho may increase cardiovascular risk by affecting the heart directly, independent of FGF-23 (27). However, in the present study, Klotho concentrations were not associated with FGF-23 levels and, unlike FGF-23, Klotho was not associated with an increased risk of adverse events. This suggests that among HF patients, the association of FGF-23 with adverse events is not mediated by Klotho. This finding is in line with findings of a recent study of patients with CKD in which soluble Klotho was not significantly related to cardiovascular outcomes, whereas FGF-23 was associated with the risk of HF development (18).

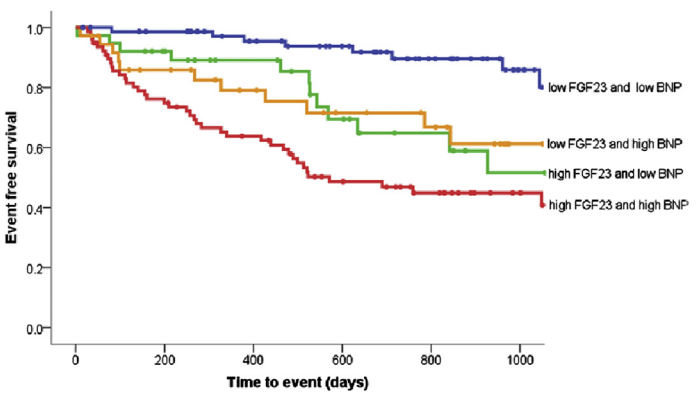
Other studies suggested that FGF-23 may have a direct effect on the heart, despite the near absence of Klotho, presumably through the PLCγ-calcineurin pathway. In an animal study, FGF-23 administration induced left ventricular hypertrophy and directly stimulated growth of isolated cardiomyocytes, whereas the FGF23-mediated increase in left ventricular mass was blocked by a fibroblast growth factor receptor inhibitor (3). In human studies, elevated FGF-23 levels were associated with increased left ventricular mass and left ventricular hypertrophy (4), although the association with cardiac structure has not been consistent across studies (28). Nonetheless, we did not observe an association between FGF-23 and left ventricular mass or geometry, suggesting that cardiac remodeling is not the

FIGURE 2 Kaplan-Meier Survival Curves by FGF-23 Level and ACEi Therapy



Kaplan-Meier survival curves stratified by FGF-23 level and angiotensin-converting enzyme therapy. ACEi = angiotensin-converting enzyme inhibitor; FGF = fibroblast growth factor.

FIGURE 3 Kaplan-Meier Survival Curves by FGF-23 and BNP Level



Kaplan-Meier survival curves by FGF-23 and BNP above and below median. BNP = B-type natriuretic peptide; FGF = fibroblast growth factor.

dominant pathway by which FGF-23 increases cardiovascular risk in systolic HF.

Another mechanism connecting FGF-23 with adverse events may be through the vitamin D level. High FGF-23 suppresses 1-alpha hydroxylase, thus decreasing the vitamin D level. Vitamin D deficiency is common in HF and is associated with an increased risk of adverse events (29). However, no clinical investigation has provided convincing evidence that vitamin D treatment yields favorable outcomes in HF. Several studies have shown that vitamin D therapy does not affect aerobic capacity or functional performance in patients with HF (30,31). Although in the present study we did not measure vitamin D concentrations, several studies in different population groups including patients with HF have shown that the association of FGF-23 with outcomes is independent of vitamin D level (2,4,6,7,10,32).

Furthermore, FGF-23 may be associated with increased risk through bone and mineral metabolism disturbances. Patients with chronic HF are at increased risk of osteoporosis and bone fractures (11), and reduced bone mineral density is associated with an increased risk of adverse events (33). Increased FGF-23 may decrease bone mineralization directly or by decreasing phosphorus and vitamin D levels. However, in a large population-based study of elderly patients, no association between FGF-23 and bone fractures was observed (34). Moreover, higher FGF-23 was associated with higher, rather than lower, bone mineral density. Similarly, in a study of patients on long-term dialysis, there was no association between FGF-23 and bone mineral density (35). These findings are in line with our results that bone mineral density and bone mass are not associated with FGF-23 levels in HF, suggesting that the association of FGF-23 with outcomes is independent of bone metabolism disturbances.

We cannot exclude a possibility that an increase in FGF-23 may not cause additional biological effects, but rather be an early marker of subclinical kidney disease (5,19). Renal impairment is one of the strongest prognostic factors in HF (36). Because FGF-23 increases in the earliest stages of renal impairment, before creatinine, it may detect early stages of renal dysfunction better than eGFR. In the present study, decreased pressure difference between the mean arterial and right atrial pressures, a proxy measure of glomerular filtration pressure, showed the strongest association with increased FGF-23 level. Interestingly, increased right atrial pressure correlated more strongly with FGF-23 levels and with the proxy measure of glomerular filtration pressure than decreased cardiac index. This may be caused by renal autoregulation, which protects kidneys from hypoperfusion due to low cardiac output, but cannot protect kidneys from central venous pressure elevation. This may also explain why the markers of hypervolemia and venous stasis, such as tricuspid regurgitation, inferior vena cava dilation, and elevated levels of γ-glutamyl transferase and alkaline phosphatase were independently associated with FGF-23, whereas left ventricular EF was not.

The precise mechanism connecting FGF-23 with renal perfusion is not known. FGF-23 is an important factor in phosphate homeostasis. We speculate that hypoperfusion of renal tubules may lead to the decrease in phosphate excretion. Hyperphosphatemia may in turn stimulate FGF-23 production, which keeps the phosphate level in the normal range in the early stages of renal dysfunction. Renal tubules are more sensitive to the decrease in perfusion pressure than the glomeruli. Consequently, glomerular filtration may not be decreased in the early stages of cardiorenal syndrome despite alterations in renal tubule functions.

Among patients with HF, moderate to severe chronic kidney disease (eGFR $<$60 ml/min/1.73 m^2) identifies a high-risk subgroup (37). However, the risk associated with eGFR is far less evident or absent in patients with an eGFR $\geq$60 ml/min/1.73 m^2 (38). This may be due to the fact that serum creatinine concentrations are relatively insensitive to early changes in renal functions, which makes biomarkers that would reflect early stages of cardiorenal syndrome highly desirable. Recently, urinary N-acetyl-β-D-glucosaminidase and other urinary markers of tubular damage were found to be related to a poor clinical outcome in HF patients (39), particularly when measured at the time of admission or discharge for acutely decompensated HF (38). However, neutrophil gelatinase-associated lipocalin did not improve the predictive value of established biomarkers such as N-terminal pro-B-type natriuretic peptide and eGFR when measured in chronic stable HF (40). In the present study in patients with chronic stable HF, we show that FGF-23 is an independent predictor of outcome in patients with normal kidney function but not in patients with CKD. This suggests that FGF-23 could be used to detect early stages of cardiorenal syndrome in HF patients, in whom renal impairment is not yet apparent from the eGFR.

We show that increased FGF-23 levels are not only a marker of increased risk, but may also identify

patients in whom ACEi therapy may provide greater clinical benefit. This finding is in line with the PEACE (Prevention of Events With Angiotensin-Converting Enzyme Therapy) trial in patients with stable coronary heart disease in which the ACEi trandolapril significantly reduced the risk of cardiovascular death or incident HF only in patients with increased FGF-23, whereas there was no clinical benefit in the remaining patients (9). Our finding confirms the results of the PEACE trial and extends them to patients with HF, suggesting that renin-angiotensin system activation or its insufficient inhibition may link FGF-23 concentrations with adverse events. Although ACEi therapy was significantly associated with FGF-23, there was no association with spironolactone therapy, suggesting that the mineralocorticoid system may not influence the FGF-23 level and its action. Similarly, we did not observe an association between an angiotensin receptor blocker and FGF-23 levels, although this could have been due to the fact that only 62 patients (17%) were treated with an angiotensin receptor blocker.

In the present study, FGF-23 was an independent predictor of outcome in patients without CKD, even after adjustment for BNP. Furthermore, combining FGF-23 with BNP allowed identification of subgroups with a substantially differing risk of adverse events. Of particular interest is the similar risk in patients with high FGF-23/low BNP and those with low FGF-23/high BNP. This confirms that FGF-23 has an additional predictive value compared with BNP, most likely due to the fact that these biomarkers reflect different biological processes, each of them being associated with an increased risk of adverse events.

STUDY LIMITATIONS AND STRENGTHS. This prospective study was an observational one, which precludes us from drawing conclusions about causality. Due to possible referral bias, our study sample may not reflect a general HF population and is limited to HF with reduced EF in the moderate to advanced phase of the disease. Patients with an EF <50% were enrolled in this study of patients with systolic HF, whereas systolic HF is commonly defined by an EF ≤40%. However, when we excluded patients with an EF in the 40% to 50% range, results did not change.

We did not have data on phosphorus and vitamin D levels, potential significant confounders of the FGF-23 association with adverse events. However, previous studies showed no association of FGF-23 with serum phosphorus level in patients with cardiovascular disease (2,4,6,7,10,32).

We did not have data on renin-angiotensin system components, which did not allow us to assess renin-angiotensin system activation as a possible pathway of FGF-23 action. The observed lack of association between FGF-23 levels and adverse events in patients with CKD may be due to the smaller sample size; the current cohort size had a 72% power to detect differences between the FGF-23 tertiles.

Due to the unavailability of renal outcomes, we cannot assess whether the risk of adverse events associated with increased FGF-23 was related to deteriorating renal function. We did not have data on emerging markers of renal damage such as N-acetyl-β-D-glucosaminidase or neutrophil gelatinase–associated lipocalin, which precluded us from comparing the predictive value of FGF-23 with that of other biomarkers.

Because we do not know the reasons for the absence of ACEi therapy, we cannot exclude that an unaccounted for confounder is responsible for the higher risk in patients with increased FGF-23 and absence of ACEi therapy. Nonetheless, it is reassuring that our data are in line with those of the PEACE study, in which patients were randomly assigned to ACEi therapy.

The main strength of the present study is the prospective follow-up of a well-characterized population of patients with HF with a decreased EF. Furthermore, detailed data on several biomarkers allowed us to evaluate different pathways by which an increased FGF-23 level may increase the risk of adverse events in patients with HF.

CONCLUSIONS

FGF-23 is a strong independent predictor of adverse events in patients with systolic HF and preserved kidney function. The addition of FGF-23 to clinical variables and BNP led to an 8.0% net reclassification improvement. The association of FGF-23 with adverse events likely reflected early changes in renal hemodynamics and an activation of the renin-angiotensin system. Importantly, our results suggest that FGF-23 concentrations identify HF patients who may benefit from ACEi therapy.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Increased FGF-23 is an independent predictor of adverse events in patients with systolic HF and preserved kidney function. The predictive value of FGF-23 in this group adds to the information provided by BNP. Increased FGF-23 likely reflects early changes in renal hemodynamics and activation of the renin-angiotensin system.

TRANSLATIONAL OUTLOOK: Angiotensin-converting enzyme inhibitors may reduce the risk associated with increased FGF-23. Studies evaluating FGF-23 as a therapeutic target are needed.

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APPENDIX For supplemental tables, please see the online version of this article.