Fibroblast growth factor 23 is related to profiles indicating volume overload, poor therapy optimization and prognosis in patients with new-onset and worsening heart failure

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Fibroblast growth factor 23 is related to volume overload, poor therapy optimization and impaired clinical outcome in patients with worsening heart failure

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Abstract

Aims

Fibroblast growth factor (FGF) 23 is a hormone that increases urinary phosphate excretion and is a key regulator of renal sodium reabsorption and plasma volume. We therefore studied the role of plasma FGF23 in therapy optimization and outcomes in patients with worsening heart failure (HF).

Methods and results

We measured plasma C-terminal FGF23 levels at baseline in 2399 of the 2516 patients included in the BIOlogy Study to Tailored Treatment in Chronic HF (BIOSTAT-CHF) trial. The association between FGF23 and outcome was evaluated by Cox regression analysis.

Median FGF23 was 218.0 [117.1-579.3] RU/ml, patients with higher FGF23 levels had a worse NYHA class, more signs of congestion, and were less likely to use ACE-inhibitors (ACEi) or angiotensin receptor blockers (ARBs) at baseline (all *P*<0.01). Higher FGF23 levels were independently associated with higher BNP, lower eGFR, the presence of edema and atrial fibrillation (all *P*<0.01????). In addition, higher FGF23 was independently associated with impaired uptitration of ACEi/ARBs, but not of beta-blockers. In multivariable Cox regression analysis, FGF23 was independently associated with all-cause mortality (hazard ratio: 1.17 (1.09-1.26) per log increase, *P*<0.001), and the combined endpoint of all-cause mortality and HF hospitalization (1.15 (1.08-1.22) per log increase, *P*<0.001). FGF23 outperformed NT-proBNP as a predictor of all-cause mortality (Harell's c-statistic: 0.689 vs. 0.679, respectively, *P*<0.001).

Conclusion

In patients with worsening HF, higher plasma FGF23 levels are independently associated with volume overload, less successful uptitration of ACEi/ARBs and an increased risk of all-cause mortality and HF hospitalization.

Abbreviations

ACEi	Angiotensin Converting Enzyme inhibitor
ARB	Angiotensin Receptor Blocker
BNP	Brain Natriuretic Peptide
BIOSTAT-CHF	BIOlogy Study to Tailored Treatment in Chronic Heart Failure
CKD	Chronic Kidney Disease
eGFR	estimated Glomerular Filtration Rate
FGF23	Fibroblast Growth Factor 23
HF	Heart Failure
JVP	Jugular Venous Pressure
NT-pro BNP	N terminal pro Brain Natriuretic Peptide
RAAS	Renin Angiotensin Aldosterone System

Introduction

The phosphaturic hormone fibroblast growth factor 23 (FGF23) is a key regulator of phosphate metabolism by inhibiting proximal tubular phosphate reabsorption in the kidney and suppressing the generation of 1,25$(OH)_2$ vitamin D.**{**ADDIN RW.CITE{{40 Kovesdy,C.P. 2013}}**}** In patients with chronic kidney disease (CKD), higher FGF23 levels have been consistently associated with an increased risk of cardiovascular morbidity and mortality.**{**ADDIN RW.CITE{{401 Isakova,T. 2011; 633 Gutierrez,O.M. 2008}}**}** In CKD, FGF23 seems particularly strongly linked with heart failure (HF) **{**ADDIN RW.CITE{{241 Scialla,J.J. 2014}}**}**, supported by mechanistic studies indicating that FGF23 is a key regulator of renal sodium reabsorption and plasma volume **{**ADDIN RW.CITE{{231 Andrukhova,O. 2014}}**}** and interacts with renin-angiotensin-aldosterone system (RAAS) activation.**{**ADDIN RW.CITE{{488 de Borst,M.H. 2013}}**}** Furthermore, high FGF23 levels were associated with an impaired response to sodium restriction and ACE-inhibition in CKD patients.**{**ADDIN RW.CITE{{479 Humalda,J.K. 2015}}**}**

Recently, elevated levels of FGF23 have been associated with cardiovascular mortality and incident HF in patients with stable ischemic heart disease.**{**ADDIN RW.CITE{{284 Udell,J.A. 2014}}**}** In chronic HF, it has been suggested that FGF23 is associated with disease severity and adverse outcome.**{**ADDIN RW.CITE{{42 Gruson,D. 2012; 400 Poelzl,G. 2014; 41 Plischke,M. 2012; 499 Wohlfahrt,P. 2015}}**}** Data on FGF23 in worsening or acute HF are scarce, even though it has been suggested that FGF23 has a direct effect on sodium homeostasis and volume handling. The present study is the first to address the relation between FGF23, congestion, and clinical outcomes in new onset or worsening HF. Also, FGF23 contributes to RAAS activation and angiotensin converting enzyme inhibitor (ACEi) therapy has been shown to be less effective in CKD patients with high FGF23 levels.(6,7) Therefore we subsequently studied whether FGF23 is associated with impaired uptitration of ACEi/angiotensin receptor blocker (ARB) therapy, a first-line therapy in patients with HF. We hypothesized that a higher plasma FGF23 level in patients with acute or worsening heart

failure is associated with less successful uptitration of guideline-recommended ACEi/ARB therapy and adverse clinical outcomes.

Methods

Patient population

The study design of the systems BIOlogy Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) has been published previously. **{**ADDIN RW.CITE{{627 Voors,A.A. 2016}}**}** In brief, 2516 patients with new onset or worsening heart failure with either an ejection fraction of ≤40% or plasma concentrations of Brain Natriuretic Peptide (BNP) >400 pg/ml and/or N terminal pro Brain Natriuretic Peptide (NT-proBNP) >2,000 pg/ml, and treated with furosemide ≥40mg/day or equivalent, who were on ≤50% of the target dose of ACEi or ARB and beta-blocker therapy were included in the BIOSTAT-CHF trial. The BIOSTAT-CHF trial is a large European, multicenter, multinational, prospective, observational study. The trial was approved by the local ethics committee at each participating center. All patients provided written informed consent.

Study design

Both inpatients and outpatients were enrolled, and had a visit at baseline and after 9 months of follow-up. During the first 3 months, the treating physician was encouraged to uptitrate ACEi/ARBs and beta-blockers to the target doses presented in the ESC heart failure guidelines.**{**ADDIN RW.CITE{{37 McMurray,J.J. 2012; 597 Ponikowski,P. 2016}}**}** Subsequently, patients were contacted every 6 months by telephone. At 9 months, reasons for not reaching the target dose were recorded. Median follow-up was 21 months.

The endpoints selected for these analyses were all-cause mortality, and the combined endpoint of all-cause mortality or first occurrence of HF hospitalization. HF hospitalization was defined as hospitalization lasting longer than one day for which the primary reason was worsening of signs or symptoms of HF, requiring intravenous medications or an increased dose of oral diuretics.

Laboratory measurements

From 2516 enrolled patients, plasma FGF23 was determined in 2399 (95.3%) baseline plasma samples using a human C-terminal FGF23 ELISA (Immutopics, Inc., San Clemente, CA, USA). More details on this specific assay, such as inter-/intra-assay variation have been described previously.**{**ADDIN RW.CITE{{632 Heijboer,A.C. 2009}}**}** Using this assay, in a cohort of 3,107 community-living persons ≥65 years of age median FGF23 was 70 [51-100] RU/mL.**{**ADDIN RW.CITE{{629 Ix,J.H. 2012}}**}** Phosphate, calcium and albumin were measured in stored samples using routine laboratory procedures. Renin and aldosterone were both measured using a RadioImmunoAssay (Renin: CisBio International; Aldosterone: IBL International). NT-proBNP was measured using Proseek Multiplex (Olink Biosciences AB, Uppsala, Sweden). Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI eGFR formula.**{**ADDIN RW.CITE{{489 Levey,A.S. 2009}}**}**

Statistical analysis

Data with a normal distribution are presented as mean ± standard deviation, and as frequencies and percentages for categorical values. Data with a skewed distribution are presented as median with interquartile ranges. Differences between quintiles of FGF23 were tested for significance with ANOVA (normal distribution), Kruskal-Wallis (skewed distribution), and Fisher's exact test (categorical variables). A linear trend was statistically tested over quintiles of FGF23, after checking

for non-linear trends. Uni- and multivariable linear regression analysis was performed with log transformed FGF23 as a dependent variable. Transformations were checked using multifractional polynomials. Multivariable linear regression analysis, including all variables with $p<0.10$ in univariable analysis were constructed via backward elimination and validated using bootstrap re-sampling with 1,000 replicates. The model was tested for collinearity and checked by plotting residuals. Cox proportional hazard regression analysis was performed to examine associations with clinical outcomes. FGF23 was investigated as a continuous variable, and by quintiles. Multivariable models were adjusted for an outcome model specifically developed and validated in the BIOSTAT index and validation cohort with addition of markers that have previously been associated with FGF23 levels (renin, aldosterone, phosphate, albumin, calcium and eGFR, if not already included in the model).**{**ADDIN RW.CITE{{628 Voors,A.A. [No Information]}}**}**The following variable were included in the BIOSTAT risk model for all-cause mortality: age, urea, NT-proBNP, hemoglobin, and use of beta-blocker at baseline.**{**ADDIN RW.CITE{{628 Voors,A.A. [No Information]}}**}** The BIOSTAT risk model for heart failure hospitalization includes age, previous HF hospitalization, peripheral edema, systolic blood pressure, and eGFR.**{**ADDIN RW.CITE{{628 Voors,A.A. [No Information]}}**}** Finally, the risk model for the combined endpoint includes age, previous HF hospitalization, peripheral edema, systolic blood pressure, NT-proBNP, hemoglobin, HDL, sodium, and use of beta-blocker at baseline.**{**ADDIN RW.CITE{{628 Voors,A.A. [No Information]}}**}** Interaction analyses were used to test significant interactions in subgroups that may affect the association between FGF23 and outcome. These were visualized using forest plots. Logistic regression was used to investigate the association between FGF23 and ACEi/ARB use, and whether target dose was reached. A two-tailed p-value <0.05 was considered statistically significant. All analyses were performed using R: a Language and Environment for Statistical Computing, version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline characteristics per quintile of FGF23 are presented in table 1. Median FGF23 was 218.0 [117.1-579.3] RU/ml. Patients with a higher FGF23 level were older, more often female, had a higher NYHA class, lower blood pressure, higher heart rate, and more signs of congestion (all $P<0.005$). All signs and symptoms of congestion, i.e. edema, orthopnea, jugular venous pressure (JVP), rales, and hepatomegaly were greater in patients with higher FGF23 levels. In addition, patients with higher FGF23 levels were more likely to be hospitalized with worsening heart failure, had worse renal function, higher (NT-pro)BNP, and renin levels (all $P<0.001$). Also, these patients less frequently used ACEi or ARBs and beta-blockers, and a significantly lower percentage used target doses of both drugs.

Correlates of log transformed FGF23 in multivariable linear regression are presented in table 2. The multivariable model had an overall r^2 of 0.466, and the variables showing the strongest association with higher log FGF23 levels were higher BNP, lower eGFR, the presence of atrial fibrillation and edema. Also, higher aldosterone levels were independently associated with higher log FGF23 levels.

FGF23 and therapy optimization

Higher levels of FGF23 were associated with lower rates of ACEi/ARB and beta-blocker use at baseline (table 1). Also, patients with a higher FGF23 level less frequently used guideline-recommended doses of ACEi/ARBs and beta-blockers (table 1). Interestingly, higher log FGF23 levels were also inversely associated with ACEi/ARB use and dosage after 3 months of uptitration (table 3). This association remained significant after adjustment for baseline ACEi/ARB use, age, sex, eGFR, aldosterone, renin, calcium, albumin, phosphate, and NYHA class. In contrast, there was no significant independent association between FGF23 levels and beta-blocker use and dosage after 3 months of uptitration (supplementary table 1).

FGF23 and outcomes

During a median follow-up of 21 [16-27] months, 631 of the 2399 patients (26%) died, and 588 patients (25%) were (re)hospitalized for heart failure. A total of 981 patients (41%) experienced the combined endpoint of all-cause mortality and/or heart failure hospitalization. In univariable Cox regression analysis, an increase in log FGF23 was significantly associated with an increased risk of the combined endpoint, and remained independently associated after multivariable adjustment (HR: 1.15 [1.08-1.22] per log increase, *P*<0.001). Similar findings were observed in a multivariable Cox regression analysis as patients in the highest three quintiles of FGF23 had a significantly increased risk of the combined endpoint, compared to the lowest (first) quintile (table 4). The Kaplan Meier curve for the combined endpoint per quintile of FGF23 is shown in figure 1. Hazard ratios were consistent across subgroups based on ejection fraction, renal function, and RAAS activation (figure 2). There was a significant interaction between FGF23 and age and NYHA class, yet consistently across these subgroups higher FGF23 levels were also associated with a higher risk of the combined endpoint. Analyses are missing when adjusted for the previously published prediction model (by Wouter), these should be added to make biostat-chf papers consistent.

Higher log FGF23 levels were also independently associated with all-cause mortality in univariable and multivariable analyses (HR: 1.17 [1.09-1.26] per log increase, *P*<0.001). The Kaplan Meier per quintile of FGF23 for mortality is shown in supplementary figure 1. The association of higher FGF23 with outcome for both endpoints persisted after additional adjustment for percentage of target dose reached after 3 months of uptitration (p<0.001 for both endpoints). The predictive value of FGF23 for all-cause mortality was greater than that for NT-proBNP (univariable Harell's c-statistic: 0.689 vs. 0.679, respectively, *P*<.001). Stats on the additive value of FGF-23 are missing. How much better in the prediction or re-classificaiton of events when added.

Discussion

In patients with worsening HF, higher FGF23 levels were strongly and independently associated with an increased risk of all-cause mortality and heart failure hospitalization. Patients with a higher FGF23 level had more severe HF, with more signs of congestion, a greater activation of the renin-angiotensin-aldosterone activation and worse renal function, yet the associations with outcomes were independent of these baseline parameters. Furthermore, patients with a higher FGF23 level were less well uptitrated with ACEi or ARBs during follow-up.

FGF23 and volume overload

FGF23 is a protein produced by osteocytes and osteoblasts, and has been identified as a phosphaturic hormone central to the regulation of phosphate homeostasis.**{**ADDIN RW.CITE{{490 Shimada,T. 2004}}**}** FGF23 is deregulated in a relatively early stage of CKD, contributing to abnormalities in bone and mineral metabolism.**{**ADDIN RW.CITE{{491 Isakova,T. 2011}}**}** Interestingly, a higher level of FGF23 has emerged as a strong risk factor for cardiovascular and all-cause mortality across the spectrum of CKD and after kidney transplantation.**{**ADDIN RW.CITE{{492 Scialla,J.J. 2014; 493 Baia,L.C. 2013}}**}** These adverse effects of FGF23 are most likely beyond its direct effects on phosphate. This is suggested by the observation that hyperphosphatemia is strongly associated with vascular calcification and ischemic cardiac events, whereas FGF23 is strongly associated with HF and not with vascular calcification.**{**ADDIN RW.CITE{{241 Scialla,J.J. 2014; 500 Scialla,J.J. 2013}}**}** Preclinical data demonstrated that FGF23 infusion leads to the development of left ventricular hypertrophy.**{**ADDIN RW.CITE{{52 Faul,C. 2011}}**}** Furthermore, FGF23 increased expression and activity of the sodium-chloride co-transporter in the distal tubule, leading to increased sodium uptake, volume expansion and cardiac hypertrophy.**{**ADDIN RW.CITE{{231 Andrukhova,O. 2014}}**}** In the current study, we demonstrate that FGF23 levels are elevated in

worsening HF (median: 218 RU/mL) compared to previous levels reported in a community cohort (median: 70 RU/mL), and that higher FGF23 levels are associated with more signs of volume overload.**{**ADDIN RW.CITE{{629 Ix,J.H. 2012}}**}** Remarkably, all signs and symptoms of volume overload and congestion, i.e. edema, orthopnea, rales, elevated JVP and hepatomegaly were more common in patients with higher FGF23 levels. Also, in the multivariable model for FGF23, higher BNP, edema, hepatomegaly, and lower hemoglobin and albumin, all signs of volume overload, were among the strongest predictors of FGF23 levels. Whether the association between FGF23 and volume overload is causal or not cannot be concluded from these observational data. Yet, in a recent study FGF23 did not markedly change in response to volume interventions, suggesting that FGF23 would more likely be a cause than a consequence of volume overload.**{**ADDIN RW.CITE{{634 Humalda,J.K. 2016}}**}** We only measured FGF23 at baseline, and were therefore not able to capture the changes of FGF23 in relation to the changes of signs of congestion and volume overload. Several studies showed an inverse relationship between renal function and FGF23 levels, consistent with our findings in worsening heart failure.**{**ADDIN RW.CITE{{483 Ix,J.H. 2010; 484 Gutierrez,O.M. 2009}}**}** Of note, renal impairment was one of strongest predictors of FGF23 in our cohort.

FGF23, circulating RAAS parameters and ACEi/ARB optimization

In the current study, we found higher renin levels in patients with higher FGF23 levels, and higher aldosterone was a predictor of higher FGF23 levels in multivariable analysis, suggesting more pronounced RAAS activation in patients with higher FGF23. This can be related to the volume expansion that is also associated with higher FGF23. Alternatively, the association between higher plasma renin levels and FGF23 levels can be explained by the finding that FGF23 increased the production of renin, by suppressing the generation of 1.25(OH)2 vitamin D, which is an established negative regulator of renin gene expression.**{**ADDIN RW.CITE{{494 Li,Y.C. 2002}}**}** The association between FGF23 and RAAS-activity is of particular interest in the light of our finding that higher FGF23 levels were also associated with lower baseline prescription rates of ACEi or ARBs and less frequent

use of guideline recommended target dose. Additionally, patients with higher FGF23 levels were also less likely to use an ACEi or ARB after 3 months of uptitration, and less frequently used target doses. In contrast, this was not observed for beta-blockers. This association between FGF23 and failure of ACEi/ARB uptitration remained significant after adjustment for baseline ACEi/ARB use, RAAS activation, renal function, and severity of heart failure, suggesting that FGF23 levels provide additional information regarding the tolerability of ACEi or ARBs. This observation may be mediated by impaired renal function in patients with high FGF23 levels, however the observed association persisted after adjustment for eGFR. Future studies should provide more insight in the relation between FGF23 and tolerability of RAAS blockade and effectiveness as this could be an incredibly interesting and applicable feature of this biomarker.

FGF23 and outcome

Our findings that higher FGF23 levels are related with poorer clinical outcome in patients with worsening signs and symptoms of heart failure are in line with three small studies in stable chronic heart failure patients.**{**ADDIN RW.CITE{{41 Plischke,M. 2012; 400 Poelzl,G. 2014; 42 Gruson,D. 2012}}**}** Recently, a larger study in patients with chronic heart failure found an increased risk of mortality in patients with a reduced ejection fraction, but not in patients with a preserved ejection fraction.**{**ADDIN RW.CITE{{495 Koller,L. 2015}}**}** In our study, we showed for the first time a strong predictive value of higher FGF23 levels for adverse clinical outcomes, all-cause mortality and heart failure hospitalization in patients with worsening signs and symptoms of heart failure. In the highest quintile of FGF23 more than 50% of patients died during a median follow-up of 21 months. In contrast with previous findings in stable chronic heart failure, we did not observe a differential association with outcomes for patients with reduced *versus* preserved ejection fraction.**{**ADDIN RW.CITE{{495 Koller,L. 2015}}**}** We recently published a risk score that was derived from BIOSTAT-CHF which included 42 demographic, clinical and biochemical variables.**{**ADDIN RW.CITE{{628 Voors,A.A. [No Information]}}**}** Interestingly, FGF23 provided significant additive predictive value of

adverse outcome on top of this risk score, and its prognostic value was greater than that of NT-proBNP.

Several mechanisms may explain the link between FGF23 and adverse outcome. FGF23 might increase fluid retention, and incomplete decongestion is associated with higher rehospitalization rates and poorer outcome. Also, higher FGF23 might reflect patients with more severe renal dysfunction, a strong predictor of outcome in heart failure. The association with outcome was however independent of markers of volume status and renal function. This observation, along with data that FGF23 is also associated with mortality in patients with normal kidney function, suggests that FGF23 is more than a marker of impaired renal function.**{**ADDIN RW.CITE{{485 Parker,B.D. 2010}}**}** As FGF23 stimulates RAAS activity, this may cause left ventricular remodeling, fluid overload, and subsequently adverse outcome. Finally, we feel FGF23 is of great interest as it may prove to be a modifiable risk factor, as illustrated by a recent study in hemodialysis patients, showing that treatment with the calcimimetic cinacalcet lowered FGF23 levels by about 50%, in association with lower risks of cardiovascular mortality and events.**{**ADDIN RW.CITE{{496 Moe,S.M. 2015}}**}** Several studies, investigating the effect of phosphate binders and diet on FGF23 in CKD patients, are currently ongoing.

Strengths and limitations

To our knowledge this is the first study to investigate the role of FGF23 in patients with worsening symptoms of heart failure. Strengths of this study are the number of patients enrolled in this observational, European, multicenter, multinational cohort, and the extensive clinical data available, such as markers of RAAS activation. Limitations of this study include the observational design of this study, in which residual confounding potentially influenced our results. Also, data during hospitalization for heart failure were not available, and we were therefore not able to establish the

association between FGF23 and efficiency of decongestion. Furthermore, we were merely able to describe associations, and were unable to establish causality.

Future directions

Our study showed a strong association between higher FGF23 levels and adverse clinical outcome, however further studies are warranted to examined the underlying pathophysiology between higher FGF23 levels, fluid overload, and RAAS activation in heart failure, and establish whether FGF23 is a cause or a consequence of fluid overload. Prospective studies targeting FGF23 levels in heart failure patients, for instance through cinacalcet, would shed more light on a potential causal role for FGF23 in fluid overload and adverse clinical outcomes in acute heart failure.

Conclusion

Higher FGF23 levels are associated with more severe heart failure, worse renal function and an increased risk of all-cause mortality and heart failure hospitalization in patients with acute and worsening heart failure. In addition, higher plasma FGF23 levels are independently associated with less successful uptitration of guideline recommended ACEi/ARB therapy.

Funding

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References

{ADDIN RW.BIB}

Figure legends

Figure 1: Kaplan-Meier survival curve for the combined endpoint of all-cause mortality and heart failure (re)hospitalization according to quintiles of FGF23

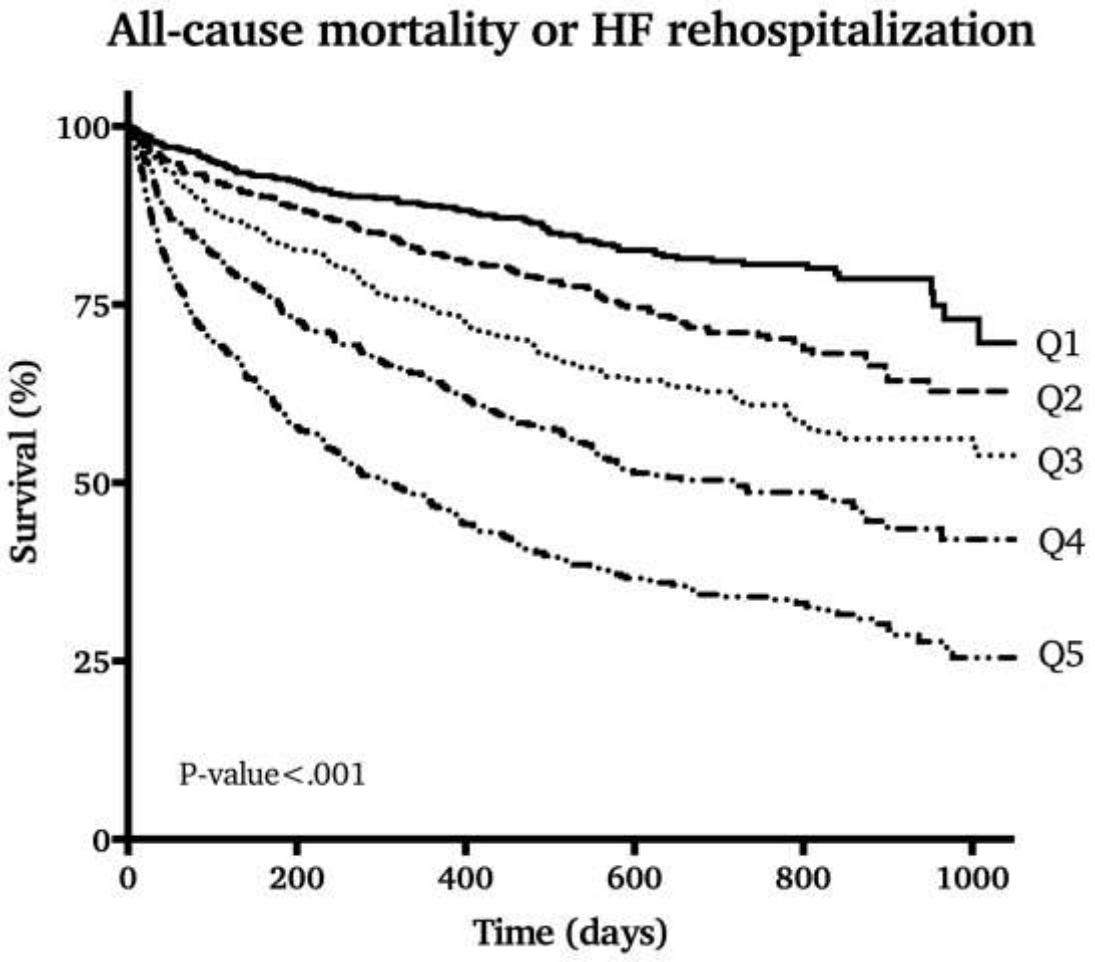
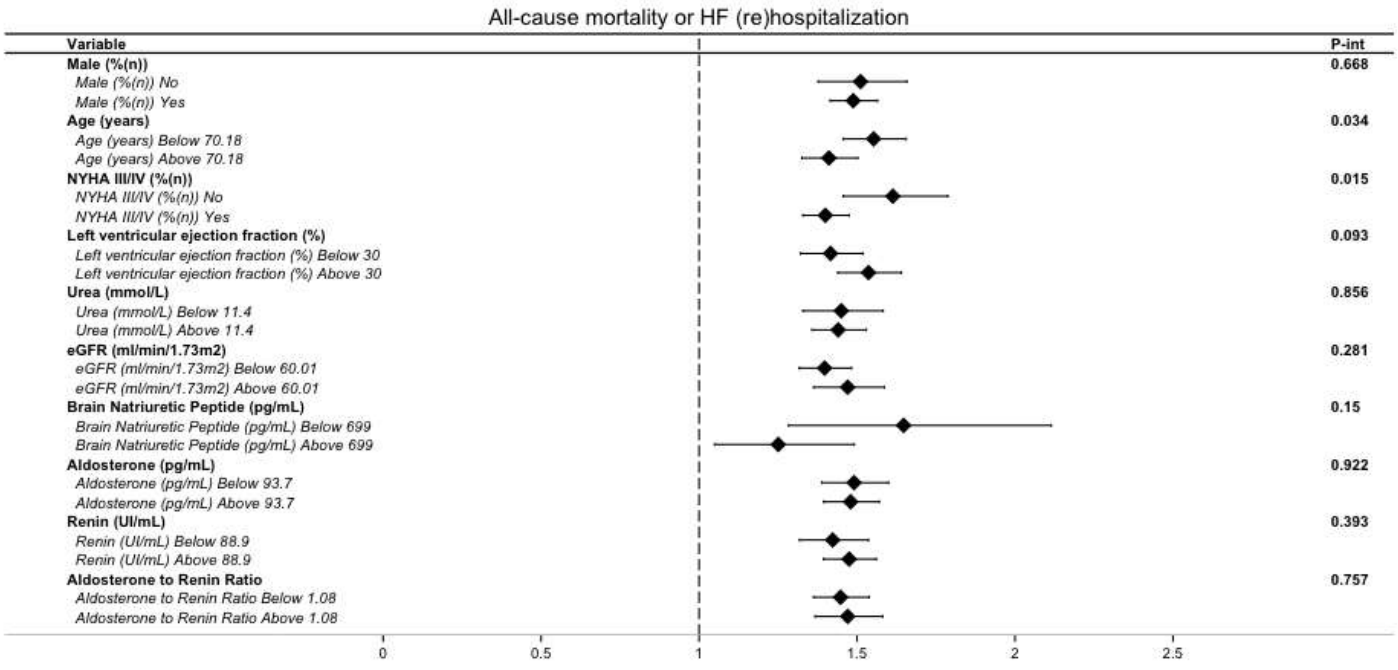


Figure 2: Subgroup analyses for the association of FGF23 with the combined endpoint



Abbreviations: eGFR: estimated Glomerular Filtration Rate; NYHA: New York Heart Association

Table 1: baseline characteristics per quintile of FGF23

Variable		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P for trend
N =		481	479	479	480	480	
FGF23		82.6 [68.2-93.7]	131.6 [117.3-147.8]	218.0 [190.2-253.3]	444.4 [364.7-579.0]	1862.0 [1118.0-3351.0]	
Demographics							
Sex (% Male(n))		80.5 (387)	74.5 (357)	76.2 (365)	65.8 (316)	70.6 (339)	<0.001
Age (years)		65.4±11.6	68.1±11.2	69.6±12	70.7±12.3	70.7±11.8	<0.001
Race (% Caucasian(n))		99.2 (477)	99.6 (477)	98.7 (473)	98.5 (473)	98.8 (474)	0.200
BMI (kg/m2)		27.8±4.6	28.2±5.4	27.7±5.8	27.8±5.5	27.8±5.8	0.720
Weight (kg)		82.3±16.1	83±17.9	81.4±19.6	80.7±18.4	81.3±19.4	0.118
Height (kg)		171.9±8.8	171.4±9	171.2±9.3	169.8±9.3	170.5±8.9	0.001
NYHA class (%(n))							<0.001
	I	5 (24)	3.5 (17)	1.7 (8)	0.8 (4)	0 (0)	
	II	52.2 (251)	41.1 (197)	34.9 (167)	28.1 (135)	17.7 (85)	
	III	35.8 (172)	44.9 (215)	48.6 (233)	51.5 (247)	61.3 (294)	
	IV	5.4 (26)	7.3 (35)	12.1 (58)	16 (77)	18.3 (88)	
LVEF (%)		31.3±8.2	31±10.5	30.4±10.7	32.2±11.3	30±11.8	0.363
HFPEF (%)		3.8 (17)	8.3 (36)	7.5 (32)	15.1 (65)	10.8 (45)	<0.001
Clinical Profile							
Edema (%(n))		9.5 (35)	22.7 (90)	24 (95)	36.4 (148)	51.7 (222)	<0.001
Orthopnea (%(n))		20.6 (99)	25.9 (124)	35.7 (171)	40.6 (194)	50.9 (244)	<0.001
Rales > 1/3 up lung fields (%(n))		12.5 (22)	20.9 (46)	21.1 (52)	17.8 (50)	22.2 (69)	0.078
Jugular venous pressure (%(n))		11.6 (36)	22.8 (74)	32.8 (104)	44.8 (138)	53.4 (171)	<0.001
Hepatomegaly (%(n))		7.5 (36)	9.8 (47)	12.6 (60)	15.7 (75)	25.1 (120)	<0.001
Third heart tone (%(n))		8.5 (41)	7.4 (35)	10 (48)	8.8 (42)	14.4 (69)	0.002

Systolic Blood Pressure (mmHg)	128±20.9	128.6±22.4	123.8±21.2	124±22.6	119.2±20.2	<0.001
Diastolic Blood Pressure (mmHg)	78±11.8	76.2±13	74.4±13.4	73.2±13.9	72.4±12.5	<0.001
Heart Rate (beats/min)	75.7±17.1	79.2±20.1	79.6±19.3	80.8±19.3	83.3±19.9	<0.001
Hospitalization						
Type of visit (%(n))						<0.001
Scheduled outpatient clinic	45.1 (217)	35.3 (169)	25.3 (121)	18.8 (90)	14 (67)	
Unscheduled outpatient clinic	9.8 (47)	5.4 (26)	4.2 (20)	3.5 (17)	4.2 (20)	
Inpatient hospitalization	45.1 (217)	59.3 (284)	70.6 (338)	77.7 (373)	81.9 (393)	
Reason for visit (%(n))						<0.001
Worsening heart failure	46.4 (223)	44.1 (211)	50.1 (240)	61.7 (296)	71.2 (342)	
New onset heart failure	28.3 (136)	34.4 (165)	30.3 (145)	24.8 (119)	19.6 (94)	
Other reason	25.4 (122)	21.5 (103)	19.6 (94)	13.5 (65)	9.2 (44)	
Diuretics iv (%(n))	95.8 (137)	99 (206)	96.6 (254)	99.1 (325)	98.6 (360)	0.074
Inotropics iv (%(n))	8.5 (12)	7.2 (15)	11.5 (30)	8.3 (27)	17.2 (63)	0.001
Nitrates iv (%(n))	34 (48)	24.6 (51)	24.8 (65)	20.2 (66)	13.9 (51)	<0.001
Heart Failure History						
Years since first diagnosis	0.4 [0.2-1.5]	3 [0.5-5.9]	0.6 [0.1-5]	3.1 [0.3-7.7]	3.9 [1-7.5]	0.287
NYHA class prior to decompensation/worsening HF (%(n))						<0.001
I	13.1 (63)	11.1 (53)	10.4 (50)	6.2 (30)	4.4 (21)	
II	52.2 (251)	46.6 (223)	43.4 (208)	44 (211)	43.8 (210)	
III	21 (101)	26.3 (126)	30.7 (147)	31.2 (150)	37.7 (181)	
IV	2.7 (13)	3.3 (16)	3.8 (18)	2.9 (14)	4.2 (20)	
Previous HF hospitalization (%(n))	24.1 (116)	26.5 (127)	34.2 (164)	35.4 (170)	39.2 (188)	<0.001
Medical History						
Hypertension (%(n))	62.8 (302)	63 (302)	63.7 (305)	63.7 (306)	58.5 (281)	0.266
Atrial fibrillation (%(n))	26 (125)	42.8 (205)	46.3 (222)	51.7 (248)	59.8 (287)	<0.001
Coronary artery disease (%(n))	41.6 (200)	40.9 (196)	44.3 (212)	49.6 (238)	49.6 (238)	0.001
Myocardial infarction (%(n))	37 (178)	34.7 (166)	38.4 (184)	40 (192)	42.1 (202)	0.027

PCI (%(n))	22.2 (107)	19.6 (94)	21.9 (105)	24.4 (117)	21.5 (103)	0.596
CABG (%(n))	13.1 (63)	14.6 (70)	15.4 (74)	20.8 (100)	22.1 (106)	<0.001
Pacemaker (%(n))	4.2 (20)	5.8 (28)	8.6 (41)	8.8 (42)	9 (43)	0.001
ICD (%(n))	7.7 (37)	5.6 (27)	5.6 (27)	9.2 (44)	12.9 (62)	<0.001
Diabetes mellitus (%(n))	27.2 (131)	30.7 (147)	29.6 (142)	36.7 (176)	37.9 (182)	<0.001
Peripheral artery disease (%(n))	6.9 (33)	9.2 (44)	10.9 (52)	14.6 (70)	14 (67)	<0.001
Medication						
ACE-inhibitors or Angiotensin receptor blockers (%(n))	78.8 (379)	76 (364)	76.4 (366)	67.1 (322)	62.1 (298)	<0.001
Target dose (%(n))	16.2 (78)	16.1 (77)	13.2 (63)	11.7 (56)	7.7 (37)	<0.001
Beta-blockers (%(n))	85.9 (413)	86.2 (413)	84.3 (404)	80.4 (386)	78.8 (378)	<0.001
Target dose (%(n))	4 (19)	4.6 (22)	5.4 (26)	7.3 (35)	5.8 (28)	0.048
Loop diuretics (%(n))	99.2 (477)	99.4 (476)	99.4 (476)	100 (480)	99.8 (479)	0.055
Aldosterone antagonists (%(n))	54.5 (262)	53.9 (258)	53.7 (257)	51.5 (247)	55.2 (265)	0.897
Laboratory values						
Hemoglobin (g/dL)	13.9 [12.9-14.8]	13.8 [12.6-14.8]	13.3 [12.1-14.6]	12.8 [11.5-14.1]	12.3 [10.9-13.7]	<0.001
Creatinine (umol/L)	87.5 [74-104]	94.4 [77.7-117.6]	100 [87-128]	113.6 [89.8-150.3]	122 [97-159.1]	0.377
Urea (mmol/L)	8.2 [6-12.9]	9.5 [6.8-15]	11.1 [7.7-17.1]	12 [8.2-19.4]	15.7 [9.6-25.3]	<0.001
eGFR (ml/min/1.73m^2)	75.9 [61.6-89.4]	64.8 [50.5-83.3]	60 [45.3-75.9]	51.2 [37.3-68.8]	47.6 [33.2-65]	<0.001
Sodium (mmol/L)	140 [138-142]	140 [138-142]	140 [138-142]	140 [137-142]	138 [136-141]	<0.001
Potassium (mmol/L)	4.3 [4-4.6]	4.3 [4-4.6]	4.2 [3.9-4.6]	4.2 [3.9-4.5]	4.2 [3.8-4.6]	0.010
BNP (pg/mL)	223.8 [81.5-503.9]	645.9 [354.5-1037.9]	637 [507.5-975]	822.5 [450.5-1517.2]	1305 [872.2-2522.5]	<0.001
NT pro-BNP (ng/L)	2365.5 [1065.8-4541.8]	3057 [1829.5-5221]	3960 [2292.5-7509.2]	5365.5 [2810.8-9914.2]	7054.5 [3397.5-11861]	<0.001
Calcium (mmol/L)	1.8 [1.6-2.1]	1.8 [1.6-2.1]	1.8 [1.5-2]	1.7 [1.5-2]	1.7 [1.4-1.9]	<0.001
Phosphate (mmol/L)	0.8 [0.7-1]	0.9 [0.7-1.1]	0.9 [0.7-1]	0.9 [0.7-1]	0.9 [0.6-1.1]	0.353
Albumine (g/L)	35 [31-40]	34 [29-40]	33 [28-38]	31 [26-36]	30 [24-34]	<0.001
Aldosterone (pg/mL)	94.9 [44-194.5]	93 [45-167]	92 [41.4-190]	88 [43-185]	106.5 [43-257]	<0.001

Renin (UI/mL)	62.8 [23.4-186.1]	81.2 [24-205.7]	90 [29.2-259.4]	95.4 [34-255.2]	134.8 [37.4-436.1]	<0.001

Abbreviations: BMI: Body Mass Index; BNP: Blood Natriuretic Peptide; CABG: Coronary Artery Bypass Graft; FGF23: Fibroblast Growth Factor 23; eGFR: estimated Glomerular Filtration Rate; HF: Heart Failure; HFpEF: Heart Failure with a Preserved Ejection Fraction; ICD: Implantable Cardiac Defibrillator; LVEF: Left Ventricular Ejection Fraction; NT-proBNP: N-terminal pro Blood Natriuretic Peptide; NYHA: New York Heart Association; PCI: Percutaneous Coronary Intervention.

Table 2: Multivariable model for log FGF23

Variable	Beta Coeff	95% CI	T value	*P-value*
BNP per SD	0.387	0.33-0.44	14.511	<0.001
eGFR per SD	-0.221	-0.28--0.17	-7.945	<0.001
Atrial fibrillation	0.366	0.27-0.47	7.170	<0.001
Edema	0.417	0.30-0.53	7.085	<0.001
Hemoglobin per SD	-0.187	-0.24--0.13	-6.589	<0.001
Aldosterone per SD	0.146	0.10-0.20	5.800	<0.001
ICD	0.519	0.34-0.70	5.594	<0.001
Albumin per SD	-0.328	-0.46--0.20	-4.994	<0.001
Calcium per SD	0.306	0.18-0.43	4.845	<0.001
NYHA III or IV	0.201	0.09-0.31	3.586	<0.001
Hepatomegaly	0.226	0.09-0.36	3.224	0.001
LVEF per SD	-0.066	-0.12--0.01	-2.496	0.013
Systolic blood pressure per SD	-0.066	-0.12--0.01	-2.444	0.015
Female sex	0.144	0.03-0.26	2.435	0.015
HF hospitalization	0.151	0.03-0.27	2.404	0.016
Heart rate per SD	0.062	0.01-0.11	2.396	0.017
Third heart tone	0.190	0.03-0.35	2.383	0.017

r^2=0.466

Abbreviations: BNP: Blood Natriuretic Peptide; eGFR: estimated Glomerular Filtration Rate; HF: Heart Failure; ICD: Implantable Cardiac Defibrillator; LVEF: Left Ventricular Ejection Fraction; NYHA: New York Heart Association Class; SD: Standard Deviation.

Table 3: FGF23 and ACEi/ARB use after 3 months of uptitration

	ACEi/ARB use		**Target dose**	
Log FGF23	OR (CI)	P-value	OR (CI)	P-value
Univariable	0.62 (0.57-0.68)	<0.001	0.69 (0.62-0.76)	<0.001
Multivariable*	0.79 (0.70-0.90)	<0.001	0.76 (0.65-0.87)	<0.001

*****Corrected for age, sex, eGFR, aldosterone, renin, calcium, albumin, phosphate, NYHA class, and ACEi/ARB use at baseline

Abbreviations: ACEi: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; CI: confidence interval; FGF23: Fibroblast Growth Factor 23; OR: odds ratio

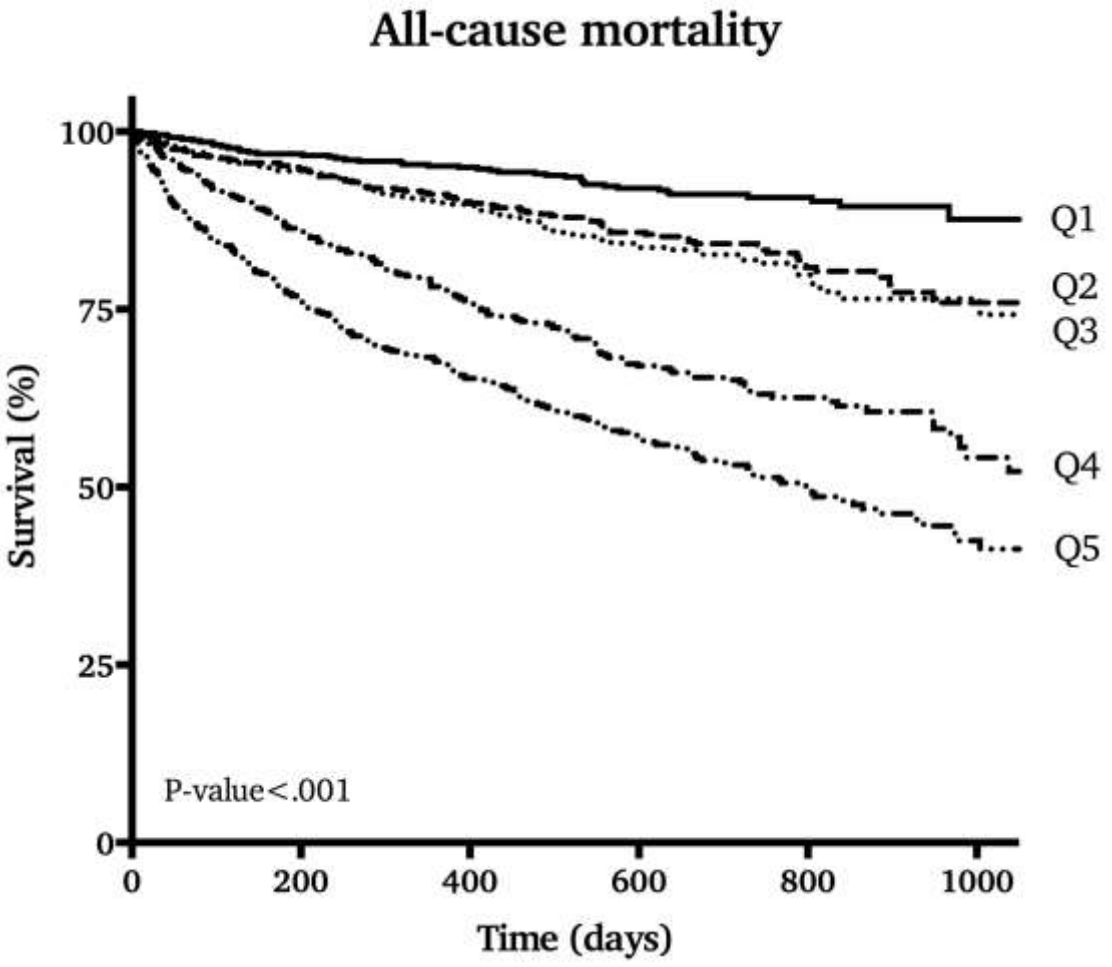
Table 4: Cox regression analysis for FGF23, and all-cause mortality, heart failure rehospitalization, and the combined endpoint

	All-cause mortality or HF hospitalization				**All-cause mortality**			
	Univariable		Multivariable*		Univariable		Multivariable*	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
Log FGF23	1.49 (1.43-1.56)	<0.001	1.15 (1.08-1.22)	<0.001	1.57 (1.49-1.66)	<0.001	1.17 (1.09-1.26)	<0.001
Quintile 1	1.0 (Reference)	Ref	1.0 (Reference)	Ref	1.0 (Reference)	Ref	1.0 (Reference)	Ref
Quintile 2	1.58 (1.22-2.06)	<0.001	1.31 (0.99-1.74)	0.059	1.93 (1.34-2.78)	<0.001	1.57 (1.06-2.34)	0.025
Quintile 3	2.28 (1.78-2.93)	<0.001	1.36 (1.03-1.79)	0.031	2.16 (1.51-3.11)	<0.001	1.25 (0.84-1.87)	0.268
Quintile 4	3.31 (2.60-4.21)	<0.001	1.56 (1.19-2.06)	0.002	4.56 (3.27-6.35)	<0.001	1.94 (1.33-2.84)	<0.001
Quintile 5	5.35 (4.24-6.75)	<0.001	1.95 (1.47-2.58)	<0.001	6.89 (4.99-9.51)	<0.001	2.10 (1.43-3.08)	<0.001

*****Corrected for the BIOSTAT risk model with addition of calcium, albumin, renin, aldosterone, phosphate, and estimated Glomerular Filtration Rate.

Abbreviations: FGF23: Fibroblast Growth Factor 23; HF: heart failure; HR: hazard ratio; ref: reference

Supplementary figure 1: Kaplan Meier survival curve for all-cause mortality according to quintiles of FGF23



Supplementary table 1: FGF23 and beta-blocker use after 3 months of uptitration

	Beta-blocker use		**Target dose**	
Log FGF23	OR (CI)	P-value	OR (CI)	P-value
Univariable	0.79 [0.72-0.88]	<0.001	0.92 (0.83-1.08)	0.112
Multivariable*	0.93 (0.47-1.08)	0.359	0.90 [0.77-1.05]	0.197

*****Corrected for age, sex, eGFR, aldosterone, renin, calcium, albumin, phosphate, NYHA class, and beta-blocker use at baseline

Abbreviations: CI: confidence interval; FGF23: Fibroblast Growth Factor 23; OR: odds ratio