# 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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## Abstract

### Background

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

### Methods

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 adjusted for potential confounders.

### Results

Median FGF23 was 218.0 [IQR: 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 an ACE-inhibitor (ACEi) or angiotensin receptor blocker (ARBs) at baseline (all $P<0.01$). Higher FGF23 levels were independently associated with higher BNP, lower eGFR, the presence of oedema and atrial fibrillation (all $P<0.001$). In addition, higher FGF23 was independently associated with impaired uptitration of ACEi/ARBs after 3 months, 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, $P<0.001$).

### Conclusions

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

**Key words:** heart failure, FGF23, volume overload, prognosis

## 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.[1] In patients with chronic kidney disease (CKD), higher FGF23 levels have been consistently associated with an increased risk of cardiovascular morbidity and mortality.[2,3] FGF23 seems particularly strongly linked with heart failure [4], supported by mechanistic studies indicating that FGF23 contributes to the development of left ventricular hypertrophy (25), regulates renal sodium reabsorption and plasma volume and interacts with renin-angiotensin-aldosterone system (RAAS) activation.[5-7] Furthermore, high FGF23 levels were associated with an impaired response to sodium restriction and ACE-inhibition in CKD patients.[8]

Recently, elevated levels of FGF23 have been associated with cardiovascular mortality and incident heart failure in patients with stable ischemic heart disease.[9] In chronic heart failure, it has been suggested that FGF23 is associated with disease severity and adverse outcome.[10-13] Data on FGF23 in worsening or acute heart failure 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 heart failure. 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) As such, or as a consequence of the suspected association with congestion and more severe heart failure, FGF23 might have an effect on the ability to receive ACEi/angiotensin receptor blocker (ARB) therapy, a first line therapy in patients with heart failure. Therefore we additionally studied whether FGF23 is associated with impaired uptitration of ACEi/ARB. Based on the above, 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.[14,15] In brief, the BIOSTAT-CHF trial is a large European, multicenter, multinational, prospective, observational study in which 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 enrolled. The trial was approved by the local ethics committee at each participating centre and complies with the declaration of Helsinki. 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.[16,17] Subsequently, patients were contacted every 6 months by telephone. At 9 months, reasons for not reaching the target dose were recorded. These unfortunately were most commonly described as "other" reason making this too unspecific for further analyses. 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%) available baseline plasma samples using a human C-terminal FGF23 ELISA (Immutopics, Inc., San Clemente, CA, USA). For the missing 117 patients (4.7%) no baseline plasma samples were available. More details on this specific assay, such as inter-/intra-assay variation have been described previously.[18] Using this assay, in a cohort of 3,107 community-living persons≥65 years of age median FGF23 was 70 [51-100] RU/mL.[19] 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) in plasma samples that had previously undergone one freeze/thaw cycle. 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.[20]

**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 (using natural logarithm) 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).[21] The following variables were included in the BIOSTAT risk model for all-cause mortality: age, urea, NT-proBNP, haemoglobin, and use of beta-blocker at baseline.[21] The BIOSTAT risk model for HF hospitalization includes age, previous HF hospitalization, peripheral oedema, systolic blood pressure, and eGFR.[21] Finally, the risk model for the combined endpoint includes age, previous HF hospitalization, peripheral oedema, systolic blood pressure, NT-proBNP, haemoglobin, HDL, sodium, and use of beta-blocker at baseline.[21] 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. For outcome analyses using data on ACEi/ARB use at 3 months, the outcome analyses were censored at 3 months. 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

Of the 2,399 patients enrolled, 1605 (67%) were hospitalized at the time of enrolment. 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 (i.e. being in a higher quintile of FGF23) 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). The prevalence of all signs and symptoms of congestion, i.e. oedema, orthopnoea, jugular venous pressure (JVP), 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 supplementary table 1. The multivariable model had an overall $r^2$ of 0.459, and the variables showing the strongest association with higher log FGF23 levels were higher NT-proBNP, the presence of atrial fibrillation and the presence of oedema. 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). Furthermore, higher FGF23 levels were also inversely associated with ACEi/ARB use and dosage after 3 months of uptitration (table 2). 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 2).

*FGF23 and outcomes*

During a median follow-up of 21 [16-27] months, 631 out of the 2399 patients (26%) died, and 588 patients (25%) were (re)hospitalized for HF. A total of 981 patients (41%) experienced the combined endpoint of all-cause mortality and/or HF hospitalization. In univariable Cox regression analysis, an increase in 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 where patients in the highest three quintiles of FGF23 had a significantly increased risk of the combined endpoint, compared to the lowest (first) quintile (table 3). The Kaplan Meier curve for the combined endpoint per quintile of FGF23 is presented in figure 1, and supplementary figure 1 shows the hazard ratio for FGF23 for the combined endpoint using a penalized spline function. Hazard ratios were consistent across subgroups based on ejection fraction, renal function, and RAAS activation (supplementary figure 2). There was also no significant difference in prognostic value in patients with new-onset or worsening heart failure or ischemic aetiology. There was a significant interaction with age, NYHA class, and new-onset or worsening heart failure, yet consistently across these subgroups higher FGF23 levels were also associated with a higher risk of the combined endpoint.

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) and with cardiovascular mortality in uni- and multivariable analyses (supplementary table 3). The Kaplan Meier per quintile of FGF23 for all-cause mortality is shown in supplementary figure 3. The association of higher FGF23 with outcome for both all-cause mortality and the combined endpoint 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 comparable to that for NT-proBNP, yet significantly higher (univariable Harrell's c-statistic: 0.689 vs. 0.679, respectively, *P*<.001). Based on ROC analysis, the optimal cut-off of FGF23 for the prediction of all-cause mortality

is 315.2 RU/mL (AUC: 0.705). When FGF23 is added to a prognostic model that includes NT-proBNP, LVEF, ischemic etiology, age and sex, there was a significant gain in c-index, yet no significant change in NRI (supplementary table 4).

## Discussion

In patients with new-onset and 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 heart failure, with more signs of congestion, a greater activation of the renin-angiotensin-aldosterone activation and worse renal function, yet the associations with outcomes were partly 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.[22] FGF23 is deregulated in a relatively early stage of CKD, contributing to abnormalities in bone and mineral metabolism.[23] 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.[24,25] These adverse effects of FGF23 are most likely beyond its direct effects on phosphate. This is suggested by the observation that hyperphosphatemia is associated with vascular calcification and ischemic cardiac events, whereas FGF23 is associated with HF and not with vascular calcifications.[4,26] Preclinical data demonstrated that FGF23 infusion leads to the development of left ventricular hypertrophy.[5] This may at least in part be corroborated with a role for FGF23 as a regulator of sodium homeostasis, through the sodium-chloride co-transporter in the distal tubule.[6] Also, recent data from a prospective observational study showed that FGF23 was independently

associated with LV remodelling after myocardial infarction.[27] In the current study we demonstrate that FGF23 levels were elevated in patients with worsening HF (median: 218 RU/mL) compared to previous levels reported in a community cohort (median: 70 RU/mL), and that higher FGF23 levels were associated with more signs of congestion.[19] Remarkably, all signs and symptoms of volume overload and congestion, i.e. oedema, orthopnoea, rales, elevated JVP and hepatomegaly were more common in patients with higher FGF23 levels. Also, in the multivariable model for FGF23, higher NT-proBNP, oedema, hepatomegaly, and lower haemoglobin 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 cannot be concluded from our observational data. It is noteworthy that in a recent study FGF23 did not markedly change in response to volume interventions that may suggest that FGF23 is a cause rather than a consequence of volume overload.[28] Plasma FGF23 levels were only measured at baseline in our study, and therefore we were not able to determine changes in plasma 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.[29,30] 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; moreover, higher plasma 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.[31] 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 association was not observed for beta-blocker therapy. 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, although 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 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 heart failure are in line with three small studies in stable chronic heart failure patients.[10-12] 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.[32] 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.[32] We recently published a risk score that was derived from BIOSTAT-CHF that included 42 demographic, clinical and biochemical variables.[21] FGF23 provided significant additive predictive value of adverse outcome on top of this risk score.

Several mechanisms may explain the link between FGF23 and adverse outcome. FGF23 might increase fluid retention, and incomplete decongestion is associated with higher rehospitalisation rates and poorer outcome. Also, higher FGF23 might reflect patients with more severe renal dysfunction, a strong predictor of outcome in HF. The association with outcome was however partly 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.[33] As FGF23 stimulates RAAS activity, this may cause left ventricular remodelling, volume overload, and subsequently adverse outcome. Additionally, higher levels of FGF23 may also indicate a more advanced disease status, as suggested by a longer disease duration, and the association with increased RAAS activation, which has shown to be an independent predictor of poor outcome in HF patients with chronic kidney disease.[34] Finally, FGF23 may prove to be a modifiable risk factor, as illustrated by a recent study in haemodialysis patients, showing that treatment with the calcimimetic cinacalcet lowered FGF23 levels by about 50%, in association with lower risks of cardiovascular mortality and events.[35] 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, multicentre, 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, both new-onset and worsening heart failure patients, as well as in- and outpatients were enrolled in our study. However, in subgroup analyses there was no difference in prognostic value. Data during hospitalization for

heart failure and repeated measures of FGF23 and other biomarkers 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, hypervolemia, 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 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 were associated with more severe heart failure, volume overload, worse renal function and an increased risk of all-cause mortality and heart failure hospitalization in patients with new-onset and worsening heart failure. In addition, higher plasma FGF23 levels are independently associated with less successful uptitration of guideline recommended ACEi/ARB therapy.

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## Conflicts of Interest (in alphabetical order):

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## References


[1] Kovesdy CP, Quarles LD. Fibroblast growth factor-23: what we know, what we don't know, and what we need to know. Nephrol Dial Transplant 2013; 28:2228-2236.

[2] Isakova T, Xie H, Yang W*, et al.* Fibroblast growth factor 23 and risks of mortality and end-stage renal disease in patients with chronic kidney disease. JAMA 2011; 305:2432-2439.

[3] Gutierrez OM, Mannstadt M, Isakova T*, et al.* Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 2008; 359:584-592.

[4] Scialla JJ, Xie H, Rahman M*, et al.* Fibroblast growth factor-23 and cardiovascular events in CKD. J Am Soc Nephrol 2014; 25:349-360.

[5] Faul C, Amaral AP, Oskouei B*, et al.* FGF23 induces left ventricular hypertrophy. J Clin Invest 2011; 121:4393-4408.

[6] Andrukhova O, Slavic S, Smorodchenko A*, et al.* FGF23 regulates renal sodium handling and blood pressure. EMBO Mol Med 2014; 6:744-759.

[7] de Borst MH, Vervloet MG, ter Wee PM, Navis G. Cross talk between the renin-angiotensin-aldosterone system and vitamin D-FGF-23-klotho in chronic kidney disease. J Am Soc Nephrol 2011; 22:1603-1609.

[8] Humalda JK, Lambers Heerspink HJ, Kwakernaak AJ*, et al.* Fibroblast growth factor 23 and the antiproteinuric response to dietary sodium restriction during renin-angiotensin-aldosterone system blockade. Am J Kidney Dis 2015; 65:259-266.

[9] Udell JA, Morrow DA, Jarolim P*, et al.* Fibroblast growth factor-23, cardiovascular prognosis, and benefit of angiotensin-converting enzyme inhibition in stable ischemic heart disease. J Am Coll Cardiol 2014; 63:2421-2428.

[10] Gruson D, Lepoutre T, Ketelslegers JM, Cumps J, Ahn SA, Rousseau MF. C-terminal FGF23 is a strong predictor of survival in systolic heart failure. Peptides 2012; 37:258-262.

[11] Poelzl G, Trenkler C, Kliebhan J*, et al.* FGF23 is associated with disease severity and prognosis in chronic heart failure. Eur J Clin Invest 2014; 44:1150-1158.

[12] Plischke M, Neuhold S, Adlbrecht C*, et al.* Inorganic phosphate and FGF-23 predict outcome in stable systolic heart failure. Eur J Clin Invest 2012; 42:649-656.

[13] Wohlfahrt P, Melenovsky V, Kotrc M*, et al.* Association of Fibroblast Growth Factor-23 Levels and Angiotensin-Converting Enzyme Inhibition in Chronic Systolic Heart Failure. JACC Heart Fail 2015; 3:829-839.

[14] Voors AA, Anker SD, Cleland JG*, et al.* A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. Eur J Heart Fail 2016; 18:716-726.

[15] Ouwerkerk W, Voors AA, Anker SD*, et al.* Determinants and clinical outcome of uptitration of ACE-inhibitor and beta-blocker in patients with heart failure: a preospective European study. European heart journal 2016; Article in press.

[16] McMurray JJ, Adamopoulos S, Anker SD*, et al.* ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur Heart J 2012; 33:1787-1847.

[17] Ponikowski P, Voors AA, Anker SD*, et al.* 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur Heart J 2016.

[18] Heijboer AC, Levitus M, Vervloet MG*, et al.* Determination of fibroblast growth factor 23. Ann Clin Biochem 2009; 46:338-340.

[19] Ix JH, Katz R, Kestenbaum BR*, et al.* Fibroblast growth factor-23 and death, heart failure, and cardiovascular events in community-living individuals: CHS (Cardiovascular Health Study). J Am Coll Cardiol 2012; 60:200-207.

[20] Levey AS, Stevens LA, Schmid CH*, et al.* A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150:604-612.

[21] Voors AA, Ouwerkerk W, Zannad F*, et al.* Development and validation of multivariate models to predict mortality and hospitalization in patients with heart failure. Submitted .

[22] Shimada T, Kakitani M, Yamazaki Y*, et al.* Targeted ablation of Fgf23 demonstrates an essential physiological role of FGF23 in phosphate and vitamin D metabolism. J Clin Invest 2004; 113:561-568.

[23] Isakova T, Wahl P, Vargas GS*, et al.* Fibroblast growth factor 23 is elevated before parathyroid hormone and phosphate in chronic kidney disease. Kidney Int 2011; 79:1370-1378.

[24] Scialla JJ, Wolf M. Roles of phosphate and fibroblast growth factor 23 in cardiovascular disease. Nat Rev Nephrol 2014; 10:268-278.

[25] Baia LC, Humalda JK, Vervloet MG*, et al.* Fibroblast growth factor 23 and cardiovascular mortality after kidney transplantation. Clin J Am Soc Nephrol 2013; 8:1968-1978.

[26] Scialla JJ, Lau WL, Reilly MP*, et al.* Fibroblast growth factor 23 is not associated with and does not induce arterial calcification. Kidney Int 2013; 83:1159-1168.

[27] Reindl M, Reinstadler SJ, Feistritzer HJ*, et al.* Fibroblast growth factor 23 as novel biomarker for early risk stratification after ST-elevation myocardial infarction. Heart 2016.

[28] Humalda JK, Seiler-Muler S, Kwakernaak AJ*, et al.* Response of fibroblast growth factor 23 to volume interventions in arterial hypertension and diabetic nephropathy. Medicine (Baltimore) 2016; 95:e5003.

[29] Ix JH, Shlipak MG, Wassel CL, Whooley MA. Fibroblast growth factor-23 and early decrements in kidney function: the Heart and Soul Study. Nephrol Dial Transplant 2010; 25:993-997.

[30] Gutierrez OM, Januzzi JL, Isakova T*, et al.* Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. Circulation 2009; 119:2545-2552.

[31] Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002; 110:229-238.

[32] Koller L, Kleber ME, Brandenburg VM*, et al.* Fibroblast Growth Factor 23 Is an Independent and Specific Predictor of Mortality in Patients With Heart Failure and Reduced Ejection Fraction. Circ Heart Fail 2015.

[33] Parker BD, Schurgers LJ, Brandenburg VM*, et al.* The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul Study. Ann Intern Med 2010; 152:640-648.

[34] Poletti R, Vergaro G, Zyw L, Prontera C, Passino C, Emdin M. Prognostic value of plasma renin activity in heart failure patients with chronic kidney disease. Int J Cardiol 2013; 167:711-715.

[35] Moe SM, Chertow GM, Parfrey PS*, et al.* Cinacalcet, Fibroblast Growth Factor-23, and Cardiovascular Disease in Hemodialysis: The Evaluation of Cinacalcet HCl Therapy to Lower Cardiovascular Events (EVOLVE) Trial. Circulation 2015; 132:27-39.

## 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**

## 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** | 5.0 (24) | 3.5 (17) | 1.7 (8) | 0.8 (4) | 0.0 (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 |
| **Interventricular wall thickness (mm)** | | 10.4±2.2 | 10.7±2.4 | 10.5±2.6 | 11.0±2.6 | 10.6±2.6 | 0.163 |
| **Posterior wall thickness (mm)** | | 10.2±2.0 | 10.2±2.2 | 10.4±2.2 | 10.5±2.7 | 10.3±2.2 | 0.269 |
| **Clinical Profile** | | | | | | | |
| **Oedema (%(n))** | | 9.5 (35) | 22.7 (90) | 24 (95) | 36.4 (148) | 51.7 (222) | <0.001 |
| **Orthopnoea (%(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 |
| **Inotropes 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** | | | | | | |
| **Haemoglobin (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 (ng/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-proBNP (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 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Albumin (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: 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 3: Cox regression analysis for FGF23, and all-cause mortality, heart failure rehospitalisation, and the combined endpoint**

| | **All-cause mortality or HF hospitalization** | | | | | **All-cause mortality** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Univariable | | Multivariable* | | | Univariable | | Multivariable* | |
| | Number of events (%(n)) | HR (95% CI) | P-value | HR (95% CI) | P-value | Number of events (%(n)) | HR (95% CI) | P-value | HR (95% CI) | P-value |
| **Log FGF23** | 981 (41%) | 1.49 (1.43-1.56) | <0.001 | 1.15 (1.08-1.22) | <0.001 | 631 (26) | 1.57 (1.49-1.66) | <0.001 | 1.17 (1.09-1.26) | <0.001 |
| | | | | | | | | | | |
| **Quintile 1** | 19.3 (93) | 1.0 (Reference) | Ref | 1.0 (Reference) | Ref | 9.4 (45) | 1.0 (Reference) | Ref | 1.0 (Reference) | Ref |
| **Quintile 2** | 29.4 (141) | 1.58 (1.22-2.06) | <0.001 | 1.31 (0.99-1.74) | 0.059 | 17.5 (84) | 1.93 (1.34-2.78) | <0.001 | 1.57 (1.06-2.34) | 0.025 |
| **Quintile 3** | 39.2 (188) | 2.28 (1.78-2.93) | <0.001 | 1.36 (1.03-1.79) | 0.031 | 19.6 (94) | 2.16 (1.51-3.11) | <0.001 | 1.25 (0.84-1.87) | 0.268 |
| **Quintile 4** | 49.6 (238) | 3.31 (2.60-4.21) | <0.001 | 1.56 (1.19-2.06) | 0.002 | 36 (173) | 4.56 (3.27-6.35) | <0.001 | 1.94 (1.33-2.84) | <0.001 |
| **Quintile 5** | 66.9 (321) | 5.35 (4.24-6.75) | <0.001 | 1.95 (1.47-2.58) | <0.001 | 49 (235) | 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*