ESC HEART FAILURE
ESC Heart Failure 2019; **6**: 536–544
Published online 25 March 2019 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.12416

ORIGINAL RESEARCH ARTICLE

Bone marrow and plasma FGF-23 in heart failure patients: novel insights into the heart–bone axis

Beatrice von Jeinsen[1,4,5], Kateryna Sopova[1,5], Lars Palapies[1], David M. Leistner[1,2,6], Stephan Fichtlscherer[1,5], Florian H. Seeger[1,5], Jörg Honold[1,5], Stefanie Dimmeler[3,5], Birgit Aßmus[1,5], Andreas M. Zeiher[1,5] and Till Keller[1,4,5]*

[1]*Division of Cardiology, Department of Internal Medicine III, Goethe University Frankfurt, Frankfurt, Germany;* [2]*Campus Benjamin Franklin, Department of Cardiology, Charité – Universitaetsmedizin Berlin, Berlin, Germany;* [3]*Institute for Cardiovascular Regeneration, Goethe University Frankfurt, Frankfurt, Germany;* [4]*Department of Cardiology, Kerckhoff Heart and Thorax Center, Benekestr. 2-8, 61231, Bad Nauheim, Germany;* [5]*German Centre for Cardiovascular Research (DZHK), partner site RheinMain, Frankfurt, Germany;* [6]*German Centre for Cardiovascular Research (DZHK), partner site Berlin, Berlin, Germany*

Abstract

Aims Fibroblast growth factor 23 (FGF-23) is known to be elevated in patients with congestive heart failure (CHF). As FGF-23 is expressed in the bone but can also be expressed in the myocardium, the origin of serum FGF-23 in CHF remains unclear. It is also unclear if FGF-23 expressed in the bone is associated with outcome in CHF. The aim of the present study was to investigate FGF-23 levels measured in bone marrow plasma (FGF-23-BM) and in peripheral blood (FGF-23-P) in CHF patients to gain further insights into the heart–bone axis of FGF-23 expression. We also investigated possible associations between FGF-23-BM as well as FGF-23-P and outcome in CHF patients.

Methods and results We determined FGF-23-P and FGF-23-BM levels in 203 CHF patients (85% male, mean age 61.3 years) with a left ventricular ejection fraction (LVEF) ≤45% and compared them with those of 48 healthy controls (48% male, mean age 39.2 years). We investigated the association between FGF-23-BM and FGF-23-P with all-cause mortality in CHF patients, 32 events, median follow-up 1673 days, interquartile range [923, 1828]. FGF-23-P (median 60.3 vs. 22.0 RU/mL, $P < 0.001$) and FGF-23-BM (median 130.7 vs. 93.1 RU/mL, $P < 0.001$) levels were higher in CHF patients compared with healthy controls. FGF-23-BM levels were significantly higher than FGF-23-P levels in both CHF patients and in healthy controls ($P < 0.001$). FGF-23-P and FGF-23-BM correlated significantly with LVEF ($r = -0.37$ and $r = -0.33$, respectively), N terminal pro brain natriuretic peptide levels ($r = 0.57$ and $r = 0.6$, respectively), New York Heart Association status ($r = 0.28$ and $r = 0.25$, respectively), and estimated glomerular filtration rate ($r = -0.43$ and $r = -0.41$, respectively) (P for all <0.001) and were independently associated with all-cause mortality in CHF patients after adjustment for LVEF, estimated glomerular filtration rate, New York Heart Association status, and N terminal pro brain natriuretic peptide, hazard ratio 2.71 [confidence interval: 1.18–6.20], $P = 0.018$, and hazard ratio 2.80 [confidence interval: 1.19–6.57], $P = 0.018$, respectively.

Conclusions In CHF patients, FGF-23 is elevated in bone marrow plasma and is independently associated with heart failure severity and all-cause mortality. The failing heart seems to interact via FGF-23 within a heart–bone axis.

Keywords Fibroblast growth factor; Heart failure; Outcome; Risk prediction

Received: 10 September 2018; Accepted: 17 January 2019
**Correspondence to: Till Keller, Department of Cardiology, Kerckhoff Heart and Thorax Center, Benekestr. 2-8, 61231 Bad Nauheim, Germany. Phone: +49 6032 996 2864; Fax: +49 6032 996 2399. Email: keller@chestpain.de*

Introduction

Fibroblast growth factor 23 (FGF-23) plays an important role in phosphate and vitamin D homeostasis and bone mineralization. FGF-23 is expressed in the bone by osteocytes and osteoblasts, and, in a so-called bone–kidney axis, its expression is stimulated by high serum phosphate or high vitamin D levels.[1,2] In patients with chronic kidney disease (CKD), circulating FGF-23 is elevated.[3,4]

An association between increased FGF-23 levels and mortality in patients suffering from CKD is well appreciated,[5,6] and several authors observed an association between FGF-23 and outcome in the general population[7,8] and under

several pathological conditions, including congestive heart failure (CHF).[9–11]

The underlying mechanism resulting in increased FGF-23 levels in CHF patients, the origin of FGF-23 in CHF (bone vs. myocardium), and the effect of FGF-23 on the cardiovascular system are incompletely understood.[9–11] While Klotho is essential for the effects of FGF-23 on the kidney, the effects of FGF-23 on the heart seem to be Klotho independent. Klotho itself is not associated with outcome in CHF patients.[10,12–14]

To gain further insights into the origin and effect of FGF-23 in CHF patients, this study investigated the distribution of FGF-23 levels both in peripheral blood (FGF-23-P) and in bone marrow plasma (FGF-23-BM) in CHF patients compared with healthy controls. Further, potential correlations between FGF-23-P or FGF-23-BM and CHF severity and the association between FGF-23-P or FGF-23-BM and all-cause mortality were evaluated. We hypothesized that FGF-23 levels are increased in CHF patients and associated with unfavourable outcome, while there are no associations between Klotho and outcome in CHF patients.

Methods

Study sample

The study sample of this retrospective analysis comprised 237 patients with systolic CHF [defined as left ventricular ejection fraction (LVEF) $\leq$45%] and 50 healthy individuals (controls) who were enrolled in the Frankfurt Bone Marrow-Derived Cell Therapy Registry. The patients and controls were included in studies assessing the effect of intracoronary bone marrow-derived mononuclear cell infusion for treatment of chronic heart failure (ClinicalTrials.gov; accession numbers: NCT 00289822, NCT 00284713, NCT 00326989, and NCT 00962364).

All patients gave written informed consent. The local ethics committee approved the study protocols, and the study was conducted in accordance with the Declaration of Helsinki.

Of the 237 CHF patients, 34 were excluded due to missing FGF-23 levels, leaving 203 CHF patients who were eligible for further analysis. Of the 50 control individuals, one individual was excluded due to missing FGF-23-P levels and one due to missing information on sex. Forty-eight controls were eligible for further analysis.

Follow-up and clinical characteristics

Patients were followed up by annual telephone contact or outpatient visit. The primary endpoint was defined as all-cause mortality and was confirmed by reviewing death certificates or contacting relatives or family physicians as previously described.[15] Median follow-up was 1673 days, interquartile range (IQR) [923, 1828].

The clinical characteristics obtained at baseline presentation were age, sex, height, weight, presence of hypertension, presence of hypercholesterolaemia, presence of diabetes, smoking status, and New York Heart Association (NYHA) class. LVEF was assessed by quantitative echocardiography as previously described.[16]

Laboratory analysis

Standard assays were applied to measure plasma cholesterol, triglycerides, HDL, LDL, creatinine, glutamic-oxaloacetic transaminase, glutamate pyruvate transaminase, and lactate dehydrogenase. The estimated glomerular filtration rate (eGFR) was calculated based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[17] N terminal pro brain natriuretic peptide (NT-proBNP) was measured by commercially available standard assays (ELECSYS2010 analyser; F. Hoffmann-La Roche Diagnostics, Basel, Switzerland) as described previously.[16]

Analysis of fibroblast growth factor 23 and Klotho

Bone marrow plasma and peripheral blood samples were obtained as described previously.[16] C terminal FGF-23-BM and FGF-23-P were measured by ELISA [Immutopics Inc. (San Clemente, California, USA) 2nd Generation Human Fibroblast Growth Factor 23 ELISA Kit; Reader: Biotek Synergy HT (Biotek Instruments, Inc., Winooski, Vermont, USA) with detection range of 1.5–1375 RU/mL]. FGF-23-P levels under the limit of detection were imputed with 0.005 RU/mL (n = 15 controls). For some analyses, we calculated the ratio between FGF-23-BM and FGF-23-P levels (FGF-23-BM/P). In controls, FGF-23-P levels under the limit of detection were excluded in calculating this ratio.

Klotho-BM and Klotho-P were measured by ELISA [IBL (Immuno-Biological Laboratories Co., Ltd, Japan) Human soluble alpha-Klotho ELISA Kit; Reader: Biotek Synergy HT (Biotek Instruments, Inc.) with detection range of 93.75–6000 pg/mL]. FGF-23 and Klotho measurements were carried out using frozen samples by experienced technical staff blinded to patient's characteristics.

Statistical analysis

Baseline characteristics with normal distribution were characterized by arithmetic mean and standard deviation (SD), whereas skewed variables were described by median and IQR. FGF-23-BM, FGF-23-P, Klotho-BM, and Klotho-P levels were compared between controls and patients and between location of assessment (Mann–Whitney U test). Pearson's

DOI: 10.1002/ehf2.12416

rank correlations analysis was used to investigate correlations between FGF-23-BM, FGF-23-P, Klotho-BM, and Klotho-P and several covariates (age, sex, Klotho-P, Klotho-BM, LVEF, eGFR, NYHA class, NT-proBNP levels, hypertension, smoking, and diabetes).

Cut-offs representing the 97.5% percentile or 2.5% percentile were derived based on the bone marrow plasma and peripheral blood levels of FGF-23 and Klotho of healthy controls. Kaplan–Meier estimator analysis was carried out to estimate event-free survival in CHF patients stratified by the cut-off levels derived from healthy controls.

For linear regression and Cox regression analyses, we created a complete cases subsample of 146 CHF patients with all used covariates (see succeeding texts). FGF-23-BM, FGF-23-P, Klotho-BM, and Klotho-P levels were transformed with natural logarithms due to their skewed distribution and standardized (to a mean of 0 and an SD of 1). We examined the associations between covariates showing a significant correlation with FGF-23-P or FGF-23-BM using linear regression, and based on the number of covariates, the *P*-value was corrected for multiple testing (Bonferroni).

The association between FGF-23-BM and FGF-23-P as categorical variables (cut-offs as described earlier) with all-cause mortality was examined using multivariable proportional hazards regression (Cox) models. Cox regression analysis was carried out unadjusted, adjusted for age and sex, and adjusted for age, sex, and additional covariates (LVEF, eGFR, NYHA class, and NT-proBNP) and in a fully adjusted model (adjusted for all covariates).

In multiple hypothesis testing (FGF-23-BM and FGF-23-P), a Bonferroni-corrected *P*-value <0.025 ($P < 0.05/2$) was considered significant. Secondary analyses were carried out for FGF-23-P and FGF-23-BM per SD increase and for FGF-23-BM/P, Klotho-P, and Klotho-BM.

If not stated differently, *P* values <0.05 were considered significant. All analyses were carried out using the R 3.4.1 software package (R Foundation for Statistical Computing, Vienna, Austria).

Results

Baseline clinical characteristics

The clinical characteristics of 203 patients with CHF included in the study are presented in *Table 1*. The majority of CHF patients were male (85%), and they presented with a mean age of 61.3 years, a mean LVEF of 30.8%, and a median NT-proBNP level of 954 ng/L (*Table 1*).

In contrast to CHF patients, men and women were equally distributed in control subjects (48% male, $P < 0.001$), and controls were significantly younger than CHF patients (mean age 39.2 years, $P < 0.001$).

Table 1 Clinical characteristics

Characteristics	Data available (*n*)	Systolic heart failure patients
Age (years)	202/203	61.3 (12.2)
Male sex	203/203	85%
Height (cm)	183/203	174.0 (8.2)
Weight (kg)	183/203	84.5 (16.7)
Hypertension	199/203	73%
Hypercholesterolaemia	194/203	73%
Diabetes	198/203	34%
Smoking	196/203	21%
NYHA class	200/203	
NYHA I		22%
NYHA II		39.5%
NYHA III		34.5%
NYHA IV		4%
LVEF (%)	181/203	30.8 (9.9)
eGFR (mL/min)	201/203	68.62 [50.54, 88.44]
NT-proBNP (ng/L)	166/203	954 [426, 1995]
Cholesterol (mg/dL)	187/203	163 [131, 187]
LDL (mg/dL)	173/203	79 [60, 109]
HDL (mg/dL)	181/203	45 [38, 54]
TG (mg/dL)	186/203	125 [95, 181]
GOT (U/L)	194/203	29 [25, 37]
GPT (U/L)	189/203	29 [21, 42]
LDH (U/L)	198/203	211 [181, 250]

Clinical characteristics of 203 patients suffering from systolic congestive heart failure. Continuous variables summarized by mean (SD) or median [interquartile range] as appropriate; binary variables presented as percentage. eGFR, estimated glomerular filtration rate; GOT, glutamic-oxaloacetic transaminase; GPT, glutamate pyruvate transaminase; LDH, lactate dehydrogenase; LVEF, left ventricular ejection fraction; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association heart failure class; TG, triglycerides.

Fibroblast growth factor 23 and Klotho in peripheral blood vs. bone marrow plasma

In both patients and controls, FGF-23 levels measured in bone marrow plasma were higher than the levels measured in peripheral blood ($P < 0.001$ for both) (*Table 2*). There were no differences in Klotho levels measured in peripheral blood or bone marrow plasma in CHF patients ($P = 0.09$) or healthy controls ($P = 0.135$) (Supporting Information, *Table S1*).

Fibroblast growth factor 23 and Klotho in patients vs. controls

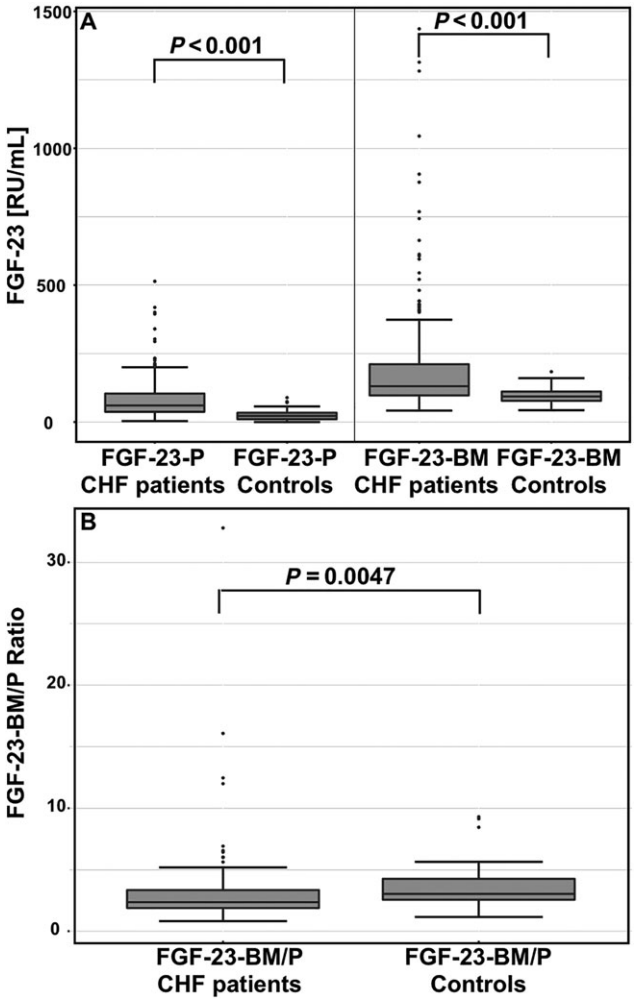
Congestive heart failure patients had significantly higher FGF-23 levels in plasma (median 60.3 vs. 22.0 RU/mL, $P < 0.001$) and bone marrow (median 130.7 vs. 93.1 RU/mL, $P < 0.001$) than controls (*Figure 1A*, Supporting Information, *Figure S1*). In CHF patients, the FGF-23-BM levels were approximately 2.4 times higher than the FGF-23-P levels, median ratio 2.4, IQR [1.9, 3.3], whereas in controls, the FGF-23-BM levels were 3 times higher than the FGF-23-P levels, median ratio

Table 2 FGF-23-P and FGF-23-BM levels in congestive heart failure patients and healthy controls

	Peripheral blood	Bone marrow plasma	*P*-value
FGF-23 (patients) (RU/mL)	60.3 [37.1, 103.6]	130.7 [97.1, 210.8]	<0.001
FGF-23 (controls) (RU/mL)	22.0 [10.1, 33.5]	93.1 [77.4, 111.1]	<0.001

Fibroblast growth factor 23 (FGF-23) levels measured in bone marrow plasma compared with levels measured in peripheral blood in patients with systolic congestive heart failure and healthy control individuals (controls). Data are displayed as median [interquartile range].

Figure 1 Distribution of fibroblast growth factor 23 (FGF-23) (A) levels measured in bone marrow plasma (BM) and peripheral blood (P) as well as FGF-23-BM/P ratio (B) in patients with systolic congestive heart failure (CHF patients) compared with healthy control individuals.



3.0, IQR [2.6, 4.4]. This difference in the FGF-23-BM/P ratio between CHF patients and controls was statistically significant (P = 0.005, *Figure 1B*).

Congestive heart failure patients had significantly lower Klotho-P levels (674 vs. 903 pg/mL, $P < 0.001$) and significantly lower Klotho-BM levels (634.12 vs. 851.78 pg/mL, $P < 0.001$) than controls (Supporting Information, *Figures S1 and S2*).

Clinical characteristics stratified by fibroblast growth factor 23 and Klotho levels

We stratified the clinical characteristics of CHF patients based on elevated or lowered FGF-23 and Klotho levels. The used threshold levels for FGF-23-P (73.75 RU/mL) and FGF-23-BM (158.07 RU/mL) represent the 97.5% percentile and for Klotho-P (509.2 pg/mL) and Klotho-BM (471.1 pg/mL) as well as for the FGF-23BM/P ratio (1.54) represent the 2.5% percentile concentration derived from our healthy controls.

Patients with increased FGF-23-P and FGF-23-BM levels were older, presented with higher NT-proBNP levels, had a lower LVEF, and had impaired renal function (Supporting Information, *Table S2A*). Patients who presented with an FGF-23-BM/P ratio less than 1.54 had higher NT-proBNP and FGF-23-P levels (Supporting Information, *Table S2B*).

Congestive heart failure patients with higher Klotho-P levels had higher Klotho-BM levels, and there were slightly more patients with lower Klotho-P levels in NYHA class II. Aside from these findings, however, there were no other differences between CHF patients with respect to Klotho-P or Klotho-BM levels (Supporting Information, *Table S3*).

Analysis of fibroblast growth factor 23 and Klotho by Pearson's correlation

FGF-23-P and FGF-23-BM levels were highly correlated, but only FGF-23-P correlated with the FGF-23-BM/P ratio. None of the measurements for FGF-23 correlated with either Klotho-P or Klotho-BM (*Table 3*). FGF-23-P and FGF-23-BM levels did not correlate with sex, hypertension, smoking, or diabetes and only weakly with age. We observed stronger correlations with impaired renal function measured by eGFR, NT-proBNP, LVEF, and NYHA class (*Table 3*). Klotho-P and Klotho-BM were strongly correlated with each other and showed a weak correlation with eGFR but not with any of the other covariates (Supporting Information, *Table S4*).

Association between elevated fibroblast growth factor 23 and covariates

FGF-23-P and FGF-23-BM levels above the defined cut-off values were associated with an older age, impaired renal function, higher NT-proBNP levels, and impaired LVEF and

DOI: 10.1002/ehf2.12416

Table 3 Correlation analysis between FGF-23-P, FGF-23-BM, and FGF-23-BM/P levels and clinical variables in congestive heart failure patients

	FGF-23-P		FGF-23-BM		Ratio	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
FGF-23-P	**1**	**<0.0001**	**0.87**	**<0.0001**	**−0.20**	**0.004**
FGF-23-BM	**0.87**	**<0.0001**	**1**	**<0.0001**	0.01	0.86
Klotho-P	0.02	0.79	0.04	0.53	−0.05	0.45
Klotho-BM	−0.02	0.84	0.04	0.60	−0.02	0.82
Age	**0.18**	**0.0090**	**0.22**	**0.002**	0.06	0.41
Sex	0.01	0.86	−0.001	0.99	−0.1	0.15
LVEF	**−0.37**	**<0.0001**	**−0.33**	**<0.0001**	0.06	0.40
eGFR	**−0.43**	**<0.0001**	**−0.41**	**<0.0001**	0.04	0.55
NYHA	**0.28**	**<0.0001**	**0.25**	**<0.0001**	−0.07	0.33
NT-proBNP	**0.57**	**<0.0001**	**0.60**	**<0.0001**	0.03	0.75
Hypertension	0.05	0.51	0.06	0.44	0.02	0.76
Smoking	−0.06	0.40	−0.07	0.32	−0.07	0.32
Diabetes	0.05	0.46	0.07	0.30	−0.05	0.47

Partial correlation coefficients (*r*) showing the correlations between FGF-23-P, FGF-23-BM, and FGF-23-BM/P ratio and several covariates. eGFR, estimated glomerular filtration rate; FGF-23-BM, fibroblast growth factor 23 measured in bone marrow plasma; FGF-23-P, fibroblast growth factor 23 measured in peripheral blood; Klotho-BM, alpha Klotho measured in bone marrow plasma; Klotho-P, alpha Klotho measured in peripheral blood; LVEF, left ventricular ejection fraction; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association heart failure class; Ratio, fibroblast growth factor 23 measured in bone marrow plasma/fibroblast growth factor 23 measured in peripheral blood. *P*-value considered significant ($P < 0.05$) are bolded.

were also correlated with age, eGFR, NT-proBNP, and LVEF. We investigated whether FGF-23-P and FGF-23-BM were independently associated with these covariates in a linear regression. FGF-23-P was independently associated with eGFR, which was statistically significant (Supporting Information, *Table S5*). There was also an association with LVEF and NT-proBNP, not reaching statistical significance after adjusting for multiple testing. In contrast, FGF-23-BM was independently and statistically significant associated with eGFR, LVEF, and NT-proBNP (Supporting Information, *Table S5*).

Association between fibroblast growth factor 23 and prognosis

In Kaplan–Meier estimator analysis stratified by cut-offs derived from our healthy controls, CHF patients with elevated FGF-23-P and FGF-23-BM had a lower survival probability (*Figure 2A* and *B*). There were no differences in survival with respect to Klotho-P, Klotho-BM, or the FGF-23-BM/P ratio (*Figure 2C*, Supporting Information, *Figure S3*).

Therefore, Cox regression analysis was carried out in primary analysis for FGF-23-P and FGF-23-BM only (n = 145, events = 32).

FGF-23-P and FGF-23-BM were both associated with all-cause mortality (treated as a categorical variable) when unadjusted and when adjusted for age and sex (*Table 4*). After additional adjustment for LVEF, eGFR, NYHA status, and NT-proBNP, FGF-23-P and FGF-23-BM remained significantly associated with all-cause mortality. Even in the fully adjusted model that included all covariates jointly, both FGF-23-P and FGF-23-BM remained significantly associated with all-cause mortality (using the Bonferroni corrected *P*-value for multiple testing).

Treated as continuous variables, FGF-23-P and FGF-23-BM showed a weaker association with all-cause mortality per SD increase in a secondary analysis (Supporting Information, *Table S6*). We also investigated the associations between the FGF-23-BM/P ratio (Supporting Information, *Table S7*) and Klotho-P and Klotho-BM levels (Supporting Information, *Table S8*) and all-cause mortality in secondary analyses but did not observe significant associations.

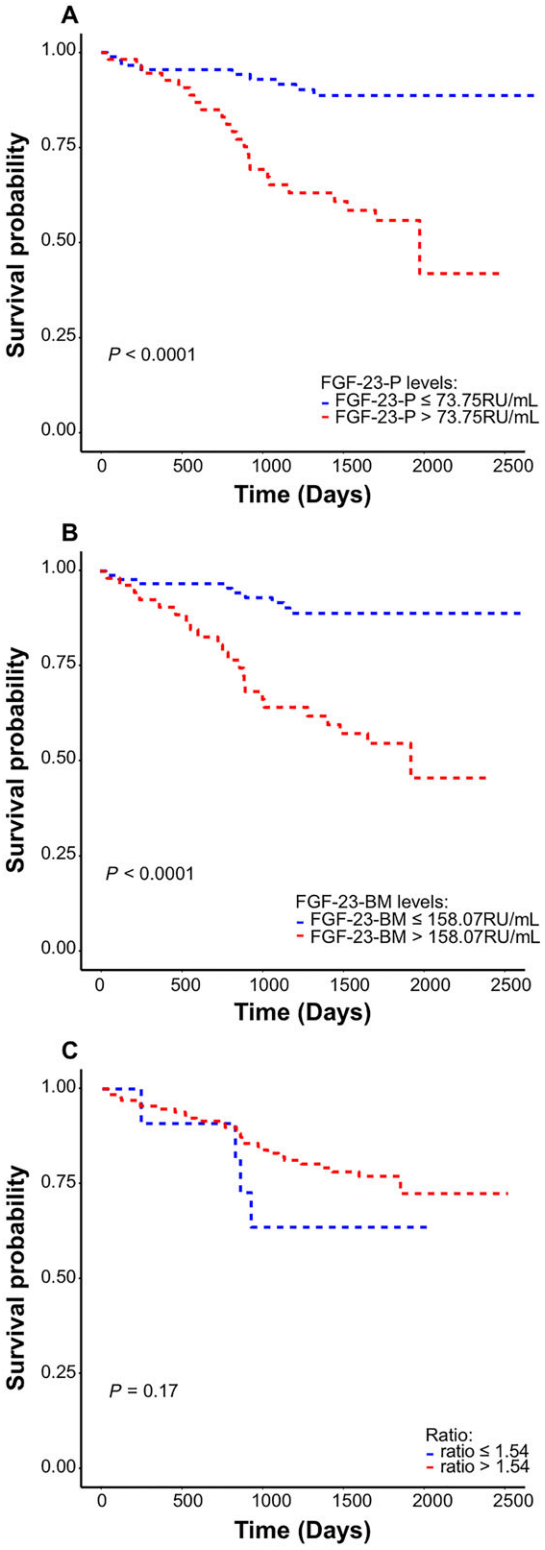
Discussion

We evaluated FGF-23 levels in peripheral blood and in bone marrow plasma of CHF patients compared with healthy individuals. We confirmed previous reports that FGF-23 levels in peripheral blood are increased in CHF patients[9–11,18] and showed for the first time that FGF-23 levels measured in bone marrow plasma are also elevated in CHF patients.

FGF-23-P and FGF-23-BM levels correlated with markers of heart failure severity (NYHA class, NT-proBNP, and LVEF) and of renal dysfunction (eGFR) and were both strong and independent risk predictors for all-cause mortality in CHF patients, especially if cut-off levels derived from healthy controls were used to stratify CHF patients.

In contrast, the FGF-23-BM/P ratio was not correlated or associated with markers of renal dysfunction, heart failure severity, or outcome in CHF patients. Similarly, neither Klotho-P nor Klotho-BM was associated with heart failure severity or outcome, which is in line with previous reports and underlines observations that FGF-23 effects on the heart are Klotho independent.[10,13,14,19,20]

Figure 2 Kaplan–Meier curves showing event-free survival (all-cause mortality). *N* = 145, events *N* = 32. (A) Patients stratified by fibroblast growth factor 23 (FGF-23) levels measured in peripheral blood (FGF-23-P). Cut-off derived from healthy control individuals. FGF-23-P levels ≤73.75 RU/mL, *n* = 89 (*n* = 9 events); FGF-23-P levels >73.75 RU/mL, *n* = 56 (*n* = 23 events). (B) Patients stratified by FGF-23 levels measured in bone marrow plasma (FGF-23-BM). Cut-off derived from healthy control individuals. FGF-23-BM levels ≤158.07 RU/mL, *n* = 91 (*n* = 9 events); FGF-23-BM levels >158.07 RU/mL, *n* = 54 (*n* = 23 events). (C) Patients stratified by FGF-23-BM/FGF-23-P ratio. Cut-off derived from healthy control individuals. FGF-23-BM/FGF-23-P ratio ≤1.54, *n* = 13 (*n* = 4 events); FGF-23-BM/FGF-23-P ratio >1.54, *n* = 132 (*n* = 28 events).



DOI: 10.1002/ehf2.12416

Table 4 Associations between FGF-23-P and FGF-23-BM and all-cause mortality

	FGF-23-P		FGF-23-BM	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Unadjusted	4.88 (2.25–10.56)	<0.001*	5.11 (2.36–11.05)	<0.001*
Age, sex	4.51 (2.05–9.90)	<0.001*	4.77 (2.16–10.53)	<0.001*
Age, sex, LVEF	3.23 (1.47–7.13)	0.004*	3.14 (1.39–7.133)	0.006*
Age, sex, eGFR	4.04 (1.75–9.29)	0.001*	4.28 (1.87–9.78)	<0.001*
Age, sex, NYHA	3.76 (1.68–8.41)	0.002*	4.29 (1.92–9.56)	<0.001*
Age, sex, NT-proBNP	4.06 (1.81–9.09)	<0.001*	4.29 (1.90–9.92)	<0.001*
Fully adjusted	2.71 (1.18–6.20)	0.018*	2.80 (1.19–6.57)	0.018*

Risk estimates for FGF-23-P and FGF-23-BM treated as a categorical variable (stratified by cut-off levels derived in a healthy population) unadjusted and adjusted for age and sex and additional covariates with respect to all-cause mortality; $n = 145$, events = 32. CI, confidence interval; eGFR, estimated glomerular filtration rate; FGF-23-P, fibroblast growth factor 23 measured in peripheral blood; FGF-23-BM, fibroblast growth factor 23 measured in bone marrow plasma; LVEF, left ventricular ejection fraction; NT-proBNP, N terminal pro brain natriuretic peptide; NYHA, New York Heart Association heart failure class; HR, hazard ratio.
*Indicates Bonferroni corrected *P*-value (for multiple testing) considered significant $P < 0.025$ ($P < 0.05/2$).

It was surprising that the FGF-23-BM/P ratio was lower in CHF patients than in controls, as FGF-23 is primarily expressed in the bone.[1,2] This might indicate that the organs other than the bone express FGF-23 in CHF, leading to relatively higher levels of FGF-23 in the circulation. Leifheit-Nestler *et al*. showed that the myocardium in patients with end-stage renal disease expresses FGF-23, and Richter *et al*. showed that FGF-23 production in cardiomyocytes can be stimulated *in vitro*.[21,22] In contrast, in a small study conducted by Andersen *et al*., FGF-23 expression in the myocardium was similar in patients with acute heart failure and in healthy controls, causing the authors to speculate that the bone might be the origin of increased FGF-23 levels in patients with acute heart failure due to a heart–bone feedback mechanism.[23]

In our investigation, the FGF-23-BM/P ratio was not correlated with markers of heart failure severity. Thus, even if FGF-23 expression takes place in the heart in CHF patients, leading to a smaller FGF-23-BM/P ratio, this expression is not correlated with heart failure severity. As FGF-23 in bone marrow plasma was significantly higher than FGF-23 in peripheral blood and was associated with heart failure severity, we hypothesize that the main origin of FGF-23 in CHF patients in our study was still the bone.

These results raise the question of how the heart 'talks' to the bone, leading to an increased expression of FGF-23 in CHF patients. It is possible that the increase in FGF-23 levels in CHF patients simply mirrors a worsened renal circulation. In a study of patients with systolic heart failure, FGF-23 levels were associated with an impaired glomerular perfusion, and the authors suggested that FGF-23 might be increased due to venous stasis in the kidney leading to an increase in FGF-23 levels in CHF patients, even in patients with a normal eGFR.[10]

Even though FGF-23-P and FGF-23-BM levels were correlated with each other and were associated with a lower eGFR in our investigation, they were also associated with a worsened LVEF and increased NT-proBNP levels independent of eGFR and, therefore, might—at least partly—reflect a worsened cardiac function independent of an impairment of renal function, which is supported by previous investigations. In humans, FGF-23 levels increase shortly after patients go into cardiogenic shock and predict outcome in these patients.[24] In rodents, FGF-23 increases to a high level after myocardial infarction, whereas serum phosphate and parathyroid hormone levels do not change, and vitamin D decreases.[25] Under these conditions, FGF-23 expression in rodents was up-regulated in the bone and—to a lesser extent—in the myocardium. The findings that FGF-23 increases shortly after cardiogenic shock independent of phosphate, parathyroid hormone, and vitamin D levels and that the FGF-23 expression is increased in the bone indicate that in heart failure, the heart might directly stimulate FGF-23 expression in the bone independent of renal dysfunction.

Two recently published review articles have proposed a potential heart–bone feedback mechanism for FGF-23, by which the injured heart might increase FGF-23 expression in the bone via pro-inflammatory cytokines.[13,26] Another possible pathway by which the heart stimulates FGF-23 expression in the bone might be sympathetic activation.[27]

Although it is not yet clear why FGF-23 levels are increased in CHF patients, these increased FGF-23 levels are associated with poor outcome. The underlying mechanisms are still a matter of debate; some authors speculate that FGF-23 reflects imbalances in bone mineralization and is therefore related to worse outcome in CHF patients[9] or that FGF-23 only reflects high serum phosphate and low vitamin D levels that are responsible for the worsened outcome.[18]

Previous investigations, however, showed that FGF-23 is not associated with bone disease in CHF patients and its association with outcome is independent of serum phosphate and vitamin D levels,[10,11,28] so FGF-23 might target the heart directly. In fact, FGF-23 causes left ventricular hypertrophy in animal models[19,29] and is independently associated with incidence and prevalence of ventricular hypertrophy in CKD patients[19,28] and in elderlies.[30]

We demonstrated here that FGF-23 levels are associated with NT-proBNP and LVEF in an independent manner and that FGF-23 is a prognostic indicator of all-cause mortality in CHF patients. This indicates that FGF-23 has a direct effect

on the cardiovascular system in CHF patients; however, further investigations are needed to uncover underlying pathophysiological mechanisms.

To the best of our knowledge, our study is the first to derive cut-off levels for FGF-23 in healthy controls. Interestingly, applying these cut-offs in CHF patients yielded a better predictive value than if FGF-23 had been used as a continuous variable per SD increase. As FGF-23 seems to be a better risk predictor if treated categorically, it might be of interest in future investigations to derive FGF-23 cut-off levels in larger populations, e.g. population-based cohorts.

The study has several potential limitations. The sample size of the healthy controls was small, so the cut-off levels derived here may not be representative of the general population; also, as the CHF patients were older than the controls, the FGF-23 levels might only be partially comparable between the two groups. There is also a lack of a control group of CHF patients not undergoing cell therapy. Furthermore, because only 15% of the CHF patients were women, our results might not be representative for women. Of note, the percentage of men in previous studies investigating FGF-23 levels in CHF patients was also high and similar to the percentage in our study.[9,10]

It has to be considered that by drawing bone marrow plasma, peripheral blood is also drawn, and therefore, the FGF-23 levels measured in bone marrow plasma might be contaminated by FGF-23 in peripheral blood.

However, as the FGF-23-BM levels were significantly higher than FGF-23-P levels, at least a significant proportion of the FGF-23 was probably derived from the bone marrow plasma itself. It would have been of interest if FGF-23-P and FGF-23-BM are associated with cardiovascular outcomes as well. Unfortunalty, we were not able to investigate further endpoints. FGF-23 is known first and foremost as a marker of phosphate and vitamin D metabolism and is closely linked to parathyroid hormone levels.[12] Unfortunately, we were not able to investigate the association between FGF-23 and these parameters; however, previous studies have indicated that FGF-23 is associated with outcome independent of phosphate and vitamin D.[11,28] Our study could not add information on a potential mechanistic link between FGF-23-P and FGF-23-BM. It is also unclear if FGF-23-BM is only secreted by osteocytes and osteoblasts or might also be secreted by bone marrow mononuclear cells. Further investigations are needed to analyse the mechanistics in FGF-23 secretion in the bone.

Conclusion

Fibroblast growth factor 23 levels are increased in CHF patients, they are higher if measured in bone marrow plasma than in peripheral blood, and they are independently associated with all-cause mortality. Our data suggest that the failing heart leads to increased bone FGF-23 expression.

As FGF-23 is independently associated with an unfavourable outcome in CHF patients, this heart–bone axis might be explored as a target of therapeutic agents in future studies.

Acknowledgement

The authors gratefully acknowledge Elizabeth Martinson for competent proof reading.

Conflict of interest

None declared.

Funding

None.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Klotho-P and Klotho-BM Levels in CHF Patients and Controls.
Table S2A Clinical Characteristics Stratified by FGF-23 Levels in Peripheral Blood and Bone Marrow Plasma.
Table S2B Clinical Characteristics Stratified by FGF-23-BM/P Ratio.
Table S3 Clinical Characteristics Stratified by Klotho Levels.
Table S4 Correlation between Klotho Measured in Peripheral Blood and Bone Marrow Plasma and Clinical Variables.
Table S5 Associations between Age, Renal Function, LVEF and NT-pro BNP levels and FGF-23-P and FGF-23-BM in CHF Patients.
Table S6 Associations between FGF-23-P and FGF-23-BM and All-cause Mortality – Treated as Continuous Variable – Secondary Analysis.
Table S7 Associations between FGF-23-BM/P and All-cause Mortality – Secondary Analysis.
Table S8 Associations between Klotho-P and Klotho-BM and All-cause Mortality – Secondary Analysis.
Figure S1 Regression Analysis for FGF-23-BM and FGF-23-P and Klotho-BM and Klotho-P in CHF Patients and Healthy Controls.
Figure S2 Distribution of Klotho-P and Klotho-BM levels in CHF patients and healthy controls.
Figure S3 Kaplan-Meier Analysis – Klotho.

References

1. Riminucci M, Collins MT, Fedarko NS, Cherman N, Corsi A, White KE, Waguespack S, Gupta A, Hannon T, Econs MJ, Bianco P, Robey PG. FGF-23 in fibrous dysplasia of bone and its relationship to renal phosphate wasting. *J Clin Invest* 2003; **112**: 683–692.
2. Liu S, Quarles LD. How fibroblast growth factor 23 works. *J Am Soc Nephrol* 2007; **18**: 1637–1647.
3. Shimada T, Urakawa I, Isakova T, Yamazaki Y, Epstein M, Wesseling-Perry K, Wolf M, Salusky IB, Jüppner H. Circulating fibroblast growth factor 23 in patients with end-stage renal disease treated by peritoneal dialysis is intact and biologically active. *J Clin Endocrinol Metab* 2010; **95**: 578–585.
4. Isakova T, Wahl P, Vargas G, Gutiérrez OM, Scialla J, Xie H, Appleby D, Nessel L, Bellovich K, Chen J, Hamm L, Gadegbeku C, Horwitz E, Townsend RR, Anderson CAM, Lash JP, Hsu C, Leonard MB, Wolf M. FGF23, PTH and phosphorus metabolism in the chronic renal insufficiency cohort. *Kidney Int* 2011; **79**: 1370–1378.
5. Negri AL. Fibroblast growth factor 23: associations with cardiovascular disease and mortality in chronic kidney disease. *Int Urol Nephrol* 2014; **46**: 9–17.
6. Yang H, Luo H, Tang X, Zeng X, Yu Y, Ma L, Fu P. Prognostic value of FGF23 among patients with end-stage renal disease: a systematic review and meta-analysis. *Biomark Med* 2016; **10**: 547–556.
7. Souma N, Isakova T, Lipiszko D, Sacco RL, Elkind MSV, DeRosa JT, Silverberg SJ, Mendez AJ, Dong C, Wright CB, Wolf M. Fibroblast growth factor 23 and cause-specific mortality in the general population: the Northern Manhattan Study. *J Clin Endocrinol Metab* 2016; **101**: 3779–3786.
8. Lutsey PL, Alonso A, Selvin E, Pankow JS, Michos ED, Agarwal SK, Loehr LR, Eckfeldt JH, Coresh J. Fibroblast growth factor-23 and incident coronary heart disease, heart failure, and cardiovascular mortality: the atherosclerosis risk in communities study. *J Am Heart Assoc* 2014: 1–12.
9. Plischke M, Neuhold S, Adlbrecht C, Bielesz B, Shayganfar S, Bieglmayer C, Szekeres T, Hörl WH, Strunk G, Vavken P, Pacher R, Hülsmann M. Inorganic phosphate and FGF-23 predict outcome in stable systolic heart failure. *Eur J Clin Invest* 2012; **42**: 649–656.
10. Wohlfahrt P, Melenovsky V, Kotrc M, Benes J, Jabor A, Franekova J, Lemaire S, Kautzner J, Jarolim P. Association of fibroblast growth factor-23 levels and angiotensin-converting enzyme inhibition in chronic systolic heart failure. *JACC Hear Fail* 2015; **3**: 829–839.
11. Gruson D, Lepoutre T, Ketelslegers J-M, Cumps J, Ahn SA, Rousseau MF. C-terminal FGF23 is a strong predictor of survival in systolic heart failure. *Peptides* Elsevier Inc 2012; **37**: 258–262.
12. Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, Fujita T, Fukumoto S, Yamashita T. Klotho converts canonical FGF receptor into a specific receptor for FGF23. *Nature* 2006; **444**: 770–774.
13. Faul C. Cardiac actions of fibroblast growth factor 23. *Bone* Elsevier Inc 2017; **100**: 69–79.
14. Seiler S, Rogacev KS, Roth HJ, Shafein P, Emrich I, Neuhaus S, Floege J, Fliser D, Heine GH. Associations of FGF-23 and sklotho with cardiovascular outcomes among patients with CKD stages 2-4. *Clin J Am Soc Nephrol* 2014; **9**: 1049–1058.
15. Honold J, Derosa S, Spyridopoulos I, Fischer-Rasokat U, Seeger FH, Leistner D, Lotz S, Levy WC, Zeiher AM, Assmus B. Comparison of the Seattle Heart Failure Model and cardiopulmonary exercise capacity for prediction of death in patients with chronic ischemic heart failure and intracoronary progenitor cell application. *Clin Cardiol* 2013; **36**: 153–159.
16. Leistner DM, Seeger FH, Fischer A, Röxe T, Klotsche J, Iekushi K, Seeger T, Assmus B, Honold J, Karakas M, Badenhoop K, Frantz S, Dimmeler S, Zeiher AM. Elevated levels of the mediator of catabolic bone remodeling rankl in the bone marrow environment link chronic heart failure with osteoporosis. *Circ Heart Fail* 2012; **5**: 769–777.
17. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI, Kusek JW, Eggers P, Van LF, Greene T, Coresh J. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604–612.
18. Seiler S, Cremers B, Rebling NM, Hornof F, Jeken J, Kersting S, Steimle C, Ege P, Fehrenz M, Rogacev KS, Scheller B, Bhm M, Fliser D, Heine GH. The phosphatonin fibroblast growth factor 23 links calciumphosphate metabolism with left-ventricular dysfunction and atrial fibrillation. *Eur Heart J* 2011; **32**: 2688–2696.
19. Faul C, Amaral AP, Oskouei B, Hu M-C, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St. John Sutton M, Ojo A, Gadegbeku C, Di MGS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro-O M, Kusek JW, Keane MG, Wolf M. FGF23 induces left ventricular hypertrophy. *J Clin Invest* 2011; **121**: 4393–4408.
20. Grabner A, Amaral AP, Schramm K, Singh S, Sloan A, Yanucil C, Li J, Shehadeh LA, Hare JM, David V, Martin A, Fornoni A, Di MGS, Kentrup D, Reuter S, Mayer AB, Pavenstädt H, Stypmann J, Kuhn C, Hille S, Frey N, Leifheit-Nestler M, Richter B, Haffner D, Abraham R, Bange J, Sperl B, Ullrich A, Brand M, Wolf M, Faul C. Activation of cardiac fibroblast growth factor receptor 4 causes left ventricular hypertrophy. *Cell Metab* 2015; **22**: 1020–1032.
21. Leifheit-Nestler M, große Siemer R, Flasbart K, Richter B, Kirchhoff F, Ziegler WH, Klintschar M, Becker JU, Erbersdobler A, Aufricht C, Seeman T, Fischer D-C, Faul C, Haffner D. Induction of cardiac FGF23/FGFR4 expression is associated with left ventricular hypertrophy in patients with chronic kidney disease. *Nephrol Dial Transplant* 2016; **31**: 1088–1099.
22. Richter M, Polyakova V, Gajawada P, Pöling J, Warnecke H, Braun T, Walther T, Kubin T. Oncostatin M induces FGF23 expression in cardiomyocytes. *J Clin Exp Cardiolog* 2012; S9:003.
23. Andersen IA, Huntley BK, Sandberg SS, Heublein DM, Burnett JC. Elevation of circulating but not myocardial FGF23 in human acute decompensated heart failure. *Nephrol Dial Transplant* 2016; **31**: 767–772.
24. Pöss J, Mahfoud F, Seiler S, Heine GH, Fliser D, Böhm M, Link A. FGF-23 is associated with increased disease severity and early mortality in cardiogenic shock. *Eur Heart J Acute Cardiovasc Care* 2013; **2**: 211–218.
25. Andrukhova O, Slavic S, Odörfer KI, Erben RG. Experimental myocardial infarction upregulates circulating fibroblast growth factor-23. *J Bone Miner Res* 2015; **30**: 1831–1839.
26. Grabner A, Faul C. The role of fibroblast growth factor 23 and Klotho in uremic cardiomyopathy. *Curr Opin Nephrol Hypertens* 2016; **25**: 314–324.
27. Kawai M, Kinoshita S, Shimba S, Ozono K, Michigami T. Sympathetic activation induces skeletal *Fgf23* expression in a circadian rhythm-dependent manner. *J Biol Chem* 2014; **289**: 1457–1466.
28. Gutierez OM, Januzzi JL, Isakova T, Laliberte K, Smith K, Collerone G, Sarwar A, Hoffmann U, Coglianese E, Christenson R, Wang TJ, DeFilippi C, Wolf M. Fibroblast growth factor 23 and left ventricular hypertrophy in chronic kidney disease. *Circulation* 2009; **119**: 2545–2552.
29. Touchberry CD, Green TM, Tchikrizov V, Mannix JE, Mao TF, Carney BW, Girgis M, Vincent RJ, Wetmore LA, Dawn B, Bonewald LF, Stubbs JR, Wacker MJ. FGF23 is a novel regulator of intracellular calcium and cardiac contractility in addition to cardiac hypertrophy. *AJP Endocrinol Metab* 2013; **304**: E863–E873.
30. Mirza MAI, Larsson A, Melhus H, Lind L, Larsson TE. Serum intact FGF23 associate with left ventricular mass, hypertrophy and geometry in an elderly population. *Atherosclerosis* 2009; **207**: 546–551.