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ORIGINAL RESEARCH ARTICLE

Fibroblast growth factor 23: a biomarker of fibrosis and prognosis in heart failure with preserved ejection fraction

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Abstract

Aims Besides regulating calcium-phosphate metabolism, fibroblast growth factor 23 (FGF-23) has been associated with incident heart failure (HF) and left ventricular hypertrophy. However, data about FGF-23 in HF and preserved ejection fraction (HFpEF) remain limited. The aim of this study was to assess the association between FGF-23 levels, clinical and imaging characteristics, particularly diffuse myocardial fibrosis, and prognosis in HFpEF patients.

Methods and results We prospectively included 143 consecutive HFpEF patients (78 ± 8 years, 61% female patients) and 31 controls of similar age and gender (75 ± 6 years, 61% female patients). All subjects underwent a complete two-dimensional echocardiography and cardiac magnetic resonance with extracellular volume (ECV) assessment by T1 mapping. FGF-23 was measured at baseline. Among the patients, differences in clinical and imaging characteristics across tertiles of FGF-23 levels were analysed with a trend test across the ordered groups. Patients were followed over time for a primary endpoint of all-cause mortality and first HF hospitalization and a secondary endpoint of all-cause mortality. Median FGF-23 was significantly higher in HFpEF patients compared with controls of similar age and gender (247 [115; 548] RU/mL vs. 61 [51; 68] RU/mL, $P < 0.001$). Among HFpEF patients, higher FGF-23 levels were associated with female sex, higher incidence of atrial fibrillation, lower haemoglobin, worse renal function, and higher N terminal pro brain natriuretic peptide levels (P for trend < 0.05 for all). Regarding imaging characteristics, patients with higher FGF-23 levels had greater left atrial volumes, worse right ventricular systolic function, and more fibrosis estimated by ECV (P for trend < 0.05 for all). FGF-23 was moderately correlated with ECV ($r = 0.46$, $P < 0.001$). Over a mean follow-up of 30 ± 8 months, 43 patients (31%) died and 69 patients (49%) were hospitalized for HF. A total of 87 patients (62%) reached the primary composite endpoint of all-cause mortality and/or first HF hospitalization. In multivariate Cox regression analysis for the primary endpoint, FGF-23 (HR: 3.44 [2.01; 5.90], $P < 0.001$) and E wave velocities (HR: 1.01 [1.00; 1.02], $P = 0.034$) were independent predictors of the primary composite endpoint. In multivariate Cox regression analysis for the secondary endpoint, ferritin (HR: 1.02 [1.01; 1.03], $P < 0.001$), FGF-23 (HR: 2.85 [1.26; 6.44], $P = 0.012$), and ECV (HR: 1.26 [1.03; 1.23], $P = 0.008$) were independent predictors of all-cause mortality.

Conclusions Fibroblast growth factor 23 (FGF-23) levels were significantly higher in HFpEF patients compared with controls of similar age and gender. FGF-23 was correlated with fibrosis evaluated by ECV. High levels of FGF-23 were significantly associated with signs of disease severity such as worse renal function, larger left atrial volumes, and right ventricular dysfunction. Moreover, FGF-23 was a strong predictor of poor outcome (mortality and first HF hospitalization).

Keywords FGF-23; Biomarker; Troponin; Mortality; NT-proBNP; Heart failure; Fibrosis; Prognosis

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Introduction

Heart failure (HF) with preserved ejection fraction (HFpEF) is a major cause of cardiovascular morbidity and mortality, especially among the elderly. Over the last years, a new paradigm has been proposed for HFpEF.[1] Systemic proinflammatory state induced by multiple co-morbidities has been identified as a potential cause of myocardial structural and functional alterations[1] and may play a key role in the genesis of vascular, skeletal muscle, and end-organ damages observed in HFpEF. Furthermore, several studies using autopsies, myocardial biopsies, or extracellular volume (ECV) measured by cardiac magnetic resonance (cMR) T1 mapping have highlighted the role of myocardial structural abnormalities and interstitial fibrosis in HFpEF.[2–5]

Fibroblast growth factor 23 (FGF-23), a bone-derived hormone regulating renal phosphate homeostasis and vitamin D metabolism, has a direct action on the cardiovascular system and has been recently related to adverse cardiovascular events in chronic HF and implicated in cardiac remodelling.[6–9] Several recent studies suggested that higher FGF-23 levels were associated with left ventricular hypertrophy[10] and worse prognosis, particularly among patients with chronic kidney disease (CKD)[11,12] but also in stable ischaemic cardiomyopathy[13] and HF with reduced ejection fraction (HFrEF).[6–8,14–16] FGF-23 has also been identified as a biomarker significantly related to atrial fibrillation.[17]

However, little is known about the association of FGF-23 with myocardial abnormalities prevalent in HFpEF, such as left ventricle (LV) diastolic dysfunction, LV hypertrophy, interstitial fibrosis, and severity of right ventricular (RV) dysfunction, nor about its role in the progression of myocardial dysfunction. In the present study, we sought to examine the relationship between FGF-23 and baseline biomarkers, demographic, and imaging characteristics, particularly fibrosis estimated by ECV using T1 mapping by cMR, and its prognostic value in a control population and in HFpEF patients.

Methods

Study population

Between December 2014 and June 2017, consecutive patients with HFpEF were prospectively evaluated for inclusion in the study.[5]

The following criteria had to be fulfilled for study inclusion: New York Heart Association functional class ≥II, typical signs of HF, N terminal pro brain natriuretic peptide (NT-proBNP) >350 pg/mL and/or a hospitalization for HF in the previous 12 months, left ventricular ejection fraction ≥ 50%, and relevant structural heart disease [LV hypertrophy/left atrial (LA) enlargement] and/or diastolic dysfunction assessed by echocardiography. The exclusion criteria were severe valvular disease, infiltrative or hypertrophic cardiomyopathy, acute coronary syndrome in the previous 30 days, chronic obstructive pulmonary disease GOLD 3 or 4, congenital heart disease, pericardial disease, atrial fibrillation with a ventricular response > 140 bpm, and severe anaemia (haemoglobin < 8 g/dL).

Patients were compared with 31 controls of similar age and gender without history of cardiovascular disease.[18] All controls underwent a full clinical exam, electrocardiogram, echocardiography, and exercise stress test, which all had to be normal prior to inclusion.

All subjects underwent blood sampling, complete transthoracic echocardiography, and cMR.

The investigation conforms with the principles outlined in Declaration of Helsinki. The local ethics committee approved the study, and all patients gave written informed consent before study enrolment (Clinical trial NCT03197350).

Echocardiography

All subjects underwent a complete two-dimensional transthoracic echocardiography at inclusion (iE33 system Philips) to assess LV and RV structure, systolic and diastolic function, and measurements of left and right atrial volumes, as well as a valvular evaluation. Pulmonary pressures were estimated using tricuspid regurgitation velocity. Strain analysis was performed on acquired images with acceptable quality in TOMTEC Software (Munich, Germany). All measurements were averaged over three beats in atrial fibrillation.

Cardiac magnetic resonance

Cardiac magnetic resonance (cMR) was performed using a three-Tesla system (Ingenia, Philips Medical Systems, Best, The Netherlands). The different sequences have been previously described.[5]

Pre-contrast and post-contrast MOLLI images were processed using the open-source software MRmap v1.4 under IDL. Pre-myocardial and post-myocardial T1 times were measured in six regions of interest in the myocardium (anterior, anterolateral, inferolateral, inferior, inferoseptal, and anteroseptal). We calculated the average T1 time of the six different region of interests. Areas of ischaemic focal fibrosis

identified by late gadolinium enhancement were excluded from the analysis. ECV was then computed according to the formula.[19]

Biomarkers analysis

Blood samples were obtained by venipuncture at inclusion. After centrifugation at 3500 rpm for 10 min, aliquots of serum and plasma were stored at −80 °C. High-sensitivity troponinT (hsTnT), NT-proBNP, and parathyroid hormone (PTH) were measured with electrochemiluminescent two sites immunoassay on Cobas 8000 platform (Roche Diagnostics, Mannheim, Germany). C-terminal FGF-23 (referred to as FGF-23 in the text) concentrations were determined with a second-generation C-terminal human enzyme-linked immunosorbent assay enzyme-linked immunosorbent assay (Immutopics, San Clemente, CA, USA). Soluble ST2 (sST2) was measured using the Presage® ST2 enzyme-linked immunosorbent assay (Critical Diagnostics, CA, USA).

Follow-up

Patients were prospectively followed by ambulatory visits and phone calls at 6-month intervals. Clinical and survival status were obtained by follow-up visits and by phone contact with the patients, their relatives, or their physician. The primary endpoint was a composite of all-cause mortality or hospitalization for HF, whichever came first. HF hospitalization was defined as patients treated in the emergency room or admitted to a hospital, diagnosed with decompensated HF, and requiring IV diuretics. The secondary endpoint was all-cause mortality.

Statistical analysis

Statistical analyses were performed using SPSS version 22 (SPSS Corp., Somers, New York). All tests were two sided, and a $P < 0.05$ was considered statistically significant. Continuous variables were expressed as mean ± 1 SD if normally distributed or as median (25th and 75th percentiles) if not normally distributed. Categorical variables were expressed as counts and percentages. Biomarkers were log-transformed to establish normality. Comparison between groups was performed using unpaired *t*-tests or chi-square test, when appropriate. The population of HFpEF patients was divided into three equal groups according to tertiles of FGF-23 levels. Clinical, biological, and imaging parameters were compared among those groups using *P* for trend analysis.

Event-free and overall survival of HFpEF patients was estimated using Kaplan–Meier method and Cox regression analysis. All baseline and imaging variables were initially proposed for inclusion in a univariate Cox proportional hazard model. Significant variables ($P < 0.10$) were entered into a stepwise forward multivariate Cox regression model. To avoid collinearity, the correlation coefficients between covariates were examined. In case of collinearity ($r > 0.50$), only the strongest of the two covariates was proposed for inclusion into the multivariate model. Kaplan Meier curves based on tertiles of FGF-23 were used to illustrate event-free and overall survival of HFpEF patients.

Results

Baseline characteristics

One hundred forty-three consecutive HFpEF patients (78 ± 8 years, 61% female patients) and 31 controls of similar age and gender (75 ± 6 years, 61% female patients) were included in the study. The baseline demographic and imaging characteristics of HFpEF patients and controls of similar age and gender are presented in *Table 1*. As expected, HFpEF patients had a higher incidence of cardiovascular risk factors and co-morbidities compared with controls. They had lower haemoglobin and lower estimated glomerular filtration rate (eGFR). Median NT-proBNP and FGF-23 were significantly higher in HFpEF (NT-proBNP: 1261 [589; 2663] pg/mL; and FGF-23: 247 [115; 548] RU/mL, respectively) than in controls (NT-proBNP: 117 [73; 158] pg/mL; and FGF-23: 61 [51; 68] RU/mL) (all $P < 0.001$) (*Figure 1*). HFpEF patients had higher LA volumes, higher E wave velocities, higher E/e' ratios, higher LV masses, higher pulmonary pressures, more signs of RV dysfunction, and higher ECV values, likely reflecting more diffuse myocardial fibrosis than controls. FGF-23 levels were significantly correlated to ECV in the whole population (*Figure 2*).

Comparison of clinical and imaging parameters across tertiles of fibroblast growth factor 23 in heart failure with preserved ejection fraction patients

Heart failure and preserved ejection fraction (HFpEF) patients in the highest FGF-23 tertile were more often female patients and had higher incidence of atrial fibrillation. They had lower haemoglobin, worse renal function, higher NT-proBNP and hsTnT, higher PTH, and higher sST2. The rise of FGF-23 was independent of the 25OH vitamin D status. Regarding imaging parameters, they had greater LA volumes with worse RV systolic function (lower RV FAC $P = 0.027$, lower TAPSE $P = 0.030$, and lower RVEF by cMR $P = 0.003$). Interestingly, ECV values significantly increased across tertiles of FGF-23 (*Table 2*).

Table 1 Baseline characteristics of HFpEF patients and controls of similar age and gender

	Controls (n = 31)	HFpEF (n = 143)	P-value
Baseline characteristics			
Age (years)	75 ± 6	78 ± 8	0.06
Female (n, %)	19 (61%)	87 (61%)	0.96
Body mass index (kg/m^2)	26 ± 4	29 ± 6	0.015
Mean blood pressure (mmHg)	113 ± 16	95 ± 14	<0.001
NYHA functional classes III and IV (n, %)	0 (0%)	62 (43%)	<0.001
Medical history			
Atrial fibrillation (n, %)	0 (0%)	88 (62%)	<0.001
Ischaemic cardiomyopathy (n, %)	0 (0%)	50 (35%)	<0.001
Previous heart failure episode (n, %)	0 (0%)	103 (72%)	<0.001
Previous valvular surgery (n, %)	0 (0%)	16 (11%)	0.051
Chronic obstructive pulmonary disease (n, %)	0 (0%)	14 (10%)	0.070
Sleep apnoea (n, %)	0 (0%)	18 (13%)	0.037
Cardiovascular risk factors			
Smoking (n, %)	7 (23%)	61 (43%)	0.038
Hypertension (n, %)	19 (61%)	134 (94%)	<0.001
Diabetes (n, %)	6 (19%)	55 (38%)	0.044
Family history of CV disease (n, %)	5 (16%)	30 (21%)	0.5
Hypercholesterolemia (n, %)	26 (84%)	95 (66%)	0,056
Medication			
Loop diuretic (n, %)	0 (0%)	101 (71%)	<0.001
Thiazide (n, %)	2 (6%)	30 (21%)	0.059
Mineralocorticoid receptor antagonist (n, %)	0 (0%)	29 (20%)	0.006
Beta-blocker (n, %)	3 (10%)	95 (66%)	<0.001
ACEI or ARB (n, %)	13 (42%)	99 (69%)	0.004
Antiaggregant (n, %)	8 (26%)	59 (42%)	0.10
Oral anticoagulant (n, %)	1 (3%)	79 (55%)	<0.001
Statins (n, %)	8 (26%)	64 (45%)	0.053
Biology			
Haemoglobin (g/dL)	13.9 ± 1.4	11.7 ± 2.0	<0.001
Total cholesterol (mg/dL)	203 ± 45	154 ± 45	<0.001
GFR (mL/min/1.73 m^2) by CK-EPI	63 ± 15	49 ± 19	<0.001
NT-proBNP (pg/mL)	117 (73; 158)	1,261 (589; 2,663)	<0.001
Hs TnT (pg/mL)	7 (6; 11)	26 (15; 38)	<0.001
Iron (μg/dL)	94 ± 33	77 ± 60	0.15
Ferritin (μg/L)	158 ± 167	231 ± 281	0.17
Calcium (mmol/L)	2.47 ± 0.14	2.39 ± 0.30	0.15
Phosphorus (mmol/L)	1.08 ± 0.17	1.19 ± 0.26	0.017
Intact PTH (pg/mL)	43 ± 23	68 ± 53	0.011
25OH-Vitamin D (ng/mL)	33 ± 15	25 ± 16	0.016
Soluble ST2 (ng/mL)	24 (21; 31)	42 (31; 60)	<0.001
FGF-23 (RU/mL)	61 (51; 68)	247 (115; 548)	<0.001
Echo study			
LA volume index (mL/m^2)	20 ± 6	46 ± 19	<0.001
LV EDV index (mL/m^2)	59 ± 10	67 ± 18	0.024
LV ejection fraction (%)	65 ± 5	63 ± 7	0.055
LV Endo GLS (%)	−21.0 ± 2.5	−16.5 ± 3.2	<0.001
LV mass index (g/m^2)	71 ± 16	96 ± 26	<0.001
E wave velocity (m/s)	55 ± 10	94 ± 31	<0.001
Septal e′ (m/s)	7.3 ± 1.8	6.9 ± 2.2	0.003
E/e′ septal ratio	9.5 ± 1.8	19.4 ± 8.7	<0.001
RV/RA gradient (mmHg)	18 ± 5	33 ± 11	<0.001
RV fractional area change (%)	47 ± 7	41 ± 9	0.001
TAPSE (mm)	24 ± 4	19 ± 5	<0.001
cMR study	n = 31	n = 121	
LA volume index (mL/m^2)	31 ± 9	67 ± 29	<0.001
LV EDV index (mL/m^2)	64 ± 11	74 ± 19	0.009
LV ejection fraction (%)	66 ± 6	63 ± 8	0.030
LV mass index (g/m^2)	57 ± 12	68 ± 15	0.001
RV EDV index (mL/m^2)	68 ± 10	82 ± 27	0.005
RV ejection fraction (%)	61 ± 7	57 ± 9	0.001
ECV (%)	27.8 ± 2.4	32.7 ± 4.9	0.001
Late gadolinium enhancement (%)	0 (0%)	1.5 ± 2.6	0.001

CK-EPI, Chronic Kidney Disease Epidemiology Collaboration; ECV, extracellular volume; EDV, end-diastolic volume; FGF-23, fibroblast growth factor 23; GFR, glomerular filtration rate; GLS, global longitudinal strain; HFpEF, heart failure with preserved ejection fraction;

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hsTnT, high-sensitivity troponinT; LA, left atrium; LV, left ventricle; NT-proBNP, N terminal pro brain natriuretuic peptide; PTH, parathormon; RV, right ventricle; ST2, soluble suppression tumourigenicity 2; TAPSE, tricuspid annular plane systolic excursion.
Values are mean ± SD or median [IQR: 0.25; 0.75].

Outcomes

Over a mean follow-up of 30 ± 8 months, 43 patients (31%) died and 69 patients (49%) were hospitalized for HF. A total of 87 patients (62%) reached the primary composite endpoint of all-cause mortality or HF hospitalization, whichever came first.

In univariate Cox regression analysis, New York Heart Association class, diabetes, haemoglobin, eGFR, HsTnT, phosphorus, intact PTH, sST2, FGF-23, treatment with loop diuretics and thiazides, E wave velocity, and E/e′ ratio were predictors of the primary endpoint. Among cMR parameters, index RV volume and ECV were also significantly associated with the primary endpoint. In multivariate Cox regression analysis, only FGF-23 (HR: 3.44 [2.01; 5.90], $P < 0.001$) and E wave velocity (HR: 1.01 [1.00; 1.02], $P = 0.034$) were independent predictors of the primary endpoint (*Table 3*).

For the secondary endpoint, BMI, eGFR, hsTnT, ferritin, sST2, FGF-23, mineralocorticoid receptor antagonist medication, RV/RA gradient, and ECV were predictors of all-cause mortality in univariate Cox regression. In multivariate Cox regression analysis, only ferritin (HR: 1.02 [1.01; 1.03], $P < 0.001$), FGF-23 (HR: 2.85 [1.26; 6.44], $P = 0.012$), and ECV (HR: 1.26 [1.03; 1.23], $P = 0.008$) were independent predictors of all-cause mortality (*Table 4*).

Figure 3A and *3B* shows the Kaplan Meier curves for the primary and secondary endpoint of HFpEF patients according to tertiles of FGF-23, respectively, illustrating that patients with the highest FGF-23 levels have the worse prognosis.

Discussion

In the present study, we sought to examine the relationship between FGF-23 and baseline biomarkers, demographic, and imaging characteristics, particularly diffuse myocardial fibrosis, in controls and HFpEF patients and the prognostic value of FGF-23 in HFpEF.

In this very well sub-phenotyped cohort of controls and HFpEF patients, FGF-23 was significantly higher in HFpEF patients compared with controls. FGF-23 was associated with some proinflammatory co-morbidities (i.e. renal dysfunction, diabetes, and atrial fibrillation), indices of cardiac dysfunction (i.e. LA dilatation and RV dysfunction), and biomarkers reflective of either ventricular stretch (NT-proBNP), myocardial necrosis (hsTnT), fibrosis (sST2), or bone and mineral metabolism (PTH). Moreover, FGF-23 was significantly correlated with myocardial fibrosis estimated by ECV. FGF-23 was

Figure 1 Box-plot of FGF-23 in heart failure with preserved ejection fraction (HFpEF) and controls of similar age and gender.

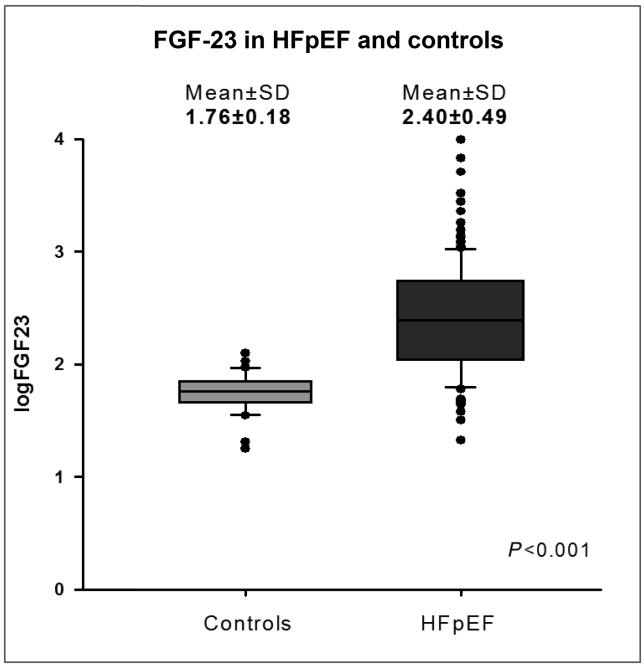
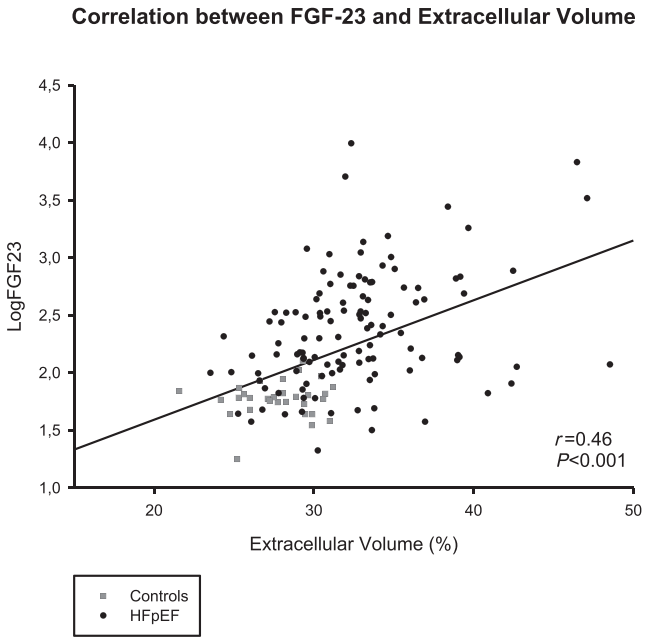


Figure 2 Correlation between fibroblast growth factor 23 (FGF-23) and extracellular volume (ECV) in heart failure with preserved ejection fraction (HFpEF) and controls of similar age and gender.



also a strong and independent predictor of the primary composite endpoint and the secondary endpoint of all-cause mortality, even after adjusting for confounding factors such as renal function and other clinically relevant variables. Interestingly, both the interplay with the other biomarkers and with the prognosis value of FGF-23 was independent of the vitamin D levels.

Mechanistic insight into the pathogenesis of disease

In HF, biomarkers are used primarily for diagnosis and risk stratification, natriuretic peptides playing a major role in this respect.[20] Nonetheless, biomarkers studies can also provide important insight into the pathophysiologic mechanisms leading to disease progression, which can then be targeted pharmacologically, such as the RAAS or the adrenergic system. However, this aspect of biomarker research is complicated by the fact that many biomarkers predictive of poor prognosis may reflect systemic organ failures rather than a specific mechanism underlying cardiac disease progression. Moreover, the plasma levels of most biomarkers are affected by multiple confounding factors such as age, sex, renal, and hepatic function. This complexity likely explains why progress has been slow in understanding the mechanisms responsible for the progression of HFpEF, as in this disease, LV systolic function remains by definition preserved to the very end while systemic organ failures inexorably progress.

Heart failure and preserved ejection fraction (HFpEF) is a clinical syndrome that has already been associated with changes in the extracellular matrix and in which fibrosis seems to be a crucial component of cardiac remodelling.[1] Among all the regulators involved in the pathophysiology of cardiac fibrosis, FGF-23 seems to play an important role, probably still underestimated and understudied. Only few studies looked at the prognostic value of this biomarker in HFpEF, although experimental and in vivo data demonstrated the implication of FGF-23 in pathophysiological mechanisms involved in the development of the disease such as cardiac hypertrophy, fibrosis, angiogenesis, and cardiac remodelling.[21–24] Indeed, in our study, we found a significant correlation between FGF-23 levels and diffuse myocardial fibrosis estimated by ECV. Whether the association between FGF-23 and ECV is causal cannot be concluded from our observational data, but previous studies demonstrated the implication of FGF-23 in pathophysiological mechanisms involved cardiac remodelling.[22–24] It was demonstrated that FGF-23 promotes hypertrophic growth of cardiac myocytes via FGF receptor 4[21] and that it contributes to myocardial fibrosis and diastolic dysfunction through the up-regulation of active β-catenin and TGF-β.[25] It was also shown that FGF-23 directly stimulates RAAS through the inhibition of ACE2[21] and that the interplay of FGF-23 with PTH and RAAS might trigger adverse cardiovascular remodelling.[26,27] Those data taken together suggest that FGF-23 is not only a marker

Table 2 Clinical and imaging parameters according to tertiles of FGF-23 in HFpEF

	FGF-23			
	Tertile 1 n = 47 (<134 RU/mL)	Tertile 2 n = 47 (134–406 RU/mL)	Tertile 3 n = 48 (>406 RU/mL)	*P*-for trend
Baseline characteristics				
Age (years)	78 ± 9	78 ± 8	79 ± 9	0.57
Female (*n*, %)	20 (43%)	33 (69%)	34 (71%)	**0.005**
Body mass index (kg/m^2)	28 ± 5	30 ± 7	27 ± 6	0.51
Mean blood pressure (mmHg)	97 ± 15	98 ± 11	90 ± 13	0.021
NYHA functional classes III and IV (*n*, %)	18 (38%)	19 (40%)	25 (52%)	0.18
Medical history				
Atrial fibrillation (*n*, %)	22 (47%)	30 (63%)	36 (75%)	**0.005**
Ischaemic cardiomyopathy (*n*, %)	18 (38%)	14 (29%)	18 (38%)	0.94
Previous heart failure episode (*n*, %)	29 (62%)	37 (77%)	37 (77%)	0.097
Previous valvular surgery (*n*, %)	5 (11%)	4 (8%)	7 (15%)	0.54
Chronic obstructive pulmonary disease (*n*, %)	5 (11%)	6 (13%)	3 (6%)	0.47
Sleep apnoea (*n*, %)	4 (9%)	8 (17%)	6 (13%)	0.57
Cardiovascular risk factors				
Smoking (*n*, %)	24 (51%)	17 (35%)	20 (42%)	0.36
Hypertension (*n*, %)	41 (87%)	47 (98%)	46 (96%)	0.087
Diabetes (*n*, %)	15 (32%)	17 (35%)	23 (48%)	0.11
Family history of CV disease (*n*, %)	12 (26%)	8 (17%)	10 (21%)	0.58
Hypercholesterolemia (*n*, %)	29 (62%)	32 (67%)	34 (71%)	0.35
Medication				
Loop diuretic (*n*, %)	27 (57%)	35 (73%)	40 (85%)	**0.011**
Thiazide (*n*, %)	13 (27%)	11(23%)	6 (13%)	0.25
Mineralocorticoid receptor antagonist (*n*, %)	5 (10%)	10 (21%)	14 (30%)	0.06
Beta-blocker (*n*, %)	31 (65%)	33 (69%)	32 (68%)	0.90
ACEI or ARB (*n*, %)	33(69%)	33 (69%)	32 (68%)	0.99
Antiaggregant (*n*, %)	23 (48%)	22 (46%)	14 (30%)	0.15
Oral anticoagulant (*n*, %)	20 (42%)	28 (58%)	32 (68%)	**0.032**
Statins (*n*, %)	24 (51%)	23 (49%)	17 (38%)	0.40
Biology				
Haemoglobin (g/dL)	12.6 ± 2.1	11.9 ± 1.7	10.8 ± 1.6	**<0.001**
GFR (mL/min/1.73 m^2) by CKD-EPI	60 ± 20	44 ± 17	44 ± 18	**<0.001**
NT-proBNP (pg/mL)	750 (360; 1289)	1201 (628; 2177)	2441 (1013; 4075)	**<0.001**
Hs TnT (pg/mL)	16 (12; 31)	28 (18; 37)	33 (18; 42)	**<0.001**
Iron (μg/dL)	84 ± 50	77 ± 49	71 ± 78	0.29
Ferritin (μg/L)	300 ± 360	230 ± 198	164 ± 254	**0.021**
Calcium (mmol/L)	2.35 ± 0.41	2.45 ± 0.23	2.37 ± 0.23	0.76
Phosphorus (mmol/L)	1.13 ± 0.15	1.23 ± 0.28	1.22 ± 0.31	0.078
Intact PTH (pg/mL)	50 ± 28	71 ± 39	84 ± 76	**0.003**
25OH-Vitamin D (ng/mL)	23 ± 15	27 ± 16	26 ± 18	0.39
Soluble ST2 (ng/mL)	35 (26; 56)	42 (32; 57)	47 (36; 69)	**0.027**
FGF-23 (RU/mL)	89 (60; 110)	245 (160; 311)	701 (549; 1131)	**<0.001**
Echo study				
LA volume index (mL/m^2)	44 ± 21	41 ± 12	52 ± 20	**0.030**
LV EDV index (mL/m^2)	69 ± 16	64 ± 16	68 ± 22	0.63
LV ejection fraction (%)	62 ± 7	63 ± 8	63 ± 8	0.58
LV Endo GLS (%)	−16.3 ± 3.5	−16.5 ± 3.1	−16.8 ± 2.9	0.45
LV mass index (g/m^2)	97 ± 26	92 ± 22	99 ± 31	0.79
E wave velocity (m/s)	89 ± 32	91 ± 29	101 ± 31	0.068
Septal e′ (m/s)	5.1 ± 1.4	5.2 ± 1.4	5.2 ± 1.3	0.93
E/e′ septal ratio	18.8 ± 9.2	18.5 ± 7.8	20.8 ± 9.2	0.27
RV/RA gradient (mmHg)	31 ± 9	32 ± 11	34 ± 12	0.17
RV/RA gradient >37 mmHg	14 (30%)	14 (29%)	18 (38%)	0.42
RV fractional area change (%)	44 ± 7	40 ± 10	40 ± 9	**0.027**
RV fractional area change<35% (*n*, %)	4 (5%)	12 (15%)	29 (35%)	<0,001
TAPSE (mm)	20 ± 5	19 ± 5	17 ± 6	**0.030**
TAPSE <17 mm (*n*, %)	6 (7%)	14 (17%)	42 (51%)	<0.001
cMR study				
LA volume index (mL/m^2)	62 ± 36	64 ± 21	76 ± 26	**0.030**
LV EDV index (mL/m^2)	76 ± 20	70 ± 18	74 ± 19	0.62
LV ejection fraction (%)	63 ± 8	62 ± 9	63 ± 7	0.94
LV mass index (g/m^2)	69 ± 15	65 ± 12	69 ± 18	0.82
RV EDV index (mL/m^2)	75 ± 20	78 ± 22	93 ± 34	0.003
RV ejection fraction (%)	59 ± 7	57 ± 8	54 ± 9	**0.003**
RV ejection fraction <45% (*n*, %)	5 (6%)	5 (6%)	11 (17%)	**0.029**
ECV (%)	31.7 ± 5.2	31.4 ± 3.3	35.5 ± 5.0	**<0.001**
Late gadolinium enhancement (%)	1.1 ± 2.6	1.7 ± 2.3	1.8 ± 2.8	0.26

CK-EPI, Chronic Kidney Disease Epidemiology Collaboration; ECV, extracellular volumeEDV, end-diastolic volume; FGF-23, fibroblast growth factor 23; GFR, glomerular filtration rate; GLS, global longitudinal strain; HFpEF, heart failure with preserved ejection fraction; hsTnT, high-sensitivity troponinT; LA, left atrium; LV, left ventricle; NT-proBNP, N terminal pro brain natriuretic peptide; PTH, parathormon; RV, right ventricle; ST2, soluble suppression tumourigenicity 2; TAPSE, tricuspid annular plane systolic excursion.
Values are mean ± SD or median [IQR 0.25; 0.75]. *P*-value obtained by *P* for trend analysis.

of risk but might contribute to the progression of the disease. The availability of FGF-23 antagonist or immunotherapy would allow testing that hypothesis.[25,28] Sodium glucose cotransporter-2 inhibitors, the most recently approved class of drug for type 2 diabetes, has recently shown benefit in HF patients, independently from its effect on diabetes.[29]

Table 3 Cox regression analysis for the primary endpoint

	Univariate			
Cox regression analysis Combined events	Hazard ratio 95% CI	*P*-value	Multivariate	
Age	1.01 [0.98; 1.04]	0.58		
Female	1.46 [0.94; 2.28]	0.088		
Body mass index	0.97 [0.94; 1.01]	0.10		
Mean blood pressure	0.99 [0.98; 1.01]	0.31		
NYHA functional classes III and IV	1.58 [1.03; 2.41]	**0.037**		
Cardiovascular risk factors				
Diabetes	1.65 [1.08; 2.52]	**0.022**		
Medical history				
Atrial fibrillation	1.25 [0.80; 1.95]	0.33		
Ischaemic cardiomyopathy	0.93 [0.60; 1.45]	0.76		
Medication				
Loop diuretic	2.16 [1.25; 3.72]	0.005		
Thiazide	0.55 [0.30; 0.99]	0.048		
Mineralocorticoid receptor antagonist	1.38 [0.85; 2.15]	0.20		
Beta-blocker	0.97 [0.62; 1.52]	0.90		
ACEI or ARB	0.90 [0.57;1.40]	0.63		
Oral anticoagulant	1.46 [0.95; 2.27]	0.088		
Biology				
Haemoglobin (g/dL)	0.86 [0.76;0.97]	**0.013**		
GFR (mL/min/1.73 m^2) by CKD-EPI	0.98 [0.97; 0.99]	**<0.001**		
NT-proBNP (pg/mL)	1.19 [0.97; 1.46]	**0.10**		
Hs TnT (pg/mL)	1.70 [1.25; 2.30]	**0.001**		
Iron (ug/dL)	1.00 [1.00; 1.00]	0.38		
Ferritin (ug/L)	1.00 [1.00; 1.00]	0.10		
Calcium (mmol/L)	0.89 [0.47; 1.68]	0.72		
Phosphorus (mmol/L)	2.31 [1.04; 5.14]	**0.051**		
Intact PTH (pf/mL)	1.01 [1.00; 1.01]	**0.003**		
25OH-Vitamin D (ng/mL)	1.00 [0.99; 1.01]	0.91		
Soluble ST2 (ng/mL)	3.46 [1.23; 9.74]	**0.020**		
FGF-23 (RU/mL)	2.21 [1.52; 3.22]	**<0.001**	**3.15 [1.88; 5.31]**	**<0.001**
Echo study				
LA volume index (mL/m^2)	1.01 [1.00; 1.02]	0.17		
LV ejection fraction (%)	1.01 [0.98; 1.04]	0.69		
LV Endo GLS (%)	1.00 [0.93; 1.07]	0.95		
E wave velocity (m/s)	1.01 [1.00; 1.02]	**0.002**	**1.01 [1.00; 1.02]**	**0.036**
E/e' septal ratio	1.03 [1.01; 1.05]	**0.018**		
RV/RA gradient (mmHg)	1.02 [1.00; 1.04]	0.093		
RV fractional area change (%)	0.27 [0.02; 3.11]	0.29		
TAPSE	0.97 [0.93; 1.01]	0.13		
cMR study				
LA volume index (mL/m^2)	1.00 [1.00; 1.01]	0.31		
LV EDV index (mL/m^2)	1.00 [0.99; 1.02]	0.83		
LV ejection fraction (%)	1.01 [0.98; 1.04]	0.55		
LV mass index (g/m^2)	1.00 [0.99; 1.02]	0.64		
RV EDV index (mL/m^2)	1.01 [1.00; 1.02]	**0.031**		
RV ejection fraction (%)	0.98 [0.95; 1.01]	0.11		
Extracellular volume (%)	1.10 [1.05; 1.15]	**<0.001**		
Late gadolinium enhancement	1.09 [0.99; 1.20]	0.11		

ACEi, angiotensin-converting-enzyme inhibitor; ARB, Angiotensin II receptor blockers; ECV, extracellular volume; EDV, end-diastolic volume; FGF-23, fibroblast growth factor 23; GFR, glomerular filtration rate; GLS, global longitudinal strain; hsTnT, high-sensitivity troponinT; LA, left atrium; LV, left ventricle; NT-proBNP, N terminal pro brain natriuretuic peptide; PTH, parathormon; RV, right ventricle; ST2, soluble suppression tumourigenicity 2; TAPSE, tricuspid annular plane systolic excursion.

Table 4 Cox regression analysis for the secondary endpoint

	Univariate		Multivariate	
Cox regression analysis All-cause mortality	Hazard ratio 95% CI	*P*-value	Hazard ratio 95% CI	*P*-value
Age	1.03 [0.99; 1.07]	0.13		
Female	1.18 [0.64; 2.20]	0.60		
Body mass index	0.91 [0.86; 0.96]	**<0.001**		
Mean blood pressure	0.98 [0.96; 1.00]	0.10		
NYHA functional classes III and IV	1.12 [0.61; 2.06]	0.72		
Cardiovascular risk factors				
Diabetes	1.53 [0.84; 2.78]	0.17		
Medical history				
Atrial fibrillation	0.85 [0.46; 1.56]	0.60		
Ischaemic cardiomyopathy	0.67 [0.34; 1.31]	0.23		
Medication				
Loop diuretic	1.71 [0.79; 3.69]	0.17		
Thiazide	0.79 [0.35; 1.79]	0.57		
Mineralocorticoid receptor antagonist	2.12 [1.12; 4.02]	0.021		
Beta-blocker	0.72 [0.39; 1.32]	0.28		
ACEI or ARB	0.62 [0.34; 1.15]	0.13		
Oral anticoagulant	1.19 [0.65; 2.20]	0.58		
Biology				
Haemoglobin	0.86 [0.73; 1.01]	**0.068**		
GFR	0.98 [0.97; 1.00]	**0.045**		
NT-proBNP	1.30 [0.98; 1.73]	**0.078**		
Hs TnT	1.62 [1.12; 2.34]	**0.014**		
Iron	1.00 [1.00; 1.01]	0.09		
Ferritin	1.00 [1.00; 1.00]	**0.004**	1.02 [1.01; 1.03]	<0.001
Calcium	0.85 [0.40; 1.78]	0.67		
Phosphorus	1.93 [0.64; 5.84]	0.26		
Intact PTH	1.00 [0.99; 1.01]	0.79		
25OH-Vitamin D	0.99 [0.97; 1.01]	0.25		
Soluble ST2	20.24 [4.88; 84.03]	**<0.001**		
FGF-23	2.12 [1.26; 3.67]	**0.010**	2.85 [1.26; 6.44]	0.012
Echo study				
LA volume index	1.01 [1.00; 1.02]	0.20		
LV ejection fraction	1.01 [0.97; 1.06]	0.53		
LV Endo GLS	1.04 [0.94; 1.15]	0.42		
E wave velocity	1.01 [1.00; 1.02]	0.11		
E/e' septal ratio	1.03 [1.00; 1.06]	**0.084**		
RV/RA gradient	1.03 [1.01; 1.06]	**0.015**		
RV fractional area change	0.16 [0.01; 4.88]	0.29		
TAPSE	0.97 [0.91; 1.02]	0.25		
cMR study				
LA volume index (mL/m^2)	1.00 [0.99; 1.01]	0.71		
LV EDV index (mL/m^2)	1.01 [0.99; 1.03]	0.26		
LV ejection fraction (%)	0.99 [0.94; 1.03]	0.60		
LV mass index (g/m^2)	1.02 [1.00; 1.04]	0.15		
RV EDV index (mL/m^2)	1.00 [0.99; 1.02]	0.66		
RV ejection fraction (%)	0.98 [0.93; 1.03]	0.39		
Extracellular volume (%)	1.12 [1.05; 1.20]	**0.003**	1.26 [1.03; 1.23]	0.008
Late gadolinium enhancement	1.13 [1.00; 1.28]	**0.069**		

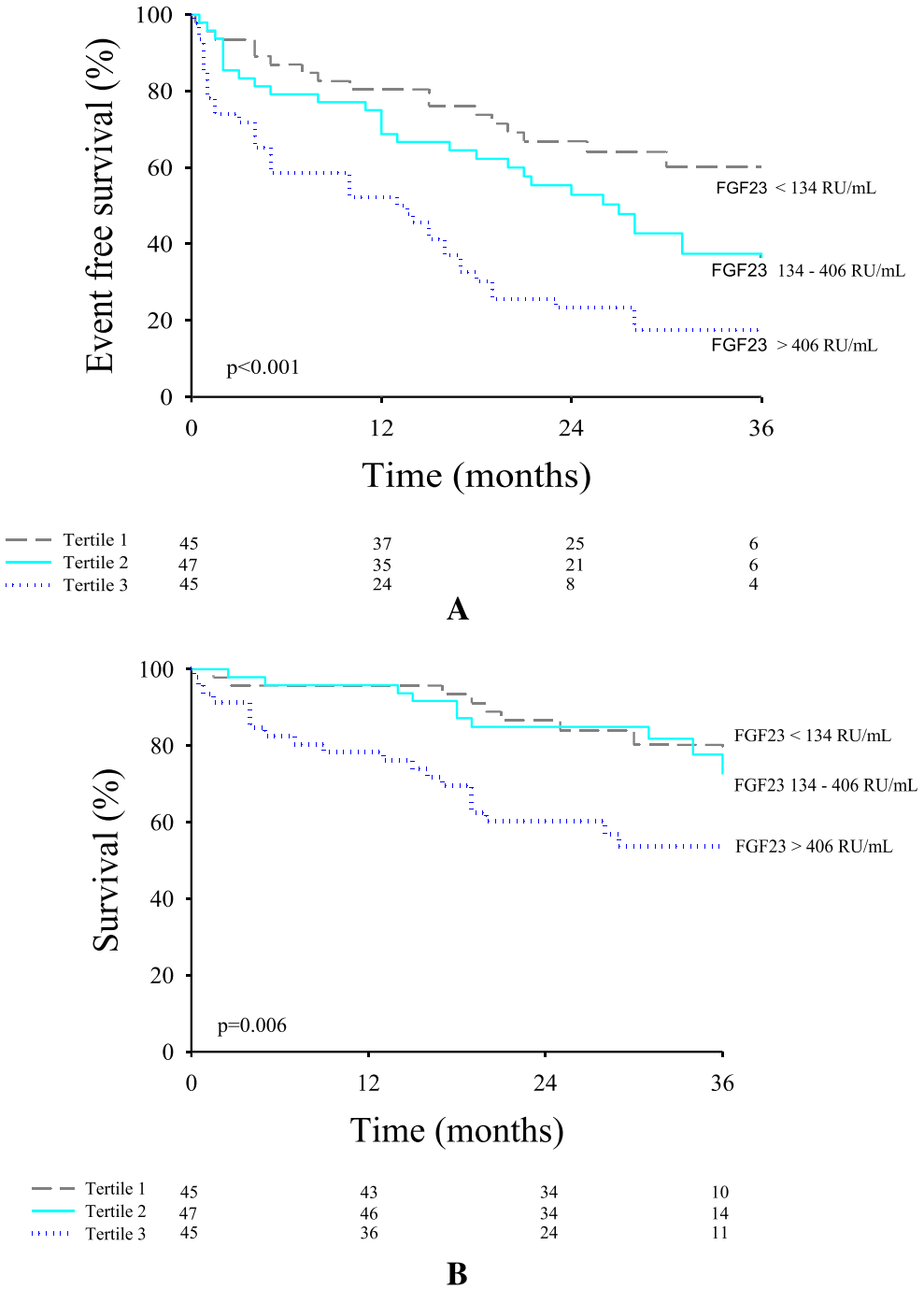
ACEi, angiotensin-converting-enzyme inhibitor; ARB, Angiotensin II receptor blockers; ECV, extracellular volume; EDV, end-diastolic volume; FGF-23, fibroblast growth factor 23; GFR, glomerular filtration rate; GLS, global longitudinal strain; hsTnT, high-sensitivity troponinT; LA, left atrium; LV, left ventricle; NT-proBNP, N terminal pro brain natriuretuic peptide; PTH, parathormon; RV, right ventricle; ST2, soluble suppression tumourigenicity 2; TAPSE, tricuspid annular plane systolic excursion.

One hypothesis to explain this effect is that canagliflozin induces a prompt increase in serum phosphorus and triggers downstream changes in FGF-23.[30,31] Studies (DELIVER and EMPEROR-Preserved) are currently going on to determine whether sodium glucose cotransporter-2 inhibitors will have a positive impact on the prognosis of HFpEF patients.[32]

Association between fibroblast growth factor 23 levels and clinical characteristics

Although the mechanisms of sex differences in FGF-23 remain unclear, the presence of higher FGF-23 levels in female patients than in male patients has been described in previous studies, both in small children[33] and in adults with

Figure 3 Kaplan Meier curves for the primary endpoint according to tertiles of fibroblast growth factor 23 (FGF-23) in heart failure with preserved ejection fraction (HFpEF) patients (A) and Kaplan Meier curves for the secondary endpoint according to tertiles of FGF-23 in HFpEF patients (B).



cardiovascular risk factors.[34,35] In the hypothesis that FGF-23 actively plays a part in the development of the disease, this might contribute to the overrepresentation of women among patients with HFpEF.

Atrial fibrillation, a highly prevalent co-morbidity in HFpEF, was also found to be associated with higher levels of FGF-23.[36–38] Two possible mechanisms were described to explain this interaction. First, FGF-23 could induce atrial fibrosis by increasing ROS production, subsequently activating STAT3 and SMAD3 signalling.[39] Second, FGF-23 increases pulmonary vein arrythmogenesis through protein kinase C signalling and dysregulation of sodium and calcium homeostatis.[40]

The association between elevated FGF-23 and impaired renal function is largely described. Levels of FGF-23 rise early in the course of CKD as part of the adaptive response to maintain neutral phosphate balance when renal excretory capacity declines. Chronic FGF-23 elevation in CKD is independently associated with development of HF and death.[9,11] We also observed that haemoglobin decreased across increasing FGF-23 levels. This is not only mediated through the interaction with renal function (where anaemia is mediated by decreased erythropoietin production, low serum active vitamin D levels, and high renin-angiotensin-aldosterone activities) but also through a direct inhibition of erythropoiesis by FGF-23.[41]

Recent data from PARAMOUNT[42] and RELAX[35] trials showed that sST2 levels were correlated with proinflammatory co-morbidities and with the severity of HFpEF, indicated by higher levels of NT-proBNP and signs of diastolic dysfunction. Authors from those trials concluded that patients with the more severe disease had a biomarker pattern associated with a more profibrotic milieu.[42] This is corroborated by our study, where FGF-23 is associated both with the presence of myocardial fibrosis evaluated by ECV and with signs of disease severity (NT-proBNP, renal function, and RV dysfunction). On the other hand, we did not find a significant association between FGF-23 levels and E wave velocities or E/e′ ratio. This is consistent with a recent study by Okamoto *et al*.[24] showing that alpha-klotho, a co-receptor of FGF-23 related to ageing suppression and organ protection, was significantly associated with E/e′ ratio, while FGF-23 was not. However, in our study, higher FGF-23 levels were associated with larger LA volumes, which is also an important marker of elevated filling pressures and diastolic dysfunction.[43]

Prognostic and risk stratification

Beyond assessment of natriuretic peptides, other biomarkers of mechanisms contributing to the pathophysiology of HF, such as inflammation (GDF-15 and sST2), LV hypertrophy (FGF-23), and myocardial necrosis (hsTnT), could add important prognostic information and identify patients at higher risk of events.[44–47] Studies have already demonstrated the superiority of different biomarkers combinations, including NT-proBNP, sST2, and Galectin-3 for risk stratification of chronic HF patients.[48,49] In 2015, we showed the additive value of FGF-23, Galectin 3, and sST2 compared with NT-proBNP for prediction of cardiovascular death in HFrEF.[50] However, in the specific CORONA-HF population, a multimarker approach using a panel of 20 inflammatory and extracellular matrix biomarkers (including sST2 and galectin-3) was of limited clinical value for identifying the risk of adverse outcomes.[51]

Although FGF-23 has been associated with adverse cardiovascular outcomes in CKD and HFrEF, data are lacking in HFpEF. Koller *et al*.[14] demonstrated that FGF-23 was independently associated with an increased risk of mortality in patients with HFrEF (n = 511) but not in those with HFpEF (n = 469). Only one large trial showed a significant prognostic value of FGF-23 compared with NT-proBNP for a combined outcome of all-cause mortality and HF hospitalization in HFrEF and in HFpEF.[7] Our data corroborate the prognostic value of FGF-23 in HFpEF, demonstrating the importance of exploring physiopathological pathways for a better understanding of this syndrome. Surprisingly, NTproBNP was not associated with the primary and the secondary endpoints in our study (borderline for the prediction of mortality HR: 1.30 [0.98; 1.73] P = 0.078), probably mainly due to the high levels of NT-proBNP on average and the limited sample size.

Fibroblast growth factor 23 (FGF-23) might increase fluid retention by stimulating RAAS, probably explaining the link between FGF-23 and HF hospitalization (in univariate Cox regression analysis: HR 2.30 [1.51–3.49], P < 0.001) and might represent a potential therapeutic target. Even after adjustment for eGFR, FGF-23 remained predictive in our population. However, the strong predictive value of FGF-23 in our study might be related to the assay we used, and it might be hypothesized that assays targeting C-terminal FGF-23 fragments have stronger value for risk estimation than intact assays.[52]

It could also be hypothesized that the rise of FGF-23 precedes the development of HF in patients at high risk, such as diabetic and hypertensive patients.[53-55] The early sub-phenotyping of these patients including measurement of FGF-23 could represent an opportunity to precociously estimate their risk for developing HF and adjust their treatment accordingly. Further studies addressing the value of FGF-23 for risk stratification in this specific population are needed to confirm this hypothesis.

Strengths and limitations

To our knowledge, this is one of the first prospective study investigating the role of FGF-23 in a cohort of HFpEF patients and in a control group well characterized by echocardiography and cMR. Our cohort is comparable with PARAMOUNT[42] and RELAX[35] biomarker analysis in terms of number of patients and demographic characteristics. However, this should be considered as exploratory and hypothesis generating and should be tested in larger mechanistic HFpEF studies.

Conclusions

Heart failure with preserved ejection fraction (HFpEF) patients showed significantly higher levels of circulating FGF-23 than controls. High FGF-23 levels were significantly

associated with LA volume (a marker of diastolic dysfunction), RV dysfunction, and with diffuse myocardial fibrosis evaluated by cMR. FGF-23 was also a strong predictor of poor outcome, even after adjusting for usual confounding factors. Sub-phenotyping of HFpEF patients with FGF-23 might therefore participate in a more personalized care.

Conflict of interest

The Cliniques St. Luc UCL has a master clinical research agreement with Philips Medical Instruments, and the MOLLI patch was supplied by Philips Medical under the terms of this agreement. The authors declare that they have no competing interests.

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