Fibroblast growth factor 23 in patients with acute dyspnea:

data from the Akershus Cardiac Examination (ACE) 2 Study

Running head: **FGF23 in acute dyspnea**

Magnus Nakrem Lyngbakken MD, PhD[a,b]; Mohammad Osman Pervez MD[a,b]; Jon Brynildsen MD[a,b]; Marit Holmefjord Pedersen BSc[a]; Janne Sølvernes MSc[a]; Geir Christensen MD, PhD, MHA[b,c]; Arne Didrik Høiseth MD, PhD[b]; Torbjørn Omland MD, PhD, MPH[a,b]; Helge Røsjø MD, PhD[a,b]

[a]Division of Medicine, Akershus University Hospital, Lørenskog, Norway

[b]Center for Heart Failure Research, University of Oslo, Oslo, Norway

[c]Institute for Experimental Medical Research, Oslo University Hospital, Ullevål, Oslo, Norway

Corresponding author: Helge Røsjø, MD, PhD, Division of Medicine, Akershus University Hospital, Sykehusveien 25, 1478 Lørenskog, Norway. Tel: +47 915 02900 Fax: +47 67962190 e-mail: helge.rosjo@medisin.uio.no

Abstract

Background: Circulating fibroblast growth factor 23 (FGF23) concentrations have been linked to left ventricular remodeling and unfavorable cardiovascular outcomes, but whether FGF23 is associated with heart failure (HF) diagnosis and outcome in unselected patients with dyspnea is unknown. Accordingly, we assessed the diagnostic and prognostic properties of FGF23 in patients presenting to the emergency department with acute dyspnea.

Methods and results: FGF23 was measured in 314 patients admitted with acute dyspnea and the diagnostic and prognostic merit was compared to that of N-terminal pro-B-type natriuretic peptide (NT-proBNP). The diagnosis of acute HF was adjudicated by two independent physicians. Circulating FGF23 concentrations on hospital admission were higher in patients with acute HF vs. patients with non-HF related dyspnea: median 3.60 (IQR 1.24-8.77) vs. 1.00 (0.43-2.20) pmol/L; $P<0.001$. The receiver-operating statistics area under the curve for acute HF diagnosis was 0.750 (0.699-0.797) for FGF23 and 0.853 (0.809-0.890) for NT-proBNP. Adjusting for clinical risk indices and cardiac biomarkers in multivariate Cox regression analysis, admission FGF23 concentrations were associated with mortality in the total study population (hazard ratio [HR] per 1 SD in $_{ln}$FGF23 1.74 [1.40-2.16]). FGF23 also reclassified patients into their correct risk strata on top of clinical variables significantly associated with outcomes in the total cohort (net reclassification index 0.386 [0.161-0.612]). In patients with acute HF, both admission FGF23 and NT-proBNP concentrations were associated with mortality.

Conclusion: Circulating FGF23 concentrations provide incremental prognostic information to established risk indices in patients with acute dyspnea, but do not improve diagnostic accuracy over NT-proBNP measurements.

Key words: FGF23; biomarkers; cardiovascular; heart failure; prognosis

1. Introduction

Acute dyspnea is a common complaint in patients admitted to hospital emergency departments (ED), causes of which range from benign conditions to potentially life-threatening disease [1]. Acute heart failure (HF) remains an important differential diagnosis in this setting and is increasingly common due to an aging population and higher prevalence of risk factors for HF development like hypertension, type 2 diabetes mellitus, and obesity [2]. Measurement of natriuretic peptides (NPs; B-type natriuretic peptide [BNP] and NT-proBNP) is a cornerstone in the evaluation of patients with acute dyspnea [3], but for prognosis novel biomarkers could add to the information provided by the NPs [4].

Fibroblast growth factor 23 (FGF23) is a hormone secreted by osteocytes and is responsible for regulation of phosphate and vitamin D (i.e. calcidiol [25(OH)D] and calcitriol [1,25(OH)2D]) homeostasis [5, 6]. Traditionally considered an indicator of declining renal function [7] and mortality [8] in patients with chronic kidney disease, concentrations of FGF23 have recently also been found associated with increased risk of cardiovascular morbidity and death, both in patients with established coronary artery disease (CAD) [9] and in the general population [10-12]. The actual mechanisms involved in relationship between concentrations of FGF23 and cardiovascular disease are yet to be resolved, as a disturbed phosphate metabolism might be both the cause and result of cardiovascular disease [13]. However, FGF23 concentrations correlate with increasing left ventricular (LV) mass and volumes [12, 14], possibly linking FGF23 directly to LV remodeling and HF development [15]. Accordingly, in this study we hypothesized that admission FGF23 concentrations could add to the information provided by NT-proBNP measurements for diagnosis and prognosis in unselected patients with acute dyspnea.

2. Methods

2.1 Akershus Cardiac Examination (ACE) 2 Study

The Akershus Cardiac Examination (ACE) 2 Study was conducted at Akershus University Hospital, Norway, from June 2009 to November 2010, and included consecutive patients presenting to the ED with acute dyspnea as their primary distress [16]. Study inclusion criteria were acute dyspnea as the cause of index hospitalization, age ≥18 years and the ability to provide informed consent. Also, patients had to be included and have blood drawn within 24 hours of hospital admission; patients with the shortest duration of stay were approached first. Patients were included only in the core working hours of the study personnel (Monday to Thursday, 08:00-14:00). Exclusion criteria were dementia or other causes rendering informed consent impossible, patients with acute myocardial infarction (AMI), coronary intervention or major surgery within the last two weeks, as well as the presence of disseminated malignant disease or other somatic disease with very short life expectancy. Also, patients with insufficient blood sampling were excluded. The study complied with the Declaration of Helsinki and was approved by the Regional Ethics Committee. All study participants provided written informed consent before study commencement.

2.2 Data collection

Data on blood pressure, heart rate and body temperature on admission, electrocardiogram (ECG), and previous medical history, were acquired from patient records. CAD was defined as prior AMI or coronary intervention. Atrial fibrillation (AF) was defined as any one of paroxysmal, persistent, or chronic atrial fibrillation. LV ejection fraction (LVEF), as well as data on LV structure and diastolic function, was assessed by routine transthoracic echocardiography (available in a total of 248 patients; 143 patients with HF, 55 patients with acute exacerbation of chronic obstructive pulmonary disease [AECOPD], and 50 patients with

other causes of acute dyspnea). Body mass index (BMI) was calculated by body weight/(height × height) (kg/m^2).

2.3 Adjudication of diagnosis and follow-up data

The index diagnosis for the patients was determined by an adjudication committee of two senior physicians, who independently reviewed all medical records, including results of supplementary examinations and follow-up data (median 464 days [interquartile range (IQR) 304-705 days] between admission and adjudication). In the case of disagreement between the adjudicators, the final diagnosis was determined by consensus. The diagnosis of HF, as well as classification of HF with reduced (HFrEF) and preserved ejection fraction (HFpEF), was based on criteria as suggested by the European Society of Cardiology [17]. The diagnosis of AECOPD was based on criteria as suggested by the Global Initiative for Chronic Obstructive Lung Disease [18]. Survival data were obtained through November 1st, 2012 from electronic hospital records, which are synchronized with Statistics Norway at monthly intervals.

2.4 Biochemical analysis

Blood samples were obtained the first working day after hospital admission (within 24 hours; *n*=314), 24 hours after initial blood sampling (*n*=234), and before discharge (*n*=96). FGF23 was measured in serum using FGF23 (C-terminal) ELISA (Biomedica, Vienna, Austria). The detection limit of the assay is 0.08 pmol/L with an intra-assay coefficient of variation of ≤12%. The median serum FGF23 concentration in apparently healthy individuals is reported to be 0.8 (range 0.2-4.2) pmol/L. Concentrations of FGF23 obtained by the assay from the current study are comparable to concentrations obtained with the Immutopics FGF23 (C-terminal) ELISA (r=0.964) [19]. Values below or above the limits of detection (0.08-220 pmol/L) were assigned a value equal to 0.08 or 220 pmol/L, respectively. NT-proBNP and cardiac troponin T (cTnT) were measured on a Cobas Platform (Roche Diagnostics, Basel,

Switzerland) using the proBNP II assay and the STAT hs Troponin T assay, respectively. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [20].

2.5 Statistical analysis

Data are given as either median (IQR) or absolute numbers (proportion) unless stated otherwise. Continuous variables were analyzed using the Related-Samples Wilcoxon Signed Rank Test or Independent-Samples Mann-Whitney U test where appropriate, and categorical variables with the Fisher exact test. Correlations were assessed by Spearman rank correlation. Univariate linear regression analyses were initially performed to identify variables associated with increasing FGF23 concentrations and these variables were subsequently explored by multivariate linear regression analysis to identify variables independently associated with high FGF23 concentrations (forward selection procedure). Kaplan-Meier survival plots were generated and survival curves compared by the log-rank test. Univariate receiver operating characteristics (ROC) analysis with area under the curve (AUC) was used to determine overall diagnostic and prognostic accuracy of biomarkers. Cox proportional hazards regression models were generated to test the relationship between biomarker concentrations and time to events. Participants were censored at the time of death or, for survivors, on November 1st, 2012. Variables significantly associated with outcomes in univariate analyses were entered in multivariate analyses using a forward selection procedure. Concentrations of NT-proBNP and FGF23 were also assessed and compared in separate models due to collinearity. Biomarker concentrations (FGF23, NT-proBNP and cTnT) were transformed using the natural logarithm prior to regression analyses due to right-skewed distributions. Continuous net reclassification index (NRI) was calculated by adding FGF23 and NT-proBNP concentrations to the basic clinical risk model obtained by the Cox models. A two-sided probability of <0.05 was considered statistically significant. IBM SPSS Statistics for Windows, version 24 (IBM Corp,

Armonk, NY, USA), was used for the statistical analyses, apart from analyses of ROC curves and C statistics, which was performed with MedCalc for Windows, version 16.2.0 (MedCalc Software, Ostend, Belgium), and calculation of NRI, which was performed with R 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1 Study population

In total, 468 patients were evaluated and 314 were found eligible for inclusion (Figure 1). The adjudicated cause of admission was HF in 143 (46%) of the patients. Of the remaining 171 patients, 84 (27% of the total study population) were adjudicated as AECOPD. The proportion of agreement between the adjudicators was 95% (κ=0.897 [0.848-0.946]). Separating the HF patients according to LVEF, 91 patients (64%) were classified as HFrEF (LVEF <50%) and 52 patients (36%) were classified as HFpEF (LVEF ≥50% and echocardiographic evidence of structural heart disease or injury and/or functional diastolic dysfunction). In our cohort, HF patients were older, and more frequently men and non-smokers (Table 1). A history of CAD, hypertension (HT), HF, AF, diabetes mellitus (DM), and renal impairment was more frequent in the HF patients, while the non-HF patients exhibited a higher prevalence of fever, leukocytosis, and increased C-reactive protein, as well as history of COPD. Also, HF patients did more frequently exhibit signs of systemic venous congestion and had lower LVEF.

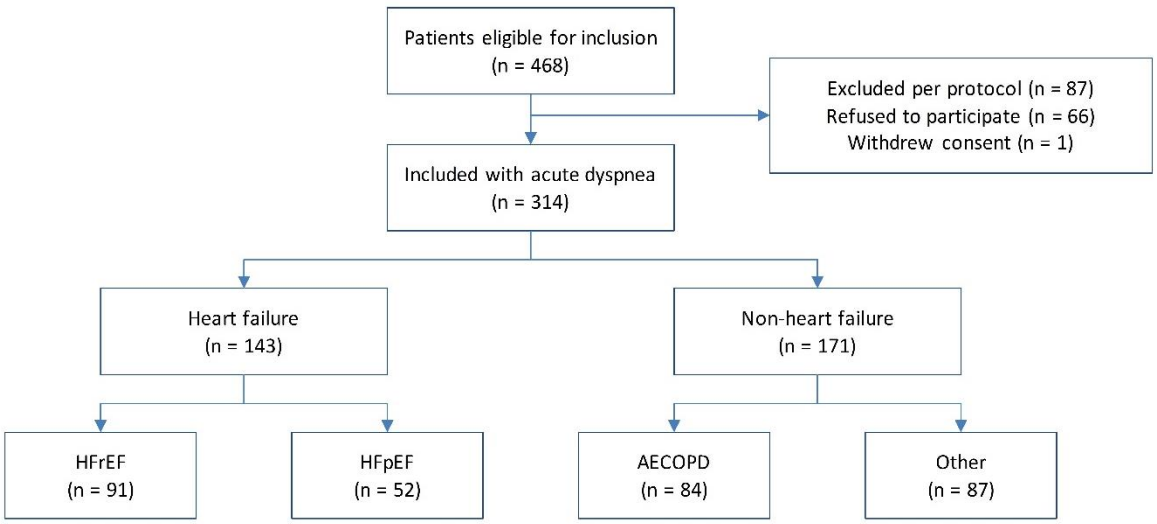

Figure 1. Flow chart of study inclusion.

Table 1. Baseline characteristics of the ACE2 Study.

	HF (*n*=143)	Non-HF (*n*=171)	*P*
Age, years (IQR)	78 (68-83)	67 (61-77)	<0.001
Male sex, *n* (%)	90 (63%)	74 (43%)	0.001
Body mass index, kg/m^2 (IQR)	26.5 (22.2-29.4)	24.8 (21.1-30.3)	0.29
Current smoking, *n* (%)	30 (21%)	55 (32%)	0.030
History of			
Coronary artery disease, *n* (%)	78 (55%)	33 (19%)	<0.001
Hypertension, *n* (%)	69 (48%)	51 (30%)	0.001
Diabetes mellitus, *n* (%)	43 (30%)	25 (15%)	0.001
Chronic obstructive pulmonary disease, *n* (%)	61 (43%)	94 (55%)	0.032
Heart failure, *n* (%)	87 (61%)	14 (8%)	<0.001
Atrial fibrillation, *n* (%)	68 (48%)	28 (16%)	<0.001
Peripheral edema, *n* (%)	77 (54%)	47 (28%)	<0.001
Pulmonary crackles, *n* (%)	83 (59%)	79 (47%)	0.041
Fever (>38°C), *n* (%)	9 (6%)	28 (16%)	0.008
Abnormal electrocardiography, *n* (%)	118 (83%)	105 (61%)	<0.001
Systolic blood pressure, mmHg (IQR)	144 (123-166)	140 (129-157)	0.67
Diastolic blood pressure, mmHg (IQR)	80 (69-92)	77 (67-89)	0.12
Heart rate, bpm (IQR)	88 (74-109)	93 (79-107)	0.19
NYHA functional class IV, *n* (%)	65 (46%)	71 (42%)	0.50
Left ventricular ejection fraction, % (IQR)	40 (30-55)	60 (50-60)	<0.001
Hemoglobin, g/mL (IQR)	13.3 (12.1-14.5)	13.6 (12.6-14.7)	0.13
Leukocytes, 10^9/L (IQR)	8.1 (6.7-11.0)	10.4 (8.1-13.2)	<0.001
C-reactive protein, mg/L (IQR)	13 (5-35)	22 (5-80)	0.026
eGFR, mL/min/1.73m^2 (IQR)	62.7 (41.9-77.5)	85.5 (68.0-97.1)	<0.001
cTnT, ng/L (IQR)	37.9 (21.8-75.3)	13.5 (4.2-25.5)	<0.001
NT-proBNP, ng/L (IQR)	3600 (1601-8396)	348 (126-1139)	<0.001
FGF23, pmol/L (IQR)	3.60 (1.24-8.77)	1.00 (0.43-2.20)	<0.001

Continuous variables are given as medians and IQR, counts are given as numbers and percentages. Differences in continuous variables were compared by the Mann-Whitney U-test and categorical data by the Fischer exact test.

eGFR, estimated glomerular filtration rate; HF, heart failure; NYHA, New York Heart Association.

3.2 FGF23 concentrations in patients with acute dyspnea

In the total study cohort (*n*=314), the median admission FGF23 concentration was 1.45 (0.60-5.09) pmol/L. Categorizing patients according to median FGF23 concentration, patients with supramedian concentrations were older with higher prevalence of cardiac comorbidities (Supplemental Table 1). Also, the prevalence of renal impairment and increased concentrations of cTnT and NT-proBNP was higher. Figure 2 displays FGF23 concentrations in all study patients, patients with adjudicated HF and AECOPD, as well as those who did and did not die during follow-up. Concentrations of FGF23 correlated with concentrations of NT-proBNP (r=0.676; P<0.001), cTnT (r=0.538; P<0.001), and LVEF (r=0.408; P<0.001), as well as a number of other clinical variables (Supplemental Table 2). eGFR, New York Heart Association (NYHA) functional class IV, and history of DM, HF, and AF were all independently associated with higher FGF23 concentrations, explaining 46% of the variance in FGF23 concentrations among patients hospitalized with acute dyspnea (Supplemental Table 3). In patients with acute HF, eGFR, NYHA functional class IV, and history of HF and AF, were independently associated with higher FGF23 concentrations (Supplemental Table 4). For patients with AECOPD, eGFR was the only variable independently associated with concentrations of FGF23 (Supplemental Table 5).

In patients with blood samples taken at all time points (admission, day 1 and discharge; *n*=89), FGF23 concentrations were significantly lower on day 1 (1.14 [0.49-3.75] pmol/L; P<0.001) and slightly increased at discharge (1.44 [0.68-3.31] pmol/L; P=0.011), compared to admission concentrations (1.38 [0.60-5.41] pmol/L). NT-proBNP concentrations decreased from admission to discharge in the total population with serial NT-proBNP measurements (*n*=88; 1807 [281-4430] vs. 837 [250-2218] ng/L; P<0.001). FGF23 concentrations remained unchanged from admission (4.61 [1.35-8.72] pmol/L) to day 1 (3.31 [1.20-6.82] pmol/L; P=0.10) in patients with acute HF (*n*=40), but were significantly

decreased on discharge (2.15 [1.13-5.01] pmol/L; *P*=0.005) compared to admission concentrations. In patients with AECOPD (*n*=27), FGF23 concentrations were reduced from admission (1.13 [0.53-2.05] pmol/L) to day 1 (0.61 [0.31-1.16] pmol/L; *P*=0.001), while FGF23 concentrations on discharge were not significantly different from admission concentrations (0.83 [0.40-1.47] pmol/L; *P*=0.24).

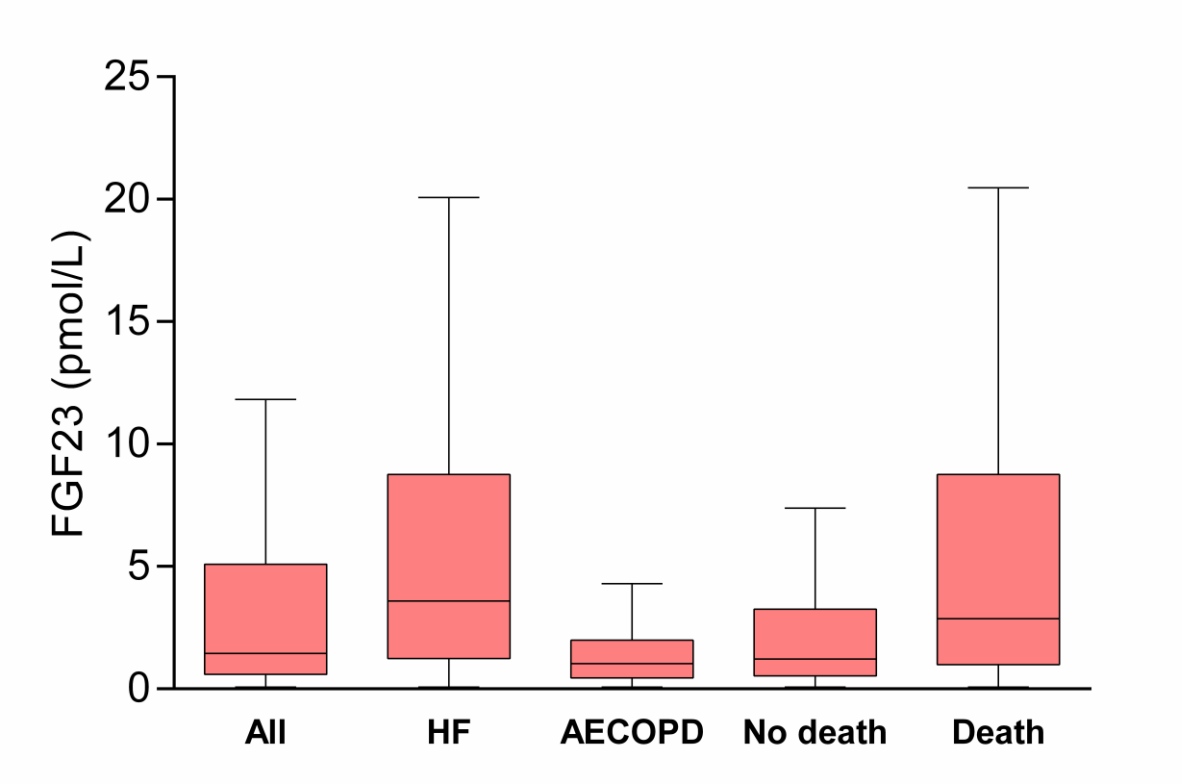

Figure 2. Concentrations of FGF23 at admission. Data presented as box (25th percentile, median, 75th percentile) and whisker (maximum of 1.5 x IQR) plots. HF, heart failure; AECOPD, acute exacerbation of chronic obstructive pulmonary disease.

3.3 FGF23 concentrations and diagnosis

Serum concentrations of FGF23 were higher in patients with acute HF compared to patients with non-HF-related dyspnea: median 3.60 (IQR 1.24-8.77) vs. 1.00 (0.43-2.20) pmol/L; *P*<0.001. The ROC-AUC of FGF23 to identify patients with acute HF in the total cohort was 0.750 (95% confidence interval [CI] 0.699-0.797) and the corresponding ROC-AUC of NT-proBNP was 0.853 (0.809-0.890). The combination of NT-proBNP and FGF23 yielded a ROC-AUC of 0.854 (0.810-0.891). In patients with an adjudicated diagnosis of HF, the ROC-

AUC to separate HFrEF from HFpEF was 0.621 (0.536-0.701) for FGF23 and 0.670 (0.587-0.747) for NT-proBNP.

3.4 FGF23 concentrations and prognosis in patients with acute dyspnea

During a median follow-up of 817 days, 114 patients died (37%). Of these, 66 deaths (46%) occurred in the HF group and 35 (42%) in the AECOPD group (*P*=0.58). Patients with the highest FGF23 concentrations had worse prognosis than patients with lower FGF23 concentrations (Figure 3; *P* by log-rank test <0.001).

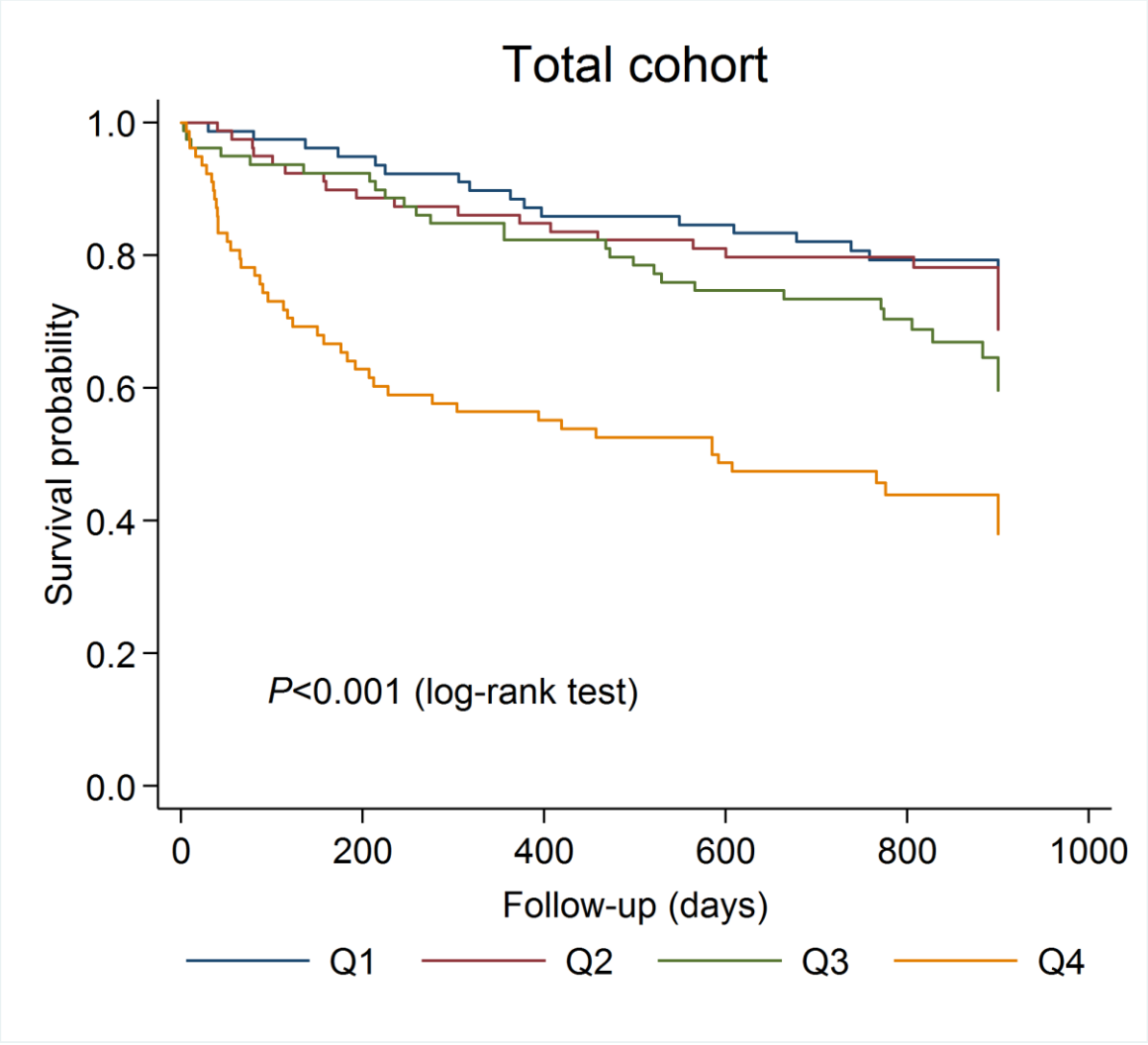

Figure 3. Cumulative survival in patients with acute dyspnea stratified by quartiles of admission FGF23 concentrations (pmol/L). Q1 <0.60, Q2 0.60-1.44, Q3 1.45-5.08, Q4 >5.08.

There was also a significant positive association between concentrations of FGF23 as a continuous variable and mortality (hazard ratio [HR] per 1 standard deviation [SD] in $_{ln}$FGF23 1.84 [95% CI] 1.54-2.22; Table 2). Adjusting for clinical variables and for cTnT and NT-proBNP concentrations in multivariate Cox regression models, admission FGF23 concentrations were still associated with mortality during follow-up: HR 1.74 (1.40-2.16). In contrast, the association between admission NT-proBNP concentrations and mortality was attenuated and no longer significant when FGF23 was included into the model (Table 2). Assessing admission FGF23 and NT-proBNP concentrations in separate models due to collinearity, both biomarkers were associated with mortality in multivariate analyses, but with higher HRs and Wald scores for FGF23 compared to NT-proBNP (Supplementary Table 6). Prognostic accuracy for mortality, as assessed by ROC-AUC, was 0.660 (0.605-0.712) for FGF23 and 0.668 (0.613-0.720) for NT-proBNP. The combination of NT-proBNP and FGF23 yielded a ROC-AUC of 0.674 (0.619-0.726). Adding admission FGF23 to a basic model of clinical variables also resulted in a significant reclassification of patients into their correct risk strata: NRI 0.386 (0.161-0.612). In contrast, admission NT-proBNP concentrations did not reclassify a significant proportion of patients on top of clinical variables: NRI 0.106 (-0.124-0.336).

Separate analyses were also performed according to adjudicated diagnoses of acute HF or AECOPD. In patients with acute HF, FGF23 concentrations were significantly higher in the patients who died during follow-up (5.54 [2.29-18.72] vs. 2.07 [0.93-7.00] pmol/L; P<0.001). Acute HF patients with FGF23 concentrations in the fourth quartile had an especially poor prognosis (Supplemental Figure 1; P by log-rank test <0.001). Admission FGF23 concentrations were also associated with mortality during follow-up in patients with acute HF as assessed by univariate (HR 1.86 [1.45-2.39]) and multivariate Cox proportional hazard regression analyses (HR 1.42 [1.07-1.89]). Admission NT-proBNP concentrations

were also retained in the final multivariate model in acute HF patients, with Wald scores of 15.2 for FGF23 and 21.6 for NT-proBNP in separate multivariate models (Supplemental Table 7). The ROC-AUC to predict mortality in patients with HF was 0.674 (0.590-0.750) for FGF23 and 0.673 (0.590-0.749) for NT-proBNP, and for the combination of both biomarkers 0.695 (0.613-0.769). The ROC-AUC of FGF23 to predict mortality in patients with HFrEF was 0.724 (0.620-0.812), and 0.642 (0.497-0.771) in patients with HFpEF. Corresponding ROC-AUCs of NT-proBNP in patients with HFrEF and HFpEF were 0.715 (0.611-0.805) and 0.709 (0.567-0.827), respectively.

Table 2. Predictors of mortality, in total cohort.

Univariate analyses	Hazard ratio	95% CI	Wald
Age	1.05	1.03 to 1.07	30.5
Male sex	1.00	0.69 to 1.44	0.0
Systolic blood pressure	0.99	0.98 to 1.00	6.3
Diastolic blood pressure	0.98	0.96 to 0.99	12.1
Heart rate	1.00	0.99 to 1.01	0.2
Body mass index	0.93	0.90 to 0.96	18.4
eGFR	0.99	0.98 to 0.99	14.0
Heart failure cause of hospitalization	1.90	1.31 to 2.75	11.4
NYHA functional class IV	1.86	1.28 to 2.69	10.8
History of:			
Coronary artery disease	1.29	0.89 to 1.88	1.80
Hypertension	1.27	0.88 to 1.84	1.60
Diabetes mellitus	1.25	0.81 to 1.93	1.03
Chronic obstructive pulmonary disease	2.15	1.46 to 3.16	14.9
Heart failure	1.95	1.35 to 2.83	12.4
Atrial fibrillation	1.71	1.18 to 2.50	7.9
$_{ln}$NT-proBNP	1.80	1.46 to 2.21	30.4
$_{ln}$cTnT	1.69	1.42 to 2.01	35.0
$_{ln}$FGF23	1.84	1.54 to 2.22	42.6

Multivariate analysis	Hazard ratio	95% CI	Wald
Age	1.03	1.01 to 1.05	6.9
Systolic blood pressure		NS	
Diastolic blood pressure	0.98	0.97 to 0.99	8.1
Body mass index	0.93	0.90 to 0.96	15.4
eGFR		NS	

Heart failure cause of hospitalization		NS	
NYHA functional class IV		NS	
History of:			
Chronic obstructive pulmonary disease	2.25	1.47 to 3.45	13.7
Heart failure		NS	
Atrial fibrillation		NS	
$_{ln}$NT-proBNP		NS	
$_{ln}$cTnT	1.39	1.11 to 1.73	8.5
$_{ln}$FGF23	1.74	1.40 to 2.16	24.8

Cox proportional hazard models. HRs per 1 SD increase in biomarkers (NT-proBNP, cTnT, and FGF23), all other variables per unit increase.
eGFR, estimated glomerular filtration rate; NS, not significant; NYHA, New York Heart Association.

None of the biomarkers (FGF23, NT-proBNP, cTnT) were associated with prognosis in patients with AECOPD (Supplemental Table 8) and admission FGF23 concentrations were not higher in non-survivors compared to survivors in AECOPD patients: 1.10 (0.44-2.31) vs. 0.89 (0.42-1.43) pmol/L; *P*=0.46). The lack of association between admission FGF23 concentrations and mortality in AECOPD patients is also demonstrated in the Supplemental Figure 1 (*P* by log-rank test=0.76).

4. Discussion

This is the first study assessing the diagnostic and prognostic properties of FGF23 in patients hospitalized with acute dyspnea. We found that concentrations of FGF23 provided robust prognostic information, especially in the subgroup of patients with acute HF, while FGF23 concentrations did not add to NT-proBNP measurements for diagnosing acute HF in unselected patients with acute dyspnea.

4.1 FGF23 in cardiovascular disease

FGF23 is a hormone produced by osteocytes in response to hyperphosphatemia, which reduces systemic phosphate concentrations by increasing renal excretion of phosphate and by lowering intestinal uptake of dietary phosphate via reduced calcitriol concentrations.[5, 6] Pertaining to cardiovascular risk factors, concentrations of FGF23 have been associated with increasing age, obesity, smoking, and HT [21], DM [22], AF, and LV dysfunction [23]. These findings are supported by the current study, as we found independent associations with DM, HF, and AF on concentrations of FGF23 in our cohort of patients hospitalized with acute dyspnea. Accordingly, increasing concentrations of FGF23 seem to be associated with processes related to the progression of cardiovascular disease, including in subjects of the general population [10] and in subjects with established cardiovascular disease [9]. However, the actual cause and effect between concentrations of FGF23 and the development of cardiovascular disease is yet to be elucidated, as disturbances in mineral metabolism, in particular phosphate, may both initiate the development of and be caused by cardiovascular disease [13]. Also, circulating FGF23 is upregulated in acute HF, but concurrent myocardial FGF23 gene expression is comparable to that of healthy individuals [24]. This further emphasizes the complex interplay between cardiac, renal and metabolic pathways in the development of cardiovascular disease.

4.2 FGF23 and prognosis

For survival analyses, concentrations of FGF23 provided strong prognostic information in the total patient cohort, including in analyses that included established cardiac biomarkers like cTnT and NT-proBNP. Of note, the association between admission NT-proBNP concentrations and mortality was attenuated and no longer significant in multivariate analysis that included FGF23. Admission FGF23 concentrations also reclassified a significant proportion of patients into their correct risk strata on top of clinical risk factors in the total patient cohort. As both FGF23 and NT-proBNP concentrations were independently associated with prognosis in patients with acute HF but not in AECOPD patients, these biomarkers primarily seem to reflect pathobiology of relevance for outcome in patients with myocardial dysfunction. The univariate prognostic accuracy was also comparable for FGF23 and NT-proBNP, both in the total cohort and in the HF patients, and we found a significant correlation between circulating FGF23 and NT-proBNP concentrations in our patients with acute dyspnea.

LV hypertrophy and dysfunction are strongly associated with unfavorable outcomes, also in asymptomatic individuals [25]. Concentrations of FGF23 have been shown to be associated with LV mass [26], and are possibly responsible for eliciting progression of LV hypertrophy [15]. The strong associations between FGF23 concentrations and mortality in our patients could therefore relate to a possible two-way relationship between LV mass and increased concentrations of FGF23. FGF23 concentrations have also been associated with increases in both end-diastolic and end-systolic volumes [14], which are characteristic features of HF, and HFrEF in particular. Both volume overload and dilatation accompany progressively failing LV systolic function, possibly reinforcing the associations between FGF23 and HFrEF. This model could explain the higher ROC-AUC for identifying high-risk patients among HFrEF patients compared to the ROC-AUC in HFpEF patients. Recently,

increased concentrations of FGF23 have also been found associated with detrimental LV remodeling following ST elevation MI [27], which supports that FGF23 concentrations correlate with LV remodeling and dilatation. FGF23 concentrations have also been associated with vascular dysfunction [28], thereby also linking FGF23 concentrations to ischemic heart disease [29] and to the development of post-AMI HF and HFrEF [30].

In our study, FGF23 concentrations were modestly correlated to LVEF compared to NT-proBNP, and comorbidities like HT, CAD, DM and AF also influenced admission FGF23 concentrations. For the patients with acute HF, eGFR, NYHA functional class, and history of HF and AF, all factors pertaining to the failing heart, were independent predictors of FGF23 concentrations. Some of the prognostic information by FGF23 concentrations may also relate to renal dysfunction and chronic inflammation, although this cannot account for all of the prognostic merit of FGF23 as these processes should have influenced outcome also in patients with AECOPD. Accordingly, our data support FGF23 primarily as a biomarker in patients with myocardial dysfunction. On the other hand, concentrations of FGF23 have been associated with inferior prognosis also in patients with HF of nonischemic origin [31], meriting further investigation of the pathobiology of FGF23 in cardiovascular disease.

4.3 FGF23 and diagnosis

Although our data support FGF23 as a biomarker primarily associated with myocardial dysfunction in patients with acute dyspnea, admission FGF23 concentrations did not improve diagnostic accuracy for HF over the information provided by NT-proBNP alone. Considering the extra-cardiac sources and non-specific physiology of FGF23 this finding was not entirely unexpected, as increased concentrations of FGF23 reflect several pathophysiological processes apart from those involving the failing myocardium. Especially, as concentrations of FGF23 are closely related to renal function, the diagnostic properties of FGF23 on HF may very well be disturbed by the cardiac-renal interplay present in patients with HF, chronic renal

disease, or both. Supramedian concentrations of FGF23 had median eGFR 60.9 (42.0-76.7) mL/min/1.73m^2, which qualifies as chronic renal disease, and this also supports the correlation between circulating FGF23 concentrations and renal dysfunction. In contrast, NT-proBNP is released in response to hemodynamic stress (ventricular dilatation, hypertrophy and/or increased wall tension), which are all hallmark features of HF [32] and therefore also explains the superior diagnostic properties of NT-proBNP over FGF23 concentrations in unselected patients with acute dyspnea.

4.4 Strengths and limitations

Several strengths and limitations of the current study merit mentioning. All diagnoses were adjudicated by a dedicated committee, as previously recommended [33]. The proportion of agreement between the adjudicators was also high, exceeding that previously reported in similar studies [34]. Both biobanking and collection of patient related information were collected in a uniform fashion by dedicated personnel. Echocardiographic data was, however, obtained from routine clinical examinations and not according to study protocols by study personnel. For this reason, more detailed analyses on the influence of other echocardiographic indices such as LV mass was also deemed inappropriate due to unstandardized protocols. The ACE2 Study was also a relatively short-lasting single-center study, resulting in a moderate sample size. It is also important to acknowledge that the associations between concentrations of FGF23 and cardiovascular disease are not yet fully elucidated. Accordingly, the present data remains hypothesis generating and should be interpreted as so. However, as both patients with HF and patients with non-HF dyspnea had increased concentrations of FGF23 compared to healthy individuals, the potential of FGF23 as a biomarker in these specific patient groups should be further explored. Moreover, larger studies with repeat blood sampling among patients with HF and non-HF dyspnea could also provide additional insight into the

pathophysiological mechanisms responsible for FGF23 release in patients hospitalized with acute dyspnea.

5. Conclusion

Concentrations of FGF23 are elevated in patients with acute HF and provide additional prognostic information to established risk indices and biomarkers in unselected patients with acute dyspnea. In contrast, FGF23 concentrations do not improve diagnostic accuracy over the information by NT-proBNP measurements alone in this patient cohort.

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Conflict of interest

FGF23 ELISA was sponsored by Biomedica (Vienna, Austria) and cTnT and NT-proBNP by Roche (Basel, Switzerland). The sponsors had no role in any of the following: design and conduct of the study, collection, management, analysis and interpretation of the data, or preparation, review and approval of the manuscript.

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Supplemental material

to

Fibroblast growth factor 23 in patients with acute dyspnea: The Akershus Cardiac Examination (ACE) 2 Study

Magnus Nakrem Lyngbakken MD, PhD[a,b]; Mohammad Osman Pervez MD[a,b]; Janne Sølvernes MSc[a]; Jon Brynildsen MD[a,b]; Arne Didrik Høiseth MD, PhD[b]; Geir Christensen MD, PhD, MHA[b,c]; Torbjørn Omland MD, PhD, MPH[a,b]; Helge Røsjø MD, PhD[a,b]

[a]Division of Medicine, Akershus University Hospital, Lørenskog, Norway

[b]Center for Heart Failure Research, University of Oslo, Oslo, Norway

[c]Institute for Experimental Medical Research, Oslo University Hospital, Ullevål, Oslo, Norway

Supplemental tables

Supplemental Table 1. Baseline characteristics according to concentrations of FGF23.

	FGF23		
	<1.45 pmol/L	≥1.45 pmol/L	*P*
Age, years (IQR)	66 (60-76)	78 (68-84)	<0.001
Male sex, *n* (%)	77 (49%)	87 (55%)	0.31
Body mass index, kg/m^2 (IQR)	25.2 (21.0-30.2)	26.5 (22.2-29.6)	0.34
Current smoking, *n* (%)	53 (34%)	32 (20%)	0.011
History of			
Coronary artery disease, *n* (%)	37 (24%)	74 (47%)	<0.001
Hypertension, *n* (%)	50 (32%)	70 (45%)	0.027
Diabetes mellitus, *n* (%)	23 (15%)	45 (29%)	0.004
Chronic obstructive pulmonary disease, *n* (%)	86 (55%)	69 (44%)	0.071
Heart failure, *n* (%)	21 (13%)	80 (51%)	<0.001
Atrial fibrillation, *n* (%)	21 (13%)	75 (48%)	<0.001
Peripheral edema, *n* (%)	36 (23%)	88 (56%)	<0.001
Pulmonary crackles, *n* (%)	63 (40%)	99 (64%)	<0.001
Fever (>38°C), *n* (%)	19 (12%)	18 (12%)	>0.99
Abnormal electrocardiography, *n* (%)	98 (62%)	125 (80%)	0.001
Systolic blood pressure, mmHg (IQR)	142 (129-161)	141 (127-162)	0.58
Diastolic blood pressure, mmHg (IQR)	80 (69-90)	77 (67-92)	0.62
Heart rate, bpm (IQR)	92 (80-108)	88 (76-108)	0.44
NYHA functional class IV, *n* (%)	57 (36%)	79 (50%)	0.017
Left ventricular ejection fraction, % (IQR)	58 (45-60)	45 (30-60)	<0.001

Hemoglobin, g/mL (IQR)	13.9 (12.9-15.0)	13.1 (12.0-14.3)	<0.001
Leukocytes, 10^9/L (IQR)	9.4 (7.6-11.9)	9.3 (7.2-12.2)	0.97
C-reactive protein, mg/L (IQR)	10 (1-45)	24 (9-50)	<0.001
eGFR, mL/min/1.73m^2 (IQR)	87.3 (70.7-98.1)	60.9 (42.0-76.7)	<0.001
cTnT, ng/L (IQR)	14.0 (4.2-26.6)	34.0 (18.4-73.8)	<0.001
NT-proBNP, ng/L (IQR)	346 (123-991)	3361 (1167-7811)	<0.001

Continuous variables are given as medians and IQR, counts are given as numbers and percentages. Differences in continuous variables were compared by the Mann-Whitney U-test and categorical data by the Fischer exact test.
eGFR, estimated glomerular filtration rate; NYHA, New York Heart Association.

Supplemental Table 2. Baseline variables associated with concentrations of FGF23.

	Correlation coefficient[a]	*P*
Age	0.419	<0.001
Male sex	0.067	0.23
Body mass index	0.003	0.96
Current smoking	-0.108	0.06
History of		
Coronary artery disease	0.314	<0.001
Hypertension	0.148	0.009
Diabetes mellitus	0.211	<0.001
Chronic obstructive pulmonary disease	-0.049	0.38
Heart failure	0.492	<0.001
Atrial fibrillation	0.414	<0.001
Systolic blood pressure	-0.071	0.21
Diastolic blood pressure	-0.063	0.27
Heart rate	0.064	0.26
Left ventricular ejection fraction	-0.408	<0.001
Hemoglobin	-0.248	<0.001
Leukocytes	-0.076	0.18
C-reactive protein	0.160	0.005
eGFR[b]	-0.579	<0.001
cTnT	0.538	<0.001
NT-proBNP	0.676	<0.001

[a] Spearman rank correlation
eGFR, estimated glomerular filtration rate

Supplemental Table 3. Determinants of increased concentrations of $_{ln}$FGF23.

	$_{ln}$FGF23		
Univariate analyses	B	95% CI	t
Age	0.044	0.033 to 0.056	7.55
Male sex	0.148	-0.206 to 0.501	0.82
Systolic blood pressure	-0.005	-0.011 to 0.001	-1.68
Diastolic blood pressure	-0.006	-0.017 to 0.005	-1.15
Heart rate	-0.003	-0.011 to 0.004	-0.85
Body mass index	-0.003	-0.028 to 0.022	-0.23
eGFR	-0.036	-0.042 to -0.030	-12.01
Heart failure cause of hospitalization	1.37	1.05 to 1.69	8.43
NYHA functional class IV	0.57	0.22 to 0.92	3.19
History of:			
Coronary artery disease	1.11	0.76 to 1.45	6.24
Hypertension	0.49	0.13 to 0.85	2.66
Diabetes mellitus	0.90	0.49 to 1.32	4.27
Chronic obstructive pulmonary disease	-0.17	-0.52 to 0.19	-0.93
Heart failure	1.68	1.35 to 2.01	10.08
Atrial fibrillation	1.38	1.03 to 1.73	7.75
	$_{ln}$FGF23 (r^2=0.46)		
Multivariate analysis	B	95% CI	t
Age		NS	
eGFR	-0.024	-0.030 to -0.018	-7.79
Heart failure cause of hospitalization		NS	
NYHA functional class IV	0.46	0.19 to 0.72	3.40
History of:			
Coronary artery disease		NS	
Hypertension		NS	
Diabetes mellitus	0.34	0.011 to 0.67	2.03
Heart failure	0.84	0.52 to 1.16	5.15
Atrial fibrillation	0.66	0.36 to 0.97	4.24

Linear regression models.
B, unstandardized coefficient; eGFR, estimated glomerular filtration rate; NS, not significant; NYHA, New York Heart Association;

Supplemental Table 4. Determinants of increased concentrations of $_{ln}$FGF23, in patients with adjudicated diagnosis of heart failure.

	$_{ln}$FGF23		
Univariate analyses	B	95% CI	t
Age	0.021	-0.003 to 0.044	1.71
Male sex	-0.30	-0.85 to 0.26	-1.06
Systolic blood pressure	-0.010	-0.019 to -0.002	-2.49
Diastolic blood pressure	-0.015	-0.030 to -0.001	-2.06
Heart rate	-0.003	-0.013 to 0.007	-0.59
Body mass index	-0.011	-0.056 to 0.034	-0.48
eGFR	-0.030	-0.041 to -0.020	-5.90
NYHA functional class IV	0.79	0.26 to 1.31	2.96
History of:			
Coronary artery disease	0.96	0.44 to 1.47	3.66
Hypertension	0.13	-0.41 to 0.66	0.46
Diabetes mellitus	0.71	0.14 to 1.29	2.45
Chronic obstructive pulmonary disease	-0.11	-0.66 to 0.43	-0.41
Heart failure	1.47	0.98 to 1.97	5.90
Atrial fibrillation	0.80	0.28 to 1.33	3.05
	$_{ln}$FGF23 (r^2=0.36)		
Multivariate analysis	B	95% CI	t
Systolic blood pressure		NS	
Diastolic blood pressure		NS	
eGFR	-0.022	-0.031 to -0.012	-4.38
NYHA functional class IV	0.60	0.16 to 1.05	2.71
History of:			
Coronary artery disease		NS	
Diabetes mellitus		NS	
Heart failure	1.00	0.52 to 1.47	4.14
Atrial fibrillation	0.50	0.05 to 0.95	2.21

Linear regression models.
B, unstandardized coefficient; eGFR, estimated glomerular filtration rate; NS, not significant; NYHA, New York Heart Association;

Supplemental Table 5. Determinants of increased concentrations of $_{ln}$FGF23, in patients with adjudicated diagnosis of AECOPD.

	$_{ln}$FGF23		
Univariate analyses	B	95% CI	t
Age	0.028	0.001 to 0.056	2.07
Male sex	0.081	-0.43 to 0.60	0.32
Systolic blood pressure	-0.007	-0.016 to 0.002	-1.48
Diastolic blood pressure	-0.006	-0.023 to 0.010	-0.76
Heart rate	-0.008	-0.022 to 0.006	-1.13
Body mass index	0.012	-0.029 to 0.053	-0.56
eGFR	-0.026	-0.038 to -0.014	-4.36
NYHA functional class IV	0.28	-0.23 to 0.79	1.09
History of:			
Coronary artery disease	0.14	-0.43 to 0.71	0.49
Hypertension	0.28	-0.27 to 0.82	1.01
Diabetes mellitus	0.66	-0.14 to 1.47	1.64
Heart failure	0.49	-0.33 to 1.30	1.19
Atrial fibrillation	0.82	0.18 to 1.46	2.56
	$_{ln}$FGF23 (r^2=0.19)		
Multivariate analysis	B	95% CI	t
Age		NS	
eGFR	-0.026	-0.038 to -0.014	-4.36
History of:			
Atrial fibrillation		NS	

Linear regression models.
B, unstandardized coefficient; eGFR, estimated glomerular filtration rate; NS, not significant; NYHA, New York Heart Association;

Supplemental Table 6. Predictors of mortality, in total cohort. Separate multivariate models for NT-proBNP and FGF23.

Multivariate analysis NT-proBNP	Hazard ratio	95% CI	Wald
Age	1.03	1.01 to 1.06	9.4
Systolic blood pressure	0.99	0.98 to 1.00	11.4
Diastolic blood pressure		NS	
Body mass index	0.94	0.91 to 0.98	10.2
eGFR		NS	
Heart failure cause of hospitalization		NS	
NYHA functional class IV		NS	
History of:			
Chronic obstructive pulmonary disease	2.05	1.36 to 3.07	11.8
Heart failure		NS	
Atrial fibrillation		NS	
$_{ln}$cTnT	1.35	1.07 to 1.69	6.5
$_{ln}$NT-proBNP	1.40	1.08 to 1.82	6.3

Multivariate analysis FGF23	Hazard ratio	95% CI	Wald
Age	1.03	1.01 to 1.05	6.9
Systolic blood pressure		NS	
Diastolic blood pressure	0.98	0.97 to 0.99	8.1
Body mass index	0.93	0.90 to 0.96	15.4
eGFR		NS	
Heart failure cause of hospitalization		NS	
NYHA functional class IV		NS	
History of:			
Chronic obstructive pulmonary disease	2.25	1.47 to 3.45	13.7
Heart failure		NS	
Atrial fibrillation		NS	
$_{ln}$cTnT	1.39	1.11 to 1.73	8.5
$_{ln}$FGF23	1.74	1.40 to 2.16	24.8

Cox proportional hazard models. HRs per 1 SD increase in biomarkers (NT-proBNP, cTnT, and FGF23), all other variables per unit increase.
eGFR, estimated glomerular filtration rate; NS, not significant; NYHA, New York Heart Association.

Supplemental Table 7. Predictors of mortality, in patients with adjudicated diagnosis of heart failure.

Univariate analysis	Hazard ratio	95% CI	Wald
Age	1.04	1.02 to 1.07	9.4
Male sex	1.90	1.17 to 3.09	6.6
Systolic blood pressure	0.99	0.98 to 1.00	8.3
Diastolic blood pressure	0.97	0.96 to 0.99	11.3
Heart rate	1.00	0.99 to 1.01	0.7
Body mass index	0.94	0.89 to 0.99	6.3
eGFR	0.98	0.97 to 0.99	9.7
NYHA functional class IV	2.01	1.23 to 3.29	7.7
Left ventricular ejection fraction	1.00	0.98 to 1.02	0.03
History of:			
Coronary artery disease	1.05	0.65 to 1.70	0.03
Hypertension	0.83	0.51 to 1.36	0.5
Diabetes mellitus	1.78	1.08 to 2.95	5.1
Chronic obstructive pulmonary disease	1.85	1.14 to 3.01	6.1
Heart failure	1.43	0.86 to 2.38	1.9
Atrial fibrillation	1.07	0.66 to 1.74	0.08
$_{ln}$NT-proBNP	2.22	1.50 to 3.28	16.0
$_{ln}$cTnT	1.45	1.12 to 1.88	8.0
$_{ln}$FGF23	1.86	1.45 to 2.39	23.7

Multivariate analysis NT-proBNP	Hazard ratio	95% CI	Wald
Age	1.05	1.02 to 1.08	9.3
Male sex	1.70	1.03 to 2.82	4.3
Systolic blood pressure	0.98	0.98 to 0.99	12.7
Diastolic blood pressure		NS	
Body mass index		NS	
eGFR		NS	
NYHA functional class IV		NS	
History of:			
Diabetes mellitus	2.62	1.51 to 4.53	11.9
Chronic obstructive pulmonary disease	2.49	1.45 to 4.28	11.0
$_{ln}$cTnT		NS	
$_{ln}$NT-proBNP	2.85	1.83 to 4.42	21.6

Multivariate analysis FGF23	Hazard ratio	95% CI	Wald
Age	1.04	1.01 to 1.07	6.0
Male sex		NS	
Systolic blood pressure		NS	

Diastolic blood pressure	0.98	0.96 to 1.00	5.6
Body mass index	0.94	0.89 to 0.99	5.8
eGFR		NS	
NYHA functional class IV		NS	
History of:			
Diabetes mellitus	1.83	1.05 to 3.19	4.5
Chronic obstructive pulmonary disease	2.19	1.26 to 3.60	7.8
$_{ln}$cTnT	1.36	1.03 to 1.79	4.6
$_{ln}$FGF23	1.75	1.32 to 2.31	15.2

Multivariate analysis NT-proBNP/FGF23	Hazard ratio	95% CI	Wald
Age	1.04	1.01 to 1.07	6.7
Male sex	1.66	1.00 to 2.77	3.8
Systolic blood pressure		NS	
Diastolic blood pressure	0.98	0.96 to 0.99	7.0
Body mass index		NS	
eGFR		NS	
NYHA functional class IV		NS	
History of:			
Diabetes mellitus	1.98	1.12 to 3.50	5.4
Chronic obstructive pulmonary disease	2.33	1.35 to 4.04	9.2
$_{ln}$NT-proBNP	2.20	1.35 to 3.58	10.1
$_{ln}$cTnT		NS	
$_{ln}$FGF23	1.42	1.07 to 1.89	5.7

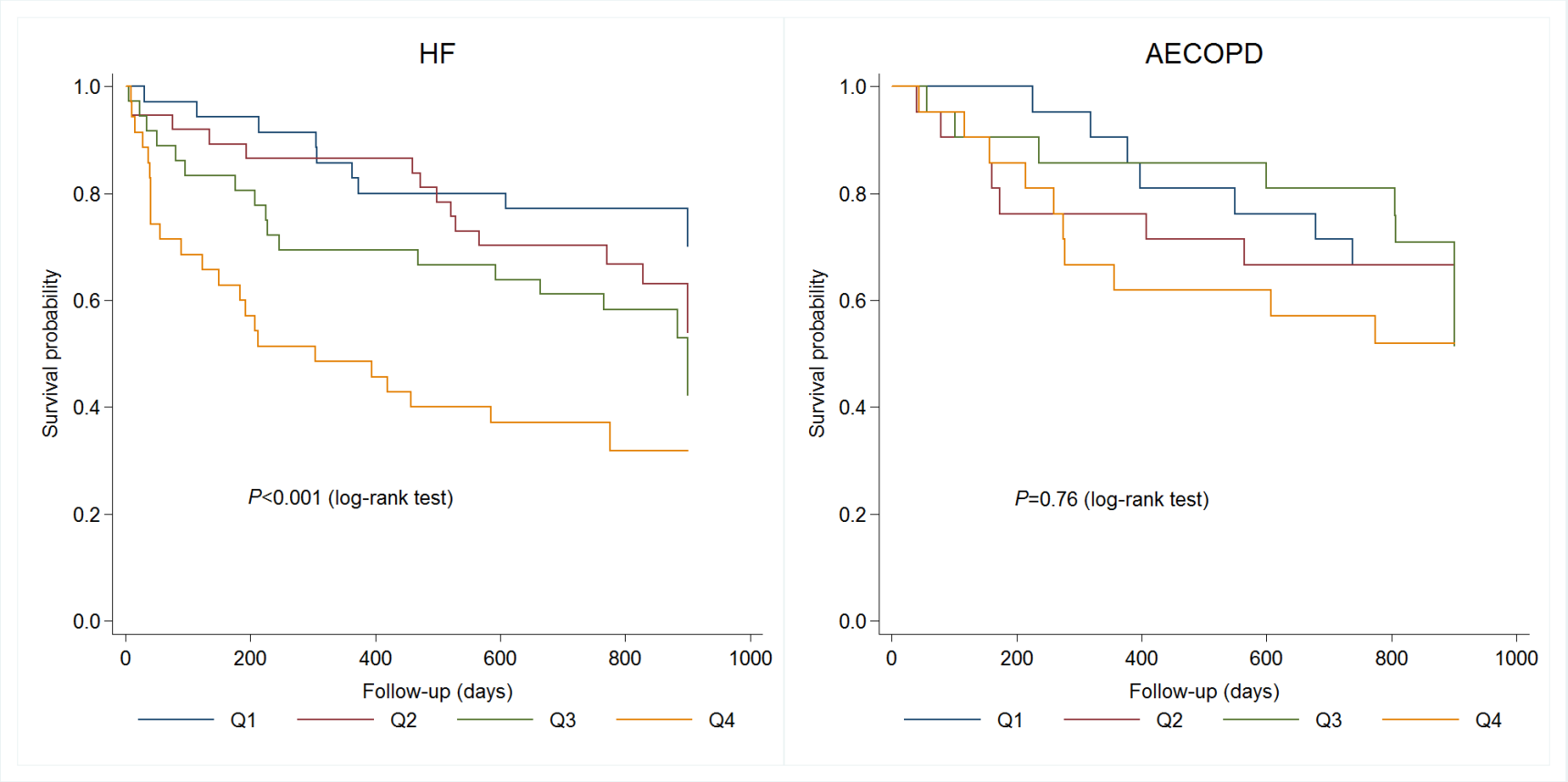
Cox proportional hazard models. HRs per 1 SD increase in biomarkers (NT-proBNP, cTnT, and FGF23), all other variables per unit increase.
eGFR, estimated glomerular filtration rate; NS, not significant; NYHA, New York Heart Association.

Supplemental Table 8. Predictors of mortality, in patients with adjudicated diagnosis of AECOPD.

Univariate analysis	Hazard ratio	95% CI	Wald
Age	1.03	0.99 to 1.07	2.7
Male sex	0.52	0.27 to 1.02	3.7
Systolic blood pressure	0.99	0.98 to 1.01	1.7
Diastolic blood pressure	0.99	0.97 to 1.01	1.1
Heart rate	1.01	0.99 to 1.03	1.0
Body mass index	0.90	0.84 to 0.96	9.3
eGFR	1.00	0.98 to 1.02	0.1
NYHA functional class IV	1.11	0.56 to 2.19	0.1
History of:			
Coronary artery disease	0.91	0.43 to 1.95	0.1
Hypertension	1.08	0.53 to 2.20	0.0
Diabetes mellitus	0.46	0.11 to 1.93	1.1
Heart failure	2.21	0.85 to 5.74	2.6
Atrial fibrillation	1.96	0.91 to 4.21	3.0
$_{ln}$NT-proBNP	1.14	0.74 to 1.76	0.3
$_{ln}$cTnT	1.49	0.91 to 2.42	2.5
$_{ln}$FGF23	1.26	0.81 to 1.97	1.1

Cox proportional hazard models. HRs per 1 SD increase in biomarkers (NT-proBNP, cTnT, and FGF23), all other variables per unit increase.
AECOPD, acute exacerbation of chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; NYHA, New York Heart Association.

Supplemental figures



Supplemental Figure 1. Cumulative survival in patients with adjudicated diagnoses of acute heart failure (*n*=143) and acute exacerbation of chronic obstructive pulmonary disease (*n*=84) stratified by quartiles of admission FGF23 concentrations (pmol/L). HF: Q1 <1.24, Q2 1.24-3.59, Q3 3.60-8.76, Q4 >8.76. AECOPD: Q1 <0.45, Q2 0.45-1.02 Q3 1.03-1.98, Q4 >1.98.